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Bioactivity of wine prepared from ripened and over-ripened kiwifruit

Research Article

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Abstract: In order to evaluate the fruit maturity value of 'Hayward' kiwifruit (*Actinidia deliciosa*) as material for wine production, ripened and over-ripened fruit samples were analyzed. In addition, the effect of pectinase enzyme treatment on physicochemical characteristics of the wine during the fermentation process was also examined. The results showed that wine production from ripening kiwifruit took almost twice as long as from over-ripened fruit. The yield of wine production was increased from 63.35% to 66.19% by using riper kiwifruits and adding pectinase before amelioration. The soluble solid content (13.5%) in the wine produced from over-ripened fruits increased in comparison with the wine produced from ripened samples (10.3%). In both groups there was a decrease to 5° Brix at the end of fermentation. Difference of fruit maturity did not have a significant effect on acidy, pH, nor color of wine. The highest scores of total acceptance estimated during fermentation were for wine made from over-ripened fruits. Total phenolics content and antioxidant activities were similar in both wine groups while the mineral content, especially potassium, in wine made from over-ripened kiwifruit was higher than wine produced from ripened fruits. In conclusion, the quality characteristics of kiwifruit wine made from over-ripened fruit treated with pectinase showed higher values of wine in many aspects such as sensory value, alcohol and total phenolics content, antioxidant activity, minerals and production yield.

Keywords: *Kiwifruit • Wine • Fermentation • Ripened • Over-ripened • Antioxidant activity • Total phenolics content • Minerals and production yield*

1. Introduction

Epidemiological studies suggest that diets supplemented with moderate quantities of certain alcoholic beverages could contribute to prevention of atherosclerosis. This phenomena was explained as the "French paradox" [1-4]. The cardioprotective effect of some alcoholic beverages is related to the presence of biologically active antioxidant compounds, ethanol, and phenolics, which are able to prevent oxidation of LDL-C [4,5]. For example, wine contains polyphenols, which are natural antioxidants, in the non-alcoholic fraction [6]. The profiles and evaluation of organic acid and polyphenol content are important parameters in winemaking. The level of organic acids is an indicator of the fermentation process, as the acids contribute to flavour balance, chemical stability and microbiological control. In addition, polyphenols and metals have effects on the organoleptic characteristics (colour, flavour and taste of wine). These parameters are affected by several factors such as ripening, variety, growing region, and fermentation process [7-12].

Kiwifruits are typically placed on market shelves when they are not yet ripe, and are sold until they become ripe. Over-ripe kiwifruits are generally not preferred and are therefore sold at a lower price. However, over-ripe

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kiwifruits are abundant in both nutrient and functional compounds and are therefore well-suited for wine processing. Over-ripe kiwifruits contain more sugar and a higher diversity of organic acids than unripe and ripe fruits, and as a result are preferable as a substrate for wine production. Enzyme treatment during wine preparation can further improve wine quality. The function of pectinase is to aid in breaking down the fruit tissues and in doing so can enhance the extraction of flavors from the mash, resulting in improved flavor and more intense colors in the final product. Pectinase also contributes to the wine-making process by accelerating the pre-fermentation stages. In addition, the presence of pectin in finished wine causes a haze or slight cloudiness, therefore pectinase is important in the overall improvement of must quality.

Kiwifruit wine quality is determined by a variety of factors, including composition of different fruit tissues (flesh, skin, and seed) as well as the winemaking process itself. Optimal maturity refers to optimal indices of moisture, protein, lipid, ash and fiber. Indicators of maturity include sugar content, acidity, fruit size, phenolic content, antioxidant levels and taste. Three types of maturity can be distinguished: technological, phenolic and aromatic. Technological maturity is the stage when sugar accumulation in flesh is high and acidity is low [13]. The extent of fruit ripening affects kiwifruit wine quality. Therefore, the maturity degree of kiwifruits selected for wine production is very important. In addition, pectinase treatment increases must yield from fruit [14,15]. There is little information about the effects of maturity level and pectinase enzyme treatment on the characteristics and quality of kiwifruit wine. Therefore, studying the effect of overripe fruit maturity on the quality of wine will contribute to our knowledge of wine production. In this report we describe comparative studies on the quality of wine made from different maturity degrees between ripening and over-ripening fruits. The technology described can be applied to white table wines with consistent high quality produced from kiwifruit.

2. Experimental Procedures

2.1 Sample preparation

Kiwifruits ('Hayward' cultivar) were harvested at a commercial maturity stage from Muan county orchard (Jeonnam, Korea) in October 2009, and immediately transferred to storage at 0°C in Pomology Laboratory, Mokpo National University. Fruits were ripened for one week at room temperature until their soluble solid content increased to 12% and 15° Brix (ripened and overripened, respectively).

The AOAC method [16] was used to analyze the proximate composition of kiwifruit flesh. Water content was measured by the atmospheric pressure heat drying method at 105°C, crude protein content by the Kjeldahl nitrogen quantitative method, crude lipid by the Soxhlet extraction method, crude fiber by the fritted glass crucible method, and crude ash by the direct-ashing method. The contents of the studied indices are expressed in percentages.

2.2 Wine processing methods

Sample preparation and pectinase treatment protocols were adapted from Towantakavanit *et al.* [17] for making wine 10 L×batch^{-1}. Firmness, SSC and pH were measured to characterize wine quality. Kiwifruits were washed and coarsely pulped in a blender to obtain a 6.3 kg sample. 4.5 g of tartaric acid was added to the sample to adjust the pH in order to increase the color intensity and aging potential of the wine (tartaric acid is not the major organic acid in kiwifruit wine). Yeast nutrients, including ammonium phosphate, were added to the blended kiwifruit and put into a primary jar. In order to study the effects of enzyme treatment on kiwifruit wine production, samples were treated with pectinase enzyme for 24 hours and then were ameliorated to standard sugar and acid contents by sucrose addition.

2.3 Amelioration

1540 g sucrose was combined with 2.7 L hot water. The resulting syrup was poured into the primary jar onto the blended kiwifruits, covered, and set aside to cool. The first sterilization of the mass used for amelioration was done with the hot water at 100°C which was used to dissolve sugar. Second sterilization was carried out after filtration by hot filling at 85°C for 3 min. In our previous experiments, 200 ppm of potassium metabisulphite was added after 8 weeks of fermentation [17]. However, because potassium metabisulphite poses a potential health hazard, a hot filling method was used in the present study.

2.4 Fermentation

Saccharomyces bayanus Lavin strain EC1118 (Prise de Mousse, Canada) was used, in dry form, as the fermenting agent. 3 g of yeast was dissolved in 15 mL of water at 40°C. The mixture was stirred and the container was then refitted with an airlock. The samples were incubated for 2 h. Water was added for a total volume of fermentation of 10 L. Samples were micro-filtered with a pore size of 0.25 μm. In order to determine the efficacy of the yeast, fermentation results were compared by using three different commercial yeast strains (*Saccharomyces cerevisiae* Gervin No.5

strain (GVN, France), *S. bayanus* Lavin EC1118 strain (Prise de Mousse, Canada) and *S. cerevisiae* Red Star Davis No.796 (Premier Cuvée) Lesaffre Yeast Corporation, Milwaukee, USA). The samples were fermented at 14°C for 2 weeks. The fermented samples were then transferred to a secondary container in order to develop the quality and reduce the risk of sluggish fermentation, and were incubated for an additional 2 weeks at 14°C. After 4 total weeks of fermentation, samples were transferred to another container and were incubated for 3 months until the concentration of reducing sugars was less than 5%. The samples were filtered, bottled, and stored at 14°C until analysis. Samples were collected every week throughout the first 4 weeks, and every 4 weeks thereafter until the end of fermentation (16 weeks). The kiwifruit juice or wine was analyzed for soluble solid content (SSC) using a refractometer (Model 0-32° Brix, Atago, Japan) and was expressed in °Brix units. The alcohol content was analyzed using the Gay Lussac Table by distilling and adjusting 100 mL of fermented sample to 15°C. pH was measured with a pH meter (Model 720A, Orion,USA). Total acidity (TA) was measured by titrating a sample (4 mL of juice or wine diluted with 20 mL of distilled water) with 0.1 N NaOH. Wine color was measured with a CR-400 Chroma meter equipped with CR-S4w utility software (Konica Minolta Sensing Inc., Japan) and expressed as ‘L’, ‘a’, and ‘b’.

2.5 Total phenols

The total phenol content was determined using a spectrophotometer (Hewlett-Packard, model 8452A, Rockvile, USA) with measurements at 765 nm according to the Folin-Ciocalteu [18] colorimetric method. The total phenol content was calculated from the calibration curve, using gallic acid as a standard. The results were expressed as mg of gallic acid equivalents per 100 g fresh fruit or per L.

2.6 Antioxidant activity

Routine chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The antioxidant activities were determined using 2,2’-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) with potassium persulfate ($K_2S_2O_8$) [19]. ABTS radical cation was generated by the interaction of ABTS (250 mM) and $K_2S_2O_8$ (40 mM). The percent decrease of absorbance at 734 nm was calculated and plotted as a function of the concentration of the extracts and of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) for the standard reference. Data in terms of the Trolox equivalent antioxidant coefficient were calculated (TEAC).

2.7 Minerals

Filtered samples were analyzed for mineral content (P, K, Ca, Mg, Na, and Fe) using an atomic absorption spectrophotometer (AVANTA, GBC Scientific Equipment Pty Ltd, Australia).

2.8 Organic acids

Organic acids were determined by HPLC. The system was equipped with Supelcogel H HPLC column (9 µm, 4.6x250 mm) and UV/Vis detector (Waters, USA) according to the method described in [12] with slight modifications. The acids were monitored at 214 nm. Isocratic elution was performed using 0.01 mol L^{-1} of potassium dihydrogen phosphate solution (pH 2.8) at a flow rate of 1.0 mL min^{-1} for 20 min at 35°C. Identification and quantitation was done by comparison of the sample peaks with those of external standard (quinic, malic, citric, succinic, lactic, and galacturonic acid). The injection volume was 10 µL for each wine sample.

2.9 Sensory evaluation

Sensory analyses were performed on the wine samples following fermentation. Sensory evaluation was carried out in a sensory laboratory by 12 qualified panellists. Taste quality of wine was evaluated by affective test of sweetness, sourness, bitterness, astringency, and total acceptation in Hedonic scale method which was 1-5 rating scale (1=severely bad, 2=bad, 3=moderate, 4=good, and 5=excellent).

2.10 Statistical analysis

To verify the statistical significance of the studied parameters, means ± SD of three measurements were determined. P-values of <0.05 were considered significant.

3. Results and Discussion

3.1 Proximate composition of kiwifruit

The physical properties of the ‘Hayward’ cultivar are shown in the Table 1. The proximate composition of ripened kiwifruit was determined to be 80.14% moisture, 1.07% protein, 0.31% lipid, 0.63% ash, and 2.87% fiber (Table 2). Over-ripened fruit contained less moisture, crude fat and crude fiber, but more crude protein and crude ash. However, the differences in all five cases were not significant (P>0.05).

Differences between the quality characteristics of wine made from ripe *versus* over-ripe kiwifruit are shown in Figure 1. The soluble solid content (SSC) started at 22° Brix for both ripening and over-ripened kiwifruit. The SSC of both decreased while the alcohol

Cultivar	Size (mm)		Weight (g)			
	Height	Diameter	Whole fruit	Flesh	Skin	Seed
'Hayward'	63.93±3.20	51.41±2.57 43.49±2.17	95.63±4.69 (100%)	82.49±3.92 (86.25%)	11.27±0.67 (11.79%)	1.87±0.09 (1.96%)

Table 1. Physical properties of 'Hayward' kiwifruit .

Values are means±SD (n=3).

production increased, especially in the first week after fermentation. The alcohol content of wine produced from over-ripe samples was 9% whereas that of ripened kiwifruit was 5.5%. Similar results were described by Withy and Lodge [20] regarding kiwifruit wine production using un-ripened and ripening fruits as raw material. The result showed that juice from un-ripened fruit took twice as long to ferment as that from ripened fruit. At the end of fermentation, the SSC of both samples was reduced to 5° Brix. However, the alcohol content of the over-ripened sample after fermentation was 12.5%, while the alcohol content of the ripening sample was only 10.3%. Difference in fruit maturity did not have a significant effect on total acidity. During fermentation, the total acidity of the samples using ripened fruit and over-ripened fruit were 0.53% and 0.59%, respectively, at initial measurement, and slightly increased until 16 weeks. At the end of fermentation the total acidity levels of ripening fruit and over-ripened fruit samples were 0.78% and 0.80%, respectively. Total acidity of kiwifruit wine samples was restored to suitable levels, ranging from 0.78 to 0.82%, which correspond to grape wine acidity levels of pH 3.46 to 3.50. The total acidity levels observed in our study are similar to those described previously. For example, Luh and Wang [21] reported that titratable acids (expressed as citric) amounted to 1.80% in kiwifruit. Heatherbell *et al*. [22] mentioned that the titratable acidity, as citric acid, amounted to 1.44% in press juice and 0.75% in wine. One exception in our study was the observation that kiwifruit must acidity was higher than the acidity of wine produced from the same sample (data not shown). This difference can be attributed to the precipitation of organic acids such as citric acid during alcoholic fermentation.

3.2 The pH

The mean pH values of ripening and over-ripened samples were not significantly different. At the initial stage of fermentation, the pH of wine from over-ripened fruit was 3.85, while the wine made using ripening fruit had a pH of 3.75. After fermentation the pH of all samples was slightly decreased to 3.46 (Figure 1). Any variation observed was likely due to the different degrees of kiwifruit ripeness. Due to the particularly acidic character of kiwifruit wine, the pH value of samples was corrected to higher levels (3.40–3.55). Luh and Wang [21] reported slightly higher pH levels of kiwifruit wine, varying between 3.1 and 3.96.

Composition	Ripened fruit (% DW)	Over-ripened fruit (% DW)
Moisture	80.14±4.01[a]	78.34±3.82[a]
Crude protein	1.07±0.05[a]	1.32±0.07[a]
Crude fat	0.31±0.02[a]	0.29±0.01[a]
Crude ash	0.63±0.03[a]	0.67±0.03[a]
Crude fiber	2.87±0.12[a]	2.63±0.13[a]

Table 2. The proximate composition of kiwifruit.

Values are means±SD (n=3). Means in columns without letters in common differ significantly (P<0.05).

3.3 The colour quality

There were no significant differences in the colour quality of both wine samples (Figure 1). At the initial state L* numbers in Hunter's colour value continually increased from 70 to 93.95, and 94.15 after 16 weeks of fermentation in ripened and over-ripened fruits, respectively. Value a* in all wine samples decreased steadily from 2.27 to -0.20 after the first week of fermentation and then remained constant until the end of the study period. Value b* showed high yellow to approximately 8.86 and then continually decreased to approximately 7.19 after 16 weeks of fermentation.

3.4 The total phenols and antioxidant activity

Changes in total phenols and antioxidant activity during fermentation of kiwifruit wine as influenced by fruit ripening degree is shown in Figure 2. During fermentation, the total phenol and antioxidant activity of both samples were slightly decreased, but the decrease was not always significant. Significantly higher amounts of polyphenols and antioxidants were

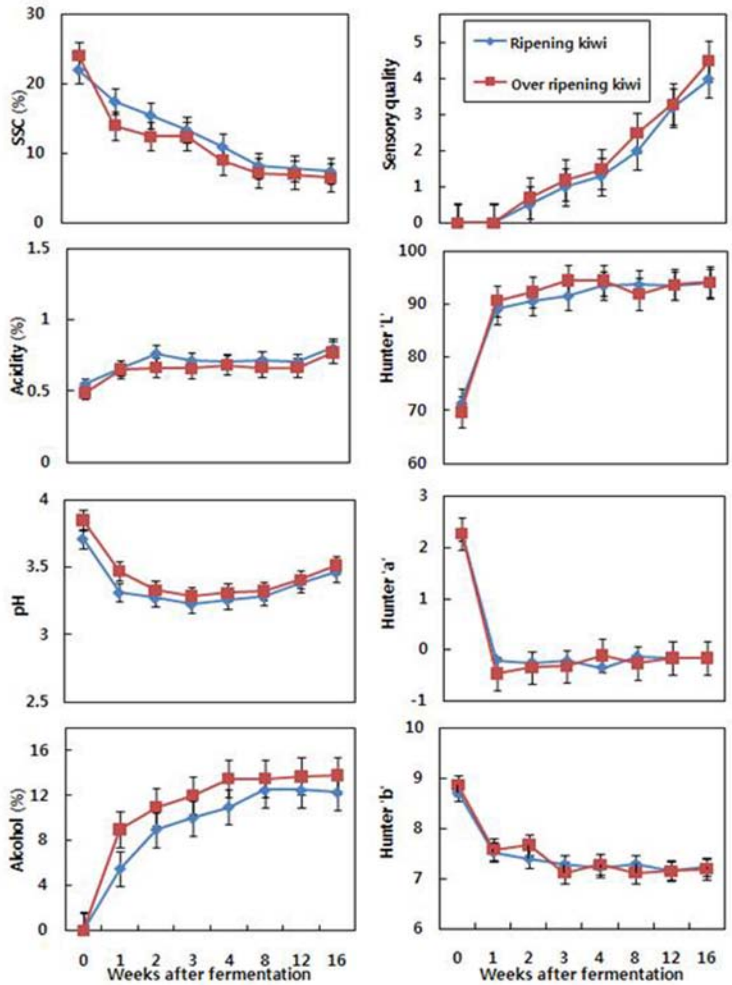

Figure 1. Changes of soluble solid content, alcohol content, pH, acidity, Hunter colours and sensory quality of kiwifruit wine fermented by Lavin EC1118 strain as influenced by fruit ripening degree. Values are means±SD (n=3).

almost always observed in wine derived from over-ripened fruit (Figure 2). The pattern of total phenolic levels correlated with levels of antioxidant activity (Figure 3). In general, the phenolic compound content increases throughout ripening of kiwifruits, which may be the reason that wine made from over-ripened fruit contained higher levels of total phenolic compounds. However, the phenolic content of wine depends not only on the maturity degree of the fruit, but also on the wine processing. Fermentation and aging could also modify the phenolic composition by processes such as oxidation, condensation and polymerisation [23]. It was shown previously that from the enological point of view, phenolic compounds mainly influence the colour, astringency, bitterness, clarity as well as the browning process [24,25]. Besides their enological attributes, polyphenols are known to have some potential health benefits due to their pharmacological activities, such as antioxidant, anti-inflammatory, antiallergic, antiviral, anticarcinogenic, and antimicrobial effects. It is generally assumed that the higher the total polyphenol content of a beverage, the greater the antioxidant activity [6]. Our previous experiments on laboratory animals and clinical investigations showed that the content of total polyphenols is higher in white wine than in beer. Total polyphenols, procyanidins, epicatechin, quercetin, ferulic, p-coumaric and gallic acids were measured in wines [8-11]. Studies in rats [2] showed that the polyphenolic component in wine dry matter favourably affect the body lipid metabolism and antioxidant activity. The main beneficial polyphenols were determined to be proanthocyanidins, epicatechin, and ferulic acid. The dry matter of South African wines had the most beneficial lipidemic and antioxidant effects. The beverages reduced total cholesterol (TC), triglyceride, and lipid peroxide levels, and elevated high-density lipoprotein

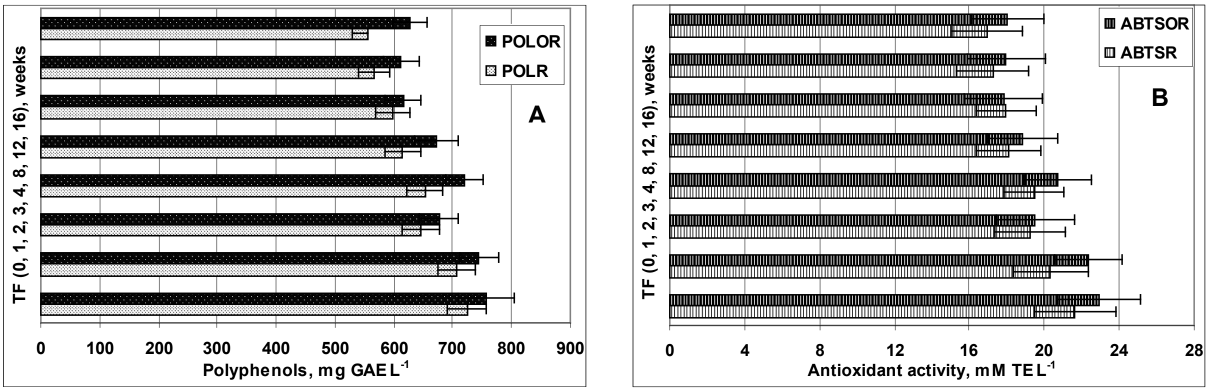

Figure 2. Changes of total phenols (A) and antioxidant activities (B) during fermentation of kiwifruit wine fermented by Lavin EC1118 strain as influenced by fruit ripening degree. Values are means±SD (n=3). Abbreviations: TF, time of fermentation, from the bottom till the top changing from 0 to 16 weeks; POLOR, polyphenols in wine from over-ripened kiwifruit; POLR, polyphenols in wine from ripened kiwifruit; GAE, gallic acid equivalent; ABTSOR, antioxidant activity determined by 2,2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging assay in wine from over-ripened kiwifruit; ABTSR, 2,2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging assay in wine from ripened kiwifruit; TE, trolox equivalent.

cholesterol/TC ratio [26]. The phenolic content of South African wines was investigated for the purpose of their classification according to variety or cultivar. Twenty-two phenolic compounds were quantified by liquid chromatography in 55 red wines of 5 varieties and in 38 white wines of 3 varieties [24]. Polyphenols ranged between 0.53 and 6.13 mg L^{-1} in fortified wines, between 0.46 and 37.26 mg L^{-1} in red wines, between 0.43 and 16.12 mg L^{-1} in white wines and between 0.38 and 11.64 mg L^{-1} in the rose wine. These values are in the range of the amounts found in other red, white and fortified wine varieties, showing that the results obtained in this study are reasonable.

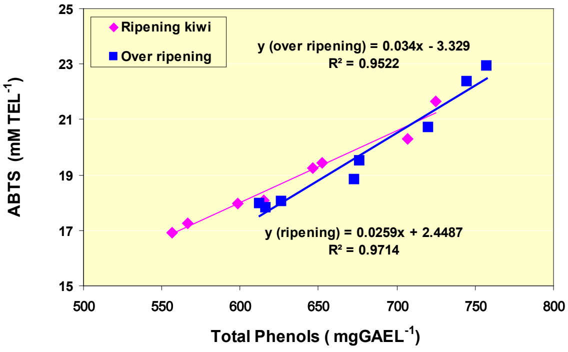

Figure 3. Relationship between the total phenols and antioxidant activities during kiwifruit wine fermentation using Lalvin EC1118 yeast strains as influenced by fruit ripening degree. Values are means ±SD (n=3).

3.5 Minerals

The concentrations of mineral elements were similar in both ripening and over-ripened fresh kiwifruit (data not shown). Changes in mineral content during fermentation in both samples are shown in Table 3. For wine produced from ripened kiwifruit, amount of potassium increased from 450 mg L^{-1} to 838 mg L^{-1}. The increase was nearly the same for the wine samples made from over ripened kiwifruit as from 522 mg L^{-1} to 1121 mg L^{-1} after 16 weeks of fermentation. Potassium and sodium levels were both significantly higher ($P<0.05$) in the over-ripened kiwifruit wine sample than in the ripening sample (Table 3). Before fermentation (time 0 weeks), the sodium content of wine made from ripened fruit was lower than of wine made from over-ripened fruit. Throughout fermentation, the sodium content increased from 4.37 to 6.60 mg L^{-1} in the ripening fruit sample, and from 8.55 to 9.95 mg L^{-1} in the over-ripened sample, after 16 weeks of fermentation. Calcium and magnesium levels of both samples slightly decreased during fermentation. Iron content decreased from 0.22 to 0.05 mg L^{-1} after 3 weeks of fermentation and then remained constant until the end of fermentation. The differences in Fe, Ca and Mg levels were not significant ($P>0.05$)

All the samples in our study have mineral concentration profiles similar to wines derived from different fruit sources, except for sodium which was much lower in our sample (6.99 mg L^{-1}) than other wines which typically have a sodium content of 23 to 66 mg L^{-1} [27]. It appears that the trace element patterns of wines from different fruit sources have a distinct composition and could be used as a fingerprint to identify some unique types. However, the influence of other factors such as geographical environment, soil chemistry, viticulture practices and processing methods needs to be evaluated. In addition, future research could involve studying the roles of minerals in the stability of wine as well as potential health impacts, possibility of toxicological risks and role in food regulations [28].

It has been shown that the stability of wines with regard to taste, colour, clarity and stability is dependent on the relationships between several components, including metals, proteins, and phenolic substances [29]. It was shown in Israeli white wines that nitrogen content, metals, and amino acids varied with respect to the type of grape, area, year of collection, and methods of vinification. The presence of aromatic C=C bonds in the infrared (1600 cm^{-1}) suggests the presence of polyphenols [8,10]. Therefore, the mineral profile of wine has also been proposed as a possible fingerprint that could be used for wine characterization based on their geographical origin. Fruit softening is associated with the disassembly of primary cell wall and the middle lamella structures [30]. Changes in cell wall composition, degrading enzyme activity, and mineral content during ripening process have been reported in detail [31].

3.6 Sensory evaluation

The score of sensory evaluation of both wine samples is displayed in Figure 1. Higher scores are seen in almost all fermentation stages of the over-ripened sample, however, the differences are not significant (P<0.05). Higher scores for the over-ripened fruit wine could be due to higher soluble solid content, lower acid, and higher alcohol concentration which may have affected the taste and flavour of this wine sample. As far as we know, there are no accredited methods to evaluate the sensory quality of wine. Several cards to score wine quality have been developed over the last decades: Davis 20-point scale [32,33], score card for international wine competitions of the International Organisation of Vine and Wine [34], and analytical procedures based on ISO 17025 [35]. Our results based on five-point scale evaluation are in correspondence with other investigators [36,37] who used 1-7 point scale (point 7 means “top quality”, point 6 “very high quality”, point 5 “high quality”, point 4 “medium quality”, point 3 “low quality”, point 2 “very low quality” and point 1 “null quality”).

3.7 Effect of pectinase on quality of kiwifruit wine

Higher must yield and total phenolic content from the fruit was obtained by using pectinase enzymes. Similar pectinase treatment procedures are used in the production of other fruit wines, especially in grape wines, to enhance the extraction of colored pigments and desirable phenolic content during fruit maceration [38-43]. The pectinase did not change the pH and total acidity throughout the wine fermentation. The concentrations of reducing sugars were significantly lower and the alcohol

Wine samples	Time (Weeks)	Mineral Contents (mg L^{-1})				
		Na	Fe	Ca	Mg	K
Ripened kiwi	0	2.93±0.15[a]	0.22±0.01[a]	47.75±2.39[a]	27.85±1.39[a]	450.22±22.51[a]
	1	3.53±0.18[a]	0.14±0.01[a]	43.59±2.18[a]	24.85±1.24[a]	525.33±26.27[a]
	2	2.85±0.14[a]	0.03±0.00[a]	48.67±2.43[a]	28.99±1.45[a]	401.33±20.07[a]
	3	2.89±0.14[a]	0.01±0.00[a]	40.54±2.03[a]	26.83±1.34[a]	365.17±18.26[a]
	4	3.24±0.16[a]	nd	38.99±1.95[a]	25.22±1.26[a]	395.96±19.80[a]
	8	4.37±0.22[a]	nd	37.69±1.88[a]	27.45±1.37[a]	442.12±22.10[a]
	12	2.91±0.15[a]	nd	21.15±1.06[a]	26.42±1.32[a]	643.73±32.19[a]
	16	6.60±0.33[a]	nd	26.9±1.35[a]	24.13±1.21[a]	838.24±41.91[a]
Over ripened kiwi	0	8.55±0.43[b]	0.28±0.01[a]	49.57±2.48[a]	29.93±1.50[a]	522.33±26.12[b]
	1	8.64±0.43[b]	0.21±0.01[b]	44.83±2.24[a]	25.55±1.28[a]	701.05±35.05[b]
	2	9.33±0.47[b]	0.12±0.01[b]	48.85±2.44[a]	28.88±1.44[a]	902.03±45.10[b]
	3	9.81±0.49[b]	0.03±0.00[b]	43.33±2.17[a]	26.65±1.33[a]	805.38±40.27[b]
	4	10.5±0.53[b]	0.03±0.00[b]	42.21±2.11[b]	22.21±1.11[a]	1102.2±55.11[b]
	8	8.55±0.43[b]	nd	39.59±1.98[a]	24.78±1.24[a]	1083.3±54.17[b]
	12	9.87±0.49[b]	nd	37.13±1.86[b]	25.68±1.28[a]	958.12±47.91[b]
	16	9.95±0.50[b]	nd	29.13±1.46[b]	24.45±1.22[a]	1120.6±56.03[b]

Table 3. Mineral contents of kiwifruit wine fermented by fruit ripening degree.

Values are means±SD (n=3). Means in columns without letters in common differ significantly (P<0.05).
nd= not detected.

concentration was higher in the pectinase-treated samples compared with untreated samples (data not shown). Our results are similar to those of Jeong *et al.* [44], who studied the effect of pectinase treatment on alcohol fermentation of persimmon wine. Pectinase enzyme preparations degrade fruit cell walls and thereby lead to a modification of the molecular weight distribution of polysaccharides released into grape wines [38-43]. The differences in sugar content observed in our study were mainly due to the different degrees of kiwifruit ripeness used in the experiments. Our results indicate that the highest yield of kiwifruit wine is obtained by using over-ripe kiwifruit with the addition of pectinase enzymes. These results support previous reports [45-47], where wines were produced from different fruits such as grapes, blackcurrants and cherries.

3.8 Organic acid in wines

Changes in organic acid content during fermentation of kiwifruit wine using Lavin EC1118 strain related to fruit ripening degree are shown in Figure 4 and Table 4. The content of quinic, succinic, and lactic acids was higher in over-ripened kiwifruit during all 16 weeks, but not always significantly (P>0.005). The content of malic acid from week 4 of fermentation was significantly higher in over-ripened kiwifruit (P<0.005). Citric acid concentration was significantly higher in over-ripened kiwifruit (P<0.005) throughout the fermentation process. The content of galacturonic acid during all weeks of fermentation was higher in ripened kiwifruit, but not significantly (P>0.005). From week 8 of fermentation, the total content of organic acids was significantly higher in over-ripened kiwifruit (P<0.005). The most frequent acids found in grape wines are tartaric, malic and citric acids. Succinic, lactic and acetic acids are also abundant and result from alcoholic and malolactic fermentations [25]. Analyses of grape wines indicated that the concentrations of oxalic and malic acids vary from 0.043 to 3.118 g L^{-1} [12,25]. Our analysis showed a slightly higher level of malic acid in kiwifruit wine, ranging from 3.58 to 3.97 g L^{-1}. Cunha *et al.* [12], Esteves *et al.* [48] and López-Tamames *et al.* [49] also reported varying concentrations of tartaric, malic, lactic, succinic and acetic acids, with values between 0.219 and 1.442 g L^{-1} in fortified wines and between 0.041 and 2.752 g L^{-1} in white wines. Similar results were observed by Villiers *et al.* [24] when determining concentrations of the same compounds in red and white wines.

Sample	Weeks	Organic acid (g L⁻¹)						
		Quinic acid	Malic acid	Citric acid	Succinic acid	Lactic acid	Galacturonic acid	Total
Ripened kiwifruit	0	7.47±0.34ᵃ	3.58±0.16ᵃ	8.47±0.38ᵃ	1.93±0.09ᵃ	0.62±0.03ᵃ	0.83±0.04ᵃ	22.89±2.03ᵃ
	1	6.52±0.29ᵃ	4.16±0.23ᵃ	6.96±0.31ᵃ	4.78±0.22ᵃ	1.08±0.04ᵃ	1.85±0.06ᵃ	25.35±1.42ᵃ
	2	5.49±0.25ᵃ	3.79±0.17ᵃ	7.39±0.26ᵃ	3.94±0.17ᵃ	0.87±0.05ᵃ	1.66±0.07ᵃ	23.13±1.32ᵃ
	3	5.53±0.29ᵃ	3.76±0.20ᵃ	6.43±0.07ᵃ	4.51±0.20ᵃ	1.81±0.10ᵃ	1.81±0.08ᵃ	23.85±1.42ᵃ
	4	5.60±0.25ᵃ	3.85±0.16ᵃ	6.17±0.32ᵃ	5.02±0.26ᵃ	2.58±0.16ᵃ	1.45±0.07ᵃ	24.66±1.85ᵃ
	8	5.29±0.19ᵃ	3.85±025ᵃ	6.38±0.25ᵃ	5.38±0.25ᵃ	2.21±0.12ᵃ	1.51±0.12ᵃ	24.63±1.62ᵃ
	12	5.51±0.25ᵃ	3.86±0.25ᵃ	7.03±0.30ᵃ	5.57±0.16ᵃ	2.16±0.11ᵃ	1.47± 0.10ᵃ	25.69±1.42ᵃ
	16	5.57±0.23ᵃ	3.97±0.19ᵃ	6.53±0.28ᵃ	5.88±0.17ᵃ	2.37±0.28ᵃ	1.46±0.04ᵃ	25.78±1.68ᵃ
Over ripened kiwifruit	0	7.64±0.34ᵃ	3.94±0.18ᵃ	9.96±0.45ᵃ	3.19±0.24ᵇ	1.15±0.17ᵇ	0.90±0.06ᵃ	26.79±2.42ᵃ
	1	7.02±0.3ᵃ	4.24±0.16ᵃ	8.17±0.42ᵇ	5.27±0.32ᵃ	1.29±0.06ᵃ	1.59±0.05ᵃ	27.59±2.01ᵃ
	2	6.24±0.31ᵃ	4.25±0.19ᵃ	8.01±0.35ᵇ	4.91±0.18ᵇ	1.39±0.12ᵇ	1.19±0.12ᵃ	25.99±1.85ᵃ
	3	6.28±0.41ᵃ	4.28±0.16ᵃ	7.28±0.40ᵇ	5.16±0.23ᵃ	2.77±0.12ᵇ	1.37± 0.06ᵃ	27.13±1.52ᵃ
	4	6.54±0.28ᵃ	4.50±0.21ᵇ	7.38±0.36ᵇ	6.17±0.32ᵃ	3.13±0.16ᵃ	0.90±0.14ᵃ	28.62±1.85ᵃ
	8	6.41±0.26ᵃ	4.56±0.12ᵇ	7.91±0.25ᵇ	6.85±0.20ᵇ	3.11±0.20ᵇ	1.19±0.12ᵃ	30.04±2.06ᵇ
	12	6.28±0.28ᵃ	4.82±0.21ᵇ	8.03±0.32ᵇ	6.58±0.23ᵇ	3.19±0.21ᵇ	1.18±0.06ᵃ	30.09±2.15ᵇ
	16	6.17±0.16ᵃ	4.91±0.15ᵇ	7.95±0.26ᵇ	6.53±0.24ᵇ	3.23±0.18ᵇ	1.28±0.09ᵃ	30.07±2.01ᵇ

Table 4. Changes of organic acid contents during fermentation of kiwifruit wine fermented by Lavin EC1118 strain as influenced by fruit ripening degree.

Values are means±SD (n=3). Means in columns without letters in common differ significantly (P<0.05).

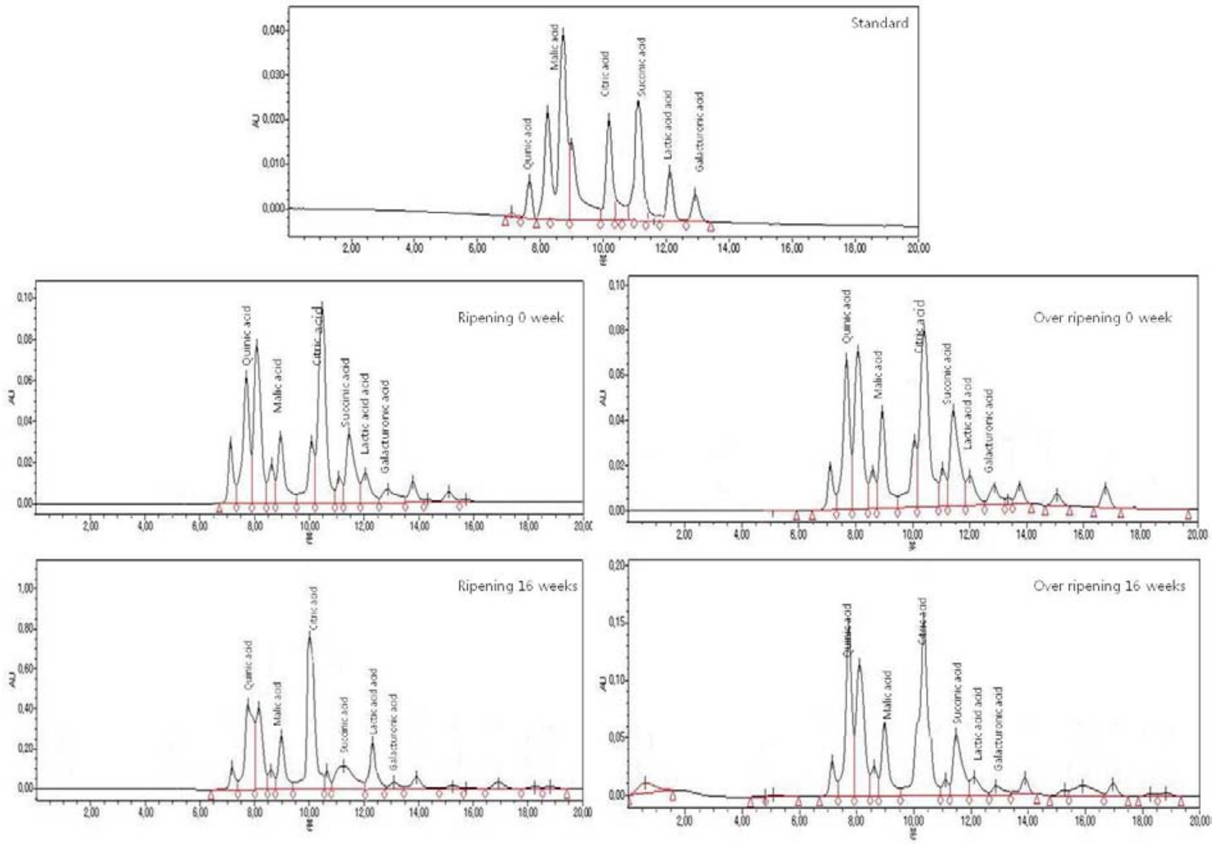

Figure 4. HPLC histograms of organic acids in kiwifruit wines made from ripened and over ripened fruits before and after 16 weeks of fermentation.

4. Conclusion

Our results indicate that kiwifruit wine made from over-ripened fruit has higher values of wine quality in many aspects such as sensory value, alcohol content, total phenol content, antioxidant activity, minerals and overall yield.

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