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PRODUCTION OF CONCOCTION WINE USING PALM SAP AND RAISIN THROUGH BATCH FERMENTATION

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ABSTRACT

Palm wine is a common cheap beverage, widely consumed in parts Africa, Asia and South America. The sap obtained from Palm tree is fermented to produce Palm wine. In the current study palm sap was blended with raisins. After processing, extract was subjected to anaerobic fermentation by inoculating *Saccharomyces cerevisiae* with an initial pH of 3.5 and initial sugar content of 22-24˚ Brix at room temperature. Wine was then subjected to malolactic fermentation using *Oenococcus oeni.* The ethanol concentration was found to be in the range of 12-13 % (v/v), and residual sugar concentration was found to be less than 2mg/ml. Fixed acidity in terms of tartaric acid equivalent was found to be in the range of 3.31g/L to 6.18g/L, radical scavenging activity in terms of trolox equivalent was found to be in the range 2.3-2.5mmol TE /L. Various metals such as Cr, Cd, Cu, Mn, Ni, Pb and Zn were estimated. In total this concoction wine was comparable with typical grape wine. This work proves that there is a potential to convert cheap palm sap into value added concoction wine.

Keywords: Concoction wine, *Oenococcus oeni*, Palm sap, Raisins

INTRODUCTION

Text Palm wine also called as palm toddy or simply toddy is an alcoholic beverage obtained by the fermentation of the sap from the palm tree **(Okafor, 1972; Lasekan *et al*., 2009).** Palm wine is a common cheap beverage, widely consumed in parts Africa, Asia and South America. Wild date *(Phoenix sylvestris),* Coconut *(Cocos nucifera* L*),* Palmyra palm *(Borassus flabellifer),* sago palm *(Metroxylan sago)* and toddy palms *(Phoenix humilis var. pedunculata)* are frequently used for this purpose. Palm wine is obtained by the natural fermentation of palm sap and collected through the tapping of unopened inflorescence. Sap obtained from coconut palm is often called as "Neera" in India **(Law *et al.*, 2011).** Palm wine has mild alcoholic flavour, sweet in taste, vigorous effervescence and milky white in colour as it contained suspension of numerous bacteria and yeast. It thus serves as a rich dietary source of vitamins of the "B" complex **(Tuley, 1965; Lasekan *et al*., 2009)**.
Fresh coconut sap contained 12-15% of sucrose (by weight) and trace amount of reducing sugar including glucose, fructose, maltose and raffinose **(Michael, 1988)**. The sap contains approximately 0.23% protein, 0.02% fat. Half of the total sugars are fermented during first 24 hours and ethanol content of the fermented palm sap reaches maximum of 5.0 - 5.28% (v/v) after 48 hours **(Sekar and Mariappan, 2005)**. Natural fermentation of fresh sap also results in the formation of various undesirable products such as acetic acid which in turn affects the taste **(Shamala, 1988).** Due to weak alcoholic strength, milky white turbid appearance and presence of off-odour palm wine is still considered as a cheap beverage and hence generate very low revenue for the palm farmers. Absence of translucent colour and characteristic taste (palate), which is the desirable characteristic feature of grape wines is absent in palm wine resulted in least preference for palm wine among wine drinkers.
The sap is collected from slits along the unexpanded flower spathes and is immediately inoculated by various wild yeasts and bacteria naturally, triggers the fermentation process. Several researchers tried to arrest fermentation by adding various antimicrobial agents **(Morah, 1994)** and reported a better quality beverage by carrying out controlled fermentation using selected yeast strains. However to achieve good alcoholic strength in the wine, it becomes imperative to increase the Brix of palm sap from 12-15 to 24. Generally table sugar is employed for increasing the initial sugar concentration.
In the current study, controlled fermentation of fresh sap of *Cocos nucifera* L and Borassus *flabellifer* was carried out using *Saccharomyces cerevisiae*. The fresh sap was supplemented with calculated quantity of cabernet sauvignon raisins so as to get a final Brix of 24 and fermented with the expectation that the raisins compliment the sap by imparting colour and palate.

MATERIAL AND METHODS

Collection of palm sap

Fresh sap was collected from *Cocos nucifera* L tree and *Borassus flabellifer* tree by professional tappers was used for the study. To avoid the fermentation by contaminating wild yeasts and bacteria, about 200 mg kg^{-1} of potassium metabisulphite was added to earthen mud pot prior to collection of palm sap **(Morah, 1994).** Palm sap was collected early morning and subsequent evening, transported immediately to the lab, stored in freezer at -20˚C till further use.

Mash preparation

Based upon the sugar content of the sap and sugar content of Cabernet Sauvignon raisin, calculated quantity of raisins were taken and soaked in palm sap and heated to 90˚ C for 15 minutes to reduce the microbial load thereby arrest the commencement of fermentation by contaminant organisms. Once steeping is over, the raisins are crushed using domestic blender. The sugar concentration of the mash was measured using refractometer and adjusted to 22 ° - 24 ° Brix. Tartaric acid crystals are used to adjust the pH to 3.5.
To determine the best possible combination of palm sap and raisins, several combinations of both were taken as given in Table 1.

Fermentation

Dry yeast (*Saccharomyces cerevisiae*) was rehydrated by adding 10 ml of water to 1 g of dry yeast, and allowed to stand for 15 min and then few drops of raisin extract was added and incubated for another 15 min. Once effervescence was visible, mash was pitched at the rate of 1 g dry yeast per litre. Fermentation was carried out at 16˚C±2 ˚C in conical flasks fitted with centrally bored cork having airlock. Fermentation was carried out till no more effervescence was noticed.
Once the yeast fermentation came to an end, malolactic fermentation was initiated by inoculating *Oenococcus oeni* bacteria, and allowed to ferment for another 21 days. After the alcoholic and malolactic fermentation, centrifugation of wine was carried out to remove the biomass and particulate matter. Centrifugation was carried out at 8000 rpm for 15 min. and clarified wine was taken for analysis.

Table 1 Combination of raisin and palm sap taken for fermentation studies.

Name	Cabernet Sauvignon raisin (g)	*Cocos nucifera* L sap (ml)	*Borassus flabellifer* Sap (ml)
N_2	350	--	500
N_3	200	500	--
N_4	100	500	--
N_5	350	500	--

Analysis

Soluble solid (Sugar)

The residual sugar concentration was estimated using Refractometer (ATAGO, RX-5000α-Plus). The use of refractometric techniques has become widespread in a number of fields due to its accuracy, reduced size, response velocity and immunity to electromagnetic interference **(Marquez *et al.*, 2013)**.

Titratable Acids Assay

Titratable acidity (TA) was determined by titration of a strong base against sample to an end point of pH 8.2 using potentiometric titration **(Jacobson, 2006).**

Volatile acid assay

Wine samples were distilled and the distillate was titrated against NaOH using phenolphthalein as indicator to determine volatile acid content **(Moura *et al.*, 2010).**

Polyphenols analysis by HPLC

Analysis of polyphenols was performed using HPLC (Dionex 3000) , using C-18 Phenomenex column with length 150mm and internal diameter 4.6mm.The mobile phase was acetonitrile–water– acetic acid at a volume ratio of 20: 82: 1 (v/v/v). Flow rate, column temperature and detection wavelength were set at 1.0 ml/min, 30°C and 280 nm **(Seruga *et al.*, 2011)** respectively and the separation time was less than 30 minutes. Extract of 20 µL volume was used as sample for injection. Pure chemicals purchased from sigma-Aldrich (India) were used for calibrating HPLC for gallic acid, caffeic acid, syringic acid and p-coumaric acid **(Budak *et al.*, 2010).**

Metal analysis by AAS

Metal analysis was carried out by Atomic absorption Spectroscopy (GBC Avanta) after carrying out acid digestion of the wine samples. 2 ml of wine sample was taken in 250 ml digestion flask, mixed with freshly prepared 10 ml HNO_3/H_2O_2 mixture and 10ml Hydrochloric acid, incubated at 60^0C. Heating was continued until the solution becomes clear and colourless **(Woldemariam *et al.*, 2011).** The cooled digest was made up to 100 ml, by adding deionised ultra-pure water, stored in refrigerator for further analysis **(Nikolakaki *et al.*, 2002)**. Standard metal solution of three different concentrations was prepared for calibration.

Antioxidant assay

Antioxidant activity of wine samples were analysed by the 2, 2-diphenylpicrylhydrazyl (DPPH) method **(Seruga *et al.*, 2011).** 50 µL of wine sample was mixed with 120µL of methanolic DPPH solution (1 mmol dm^{-3}) and 1880µL of methanol to attain a final concentration of DPPH in the reaction mixture of 5.85 x 10^{-5} mol dm^{-3}. The reaction mixture was kept in the dark at room temperature for 15 min and then the absorbance at 517 nm of this mixture (A wine) was measured against the blank sample (50µL of diluted wine, 2000µL of methanol). The DPPH blank solution was prepared fresh (120µL of 1 mmol dm -3 DPPH, 1930 micro L of methanol) and its absorbance at 517 nm (A DPPH) was measured. Trolox standards with final concentration 0 – 2550µmol/L in methanol were assayed under the same conditions as those used for the wine samples; i.e. 50µL of Trolox was mixed with 120µL of methanolic 1 mmol/L DPPH solution and 1880 µL of methanol. After 15 min, the absorbance at 517 nm (A Trolox) against the prepared blank sample was measured. The calibration curve for Trolox, constructed by linear regression of absorbance value (A Trolox) vs. Trolox concentration, was used to calculate the antioxidant activity of wine sample and to express their anti oxidant value in mmol of Trolox equivalents (mmol TE/L) **(Seruga *et al.*, 2011)**

Residual sugar estimation

The concentration of residual sugars was estimated by the colorimetric method using the UV Vis spectrophotometer at 540 nm with 3, 5- DNSA reagent **(Miller, 1959).**

Quantitative estimation of ethanol

Ethanol concentrations in wine samples were estimated using gas chromatography (Shimadzu GC-2014). Injection port; column and FID detector temperature was set 235^0C, 55^0C and 250^0C respectively. Column temperature was varied from 55^0C to 80^0C at a rate of 5^0C per min and maintained for 3min. Flow rate of carrier gas was maintained to 8 ml/min.

RESULTS AND DISCUSSION

Estimation of soluble solids

The estimation of the soluble solids was evaluated in order to check the progress of batch fermentation process. Grape soluble solids are expressed as Brix of sugar and it is estimated using refractometer **(Bindon *et al.*, 2013).** In the current investigation initial soluble solids were maintained between 22 ° - 24 ° Brix. It was observed that there was a steep decrease in Brix value during fermentation **(Jacobson, 2006).** After fermentation, a constant value of Brix was observed, which was possibly due to the presence of by products present during the palm sap collection, which further must have inhibited the growth of yeast. Secondly, the blend of palm sap and raisin contain sugars like raffinose, rhamnose, xylose and arabinose, which cannot be metabolised by yeast, contributes Brix value along with other dissolved molecules present in the broth. Initial soluble solids of fresh toddy of 5 ° Brix reported **(Shamala *et al.*, 1988)** and Cabernet Sauvignon grape was 20 to 26 ° Brix **(Bindon *et al.*, 2013).**

Acidity

Volatile acidity (VA)

VA is expressed as amount of acetic acid present in wine. The result reveals the presence of acetic acid in the wine. *Oenococcus oeni* produces acetic acid as by product **(Olguin *et al.*, 2009).**In the current investigation volatile acidity estimated was 0.7 to 2.3 g/L acetic acid. N2 combination was reported higher VA of 2.3 g/L acetic acid. N4 combination reported low VA of 0.7 g/L acetic acid. Low value of VA signifies that during the malolactic fermentation using *Oenococcus oeni* has reached the death phase soon compare to other combinations. N2 combination has higher value of VA and low value of residual sugar. The results clearly indicate that *Oenococcus oeni* has metabolized soluble solids to maximum extent during malolactic fermentation and produced more acetic acid as by product. Volatile acidity was reported **(Moura *et al.*, 2010)** to be 1.12 to 2.80 g/L acetic acid for red wine. VA of pomegranate wine was reported between 0.26 to 0.36 g/L acetic acid **(Mena *et al.*, 2012)**

Titratable acidity (TA)

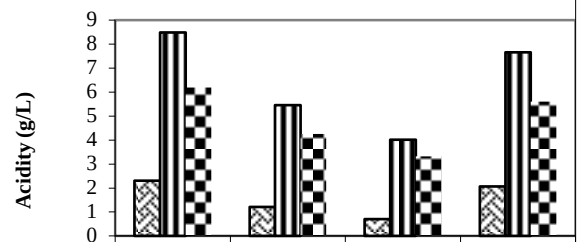
TA (g/L) =75 x N x (T/S) (1)

Where N is the normality of NaOH, T is the titer volume (in ml), S is the sample volume (in ml), and 75 is a constant. **Moura *et al.*, (2010)** reported TA of 7.05 to 9.83 g/L tartaric acid. **Vahl *et al.*, (2013)** reported TA in the range of 4.80 to 6.24 g/L tartaric acid. TA estimated in the current investigation is in the range of 4.01 to 9.99 g/L tartaric acid.

Fixed acidity (FA)

Total acidity= Fixed acidity + Volatile acidity (2)

As per the Organisation of Vine and Wine (OIV) norms FA should not be less than 5g/L.Fig.1 clearly indicates N2 and N5 contain FA as per the OIV. N3, N4 blending combination was found to have FA less than 5g/L as shown in Fig.1.



	N2	N3	N4	N5
Volatile acidity (g/L)	2,3	1,2	0,7	2,06
Total acidity (g/L)	8,48	5,45	4,01	7,65
Fixed acidity (g/L)	6,18	4,25	3,31	5,59

Figure 1 - Acidity of blended combination

Polyphenols

Syringic acid, caffeic acid, P-Glucinol, gallic acid were estimated in blended palm wine. In the present investigation concentration of polyphenols were estimated as follows: Gallic acid (5.42-105 mg/L), P-Glucinol (499.99-9678.22 mg/L), Caffeic acid (0.07-2.94 mg/L), Syringic acid (0.32- 1.19 mg/L). Concentration of gallic acid was estimated maximum in N2. Similarly P-Glucinol, Caffeic acid, Syringic acid in N1, N2, N3 respectively as shown in Table 2.

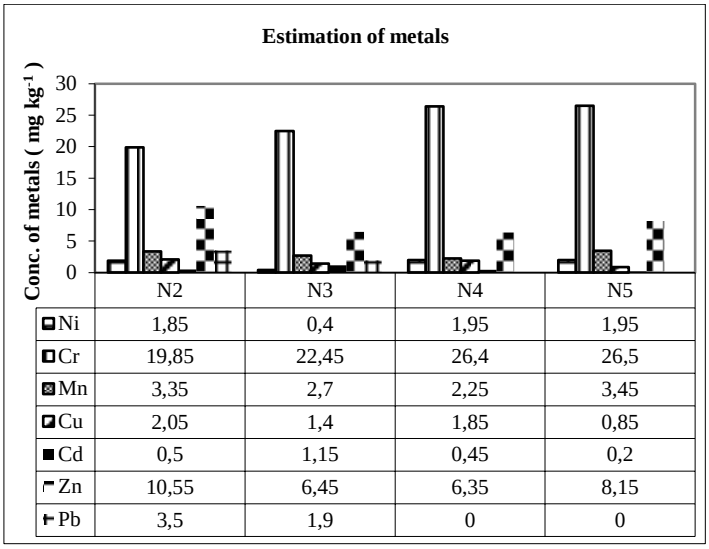
Presence of gallic acid in red wines from New Zeland and Australia were 37 to 108 mg/L. Similarly in Italian red wines 47.21–325.48 mg/L and Hungarian red wines gallic acid content of 29.7–79.2 mg/L **(Seruga *et al.*, 2011)**. N2 has 105 mg/L almost equivalent to the concentration of gallic acid reported Australian red wine. Caffeic acid concentration in white wine was reported 27.2 to 49.6 mg/L **(Fracassetti *et al.*, 2011)**. All the combination of current investigation is having lower concentration of Caffeic acid. It is probably due to the raisin must had lower concentration of gallic acid. Syringic acid was reported 6.0 – 23.9 mg/L in red wines **(Garaguso *et al.*, 2015)**. It is much higher than N2 to N5 combination investigated in current study. Phloroglucinol estimated in N2 was 9678.22 mg/L. It is the highest among all the four combination. N4 was found lowest concentration of 499.99 mg/L. N3 and N5 was estimated 9090.4 mg/L and 2569.83 mg/L respectively.

Table 2 Concentration of polyphenols (mg /L) estimated by HPLC method.

Wine	Syringic acid	Caffeic acid	P-Glucinol	Gallic acid
N2	0.88	2.94	9678.22	105.01
N3	1.19	2.24	9090.4	98.64
N4	ND	0.07	499.99	5.42
N5	0.32	0.38	2569.83	27.88

Metals in blended wine

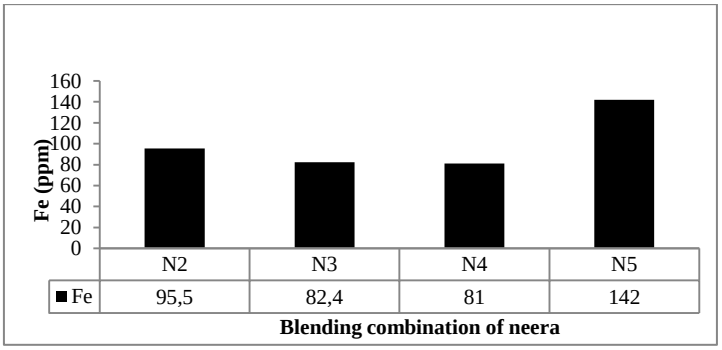
Most metals are important for efficient alcoholic fermentation **(Pohl 2007)**. Cu, Fe and Mn are responsible for changes in stability of old wine and modification of the sensory quality of wine after bottling. Palm wine contains following metals Cu (0.85 to 2.05 mg kg^{-1}), Fe (95.15 to142 mg kg^{-1}), Mn (2.25 to 3.45 mg kg^{-1}), Ni (1.85 to 1.95 mg kg^{-1}), Cr (13.85 to 31.5 mg kg^{-1}), Cd (0 to 1.15 mg kg^{-1}), Zn (6.35 to 10.55 mg kg^{-1}), Pb (1.9 to 3.5 mg kg^{-1}) as shown in Fig.2 and Fig.3.The level of metals in the blended palm wine was found in the decreasing concentration order of metals Fe>Cr>Zn>Cu>Mn>Pb>Ni>Cd. Elements estimated in palm sap blended wine exceed Organisation of Vine and Wine (OIV) recommendations except for copper. This may be due to presence of elements in palm sap itself.



	N2	N3	N4	N5
Ni	1,85	0,4	1,95	1,95
Cr	19,85	22,45	26,4	26,5
Mn	3,35	2,7	2,25	3,45
Cu	2,05	1,4	1,85	0,85
Cd	0,5	1,15	0,45	0,2
Zn	10,55	6,45	6,35	8,15
Pb	3,5	1,9	0	0

Figure 2 - Concentration of metals in wine

Fe concentration of 142 mg kg^{-1} was found in blended palm wine N5 compare to 3.16 mg kg^{-1} **(Woldemariam, 2011)** and 12.5 mg kg^{-1} **(Nikolakaki, 2002)**. In case of Zn higher concentration of 10.55 mg kg^{-1} in N2 blended combination was estimated compare to 2.7 mg kg^{-1} **(Woldemariam, 2011)** and 5.5 mg kg^{-1} **(Nikolakaki, 2002).** Maximum Mn concentration was estimated to be 3.45 mg kg^{-1} in N5, compare to 1.88 mg kg^{-1} **(Woldemariam 2011)** and 2.2 mg kg^{-1} **(Nikolakaki, 2002).** Ni concentration found less in N3 (0.4mg kg^{-1}) and maximum in N4, N5 compare to less than 10mg kg^{-1} **(Nikolakaki ,2002)**. Maximum concentration of Cd estimated was 1.15mg kg^{-1}. Ethiopian wines reported less than 0.01 mg kg^{-1} of Cd **(Woldemariam, 2011).**Cu element was found in N2 was 2.05 mg kg^{-1}. Ethiopian wines reported concentration Cu 1.5 mg kg^{-1} **(Woldemariam, 2011).** Cr concentration of estimated to be 26.5 mg kg^{-1} in N5 blending combination.



	N2	N3	N4	N5
Fe	95,5	82,4	81	142

Figure 3 Concentration of Fe in wine

Antioxidant assay

All the blending combination exhibited radical scavenging activity. The stronger radical scavenging activity of 2.5 mmol TE/L was estimated in N3 blending combination as shown in Fig.4. Least antioxidant activity of 2.3 mmol TE/L was estimated in N2. 2.4 mmol TE/L was estimated in N4 and N5 respectively. Market wine of Cabernet Sauvignon was exhibiting antioxidant activity of 2.1mmol TE/L. It is low compare to other wine produced for the current investigation. Antioxidant activity of 8.22–11.43 mmol TE/L was determined in Croatian red wines **(Piljac *et al.*, 2007).** Antioxidant activity of 13.2–17.8 mmol TE/L (DPPH method) for Slovak and Austrian red wines **(Stasko *et al.*, 2008).**Conventional wines was exhibiting antioxidant activity between 14.6 to 26.4 mmol TE/L **(Garaguso *et al.*, 2015)**. All the combination of wines produced in the current investigation is exhibiting strong antioxidant activity.

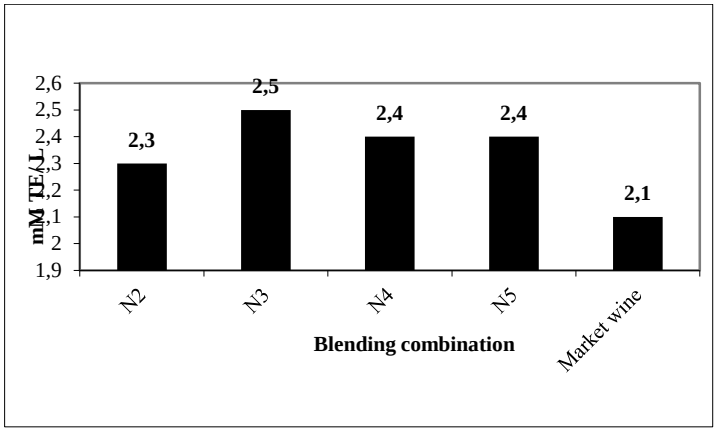


Figure 4 - Radical scavenging activity of wine

Residual sugar

Amount of residual sugar present is within 2 mg/ml as shown in Fig.5. As the weight of raisin in blended combination decreased, residual sugar concentration increased. In case of N2 and N3 combination *Oenococcus oeni* must have reached death phase and couldn't metabolize carbohydrates completely. N4 combination shows maximum residual sugar concentration of 1.73mg/ml. In case of N4 combination *Oenococcus oeni* probably has reached death phase soon, it is supported by lower VA value of N4 combination, i.e. 0.7 g/L acetic acid. *Oenococcus oeni* initially metabolized sucrose and further raffinose, arabinose present in the wine, since yeast cannot metabolize these sugars. During the raffinose and arabinose metabolism due to alpha-galactosidase metabolism sucrose and galactose will be released. N5 combination has 0.54 mg/ml of residual sugar.

Ethanol

Ethanol concentration was found to be in the range of 12 to 13 % (v/v).

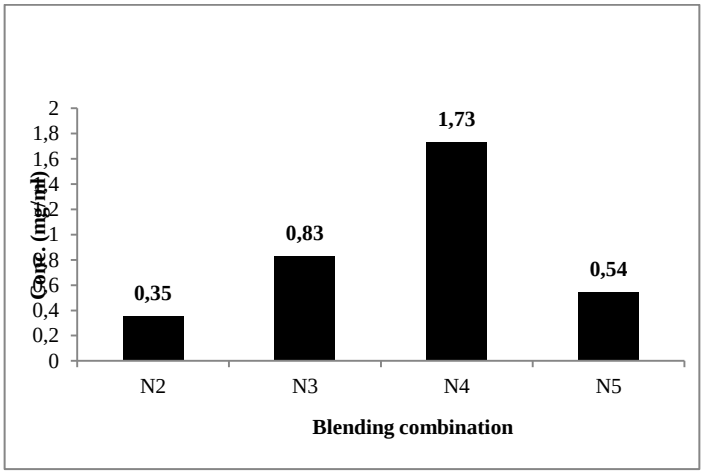


Figure 5 - Residual sugar concentration

CONCLUSION

The Current investigation has revealed that concoction palm wine is a promising alternative for the existing low cost palm wine. Fixed acidity in terms of tartaric acid equivalent was found to be in the range of 3.31g/L to 6.18g/L. All the blended wine had revealed potential radical scavenging activity and maximum activity of 2.5mM TE/L was estimated in N3. Polyphenols were present in higher concentration. Further research is desirable in documenting oragnoleptic properties and scale-up of the process.

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