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Possibility of Red Wine Fermentation from Watermelon (*Citrullus vulgaris* L.) Juice and Roselle (*Hibiscus sabdariffa*) Extract

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Watermelon (*Citrullus vulgaris* L.) and roselle (*Hibiscus sabdariffa*) are rich source of phenolic antioxidants beneficial for human health. In order to exploit the phytochemical constituents and diversify processing products from these materials this research focused on the possibility of red wine fermentation from watermelon juice and roselle extract. There were 3 exprimentsverfified influence of blending ratio (watermelon juice: roselle extract, 2.0:1.0, 1.5:1.0, 1.0:1.0, 1.0:1.5, 1.0:2.0 v/v), initial total soluble solid (11-19 ºBrix), yeast inoculum size (0.01-0.05%). Results showed that under the fermentation of watermelon juice: roselle extract (1.5:1.0 v/v), initial total soluble solid 15 ºBrix, 0.04% *Saccharomyces cerevisiae* (9 log cfu/g), the mixed fruit wine of watermelon-roselle would be obtained at the high ethanol content (14.54±0.03g/L), low residual sugar (0.51±0.01g/L), high total phenolic content (781.30±0.08 mg GAE/100 mL) and high overall acceptance (8.72±0.00). Roselle calyx and watermelon could be exploited to produce functional red wine.

Keywords: ***Blending rati, roselle extract, total soluble solid, watermelon juice, wine, yeast inoculum size***

INTRODUCTION

Watermelon (*Citrullus vulgaris* L.) belongs to the family of cucumbitacea. It's rich in carbohydrate and fibre. Carotenoids including lycopene, phytofluene, phytoene, beta carotene, lutein and neurospnene existed in watermelon fruit (Aminu et al. 2018). Flesh occupies 60% watermelon fruit and 90% of the flesh contains 7 to 10% (w/v) sugar (Altas et al. 2011). Watermelon fruit can be processed into juice, nectar, cocktail (Enukainure et al. 2010). Red wine was produced from watermelon (Roger et al. 2010). Mixed fruit wine was produced from pineapple and watermelon using *Saccharomyces cerevisiae* (Okeke et al. 2015). One study investigated the possibility of water melon juice as substrate for wine fermentation (Aminu et al. 2018). Wine was produced from watermelon (*Citrulluslanatus*) and pawpaw (*Carica papaya*).

Roselle (*Hibiscus sabdariffa*) belongs to the family of Malvaceae. It's one of the most locally available herbs that is under-utilized in Vietnam. Roselle calyx extract has a unique red pigment of anthocyanin involving in various therapeutic benefits such as antimicrobial, diuretic, febrifugal, antihypertensive, and antihelminthic (Delgado-Vargas and Paredes-Lopez, 2002). Bioactive components of the roselle calyces included flavonoids, organic acids, anthocyanin, and polysaccharides. Roselle calyx is commonly exploited in the production of beverage, jam, and sauce (Serifat and Anthony, 2020). The nonalcoholic beverage manufactured from roselle calyx extract is commonly added with sweetener and flavorings to create a pleasant feeling (Omemu et al. 2006).

Fermentation is basic processing technique to alter the original form of fruits into alcoholic drink.Wine having low alcohol content can be classified as a healthy beverage with diversified functional purposes (Pongkan et al. 2018)**.**Various types of fruits and herbs can be utilized to manufacture wine to obtain specific flavor, aroma and taste originated from their sources (Obaedo and Ikenebomeh, 2009; Archibong et al. 2015; Ogodo et al. 2015; Lowor et al. 2016). The wine is simply prepared by supplementing sucrose and *Saccharomyces cerevisiae* into fruit juice to alter dry matter into alcohol, CO_2, flavor (Kunkee and Vilas, 2014; Phonesavard et al. 2010; Barrajon et al. 2011; Capece et al. 2010). During wine fermentation, pH goes down while accelerating specific gravity and alcohol (Giri et al. 2013). Idolo et al. (2016) examined the effect of temperature during roselle wine production. Wine prepared from agricultural materials minimizing the post-harvest losses and diversifying the wine variety (Alobo et al. 2009).

In order to exploit the phytochemical constituents and diversify processing products from these materials, our research focused on the possibility of red wine fermentation from watermelon juice and roselle extract by investigating the influence of blending ratio (watermelon juice: roselle extract), initial total soluble solid, yeast inoculum size

MATERIALS AND METHODS

2.1 Material

Watermelon fruits at ripen maturity were collected from TienGiang province, Vietnam. Roselle calyces were harvested from SocTrang province, Vietnam. Active dried wine yeast, *Saccharomyces cerevisiae*was supplied from Rainbow Trading Co. Ltd., Ho Chi Minh city, Vietnam. Chemical reagents were all analytical grade. Watermelon fruits and roselle calyces were all washed under clean water to remove foreign matter before experiments.

2.2 Researching method

Dried yeast *Saccharomyces cerevisiae* was preliminarily cultured at temperature 30 °C and shaking 200 rpm for 20 hours in 500 mL Erlenmeyer flask containing propagation medium prepared from ammonium sulfate and ammonium phosphate monobasic. Watermelon fruits were chopped to collect their flesh ready for mashing to receive its juice. Roselle calyces (*Hibiscus sabdariffa*) were heated in hot water (1: 2, calyx:water) at 100 ^{o}C for 15 minutes to collect extract with specific anthocyanin pigment. Roselle extract was cooled to ambient temperature. Watermelon juice was combined with roselle extract at different ratio (2.0:1.0, 1.5:1.0, 1.0:1.0, 1.0:1.5, 1.0:2.0, v/v). This juice mixture was then adjusted by sucrose to get the total soluble solid (11-19 ^{o}Brix), pasteurized by 50 ppm sodium metabisulphite to eliminate bacteria and wild yeast before being fermented by *Saccharomyces cerevisiae* (10^9cfu/g) at different inoculum size (0.01-0.05%) at ambient temperature for 12 weeks in 10 L stainless steel fermenter. Racking in 3 week-interval, sample was periodically taken to measure alcohol accumulation (g/L), residual sugar (g/L), total phenolic content (mg GAE/100 mL) and overall acceptance of wine. At the end of fermentation, filtration and bottling were applied to preserve red wine.

Total soluble solid (TSS, ^{o}Brix) was measured by refractometer (model: Atago). Ethanol (g/L) was determined by reversed-phase liquid chromatography using the UV detector (Joseph and John, 1987). Residual sugar (g/L) was analyzed by Fiber-optic FT-NIR spectrometry (Bingqian and Bangzhu, 2017). Total phenolic content (mg GAE/100 mL) was quantified by the Folin-Cicalteau method (Abdullakasim et al., 2007). Overall acceptance (sensory score) was evaluated by a group of panelists using 9 point-Hedonic scale.

2.3 Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS AND DISCUSSION

Ethanol content, residual sugar, total phenolic content and overall acceptance under different ratio of watermelon juice: Roselle extract were presented in table 1-4. During fermentation, total soluble solid was utilized by *Saccharomyces cerevisiae*. High rate of sugar fermentation occurred at the primary stage, while secondary stage showed a slowdown of the fermentation rate (Idolo et al. 2012). The change of watermelon juice:roselle extract ratio propably resulted to difference of the total soluble solids, leading to the accumulation of ethanol (1.03-10.17 g/L), total phenolic content (69.50-527.43 mg GAE/100 mL), residual sugar (0.61-12.33 g/L), sensory score (1.79-6.19). At ratio 1.5:1.0 of watermelon juice:roselle extract, the red wine obtained the highest ethanol content (9.93±0.01g/L), low residual sugar (3.79±0.02g/L), fair total phenolic content (354.19±0.15 mg GAE/100 mL), high sensory score (6.09±0.04) at the end of 12 weeks of fermentation. This data revealed the high efficiency of *Saccharomyces cerevisiae*in utilizing watermelon juice and roselle extract. Roselle extract was mixed with pawpaw juice at the rate of 3:1 to produce red wine (Okoro and Casmi, 2007). Pawpaw (*Carica papaya*) and watermelon (*Citrulluslanatus*) blend was prepared to make wine (Adedeji and Oluwalana, 2013).

Ethanol content, residual sugar, total phenolic content and overall acceptance under different concentration of total soluble solid (11-19 ºBrix) were presented in table 5-8. *Saccharomyces cerevisiae* was strongly adapted to high total soluble solid content. There was significant difference of ethanol content, residual sugar content, total phenolic content and overall acceptance by different initial dry matter. 15 ºBrix was optimal for initial fermentation to obtain the highest ethanol content (12.69±0.01g/L), low residual sugar (4.50±0.00g/L), high total phenolic content (547.33±0.19GAE/100 mL) and high sensory score (7.45±0.01). High total soluble solid limited the growth and proliferation of yeast (Pino and Queris, 2015). Sucrose should be incorporated into the fermentation medium to facilitate for the fruit having low natural solid content (Pazhani et al. 2017).

There was significant difference of ethanol content, residual sugar, total phenolic content and sensory score in wine by the inoculated yeast ratio (table 9-12). Yeast was one of key factors in wine fermentation as its capacity to ferment quickly (Pazhani et al., 2017). *Saccharomyces cerevisiae* required optimal inoculum ratio for its growth and proliferation. Appropriate density of inoculated *Saccharomyces cerevisiae* created dominating ability over the wild bacteria (Maurizio et al., 2016). At 0.04% or 0.05% yeast ratio (9 log cfu/ml) inoculated into fermenter, wine obtained the high ethanol content (14.54±0.03g/L), low residual sugar (0.51±0.01g/L), high total phenolic content (781.30±0.08 mg GAE/100 mL) and high sensory score (8.72±0.00). The optimal yeast inoculum ratio had key role in balance between biomass transformation and ethanol production. An appropriate inoculum ratio created a compromise on ethanol content (Samson et al. 2017). High inoculum size induced better fermentation efficiency, especially glycerol and ethyl alcohol (Francisco et al. 2010; Radler and Schütz, 1982). However yeast grew not well under the limited nutrient and not profitable to metabolize more sugar into ethanol (Pongkan et al. 2018). Ethyl acetate, higher alcohols, iso-acids, and fatty acids were harmful components in wine by high yeast inoculum size (Carrau et al. 2008; Erten et al. 2006; Mateo et al. 2001). Total diacetyl content remarkably increased by inoculum size (Verbelen et al. 2009; Nguyen and Viet, 2009). One study investigated the possibility of water melon juice as substrate for wine fermentation. The total yeast inoculum ranged between 1.9-8.2 × 10^7 CFU/mL (Aminu et al. 2018).

Table 1: Watermelon juice: roselle extract to ethanol formation (g/L) of red wine

Fermentation time (weeks)	Watermelon juice: roselle extract (v/v)				
	2.0:1.0	**1.5:1.0**	**1.0:1.0**	**1.0:1.5**	**1.0:2.0**
0	0	0	0	0	0
3	3.75±0.01^{a}	3.50±0.00^{a}	2.97±0.02^{ab}	2.14±0.01^{b}	1.03±0.00^{c}
6	6.52±0.02^{a}	6.39±0.03^{a}	5.24±0.01^{ab}	4.66±0.02^{b}	2.30±0.03^{c}
9	8.69±0.00^{a}	8.48±0.02^{a}	7.73±0.03^{ab}	7.01±0.00^{b}	5.12±0.02^{c}
12	10.17±0.03^{a}	9.93±0.01^{a}	9.04±0.02^{ab}	8.22±0.03^{b}	7.17±0.01^{c}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2: Watermelon juice: roselle extract to residual sugar (g/L) of red wine

Fermentation time (weeks)	Watermelon juice: roselle extract (v/v)				
	2.0:1.0	1.5:1.0	1.0:1.0	1.0:1.5	1.0:2.0
0	12.33±0.04^{a}	11.06±0.03^{ab}	10.23±0.01^{b}	9.31±0.02^{bc}	8.58±0.03^{c}
3	10.37±0.02^{a}	8.71±0.01^{ab}	6.15±0.03^{b}	5.29±0.00^{bc}	4.17±0.02^{c}
6	7.55±0.01^{a}	6.93±0.00^{ab}	4.96±0.02^{b}	3.48±0.01^{bc}	2.06±0.00^{c}
9	6.16±0.03^{a}	5.37±0.00^{ab}	3.64±0.01^{b}	2.93±0.03^{bc}	1.30±0.03^{c}
12	5.02±0.00^{a}	3.79±0.02^{ab}	2.97±0.03^{b}	2.08±0.00^{bc}	0.61±0.02^{c}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3: Watermelon juice: roselle extract to total phenolic content (mg GAE/100 mL) of red wine

Fermentation time (weeks)	Watermelon juice: roselle extract (v/v)				
	2.0:1.0	1.5:1.0	1.0:1.0	1.0:1.5	1.0:2.0
0	69.50±0.17c	112.63±0.14bc	167.92±0.11b	221.53±0.05ab	279.17±0.09a
3	111.84±0.23c	178.24±0.29bc	213.09±0.30b	284.60±0.18ab	321.34±0.17a
6	163.46±0.09c	215.31±0.12bc	290.33±0.16b	347.18±0.13ab	401.50±0.06a
9	207.22±0.14c	294.07±0.27bc	341.78±0.08b	401.26±0.09ab	486.11±0.13a
12	286.45±0.32c	354.19±0.15bc	402.30±0.14b	485.67±0.22ab	527.43±0.19a

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 4: Watermelon juice: roselle extract to overall acceptance (sensory score) of red wine

Fermentation time (weeks)	Watermelon juice: roselle extract (v/v)				
	2.0:1.0	1.5:1.0	1.0:1.0	1.0:1.5	1.0:2.0
0	3.83±0.03a	3.67±0.02ab	2.93±0.02b	2.22±0.03bc	1.79±0.04c
3	4.76±0.07a	4.50±0.03ab	3.87±0.01b	3.16±0.04bc	2.66±0.01c
6	5.15±0.05a	5.03±0.07ab	4.44±0.04b	3.95±0.02bc	3.07±0.05c
9	6.02±0.03a	5.89±0.01ab	5.07±0.02b	4.60±0.01bc	3.95±0.03c
12	6.19±0.06a	6.09±0.04ab	5.28±0.06b	4.97±0.02bc	4.38±0.04c

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%)

Table 5: Initial total soluble solid (ºBrix) to ethanol formation (g/L) of red wine

Fermentation time (weeks)	Total soluble solid (ºBrix)				
	11	13	15	17	19
0	0	0	0	0	0
3	3.50±0.00c	4.65±0.02bc	6.94±0.03a	6.13±0.00ab	5.41±0.02b
6	6.39±0.03c	7.47±0.01bc	9.03±0.02a	8.41±0.03ab	8.06±0.01b
9	8.48±0.02c	9.11±0.03bc	10.72±0.00a	10.29±0.01ab	9.83±0.00b
12	9.93±0.01c	10.45±0.00bc	12.69±0.01a	12.01±0.02ab	11.22±0.03b

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 6: Initial total soluble solid (ºBrix) to residual sugar (g/L) of red wine

Fermentation time (weeks)	Total soluble solid (ºBrix)				
	11	**13**	**15**	**17**	**19**
0	11.06±0.03c	13.02±0.01bc	14.99±0.00b	16.97±0.03ab	19.02±0.01a
3	8.71±0.01c	9.25±0.02bc	10.08±0.01b	11.63±0.02ab	12.10±0.00a
6	6.93±0.00c	7.31±0.03bc	8.45±0.00b	9.01±0.01ab	9.77±0.03a
9	5.37±0.00c	6.04±0.01bc	6.82±0.03b	7.30±0.02ab	7.61±0.01a
12	3.79±0.02c	4.15±0.03bc	4.50±0.00b	5.01±0.03ab	5.24±0.00a

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 7: Initial total soluble solid (ºBrix) to total phenolic content (mg GAE/100 mL) of red wine

Fermentation time (weeks)	Total soluble solid (ºBrix)				
	11	13	15	17	19
0	112.63±0.14a	112.29±0.07a	112.21±0.08a	112.16±0.12a	112.10±0.04a
3	178.24±0.29c	196.15±0.13bc	293.61±0.21a	255.04±0.07ab	221.64±0.09b
6	215.31±0.12c	268.54±0.08bc	387.20±0.05a	329.55±0.11ab	297.82±0.07b
9	294.07±0.27c	347.25±0.16bc	458.97±0.09a	410.31±0.04ab	384.63±0.14b
12	354.19±0.15c	398.17±0.12bc	547.33±0.19a	504.16±0.17ab	457.04±0.11b

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 8: Initial total soluble solid (ºBrix) to overall acceptance (sensory score) of red wine

Fermentation time (weeks)	Total soluble solid (ºBrix)				
	11	**13**	**15**	**17**	**19**
0	3.67±0.02[a]	3.65±0.03[a]	3.69±0.01[a]	3.67±0.00[a]	3.68±0.02[a]
3	4.50±0.03[c]	4.86±0.00[bc]	5.46±0.03[a]	5.20±0.02[ab]	5.01±0.03[b]
6	5.03±0.07[c]	5.27±0.01[bc]	5.98±0.02[a]	5.63±0.01[ab]	5.42±0.00[b]
9	5.89±0.01[c]	6.13±0.02[bc]	6.79±0.03[a]	6.54±0.00[ab]	6.36±0.02[b]
12	6.09±0.04[c]	6.51±0.00[bc]	7.45±0.01[a]	7.14±0.03[ab]	6.84±0.01[b]

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 9: Yeast inoculum size (%) to ethanol formation (g/L) of red wine

Fermentation time (weeks)	Yeast inoculum size (%)				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0	0	0	0	0	0
3	6.94±0.03[c]	7.85±0.00[b]	7.99±0.02[ab]	8.13±0.01[a]	8.16±0.00[a]
6	9.03±0.02[c]	10.21±0.03[b]	10.63±0.00[ab]	10.91±0.00[a]	10.96±0.03[a]
9	10.72±0.00[c]	11.87±0.02[b]	12.08±0.03[ab]	12.33±0.02[a]	12.39±0.01[a]
12	12.69±0.01[c]	13.95±0.03[b]	14.19±0.02[ab]	14.54±0.03[a]	14.60±0.02[a]

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 10: Yeast inoculum size (%) to residual sugar (g/L) of red wine

Fermentation time (weeks)	Yeast inoculum size (%)				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0	14.99±0.00[a]	14.95±0.02[a]	14.97±0.03[a]	14.96±0.01[a]	14.93±0.00[a]
3	10.08±0.01[a]	8.11±0.03[b]	7.23±0.02[bc]	6.81±0.00[c]	6.75±0.03[c]
6	8.45±0.00[a]	6.09±0.01[b]	5.47±0.03[bc]	4.94±0.02[c]	4.86±0.01[c]
9	6.82±0.03[a]	4.15±0.02[b]	3.29±0.01[bc]	2.60±0.03[c]	2.48±0.02[c]
12	4.50±0.00[a]	2.07±0.04[b]	1.26±0.02[bc]	0.51±0.01[c]	0.40±0.03[c]

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 11: Yeast inoculum size (%) to total phenolic content (mg GAE/100 mL) of red wine

Fermentation time (weeks)	Yeast inoculum size (%)				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0	112.21±0.08[a]	112.24±0.04[a]	112.20±0.04[a]	112.19±0.10[a]	112.23±0.05[a]
3	293.61±0.21[c]	407.38±0.07[b]	456.91±0.12[ab]	496.75±0.05[a]	501.04±0.11[a]
6	387.20±0.05[c]	458.12±0.11[b]	497.04±0.08[ab]	527.13±0.08[a]	530.21±0.04[a]
9	458.97±0.09[c]	670.25±0.13[b]	695.41±0.07[ab]	733.19±0.03[a]	735.02±0.12[a]
12	547.33±0.19[c]	698.07±0.08[b]	736.92±0.13[ab]	781.30±0.08[a]	786.53±0.07[a]

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 12: Yeast inoculum size (%) to overall acceptance (sensory score) of red wine

Fermentation time (weeks)	Yeast inoculum size (%)				
	0.01	**0.02**	**0.03**	**0.04**	**0.05**
0	3.69±0.01[a]	3.64±0.00[a]	3.67±0.02[a]	3.65±0.01[a]	3.68±0.03[a]
3	5.46±0.03[c]	6.75±0.02[b]	6.93±0.01[ab]	7.21±0.03[a]	7.25±0.01[a]
6	5.98±0.02[c]	6.98±0.00[b]	7.15±0.03[ab]	7.52±0.02[a]	7.60±0.02[a]
9	6.79±0.03[c]	7.46±0.01[b]	7.79±0.00[ab]	7.93±0.01[a]	7.99±0.03[a]
12	7.45±0.01[c]	8.03±0.03[b]	8.36±0.02[ab]	8.72±0.00[a]	8.80±0.02[a]

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Idolo et al. (2012) exploited red table wine originated from roselle calyx. They also found the decrease of soluble solids and increase of ethanol during fermentation. Wine was stored for 4 months to facilitate the formation of distintive flavor. There was no significant difference between roselle wine and commercial wine. Alobo and Offonry (2009) manufactured wine from roselle (*Hibiscus sabdariffa*) calyx extract using 3% *Saccharomyces cerevisiae.*

CONCLUSION

Roselle calyx contained numerous phytochemical constituents beneficial for health. It would be considered as a potential raw material substitute for watermelon juice in red wine fermentation. This research successfully found out three main variables of fermentation: ratio of watermelon juice/roselle extract, total soluble solid, yeast inoculum size. This wine had a brilliant red colour. Roselle-watermelon wine was highly valued as a refreshing medicinal drink.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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