



RESEARCH ARTICLE

Possibility of wine production from karanda (*Carissa carandas* L.) fruit

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ABSTRACT

Karanda (*Carissa carandas* L.) is an underutilized fruit with plenty therapeutic and functional properties. In fermentation, yeast metabolites soluble solids in fruit juice to produce energy, ethanol and carbon dioxide. The present research aimed to examine various parameters such as temperature, pH, sugar addition and inoculum size in the primary fermentation, and aging time in storage affecting to karanda wine quality. This research was conducted from 2019 to 2020. Results showed that supplementation of sugar 9 %, pH 3.6, temperature 28 °C, inoculum size 14% and aging in 10 weeks, karanda wine obtained high ethanol content (8.19±0.02 % v/v), total phenolic content (184.32±1.17 mg GAE/100 g) and overall acceptability (8.01±0.02). Wine from this valuable karanda fruit would be beneficial for health by moderate consumption.

Introduction

Ethanol is one of the main constituents in wine production from fruit juice under dynamic reaction of *Saccharomyces cerevisiae* (1). Polyphenols, organic acids, mineral salts, pectins and volatiles are also important in improving functional and organoleptic properties of wine (2, 3). Temperature, pH, soluble solid content, yeast inoculum size, secondary fermentation duration are vital variables greatly influencing to the growth and proliferation of yeast to synthesize beneficial component in wine (4).

Karanda (*Carissa carandas* L.) is an exploited fruit closely related to *Carissa spinarum*. It contains a huge amount of vitamins, phenolics, minerals (5-7). It has been used as folk medicine with various therapeutic properties like antidiabetic, antimicrobial, cytotoxicity, hepatoprotective, anti-inflammatory (8-12). It's commonly converted to jam, jellies and pickles (13). There has been a trend in doing research related to wine production from different varieties of fruit source (14, 15). The fermentation was influenced by numerous variables such as the initial soluble solid, yeast inoculation ratio, and fermentation duration (16). The main objective of our study was to optimize the fermentation parameters such as temperature, pH, sugar addition, inoculum size and aging time to achieve good quality wine with respect to high ethanol

content, total phenolic content and overall acceptability.

Materials and Methods

Material

Carissa carandas L. fruits were collected from the gardens in Soc Trang province, Vietnam. They were cleaned under tap water to remove debris and other matters. *Saccharomyces cerevisiae* was supplied from Pasteur Institute, Ho Chi Minh city, Vietnam. It was cultured in stocking pepton media before inoculating to must fermentation. Chemical reagents such as NaHCO₃, ascorbic acid, Folin-Ciocalteu reagent, standard gallic acid were all analytical grade (> 99 % in purity) purchased from Merck (Darmstadt, Germany).

Experiments

Seeds were removed from the fruits by hand. Seedless fruits were sanitized with peracetic acid 50 ppm to retard the growth of wild microorganism.

Experiment #1

Effect of temperature to wine quality attributes

The preliminary treated fruits were added with 5% sugar, initial pH 3.8 adjusted by NaHCO₃ or ascorbic acid, yeast inoculum size 8% (12 log cfu/ml). The

main fermentation was performed in 15 days at different temperatures (24, 26, 28, 30, 32 °C). Secondary fermentation continued for 4 weeks at cool storage 25 °C as ageing. Turbidity in wine was overcome by flocculation with 0.5% gelatin and filtered through Whatman paper No. 4. Wine was ready for evaluation of ethanol content (% v/v), total phenolic content (mg GAE/100g) and overall acceptability.

Experiment #2

Effect of pH to wine quality attributes

The preliminary treated fruits were added with 5% sugar, initial pH adjusted to different values (3.4, 3.5, 3.6, 3.7, 3.8) by NaHCO₃ or ascorbic acid, yeast inoculum size 8% (12 log cfu/ml). The main fermentation was performed in 15 days at temperature 28 °C. Secondary fermentation continued for 4 weeks at cool storage 25 °C as ageing. Turbidity in wine was overcome by flocculation with 0.5% gelatin and filtered through Whatman paper No. 4. Wine was ready for evaluation of ethanol content (% v/v), total phenolic content (mg GAE/100 gm) and overall acceptability.

Experiment #3

Effect of sugar addition to wine quality attributes

The preliminary treated fruits were added with sugar in different amounts (5, 7, 9, 11, 13 %), initial pH adjusted to 3.6 by NaHCO₃ or ascorbic acid, yeast inoculum size 8% (12 log cfu/ml). The main fermentation was performed in 15 days at temperature 28 °C. Secondary fermentation continued for 4 weeks at cool storage 25 °C as ageing. Turbidity in wine was overcome by flocculation with 0.5% gelatin and filtered through Whatman paper No. 4. Wine was ready for evaluation of ethanol content (% v/v), total phenolic content (mg GAE/100 gm) and overall acceptability.

Experiment #4

Effect of inoculum size to wine quality attributes

The preliminary treated fruits were added with sugar 9 %, initial pH adjusted to 3.6 by NaHCO₃ or ascorbic acid, different yeast inoculum sizes 8, 10, 12, 14, 16 % (12 log cfu/ml). The main fermentation was performed in 15 days at temperature 28 °C. Secondary fermentation continued for 4 weeks at cool storage 25 °C as ageing. Turbidity in wine was overcome by flocculation with 0.5% gelatin and filtered through Whatman paper No. 4. Wine was ready for evaluation of ethanol content (% v/v),

total phenolic content (mg GAE/100 gm) and overall acceptability.

Experiment #5

Effect of aging time to wine quality

The preliminary treated fruits were added with sugar 9 %, initial pH adjusted to 3.6 by NaHCO₃ or ascorbic acid, yeast inoculum sizes 14 % (12 log cfu/ml). The main fermentation was performed in 15 days at temperature 28 °C. Secondary fermentation continued for different durations (4, 6, 8, 10, 12 weeks) at cool storage 25 °C as ageing. Turbidity in wine was overcome by flocculation with 0.5% gelatin and filtered through Whatman paper No. 4. Wine was ready for evaluation of ethanol content (% v/v), total phenolic content (mg GAE/100 gm) and overall acceptability.

Physicochemical evaluation

Ethanol content (% v/v) was determined by capillary gas chromatography using megapore polar column (17). Total phenolic content (mg GAE/100 gm) was estimated by Folin-Ciocalteu reagent assay (18). Overall acceptability was evaluated by a group of panelists using 9-point Hedonic scale.

Statistical summary

The demonstrations were prepared as 3 replicates for various sample groups. The values were expressed as mean ± standard deviation. Statistical summary was done using Statgraphics version XVI.

Results and Discussion

Effect of Fermentation Temperature to Karanda Wine Quality Attributes

Fermentation temperature has been greatly affected the growth and proliferation of yeast hence, it directly influenced the ethanol formation, total phenolic content and sensory characteristics. Influence of fermentation temperature to ethanol formation (% v/v), total phenolic (mg GAE/100 ml) and overall acceptability of *Carissa carandas* wine was presented in Table 1. When temperature increased from 24 to 28 °C, ethanol formation (3.27±0.00 to 4.87±0.02 % v/v), total phenolic (124.08±2.19 to 146.25±1.38 mg GAE/100 ml) and overall acceptability (3.56±0.02 to 4.69±0.03) accumulated respectively. However, at temperature 30 or 32 °C, these parameters degraded significantly. Therefore, 28 °C was optimal for *Carissa carandas* wine fermentation. Similarly, wine prepared from *C. spinarum* fruit at the fermentation temperature of 25 °C, resulted to have 8.3% (v/v) of ethanol, 134.9 mg GAE/100 ml of total phenolic content and 7.2 out of 10 in sensory attributes (19). High fermentation temperature

Table 1. Effect of fermentation temperature to wine quality attributes

Fermentation temperature (°C)	24	26	28	30	32
Ethanol (% v/v)	3.27±0.00 ^c	3.95±0.01 ^{bc}	4.87±0.02 ^a	4.52±0.00 ^{ab}	4.26±0.03 ^b
Total phenolic content (mg GAE/100 ml)	124.08±2.19 ^c	130.18±1.63 ^{bc}	146.25±1.38 ^a	140.73±1.27 ^{ab}	136.02±1.09 ^b
Overall acceptability	3.56±0.02 ^c	3.75±0.01 ^{bc}	4.69±0.03 ^a	4.22±0.00 ^{ab}	4.01±0.01 ^b

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

Table 2. Effect of pH in fermentation to wine quality attributes

pH of fermentation	3.4	3.5	3.6	3.7	3.8
Ethanol (% v/v)	3.81±0.02 ^c	4.25±0.03 ^{bc}	6.43±0.01 ^a	5.14±0.03 ^{ab}	4.87±0.02 ^b
Total phenolic content (mg GAE/100 ml)	137.53±1.42 ^c	141.06±1.17 ^{bc}	165.02±1.15 ^a	159.34±1.19 ^{ab}	146.25±1.38 ^b
Overall acceptability	4.01±0.03 ^c	4.24±0.00 ^{bc}	5.26±0.02 ^a	4.95±0.03 ^{ab}	4.69±0.03 ^b

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

may induce to much more glycerol and higher alcohols. 22.3 °C was appropriate for mango wine fermentation (20). For guava wine production, 25 °C was found to be suitable (21).

Influence of pH to Karanda Wine Quality Attributes

pH is a major variable greatly affecting to the yeast proliferation and sensory quality of wine (4). Effect of pH to ethanol formation (% v/v), total phenolic content (mg GAE/100 ml) and overall acceptability of *Carissa carandas* wine was presented in Table 2. Among different pH values (3.4-3.8), pH 3.6 revealed the highest ethanol content (6.43±0.01 % v/v), total phenolic content (165.02±1.15 mg GAE/100 ml) and overall acceptability (5.26±0.02) of *Carissa carandas* wine. In another report, winemaking prepared from *C. spinarum* fruit at pH of 3.5 resulted to 8.3% (v/v) of ethanol, 134.9 mg GAE/100 ml of total phenolic content and 7.2 out of 10 in sensory attributes (19). pH 2.47±0.06 was ideal for karanda wine fermentation (22). Effect of pH on different kinds fruit wine (mango 3.8, guava 3.5 and plum 3.0) was reported such as (20, 21, 23).

Impact of Sugar Addition to Karanda Wine Quality Attributes

Karanda had low total soluble solid. It's necessary to increase the soluble solid content of initial must to produce more alcohol. However, too much sugar creating osmotic pressure difference also retarded the growth, performance and viability of yeast, directly affecting to fermentation efficiency to release ethanol (24, 25). Effect of sugar supplementation to ethanol formation (% v/v), total phenolic content (mg GAE/100 ml) and overall acceptability of *Carissa carandas* wine was presented in Table 3. By different percentages of sugar addition (5-13 %), 9% sugar resulted the highest ethanol content (7.25±0.03 % v/v), total

phenolic (186.23±1.04 mg GAE/100 ml) and overall acceptability (6.72±0.03). In one report, karanda juice supplemented with sugar to 24°Brix before fermentation resulted the total phenolic content 746.64±3.10 mg GAE/100 ml, ethanol 12.50±0.35% (22). Total phenolics 5.31 ± 0.21 mg TAE/gm was noticed on *C. spinarum* fruits (26). The initial soluble solid 18 °Brix in juice was beneficial for dragon wine production to obtain 3.54% v/v ethanol (16).

Effect of Inoculum Size to Karanda Wine Quality Attributes

Influence of inoculum size to ethanol formation (% v/v), total phenolic (mg GAE/100 ml) and overall acceptability of *Carissa carandas* wine was presented in Table 4. There was significant difference of quality indicators by varied inoculum size from 8-14%. At 14% and 16%, there was not significant difference of ethanol, total phenolic content and sensory attributes. Inoculum size 14% was optimal to achieve the highest ethanol (8.03±0.03 % v/v), total phenolic content (201.03±1.26 mg GAE/100 ml) and overall acceptability (7.13±0.03) of *Carissa carandas* wine. Yeast greatly affected to the character and aroma of wine (27). In theory, the higher inoculum size implemented the higher ethanol content obtained. Wine prepared from *C. spinarum* fruit at inoculum size of 10% (v/v) resulted to 8.3% (v/v) of ethanol, 134.9 mg GAE/100 ml of total phenolic and 7.2 out of 10 in sensory attributes (19). Inoculum size of 11.9% was optimal for mango wine fermentation (20). Inoculum size of 12% was appropriate for guava wine production (21). Accumulation of high ethanol content limited yeast viability and growth (28). 2% v/v of the supplemented yeast inoculation size was optimal for dragon wine fermentation to obtain 3.54% v/v ethanol and 14.6 °Brix residual sugar (16).

Table 3. Effect of sugar addition to wine quality attributes

Sugar addition (%)	5	7	9	11	13
Ethanol (% v/v)	6.43±0.01 ^c	6.79±0.02 ^{bc}	7.25±0.03 ^a	7.01±0.01 ^{ab}	6.86±0.03 ^b
Total phenolic content (mg GAE/100 ml)	165.02±1.15 ^c	171.14±1.29 ^{bc}	186.23±1.04 ^a	180.37±1.03 ^{ab}	176.49±1.09 ^b
Overall acceptability	5.26±0.02 ^c	5.53±0.03 ^{bc}	6.72±0.03 ^a	6.15±0.00 ^{ab}	5.89±0.02 ^b

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

Table 4. Effect of inoculum size to wine quality attributes

Inoculum size (%)	8	10	12	14	16
Ethanol (% v/v)	7.25±0.03 ^c	7.69±0.00 ^b	7.91±0.02 ^{ab}	8.03±0.03 ^a	8.07±0.02 ^a
Total phenolic content (mg GAE/100 ml)	186.23±1.04 ^c	194.17±1.04 ^b	197.05±1.11 ^{ab}	201.03±1.26 ^a	202.14±1.12 ^a
Overall acceptability	6.72±0.03 ^c	6.95±0.01 ^b	7.06±0.02 ^{ab}	7.13±0.03 ^a	7.15±0.00 ^a

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$).

Table 5. Effect of aging time to wine quality attributes

Aging time (weeks)	4	6	8	10	12
Ethanol (% v/v)	8.03±0.03 ^b	8.09±0.01 ^{ab}	8.13±0.00 ^{ab}	8.19±0.02 ^a	8.20±0.03 ^a
Total phenolic content (mg GAE/100 ml)	201.03±1.26 ^a	197.68±1.15 ^{ab}	192.07±1.54 ^b	184.32±1.17 ^{bc}	176.92±1.04 ^c
Overall acceptability	7.13±0.03 ^c	7.52±0.03 ^{bc}	7.89±0.01 ^b	8.01±0.02 ^{ab}	8.29±0.03 ^a

Figures are the mean of three replications; Figures in column followed by the same letter/s are not differed significantly ($\alpha = P=0.05$)

Effect of Aging Time to Karanda Wine Quality Attributes

Effect of aging time to *Carissa carandas* wine quality was presented in Table 5. There were not much changes of ethanol content from 4 to 12 weeks of storage. Total phenolic content decreased slightly from 4-12 weeks. Meanwhile, overall acceptability improved greatly after 10 or 12 weeks. Aging in 10 weeks resulted *Carissa carandas* wine with ethanol (8.19±0.02 % v/v), total phenolic (184.32±1.17 mg GAE/100 gm), overall acceptability (8.01±0.02). In the present study, the main fermentation time lasted 15 days and secondary period was 10 weeks. In another research, karanda fruit was fermented by *Saccharomyces cerevisiae* in 22 days to release ethanol (22). Fermentation for 44 hrs was enough for the dragon wine fermentation to obtain 3.54% v/v ethanol and 14.6 °Brix residual sugar (16). A remarkable degradation of the total phenolic content during fermentation could be due to the adsorption of phenolics onto yeast cell wall as well as condensation and polymerization (3). However, several studies proved that bioactive substances were formed in wine during fermentation (29, 30).

Conclusion

Wine from fruits has multifunctional health benefits as a common beverage being enjoyed widely. *Carissa carandas* L. (Karanda) fruit contains numerous vitamins, minerals and soluble solids essential to yeast proliferation and performance. The present study verified different aspects influencing to karanda wine quality. With supplemented sugar 9 %, pH 3.6, temperature 28 °C, inoculum size 14% and aging 10 weeks, karanda wine obtained high ethanol content, total phenolic content and sensory attribute. By diversifying processing products from karanda fruit, added value of this fruit could be improved. Farmer would have more chance to escape hunger and poverty in cultivation of this plant.

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Authors' contributions

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

Conflict of interests

The author strongly confirm that this research was conducted with no conflict of interest.

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