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Research **Note**

Infusion of Pineapple (*Ananas comosus* (L.) Merr.) Wine with Rangoon Creeper (*Quisqualis indica* L.) Flower

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To develop food use for underutilized Philippine flowers, the potential of Rangoon creeper (*Quisqualis indica* L.) flowers (RCFs) as a natural preservative applied through infusion of pineapple wine making was studied. Cabinet-dried RCFs were added at varying concentrations (0.1%, 1% and 2%) to the fermenting pineapple must at different stages (i.e., fermentation, aging and both). During fermentation, RCFs exerted an antibacterial effect regardless of concentration and an anti-yeast effect except at 0.1%. However, the RCFs showed no effect on the physicochemical properties of the fermenting must with final means of 6.0 °Bx total soluble solids, 10% alcohol content (v/v), and pH 3.0. Interestingly, the RCF-infused fermenting musts completed fermentation at an earlier time (2–3 d) compared with the control. DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu assays of the completely fermented pineapple wine showed that RCFs generally increased the antioxidant activities of the wine, reaching values comparable to those of commercial red wine and better than those of conventional pineapple and white wines. A cost and benefit analysis of RCF infusion in pineapple wine making showed a 51.80% return on investment. With today's trend in food innovation geared toward the importance of health and wellness, RCF infusion can provide a huge market advantage targeting niche markets of health enthusiasts and wine connoisseurs and at the same time address the problems of wine manufacturers.

Key Words: antioxidant activity, infusion, pineapple wine, Rangoon creeper

Abbreviations: A – aging, DPPH – 2,2-diphenyl-1-picrylhydrazyl, F – fermentation, FA – fermentation and aging, GAE – gallic acid equivalent, RCF – Rangoon creeper flower, SA – scavenging activity, TPC – total phenolic content

INTRODUCTION

As a tropical country, the Philippines boasts of a wide array of flowers that have little use except as ornamentals. One unique aromatic flower species commonly propagated in the country is Rangoon creeper (*Quisqualis indica* L.). Rangoon creeper flowers (RCFs) are bright pink to reddish purple when mature and give out a strong floral scent particularly at night. They are rich in bioactive compounds with anti-inflammatory, immunomodulatory, antipyretic, antimicrobial and antioxidant properties (Sahu et al. 2012). However, RCFs have no known applications in the country except for ornamental purposes, in spite of the fact that the roots, seeds and fruits of Rangoon creeper are used as a remedy against parasitic worms, cough, toothache, diarrhea and rheumatism.

With the common practice of using plants (whole or parts) in fermented beverage preservation in the Philippines, such as the addition of *samak* (*Macaranga tanarius*) leaf in *basi* (sugarcane wine) and *tungog* (*Ceriops tagal*) bark in *bahalina* (fermented coconut sap), the potential use of RCFs as a natural preservative in fruit wine making may be worth investigating. The addition of plants and their parts with antimicrobial activity is a common practice in alcoholic beverage production because of the huge threat of microbial spoilage and oxidative browning. These added plants serve as natural preservatives and processing aids, as well as flavor and color enhancers, functions that are strongly linked to the bioactive compounds present in the plants. For instance, studies have shown that *M. tanarius* leaves contain glucosides that have potent radical-scavenging activity (Matsunami et al. 2006), while *C. tagal* extract contains tripenoids and diterpenoids that exhibit anti-fouling, antibacterial, antihyperglycemic, antifeedant, cytotoxic, and α-glucosidase inhibitory bioactivities (Chan et al. 2015). The composition and functionality of bioactive compounds vary among the different plant parts, with flowers considered to have the least. However, Mishra et al. (2016) and Uddin (2010), respectively, showed that flowers of *Rosa indica* and *Hibiscus rosa-sinensis* have more potent antibacterial capacities than other parts of the plant. Bungihan and Matias (2013) also revealed that underutilized Philippine flowers such as *Mussaenda philippica, Ixora coccinea* and *Caesalpinia pulcherrima* have potential for pharmaceutical and nutraceutical applications because they are rich in phytochemicals with antioxidant and antibacterial properties. It is therefore important to determine the potential of RCFs in fruit wine preservation.

Fruit wine is considered a value-added product of fruits. In Asia, one popular fruit wine is pineapple wine, the Philippines being the top exporter of pineapple (fresh and processed) worldwide next to Thailand (Gaille 2018). Pineapple wine is well-enjoyed because of its pleasing color and aroma, and distinctive taste and mouth feel. However, Barcelo et al. (2015) have shown that pineapple wine has the lowest antioxidant activity (i.e., DPPH radical scavenging activity and total phenolic content) among other fruit wines such as *ayosep* (*Vaccinium myrtoides*), soursop (*Annona muricata*), mangosteen (*Garcinia mangostana*), and strawberry (*Fragaria x ananassa*). In addition, pineapple wine is highly susceptible to oxidative browning — the biggest threat to winemaking (Joshi et al. 2017). To address these problems and at the same time develop food use for RCFs, we looked into the unexplored idea of infusing pineapple wine with RCFs. Infusing wine with a natural preservative (e.g., RCFs) will bring huge competitive advantage over conventional fruit wine making with today's growing focus on health and wellness.

This study was conducted to determine the effect of RCF infusion on pineapple wine making. Specifically, we evaluated how the addition of RCFs at various concentrations (i.e., 0.1%, 1% and 2% based on the standard 1% in tea making) at different winemaking stages (i.e., fermentation, aging and both) affects the microbiological and physicochemical properties of the pineapple fermenting must during fermentation as well as the antioxidant properties of the pineapple wine after aging. We also determined the market viability of the resulting RCF-infused pineapple wine compared with its uninfused counterpart through cost and benefit analysis.

MATERIALS AND METHODS

The study was conducted at the Food Microbiology Laboratory, Institute of Food Science and Technology, University of the Philippines Los Baños from November 2016 to January 2018.

RCF Preparation

Fresh RCFs were collected from organic flower gardens in the towns of Sta. Cruz, Los Baños and Calamba, in Laguna, Philippines. The RCFs were cut at the pedicel and dried in a cabinet dryer (BNE Model, Food Machineries and Technology Inc., Manila, Philippines) at 42°C for 20–24 h (Mabilin 2015). Drying prolongs the shelf-life and prevents microbial contamination from any adhering dirt or insect in the flower. The dried RCFs were packed in resealable bags and refrigerated at 4°C until use.

Infusion of Pineapple Wine with RCFs

Pineapple wine was produced using the method described by Dizon (2010). Fully ripe pineapples purchased from the local market in Calauan, Laguna were peeled (crown, core, and eyes were removed), chopped into small pieces, and blended using a Waring blender (Model 4172-051, Oster, Queretaro, Mexico). The resulting puree was filtered using cheese cloth and diluted with distilled water (1:2 pineapple juice: water ratio). The total soluble solids (TSS) of the mixture was adjusted to 20.0°Bx by adding refined sugar.

Ten percent of the mixture was pasteurized in boiling water for 30 min, cooled to 40–45°C, inoculated with a stocked strain of *Saccharomyces ellipsoideus*, and allowed to ferment for 18–24 h. This was used as a starter for wine fermentation.

The remaining 90% must was transferred to a large fermentation jar, into which 5 mL of 10% sodium metabisulfite solution per gallon of must was added. The jar was covered with a cotton plug, and the fermenting mixture was left to stand for 18–24 h. The previously prepared starter culture was added to the must, which was then dispensed into 20 fermentation jars (one control and three RFCT concentrations, i.e., 0.1%, 1% and 2% designated for different winemaking stages, i.e., fermentation, aging, and both). The jars were again covered with a cotton plug, and the musts were allowed to ferment for 2 d, after which the cotton plug was replaced with a fermentation lock. The musts were then left to ferment to completion at 25°C.

After 3 wk of fermentation, the samples were carefully siphoned using a flexible polymer tubing into clean bottles lined with clean muslin. Five milliliters (5 mL) of 10% sodium metabisulfite per gallon of harvested solution was added. RCFs were again added depending on the designated treatment. The samples were aged in a cool, dry place at approximately 25°C. After 6 mo of aging, the samples were carefully harvested by siphoning and filtered. Their final TSS was adjusted to 12.0°Bx as standard for sweet wine.

Microbiological and Physicochemical Analyses

Throughout the fermentation period, a weekly evaluation of the fermenting musts using the following analyses was conducted to determine the effects of RCF infusion on pineapple wine making.

Total viable and yeast counts were determined by spread plating 0.1 mL of appropriate dilutions of the fermenting musts in nutrient agar (NA) for bacteria (Weenk 1995) and in potato dextrose agar (PDA) for yeasts (Beuchat 1995).

pH and total soluble solids (TSS, °Bx) were determined in triplicate using a pH pen (pH 600 M-21919, Milwaukee, North Carolina, USA) and a hand refractometer (Master-2M Cat. No. 2323, Atago, Tokyo, Japan), respectively. Percent alcohol content determination was done through simple distillation and the use of an alcohol hydrometer (Model 08285-58, Cole-Parmer Instrument Co., Wertheim-Mondfel, Germany) (AOAC 1975).

Antioxidant Tests

To determine the effect of RCF infusion on the antioxidant properties of pineapple wine, samples of the RCF-infused and control wines were subjected to antioxidant tests using a UV-vis spectrophotometer (Thermo Scientific Multiskan GO, Leicester, UK) after 1 yr of aging. Scavenging ability (SA) was determined as described by Brand-Williams et al. (1995) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A solution of 42 μL of sample, 167 μL of distilled water, and 42 μL of DPPH solution was prepared and allowed to stand in the dark for 30 min; its absorbance was read at 517 nm. % DPPH scavenging activity was computed based on the formula:

$$\%\text{DPPH Scavenging Activity} = \left(1 - \frac{\text{sample absorbance}}{\text{blank absorbance}}\right) \times \text{DF}$$

Total phenolic content (TPC) was determined using Folin-Ciocalteu assay as described by Singleton and Rossi (1965) with slight modifications. Thirty-two microliters (32 μL) of each sample was obtained, to which 8 μL of 50% Folin-Ciocalteu reagent and 48 μL of 10% Na_2CO_3 were added. The solution was then vortexed, incubated in the dark for 15 min, diluted with 161 μL of distilled water, and finally read of its absorbance at 725 nm. Gallic acid was used in plotting the standard calibration curve at concentrations ranging from 0 to 0.80 mg mL^{-1}. TPC was computed using the formula:

$$\text{Total Phenolic Content (GAE mgmL}^{-1}) = \frac{\text{Conc from curve} \times \text{Vol Solution} \times \text{DF}}{\text{Vol Sample}}$$

and expressed in gallic acid equivalent (GAE mg mL^{-1}).

Both antioxidant tests were done in triplicate with three controls (all sweet wines). Aside from the uninfused pineapple wine, red (Spanish Gate sweet red wine) and white (Carlo Rossi Moscato sweet white wine) wines served as control in evaluating the effect of RCF infusion on the antioxidant properties of pineapple wine. Wine is the most commonly consumed alcoholic beverage known to have high antioxidant properties.

Cost and Benefit Analysis

A standard cost and benefit analysis for the production of pineapple wine and pineapple wine infused with RCFs was done to determine and compare market viabilities.

Statistical Analyses

Data obtained from the antioxidant tests were analyzed using Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI 2013) through one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test when a significant difference was detected. Means were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Microbiological and Physicochemical Properties of Fermenting Musts

The microbial ecology of the pineapple must was affected by the RCF infusion. Figure 1 shows that the control fermenting must generally had higher peak bacterial and yeast counts than the RCF-infused fermenting musts. Only the 0.1% RCF-infused fermenting must showed a trend similar to that of the control fermenting must. This result can be explained by the presence of bioactive compounds in RCFs that have very potent antimicrobial properties. According to Mukherjee and Chandra (2017), Agarwal et al. (2016) and Kumar and Sharma (2014), RCF extract is highly antagonistic against gram-positive and gram-negative bacterial pathogens (e.g., *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*) compared with commercial antibiotics. Bioactive compounds such as flavones, flavonoids and flavonols can form a complex with bacterial cell walls that can cause damage and decrease bacterial viability (Papuc et al. 2017). However, the anti-yeast effect of RCFs cannot be unequivocally concluded owing to overly excessive carbon dioxide production, which led to overflow of the 1% and 2% RCF-infused fermenting musts during the initial fermentation period. This could have resulted in the loss of some of the growing microorganisms, and thus in the lower initial yeast counts recorded in the RCF-infused fermenting musts (Fig. 1b).

On the other hand, the physicochemical properties were not affected by the RCF infusion. Figure 2 shows that there was no significant difference in TSS, alcohol content, or pH between each of the RCF-infused fermenting musts and the control. The final mean TSS, alcohol content, and pH were 6.0°Bx, 10% (v/v), and approximately 3.0, respectively, for all the treatments and the control.

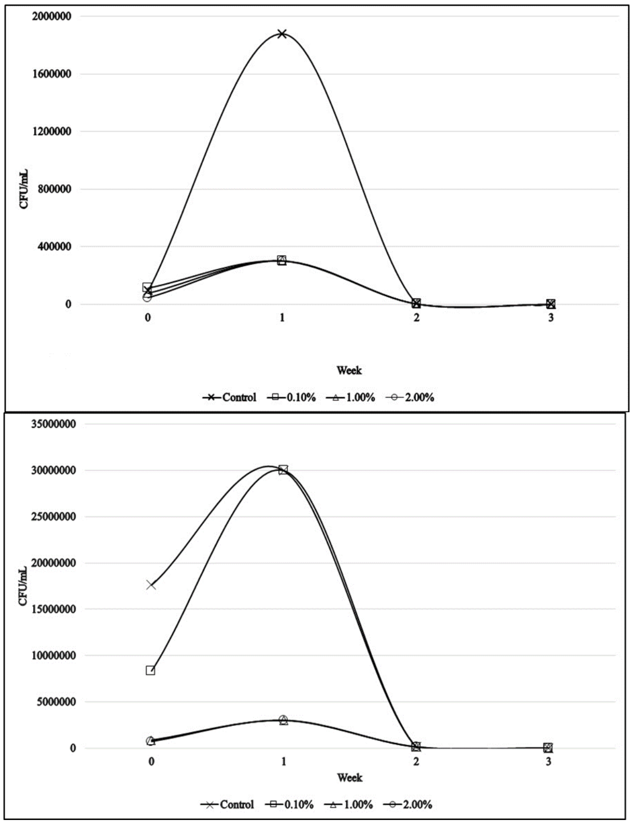

Fig. 1. Changes in (a) total bacterial and (b) yeast counts of the control and Rangoon creeper flower (RCF)-infused fermenting musts with different RCF concentrations over time.

The above result can be primarily explained by the promotive effect of RCF infusion on pineapple wine fermentation. RCFs have antibacterial properties which could have inhibited non-fermentative microorganisms, resulting in the predominance and hence in the proliferation of yeasts, thereby enhancing the yeasts' fermentative action. As observed, the fermentation of the RCF-infused musts generally ceased 2–3 d ahead of that of the control must. For instance, the 2% RCF-infused must exhibited the most vigorous carbon dioxide production during fermentation, being the first sample to reach the maximum alcohol content of 10% in the 1st week (Fig. 2b). It is therefore conjectured that aside from suppressing the growth of other microorganisms, RCFs facilitate the transport of essential nutrients necessary for faster yeast growth. Furthermore, there may be synergistic reactions among the bioactive compounds present in the must and RCFs that enhance yeast metabolism. Notably, the fermentative activity of yeasts is not exclusively dependent on yeast population size, but rather on the quantity and utilization of nitrogen and on the presence of other nutrients or compounds during yeast growth (Brice et al. 2014). These factors should be looked into in future research.

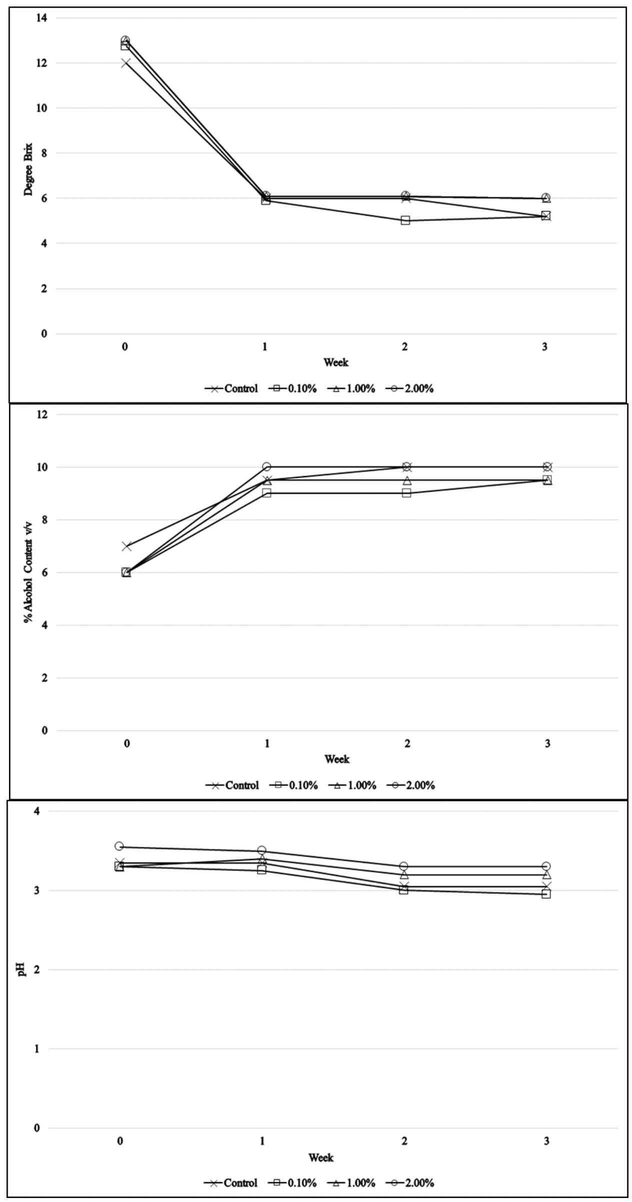

Fig. 2. Changes in (a) TSS, (b) alcohol content and (c) pH values of the control and Rangoon creeper flower (RCF)-infused fermenting musts with different RCF concentrations over time.

Studies of Navarro and Capelo (2017) and Puhawan (2017) also showed the same effects of RCF infusion on the physicochemical and microbiological parameters of the fermenting musts of mango and guyabano wines, respectively.

Antioxidant Activities of Wine

RCF infusion generally enhanced the antioxidant properties of pineapple wine. Table 1 shows that the scavenging activity of each RCF-infused wine significantly increased independent of RCF concentration, while the total phenolic content of each RCF-infused wine significantly increased only with increasing RCF concentration compared with those of the control pineapple wine, which have the lowest values reported, i.e., 80.8454% and 2.3626 GAE mg mL^{-1}, respectively. It can be observed that among the uninfused samples, the red wine had the highest % DPPH scavenging activity and total phenolic content (88.4430% and 5.7604 GAE mg mL^{-1}, respectively), which are lower than, although statistically insignificantly different from, those of the fermentation and aging (FA) and aging (A) treatments (90.3157% and 6.1751 GAE mg mL^{-1}), respectively. This result clearly points to the substantial amounts of phenolic compounds and potent scavenging ability of the RCF-infused wines comparable to those of commercial red wine and better than those of conventional pineapple and white wines.

The higher antioxidant activity and total phenolic content of the RCF-infused pineapple wines than of the conventional pineapple wine and commercial white wine (sweet wine) are presumed to be due to the bioavailability of bioactive compounds in RCFs as a result of fermentation. Aromatic and medicinal plants, such as Rangoon creeper, are natural sources of functional bioactive compounds with excellent antioxidant and antimicrobial properties (Tawaha et al. 2007). Phenolic compounds specifically contribute to the enhancement of the color, flavor, and aroma of food products, as well as provide nutritional benefits to the consumers.

Cost and Benefit Analysis of Pineapple Wine Infusion with RCFs

A cost and benefit analysis for pineapple wine and pineapple wine infused with RCF per 100 kg of fruit is shown in Table 2. Even with the added expense of RCF addition, pineapple wine infused with RCFs had a higher return on investment (ROI) (51.80%) than the uninfused pineapple wine (37.07%). RCFs imparted a natural golden-brown color (almost like the color of brandy) and sweet aroma to the infused wines which deepened with increasing RCF concentration. Hence, RCF infusion, as a value addition process, makes the increase in product cost (Php 350.00 versus Php 200, for pineapple wine with RCF and for pineapple wine, respectively) reasonable. The positive ROI for both products supports the profitability of tropical fruit wine processing in the Philippines with additional mark up from RCF infusion.

Table 1. Mean 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities (SA), and mean total phenolic content (TPC) of the control, red wine, white wine, and RCF-infused pineapple wines.

Treatment	Mean Score*	
	%DPPH SA	TPC (GAE mg mL^{-1})
Control	80.8454^{c}	2.3626^{ef}
Red wine	88.4430^{ab}	5.7604^{a}
White wine	86.0353^{abc}	3.1697^{de}
F, 0.1%	84.9117^{abc}	1.7237^{f}
F, 1%	88.8175^{ab}	3.8742^{bcd}
F, 2%	87.1054^{ab}	4.2470^{b}
FA, 0.1%	88.3895^{ab}	3.1369^{de}
FA, 1%	90.3157^{a}	3.9628^{bcd}
FA, 2%	87.1589^{ab}	4.0812^{bc}
A, 0.1%	84.1894^{bc}	1.5637^{f}
A, 1%	86.8914^{ab}	3.3790^{cd}
A, 2%	87.8812^{ab}	6.1751^{a}

*N=3, mean scores with the same letter are not significantly different, α=0.05 using Tukey's HSD.

CONCLUSION

Generally, pineapple wine infusion with RCFs showed the huge benefits of floral infusion for both wine manufacturers and consumers. For wine manufacturers, the antimicrobial and fermentation-hastening effects of RCFs can eliminate the use of potentially harmful chemical preservatives such as sulfites. With these results, the time and cost of production can be reduced as a result of shortened fermentation, hence, less input and a higher profit. At the same time, RCF infusion is effective in enhancing the antioxidant property of pineapple wine, which can also solve one of the biggest threats to winemaking—oxidative browning.

With RCF infusion, consumers can experience wine drinking beyond what conventional wines offer. RCF-infused wine has a striking but very appealing color (i.e., bright yellow to an almost brandy-like color depending on the RCF concentration), a sweet floral aroma, and enhanced overall sensory properties. Thus, infusion can equal or even better the effects of using oak barrels for aging, which are not readily available in the Philippines. More importantly, RCF-infused wine inclusion in the diet as an antioxidant-rich beverage can potentially

Table 2. Cost and benefit analysis of pineapple wine and pineapple wine infused with Rangoon creeper flower (RCF).

Item Description	Quantity/Unit	Cost/Unit (Php)	Total Cost, Php	
			Pineapple Wine	Pineapple Wine with RCF
Pineapple fruit	100 kg	50.00/kg	5,000.00	5,000.00
Sugar, refined	50 kg	60.00/kg	3,000.00	3,000.00
Dried Rangoon creeper flower	30 kg	500.00/kg	NA	15,000.00
[a]Fermentation vat, 5-gal capacity	14 units	250.00/unit	3,500.00	3,500.00
[b]Aging vessel, 5-gal capacity	12 units	250.00/unit	3,000.00	3,000.00
[a]Mixing vessel, 100-L capacity	2 units	1,000.00/unit	2,000.00	2,000.00
[c]Fermentation lock	14 units	100.00/unit	1,400.00	1,400.00
Sodium metabisulfite			100.00	100.00
Yeast nutrients/culture medium			100.00	100.00
Wine bottles, 750-mL capacity	350 units	25.00/unit	8,750.00	8,750.00
Aluminum cap	350 units	5.00/unit	1,750.00	1,750.00
Capsule, heat shrink	350 units	10.00/unit	3,500.00	3,500.00
Label	350 pairs	12.00/pair	4,200.00	4,200.00
Other consumables			350.00	350.00
Other fixed capital			700.00	700.00
[f]Labor cost, Php 400/day			6,400.00	6,400.00
Water and electricity			300.00	300.00
Total cost			44,050.00	59,050.00
Cost of production per bottle			125.86	168.71
Total sales, Php 200/bottle (pineapple wine), 350/bottle (pineapple wine with RCF)			70,000.00	122,500.00
Return on investment, ROI			25,950.00	63,450.00
%ROI			37.07	51.80
[a]Assuming 3 yr of useful life at 6 times usage/annum				
[b]Assuming 3 yr of useful life at 2 times usage/annum				
[c]Assuming 2 yr of useful life at 6 times usage/annum				
[f] 3 man days x 2 days (preparation of wine)			2,400.00	2,400.00
2 man days x 2 days (harvesting of wine)			1,600.00	1,600.00
3 man days x 2 days (bottling and packaging)			2,400.00	2,400.00
Total labor cost			6,400.00	6,400.00

Assumption: Basic test equipment such as pH meter, hand refractometer, and ebulliometer are available.

alleviate the risks posed by oxidative stress, as well as reverse the increasing prevalence of noncommunicable diseases and degenerative diseases. However, in vivo studies are necessary to unequivocally draw this conclusion.

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