International Journal of Agriculture, Environment and Bioresearch

Vol. 2, No. 06; 2017

ISSN: 2456-8643

INFUSION OF MANGO (Mangiferaindica) WINEWITH RANGOON CREEPER (Quisqualisindica) FLOWER TEA

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ABSTRACT

Recently, there has been a shift to a healthier lifestyle, fuelling the demand for healthier food products. This is a welcome development for the Philippines, which has a high rate of non-communicable diseases. Therefore, here, we aimed to develop a new healthy beverage by infusing mango (Mangiferaindica) wine with Rangoon creeper (Quisqualisindica) flower tea (RCFT). The effects of floral infusion on the fermentation, physicochemical properties and sensory characteristics of mango wine were determined through microbiological and physicochemical tests, and sensory evaluation. The factors were time of infusion (i.e., fermentation, racking, and both fermentation and racking) and RCFT concentration [i.e., 0.1, 1.0, and 2.0% (w/v)]. Significantly lower total microbial and yeast counts were observed for the RCFT-infused fermenting musts than for the control, regardless of RCFT concentration. However, no significant differences were observed in the physicochemical properties between the control and RCFT-infused fermenting musts. Fermentation duration was observed to be shorter for the infused wines than for the control. RCFT infusion at 0.1% generally increased the antioxidant activity of the infused mango wine, although not at levels significantly different from that of the control wine; the same process at 1 and 2% generally decreased it. Moreover, RCFT infusion significantly increased the aroma, color, clarity, and astringency ratings of the infused wine. In spite of these, sweetness, alcoholic taste or general acceptability showed no significant difference among the wine samples. RCFT infusion clearly improves some of the functional and sensory properties of mango wine, without significantly affecting the physicochemical properties of the fermenting musts. Therefore, with optimization of the infusion process, floral infusion may show high potential in improving the commerciability of mango wine for the consumer market owing to its ability to improve the sensory attributes as well as to shorten the production duration of the wine.

Keywords: mango wine, Rangoon creeper, infusion, fermentation

Introduction

The busy lifestyle due to work or studies makes people choose convenience over health in terms of food choices, eating and drinking habits, and physical activities. Most people now opt for instant and ready-to-eat foods or ready-to-drink beverages because of time and money considerations. They live sedentary lives,with little physical activities or exercise. This kind of lifestyle leads to an increase in the incidence of diseases, which are the major cause of death not only domestically but also globally.

Lifestyle diseases are those linked topoor daily habits. These daily habits include unhealthy eating, lack of physical activity, incorrect body posture, and a disrupted biological clock, which are the major factors contributing to the occurrence of lifestyle diseases [1] These lifestyle diseases are called non-communicable diseases (NCDs), also known as chronic diseases. The major NCDs include cardiovascular diseases, chronic respiratory diseases, diabetes, and cancers. According to the World Health Organization [2] NCDs account for 67% of the total death in the Philippines, wherein 33% is due to cardiovascular diseases.

The increasing number of NCD cases not only in the Philippines but also globally has been very alarming that it caught the attention of different health organizations. These organizations are trying to at least lessen the risk factors that markedly influence the occurrence of NCDs. They look for ways and make action plans to reduce the harmful use of alcohol and lessen tobacco use or cigarette smoking. They also promote healthy diet and eating habits as well as regular physical activities.

One way of promoting a healthy diet and healthy eating and drinking habits is by creating and developing food or beverage products that does not only satisfy the taste buds but also provide health benefits to the consumers. Creating and promotingfood products that diminish the risk factors of NCDs such as harmful alcohol consumption and high glucose levels can be a way of reducing the occurrence of NCDs.

Some beverages reported to have health benefits when consumed moderately are wine and tea. Wine is fermented grape juice. However, there has been a recent proliferation of wine made from different fruits and hence the birth of the term “fruit wine”, which carries the same benefit as the original wine. According to Fischer (2006) [3], moderate consumption of wine at less than half a bottle for men and one-fourth of a bottle for women may decrease the prevalence of NCDs, such as cancers, cardiovascular diseases, and coronary spasms. Moreover, it increasesthe level of high-density lipoprotein (HDL) cholesterol, also known as “good” cholesterol, in our body and decreases that of low-density lipoprotein (LDL) cholesterol, also known as “bad” cholesterol. It also increases the incidence of type 2 diabetes. On the other hand, tea, a popular beverage consumed in many countries such as China, Japan, Turkey, and the United Kingdom, has medicinal and health benefits including the reduction in the risks of cancer, heart disease, and

Vol. 2, No. 06; 2017

ISSN: 2456-8643

diabetes [4]. These physiological functions are attributed to tea components, such as catechins, caffeine, flavonols, and vitamins [5]. Moreover, tea boosts the immune system, reduces the risk of heart attack and stroke, and provides antioxidants [6],[7] Although originally prepared from leaves of *Camellia sinensis*, recently, like wine, it has evolved to include concoctions made by steeping different parts of a plant, including flowers, and thus the coining of the term “flower tea”.

Given the health benefits of tea and wine, these beverages may alleviate the risk of NCDs if their benefits are properly harnessed with proper and moderate consumption. Thus, developing a product with the benefits of both tea and wine could be a more potent beverage against NCD prevention. Creating a product with the taste and aroma of both tea and wine may encourage wine drinkers to drink tea and tea drinkers to drink wine. Moreover, a combination of these two may result in a unique flavor that regular drinkers of soda, juice, and coffee may consider appealing.

The main objective of this study is to develop a new beverage by infusing mango wine with Rangoon creeper (*Quisqualisindica*) flower tea (RCFT). The specific objectives are (1) to determine the effect of RCFT infusion on mango wine fermentation; (2) to determine the effect of RCFT infusion on the physicochemical properties of mango wine; (3) to determine the effects of RCFT infusion on the aroma and flavor of mango wine; and (4) to compare consumer preference between RCFT-infused mango wine and conventional mango wine

1. Materials and Methods

1.1. RCFT preparation

Rangoon creeper flowers were harvested and cut at the receptacle to separate the petal from the stalk. They were spread thinly and evenly on plastic meshed trays and dried inside in a cabinet drier at 42°C to constant weight. The dried flowers were packed in resealable plastic bags and stored in a refrigerator until use.

1.2. Mango wine fermenting mustpreparation

Fully ripe mangoes were washed and cut, and their flesh was scooped. The collected flesh was pureed using a blender for 3-5 min. The puree was diluted with water in a 1:3 ratio.

The total soluble solids (TSS) of the prepared must was measured using a hand refract meter and then adjusted to 20° Brix by adding sugar. After TSS adjustment, 10% of the total volume was placed in an Erlenmeyer flask for starter preparation. The flask was covered with a cotton plug, pasteurized in boiling water for 30 min, and cooled to 40-45°C. After cooling, the sample was inoculated with *Saccharomyces ellipsoideus*and left to ferment for 18-24 h.

To the remaining 90% of the original must, 5 mL of 10% sodium metabisulfite solution was added per gallon. This mixture was cotton-plugged and left undisturbed for 18-24 h, after which it was inoculated with the previously prepared starter and uniformly mixed. The resulting mixture was distributed evenly in 20 separate fermentation bottles to prepare three treatment setups (floral infusion during fermentation, during racking, and during both fermentation and racking) and two controls, wherein each setup comprised 6 bottles (2 each for 0.1%, 1.0% and 2.0% RCFT infusions). Each bottle was cotton-plugged and left to ferment underpartially anaerobic condition for 24-48 h, after which the cotton plug was replaced with a fermentation lock. Fermentation was carried outanaerobically for 3-4 weeks at around 25°C.

1.3. Microbiological analyses

Microbial counts were monitored weekly for the entire fermentation durationby the spread plate technique. For total microbial count, nutrient agar (NA) was used; for yeast count, malt yeast extract agar (MYA) was used, with filter-sterilized 10% tartaric acid added before plating the medium. Both media were sterilized by autoclaving at 15 psi and 121°C for 15 min and plated prior to inoculation with appropriate dilutions of each fermenting must. The inoculated NA and MYA plates were incubated in an inverted position at 30-32°C for 1-2 and 2-3 days, respectively, and colonies observed after the incubation period were counted.

1.4. Physicochemical analyses

pH, TSS, and alcohol content were also monitored weekly throughout the entire fermentation duration. About 5 mL of each sample was obtained for the pH and TSS measurements. pH was measured using a pH meter. TSS was measured using a hand refract meter. To determine alcohol content, 100 mL of each sample was transferred into an Erlenmeyer flask and then added with 50 mL of distilled water. The resulting solution was distilled using an alcohol distillation setup and 75 mL of alcohol was collected. The collected sample was diluted to 100 mL and then chilled to 15°C. After chilling, alcohol content was measured using an alcohol meter.

1.5. Harvesting and aging

After the fermentation, the wine was carefully siphoned without disturbing the sediment using a Tygon tubing and transferred into a clean bottle through a funnel lined with clean Muslin. 5 ml of 10% sodium metabisulfiteper gallon was again added to the harvested wine, which was then stored in a cool dry place at approximately 25°C before bottling after racking for 6 months.

1.6. Sensory evaluation

Differentwine attributes, i.e., color, clarity, aroma, astringency, sweetness and alcoholic taste, were evaluated using quality scoring. The experimental design used for the sensory evaluation was balanced incomplete block design. The data gathered were analyzed using Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) Test. The analysis was

ISSN: 2456-8643

done using the Statistical Tool for Agricultural Research (STAR). Ranking test for general acceptability was performed using the Newell and MacFarlane (1987) table [8].

2. Results and Discussion

2.1. Microbiological analyses

Figure 1(a) shows the yeast counts of the fermenting musts. In the first week, the yeast count of the control was 5.45 × 10^6 cfu/mL and that of the fermenting must with 0.1% RCFT was 2.52 × 10^6 cfu/mL. For the fermenting musts with 1 and 2% RCFT, either no colonies or less than 25 colonies were observed, and hence the yeast count was estimated using the rules and guidelines in the Bacteriological Analytical Manual (BAM). The yeast counts of the control and 0.1% RCFT-infused musts peaked in the second week and decreased in the third week. The final yeast counts were 7.25 × 10^5 cfu/mL for the control and 1.01 × 10^5 cfu/mL for the fermenting must with 0.1% RCFT.

The same growth pattern was observed for the total microbial count [Fig. 1(b)]. However, the initial counts were zero for all the treatments. They all peaked in the second week and decreased in the third week. No growth was observed in the mango fermenting must with 1 or 2% RCFT. Figure 1(b) shows that the total microbial counts in the second week were 7.37 × 10^6 cfu/mL for the control and 5.35 × 10^6 cfu/mL for the fermenting must with 0.1% RCFT. The total microbial counts in the third week were 2.76 × 10^5 cfu/mL for the control and 7.69 × 10^5 cfu/mL for the wines with 0.1% RCFT.

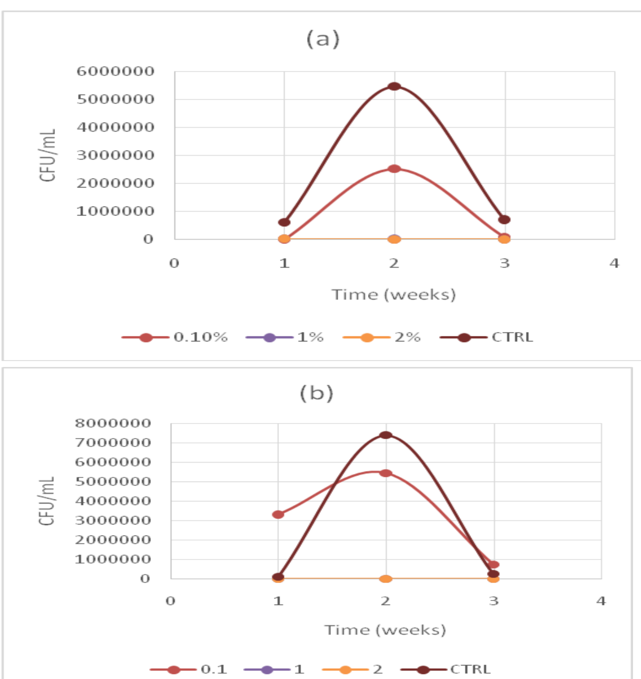

Figure 1: (a) Changes in yeast counts of control and RCFT-induced fermenting musts with different RCFT concentrations over time. (b) Changes in total microbial counts of control and RCFT-induced fermenting musts with different RCFT concentrations over time.

From the above results, the RCFT-infused fermenting musts have lower yeast and total microbial counts than the control. This could be attributed to the antimicrobial property of Rangoon creeper flowers. Rangoon creeper is an herbal plant used for the treatment of various ailments. Studies have been conducted to determine its therapeutic properties, and results have shown that secondary metabolites such as flavonoids, tannins, terpenoids, and alkaloids present in the plant have high antimicrobial properties, which are attributed to flavonoids that disrupt the membrane and cell wall of microbial cells as well as totanninsthat inactivate adhesins and enzymes in cells[9]. Rangoon creeper flower extract hasbeen reported to have high polyphenol contents and strong antioxidant activity.

2.2. Physicochemicalanalyses

Just like the yeast and total microbial counts, the physicochemical properties of the mango fermenting must, namely,TSS, pH and alcohol content showed certain trends during fermentation (Fig. 2).

2.2.1. TSS.

TSS is a very important parameter in determining the type and quality of wine. Dry wine hasan initial TSS of 20°Brix, while sweet wine has an initial TSS of 25°Brix. TSS is one of the most important factors considered in winemaking because it dictates the rate and extent of fermentation as well as of ethanol production. According to Paul et al. (2014) [10], optimum ethanol productionoccurs in the TSS range of 18-22°Brix; it decreases above this range.

TSS was initially adjusted to20°Brix for all fermenting musts. After seven days (week 2), the TSS valuesof the control and fermenting must with 0.1% RCFT decreased to 11°Brix, while those of the fermenting musts with 1 and 2% RCFT decreased to 10.5°Brix. In the third week, the TSS values of the control and fermenting must with 1% RCFT were 6.7°Brix, while those of the fermenting musts with 1 and 2% RCFTwere 6.8 and 7°Brix, respectively. Figure2(a) shows that TSS, which approximately represents the amount of fermentable sugars in the wines, decreases over time. This pattern follows the expected trend of TSS during fermentationbecause yeast metabolizes fermentable sugars (glucose and fructose) into ethanol via glycolysis. It can be observed from Fig. 2(a) that the TSSvalues of the fermenting musts with 1and 2% RCFT are slightly higher than those of the control and fermenting must with 0.1% RCFT. This is because the reducing sugars in the flower slightly increase the initially available sugars in the mango wine. In terms of fermentation rate, the graph shows that the control is not different from the RCFT-infused wines in terms of actual TSS values and the trend of TSS over time.

2.2.2. Alcohol content.

Alcohol is one of the most important factors considered in winemaking. It is used in characterizing wine into either table wine (10-11% alcohol) or dessert wine (16-23% alcohol) (Pandey et al.,2016).The alcohol contentof wine influences wine aroma and taste because the solubility of aromatic compounds is affected by alcohol concentration. In low- or normal-alcohol wines (10-14%), high-molecular-density aromatic compounds are not easily noticeable because they volatilize more slowly, and hence low-molecular-density aromatic compounds are perceived first. In high-alcohol wines (15.5%-20%), low-molecular-density aromatic compounds easily volatilizetogether with high-molecular-density aromatic compounds, and hencea more complex aroma is perceived [11].

Figure 2(b) shows that the initial alcohol contents of the control and RCFT-infused fermenting mustsare 0.0%, which increases to 10% (v/v) in the second week. For the control and fermenting must with 2% RCFT, the alcohol contents reach 15.0 and 16.0% (v/v), respectively, in the third week. The alcohol contents of the fermenting musts with 0.1 and 1% RCFT concentrations are14.5 and14.0%, respectively. The highest alcohol content is observed in the fermenting must with 2% RFCT, which may be caused by the sugar present in the flower, although at very low levels.

2.2.3. pH.

pH can affect the microbial and chemical stabilities of wine as well as wine sensory atributessuch as color, flavor, and aroma. The shelf-life of wine is mainly attributed to wine pH.Figure 2(c) shows that pH decreases over time. The initial pHs of the control and RCFT-infused fermenting mustsare all 4.3, which showed a steady decline over time. The pHs of all the wines from the control and RCFT-infused fermenting mixture after racking are found to range from 3.6 to 3.9. The optimum pH range of wine is 2.9-4, above which wine becomes chemically and microbiologically unstable [12] and tends to taste flat and lose character [13]. Low pH can increase the color and clarity of wine, improve enzyme activity, and suppress oxidation [14]. Based on the optimum pH range, the RCFT-infused wines are chemically and microbiologically stable.

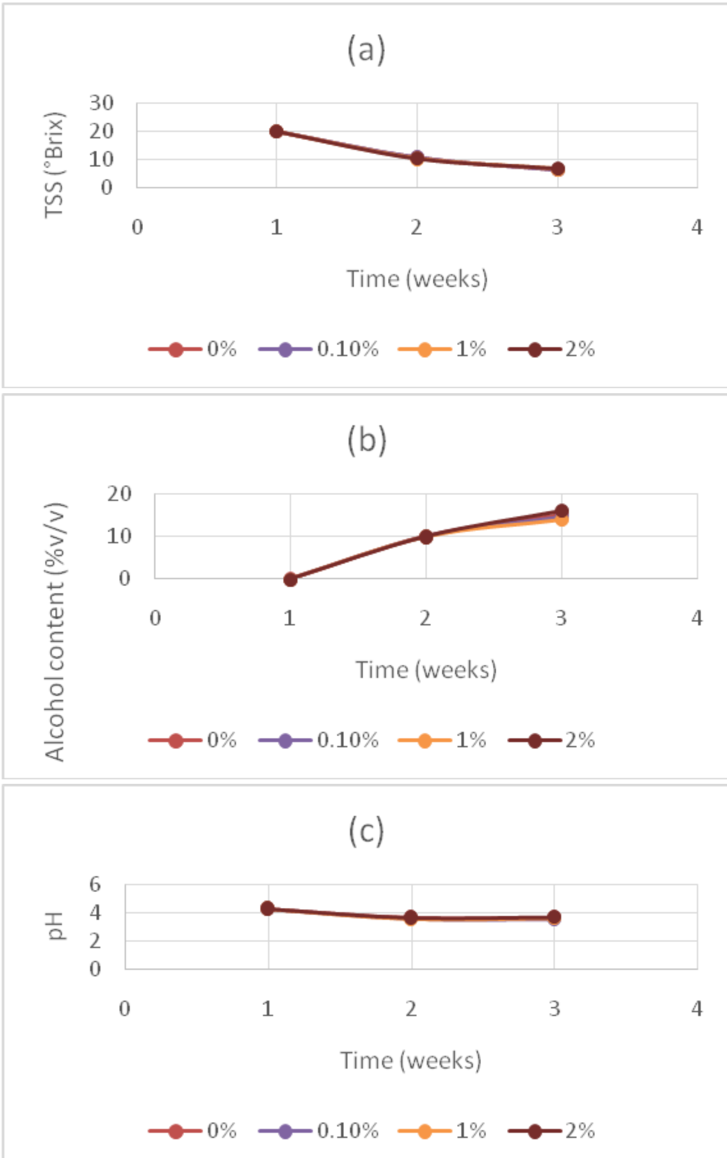

Figure 2: (a) Changes in TSS of control and RCFT-induced fermenting musts with different RCFT concentrations over time. (b) Changes in Alcohol content of control andRCFT-induced fermenting musts with different RCFT concentrations over time. (c) Changes in the pH values of the control and RCFT-infused fermenting musts with different RCFT concentrations over time.

2.3. Sensory Analysis

The analysis of variance (ANOVA) results showed that the wine sampleshave significantly different color, aroma, clarity and astringency ratings. However, they also showed that the wine samples are not significantly different in sweetness and alcoholic taste.

2.3.1. Color.

Figure 3 showsthe colors of all the wines tested. It is evident that flower infusion has a marked effect on the color of mango wine, possibly a result of the flower pigment that diffused into the product during the infusion stage. Table 1 shows that the colors of all the wine samples except Samples 2 and 5, which are both 0.1% RCFT-infused, are significantly different. Samples 9 and 10 have the highest mean color ratings because they were infused with the highest RCFT concentration and for the longest duration. The colors of Samples 2 and 5 not being significantly different suggests that RCFT-infused mango wine color is not influenced by infusion timing, only by infusion concentration. However, Samples 3 and 4 being significantly different in color from Samples 6 and 7 suggests that infusion duration affects wine color. Hence, RCFT concentration and infusion duration generally intensify the color of mango wine.

Figure 3: Photographs of the control and RCFT-infused mango winestable

2.3.2. Aroma.

Table 1 shows that wine aroma rating generally increases with increasing RCFT concentration up to 1% and then decreases. The aroma rating of the samples that were RCFT-infused dR decreases with increasing RCFT concentration. Hence, wine aroma is affected by RCFT concentration and infusion timing. Samples 3, 9 and 10 are not significantly different in aroma (Table 1). Hence, infusion duration does not affect mango wine aroma. Based on aroma alone, the optimum infusion concentration and timing for the RCFT infusion of mango wine are 1% dF.

2.3.3. Clarity.

Table 1shows that the control has the lowest mean clarity, which shows that RCFT infusion can significantly improve mango wine clarity. Table 1shows that wine clarity decreases with increasing RCFT concentration. However, theRCFT-infused winesstill have significantly higher clarity ratings than the control, suggesting that certain compounds in the RCFT serve as clarifying agents. These compounds can possibly be pectic enzymes, which are widely used for clarifying wine and juices. Further study can be conducted to identify the components responsible for the clarification property of RCFT as well as the mechanism of such clarification.The decreasing clarity with increasing RCFT concentration can be caused by either the panelist associating clarity with the darkness of color or RCFT concentrations of 1 and 2% producing too much sediments that contribute to the cloudiness of the RCFT-infused wines. Samples 2, 5 and 8 have the highest mean clarity ratings. This suggests that the highest degree of clarity can be achieved by infusion at an RCFT concentration of 0.1%. Samples 2, 8, and 9 are not

significantly different in clarity. Hence, clarity is not influenced by infusion timing. Based on clarity alone, the optimum infusion concentration and timing for the RCFT infusion of mango wine are 0.1% dF.

2.3.4. Astringency.

Table 1 shows that wine astringency generally increases with increasing RCFT concentration. There is no significant difference between the samples that were 1 and 2% RCFT-infused dF, dR or dF. The perception of astringency takes time, which is why it is the last sensation perceived. Astringency intensity also builds up as the number of samples tasted becomes larger. Differences in the inherent characteristics of the judges also influence the perception of the astringency [15]. These can be the possible reasons for the fluctuations in the results observed.

Astringency is one of the most important attributes of wine. It is defined as the combination of different sensations perceptible by touch. It is often characterized as the drying of the oral surfaces, roughness and puckering in the mouth. Wine astringency is influenced by various factors including pH, sweetness, and ethanol concentration as well as the presence of phenolic compounds and tannins. [15].

Table 1. Results of the Tukey's Honestly Significant (HSD) test on the different attributes of the control and RCFT-infused mango wines

Sample	Mean Scores of the control and RCFT-infused wine samples			
	Color	Aroma	Clarity	Astringency
1	1.55^{I}	4.79^{E}	7.47^{D}	3.43^{F}
2	2.44^{H}	7.75^{C}	11.92^{AB}	3.69^{F}
3	7.30^{F}	10.37^{A}	11.11^{BC}	9.52^{BC}
4	9.87^{D}	8.20^{BC}	11.48^{AB}	9.00^{BCD}
5	2.72^{H}	7.67^{C}	11.08^{BC}	7.54^{DE}
6	9.04^{E}	6.95^{CD}	10.69^{BC}	8.44^{CD}
7	10.75^{C}	4.71^{E}	10.05^{C}	9.26^{BC}
8	4.33^{G}	5.24^{DE}	12.67^{A}	6.25^{E}
9	12.42^{B}	10.82^{A}	11.88^{AB}	11.26^{A}
10	14.12^{A}	9.91^{AB}	9.67^{C}	10.62^{AB}

*Means with the same superscript are not significantly different.

2.3.5. Sweetness.

All the wine samples do not significantly differ in sweetness (P<0.05). This is expected because the TSS levels of all the samples were all the same, having been adjusted to 12°Brixwhen the initially obtained dry wines were all converted into sweet wines.

2.3.6. Alcoholic taste.

All the control and RCFT-infused wines did not significantly differ in alcoholic taste (P<0.05). This is because the alcohol contents of all the wines did not significantly differ, being in the range of 14-16% (v/v).

2.3.7. General acceptability.

Table 2 shows the rank sum and significance group of each of the wine samples. The most acceptable wine is ranked 1 while the least acceptable is ranked 4. The significance group is generated by getting the differences in the rank totals of the different treatments and comparing them to the critical value indicated in the Newell and MacFarlane table. Treatments belonging to the same significance group indicate that none of the treatments are more preferred over the other. For a rank test with 4 samples and 10 panelists, the indicated critical value is 15. This means that the difference in the rank total of two treatments should be greater than 15 to be considered significantly different. Results showed that all the differences of the rank totals did not exceed 15. Hence, no treatment is more preferred over the other. Since no treatment is more preferred over the other, this means that RCFT infused mango wine is comparable to the traditional mango wine.

Table 2. Results of the ranking test on the acceptability of the control and the mango wines with different RCFT concentrations.

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[1]	[2] Sample									
	[3] 1	[4] 2	[5] 3	[6] 4	[7] 5	[8] 6	[9] 7	[10]	[11]	[12] 0
[13] Rank sum *	[14] 3	[15] 1	[16] 9	[17] 4	[18] 2	[19] 4	[20] 7	[21] 7	[22] 4	[23] 9
[24] Significance group	[25]	[26]	[27]	[28]	[29]	[30]	[31]	[32]	[33]	[34]

* Analyzed using the Newell and MacFarlane (1987) table

Considering all the data and results gathered, the RCFT infusion of mango wine can improve the sensory attributes of the wine specifically in terms of clarity, aroma, and color. The positive effect of floral infusion on the clarity of the wines can result in a more economical and convenient wine production. Considering all the microbiological aspects, physicochemical aspects, and sensory characteristics, it is concluded that RCFT infusion at a concentration of 0.1% during fermentation can markedly enhance the sensory attributes and hasten the fermentation and clarification of mango wine. Hence, there is high potential for the infusion of mango wine using Rangoon creeper flowers.

3. Conclusion

With the main objective of developing a new beverage by the floral infusion of wine to help alleviate the risk posed by NCDs, mango wine was infused with RCFT, and the effects of floral infusion on the fermentation, physicochemical properties and sensory characteristics of mango wine were determined by microbiological and physicochemical analyses of the fermenting musts, and by sensory evaluation of the resulting products using a balanced incomplete block design with 25 panelists. RCFT infusion at 1% and higher decreased the microbial load of the mango fermenting must. No significant changes were observed in the physicochemical properties of the control or RCFT-infused fermenting must. Moreover, RCFT infusion of mango wine increased the aroma, color, clarity and astringency ratings. The sweetness, alcoholic taste or general acceptability did not significantly differ among all the wine samples. Based on the data gathered and analyzed, it is concluded that the floral infusion has high potential in improving the commerciability of mango wine for the consumer market owing to its ability to improve the sensory attributes and antioxidant activity, as well as to shorten the production duration of the wine.

Floral infusion of wine is a relatively new area in product development studies. Hence, its methodology needs to be improved to obtain better experimental results, facilitate production, and ensure uniform wine quality. During production of RCFT-infused wine, RCFT tends to float and clump at the surface of the fermentation bottle which may alter the conditions necessary for fermentation. Also, RCFT greatly contributes to the sediments that result in the turbidity of the final product. Considering these problems, the development and design of a floral infusion container are highly recommended as it will not only preserve the desired conditions for fermentation but also enable convenient harvesting of wine. It is also recommended to explore and study the possibility of using flowers in powdered form similar to commercial tea during infusion to enhance the diffusion of aromatic compounds, antioxidants, pigments and other components into the wine. Moreover, the flower-infused wine should also be determined of its antioxidant and phytochemicals components such as anthocyanidins, tannins, and phenolic

ISSN: 2456-8643

compounds to clarify its health benefits. Finally, to further improve this study, the antimicrobial property and clarifying ability of Rangoon creeper flowers should be evaluated as they are highly expected to have direct correlations with the cost of wine production. This can be done by identifying the components responsible for the antimicrobial and clarification actions of the flowers.

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