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Physico-chemical, antioxidant and sensory properties of artificially-carbonated fruit wine blends

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Abstract

With the aim of developing artificially-carbonated fruit wine blends, three locally available fruits (mango, pineapple and passion fruit) were fermented, blended and carbonated, and were evaluated for their physico-chemical, antioxidant and sensory properties. Physico-chemical tests conducted include pH, titratable acidity (TA; as citric acid), total soluble solids (TSS), total sugar and alcohol content. Total phenolic content (TPC) was measured by Folin-Ciocalteau Assay and antioxidant activity (free-radical scavenging activity) was evaluated by DPPH assay. Four formulations were created from the mixture of the three wine flavours. Based on overall acceptability, best wine blend formulation for carbonation consisted of 50% mango wine, 25% pineapple wine and 25% passion fruit wine. This formulation exhibited the highest TSS (6.3°Brix), total sugar content (4.5 mg/mL), total phenolic content (256 mg/L GAE) and antioxidant activity (44.47% inhibition). This blend was perceived as the sweetest, least bitter and least sour as compared to the other treatments evaluated. This blend was subjected to carbonation at -4°C.

Introduction

Wine is an alcoholic beverage produced from the anaerobic fermentation of a fruit must. It has been scientifically proven in many studies that wine is beneficial for human's health if consumed in moderation. A study by Weisse *et al*. (1995) proved that controlled wine consumption reduced the risk of getting sick from diseases caused by *Salmonella*, *Shigella* and *Escherichia coli*. Some studies have shown evidence that consumption of wine had a protective effect against the development of cardiovascular diseases. This is believed to be due to the antioxidant properties of phenolic compounds present in the wines (Wollin and Jones, 2001).

Wine can be made from any plant extract or fruit juice that contains sufficient levels of fermentable sugars. Yeasts breakdown glucose via glycolysis, a multi-step process in which one glucose molecule is converted to two molecules of pyruvate. In the absence of oxygen, pyruvate is then converted to acetaldehyde by the enzyme alcohol dehydrogenase, thereby releasing carbon dioxide as a by-product (Alcamo and Warner, 2010). There are several factors that influence the fermentation process and among these are yeast strains, pH, temperature, concentration and nutritional components of the batch of fruit must (Waites *et al*., 2001), whereas the resulting amount of alcohol produced is determined by the initial sugar level and the strain of yeast used (Buglass, 2010).

In making wine, grape is most commonly used because it produces high quality wines from its high levels of fermentable sugars consisting mainly of glucose and fructose. But non-viniferous fruits and berries can also be used. Although fruit wines are considered less noble, their quality can be comparable to grape wine (Tarko *et al*., 2008). In the Philippines, juice from fruits such as pineapple, mango, *bignay* (wild berry), *duhat* (plum), passion fruit, tamarind, *guyabano* and banana are used in wine production. Some of these fruits are rich in phenolics, compounds that contribute to wine's antioxidant activity. The type of fruit used in winemaking is often based on what is grown locally. Fully mature and ripe fruits, with good colour and flavour should be considered (Rivard, 2009). Ripe fruits usually have the optimum level of sugar concentration, acidity, pH and aromatic/flavour profile which is ideal to achieve a good quality wine.

Several studies were already undertaken to investigate the potential of different fruits in winemaking. So far, no other study had been conducted yet on blending different fruit wines to come up with a new wine product. Blending of local fruit wines would not only offer variants to wine

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products but it can also improve the quality and increase the product's consumer appeal. Blending is usually done to balance out acids, sugars and colour making a better or more complex wine (Rivard, 2009).

Another way to increase a wine's consumer appeal is by the introduction of carbon dioxide which can be done either through secondary fermentation or by artificial carbonation. Artificial carbonation is quick, simple, and is the least expensive method. This method also leaves the aromatic and taste profile of the wine unmodified (Jackson, 2000). In artificial carbonation, carbon dioxide is dissolved in the base wine by injection under pressure to impart a sparkle and a tangy taste to the wine. The base wine that will be used should be of high quality because according to Jackson (2009) carbonation may accentuate flaws that the wine may possess.

The present work thus aimed to develop an artificially-carbonated wine blend from a combination of locally available fruits that will serve as an alternative to imported sparkling wines. This was carried out through the following: formulation of a fruit wine blend from mango, pineapple and passion fruit wines, the establishment of carbonation parameters and evaluation of the physico-chemical, sensory and antioxidant properties of the fruit wine blend formulations and the carbonated blends.

Materials and methods

Fermentation of base wine

Fermentation of base wine was conducted following the procedure of Dizon (2010). *Saccharomyces ellipsoideus* from Food Science Cluster, UP Los Baños was used as the starter culture for the fermentation of mango, pineapple and passion fruit. Mango and pineapple were purchased in Los Baños and Calauan, Laguna, Philippines, respectively while passion fruit was from Lucban, Quezon, Philippines. Fully ripened, unspoiled fruits were washed with water and drained. Mango and pineapple were peeled, and the fruit pulps were cut before crushing the edible portions with a Waring blender. The homogenised pulp of pineapple was added with water at one part pineapple puree to two parts water (1:2). Mango puree was diluted at 1:2 mango puree:water as well. Passion fruit was cut into half and the pulp was scooped out. The flesh containing seeds was mashed manually and added with water at the rate of one part flesh (with seeds) to five parts water (1:5). TSS of each must was adjusted to 20°Brix using refined sugar.

Ten percent (10%) of the total volume of each of the TSS-adjusted must was set aside and transferred to Erlenmeyer flasks, and plugged with cotton. The flasks were pasteurised in boiling water bath for 30 min, and cooled to 40-45°C prior to the inoculation with cell suspension of *S. ellipsoideus*. Then, the inoculated musts were fermented at ambient temperature for 24 h.

The remaining must was placed in fermentation jars and added with 5 mL 10% sodium metabisulphite (Univar, AJAX Finechem, Auburn, NSW, Australia) for every gallon. Jars were covered with cotton plug and allowed to stand for 24 h. Then, the previously prepared starter culture was added into the must and allowed to undergo aerobic fermentation for 2 d to allow for yeast propagation. Next, the jars were covered with fermentation locks for the musts to undergo 3 to 4 w anaerobic fermentation at 28-30°C.

Fruit wine blend formulation

Following 3 w alcoholic fermentation, the raw wines were siphoned off and added with 5 mL 10% sodium metabisulphite for every gallon. The wines were aged for three months in gallon jars. Aged and clarified mango, pineapple and passion fruit wines were blended at different proportions and coded as MPAPF = 33.33% mango wine + 33.33% pineapple wine + 33.33% passion fruit wine; 2M = 50.00% mango wine + 25.00% pineapple wine + 25.00% passion fruit wine; 2PA = 25.00% mango wine + 50.00% pineapple wine + 25.00% passion fruit wine; and 2PF = 25.00% mango wine + 25.00% pineapple wine + 50.00% passion fruit wine. Sensory attributes evaluated were colour, clarity, aroma, sweetness, bitterness, sourness and overall acceptability.

Determination of optimum carbonation temperature

Using a soda siphon (Mosa, Taiwan) with 1-liter capacity and a soda charger (Mosa, Taiwan) containing 8 g CO_2, carbonation of the wine blend was done at 4°C, 0°C and -4°C. The carbonated fruit wine was then evaluated for dissolved CO_2 content using Zahm and Nagel CO_2 gas analyser (Zahm and Nagel Co., Inc., Holland, New York, USA).

Determination of sweetness preference

To determine the sweetness preference of the panellists as well as the effect of TSS on the degree of carbonation, the wine was adjusted to 6.5°Brix, 10°Brix and 12°Brix. Effervescence, bubble size, clarity, sweetness, sourness and overall acceptability were evaluated. Dissolved CO_2 content was also analysed.

Physico-chemical analyses

Analyses performed were pH, total soluble solids (TSS), titratable acidity (TA), alcohol content and total sugar content. The pH and TSS of the wine samples were measured using a pH meter (Eutech-pH 510) and hand-held refractometer (Atago), respectively. Estimation of TA (as citric acid) was done by titration of wine sample with standardised sodium hydroxide (NaOH) solution to pH 8.2. TA was calculated as follows:

$$\% \text{ TA (as citric)} = \frac{(\text{Normality NaOH} \times \text{Volume NaOH}) \times 64 \times 100}{\text{Volume sample} \times 1000}$$

The alcohol contents of the wine samples were determined using an ebulliometer (DOST-ITDI, Parañaque City, Philippines) while total sugars were analysed by phenol-sulfuric acid method (DuBois *et al*., 1956). All tests were done in triplicate.

Antioxidant properties

The total phenolic contents of the wine samples were estimated by Folin-Ciocalteu method using gallic acid as standard (Tanqueco *et al*., 2007). The results were expressed in mg L^{-1} gallic acid equivalent (GAE).

The total antioxidant activities of the wine samples were determined by 2,2' diphenylpicrylhydrazyl (DPPH) assay where 1000 µL of wine sample was added to 4 mL 0.004% (w/v) of DPPH in methanol. The solution was then allowed to stand at room temperature for 60 min, and absorbance was read at 517 nm against a reagent blank. The percent inhibition of free radical formation was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(A_{blank} - A_{sample}) \times 100}{A_{blank}}$$

where A_{blank} was absorbance of DPPH radical in methanol and A_{sample} was absorbance of DPPH radical mixed with sample.

Sensory evaluation

The fruit wine samples were evaluated by 15 panellists familiar with drinking wines. They were composed mostly of graduate students and staff of the Food Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna. Panellists evaluated the samples by quality scoring on a 7-point Hedonic scale to rate the intensity of each attribute.

Data analysis

Analysis of Variance (ANOVA) using Microsoft Excel, version 2007 was used to statistically analyse the data at $p < 0.05$ level of significance. To locate the differences among means where differences in treatments were found significant, Duncan's New Multiple Range Test (DNMRT) was carried out.

Results and discussion

Physico-chemical and antioxidant properties of fruit wine blends

Mango, pineapple, and passion fruit wines were mixed at different proportions and the resulting products were analysed for their physico-chemical and antioxidant properties (Table 1).

Table 1. Physico-chemical and antioxidant properties of blended fruit wines.

Parameter	Blend formulation			
	MPAPF	2M	2PA	2PF
pH	3.20^{b}	3.10^{c}	3.43^{a}	3.13^{bc}
TTA (% citric acid)	0.540^{b}	0.566^{a}	0.446^{c}	0.577^{a}
TSS (°Brix)	6.3^{b}	6.5^{a}	6.1^{b}	6.1^{b}
Total Sugar (mg mL^{-1})	4.50^{b}	5.16^{a}	4.10^{c}	3.75^{c}
Ethanol Content (%, v/v)	13.33^{c}	12.53^{a}	12.93^{b}	12.99^{b}
Total Phenolic Content (mg L^{-1} GAE)	256^{bc}	275^{b}	230^{a}	239^{ac}
Antioxidant Activity (% inhibition)	44.47^{bc}	45.26^{c}	42.67^{ab}	41.27^{a}

[1]Means within rows followed by the same letter(s) are not significantly different from each other at $p < 0.05$.

Treatment MPAPF = 33.33% mango, 33.33% pineapple, 33.33% passion fruit; 2M = 50% mango, 25% pineapple, 25% passion fruit; 2PA = 25% mango, 50% pineapple, 25% passion fruit; 2PF = 25% mango, 25 pineapple, 50% passion fruit

Blending mango, pineapple and passion fruit wines at different proportions yielded pH ranging from 3.10 to 3.43 with 2M having the lowest and 2PF the highest. Normally, wine pH ranges from 2.8 to 4.0 (Ribéreau-Gayon *et al*., 2006) depending on the type and health of the fruit used. But for white wines, pH of 3.1 to 3.4 is recommended (Jackson, 2009). Lower pH is desired because it enhances microbiological stability by inhibiting the growth of unwanted bacteria. It also promotes physico-chemical stability due to its effect on the solubility of tartrates (Ribéreau-Gayon *et al*., 2006). The importance of pH is not limited to its effect on the stability of wine. It also has a great impact on sensory aspects of wine because it affects the aroma, colour and clarity.

Titratable acidity is a measure of the total amount of acid present determined by titration with a strong base until the endpoint. 2PF yielded the highest level of acidity (0.577%), while 2M, MPAPF and 2PA had 0.556%, 0.540% and 0.446% total acidity, respectively. In wine, the organic acids present can enhance flavour and add to its palatability when in acceptable levels. They also aid in the precipitation of pectins and proteins which is essential to achieve a clear wine.

Sugars present in the fruit contribute the greatest to the total soluble solids (TSS) present in wine. The most common method of TSS determination is by refractometry. Aside from sugar, soluble solids measured by a refractometer also include organic acids, soluble pectins and other phenolic compounds (Kader, 2008). In the wine samples, TSS content varied from 6.1°Brix to 6.5°Brix. The highest level of TSS was observed in 2M which contained the greatest concentration of mango wine. The TSS in the wine blends ranged from 3.75 mg mL^{-1} to 5.16 mg mL^{-1}. The lowest TSS was recorded in 2PF.

The amount of alcohol produced is related to the amount of sugar in the must, thus, the amount of alcohol can be controlled by controlling the sugar level. According to the Association of Cider and Fruit Wines Producers in Europe, fruit wines have alcoholic strength of 8% to 14% alcohol by volume. All four samples fell within this recommended range for fruit wines. Highest ethanol content (13.33%) was observed in sample blended with equal portions of the component wine (MPAPF) while 2M had 12.53% alcohol which was lowest among the four samples.

Phenolic compounds are a large class of plant secondary metabolites that are structurally diverse (Cheynier, 2012). They contribute to the sensory qualities of wine mainly on the colour, flavour (odour and taste), astringency and bitterness due to their interaction with other molecular types such as proteins, polysaccharides or other polyphenols (Macheix and Fleuriet, 1990). Phenolic contents of the fruit wine formulations assayed were from 230 to 275 mg L-1 GAE. The highest phenolic content was observed in 2M which had the highest portion of mango wine followed by MPAPF and the least was observed in 2PA. Ngereza *et al*. (2008) studied the phenolic contents of mango, yellow passion fruit and pineapple in Tanzania and found out that mango had the highest concentration of phenolic contents in 100 mL of juice (2.80 mg) followed by pineapple (2.52 mg) and by passion fruit (2.11 mg). In another study conducted to Indian mango wines, total phenolic levels were at 610 mg/L and 725 mg/L for Banginapalli and Alphonso variety, respectively (Reddy *et al*., 2010). Phenolic composition of wine depends on the extraction and degree of maturation of the fruit, nature of soil and climate, winemaking procedure and the chemical reactions during the aging of wine. Other than the impact phenolics on the sensory properties of food, they are also associated with antioxidant properties.

Antioxidants are compounds that have the ability to scavenge free radicals. Free radicals can cause oxidative damage which might build up over time and lead to degenerative diseases. The values of antioxidant activity in this method are classified as high (>70% inhibition), intermediate (40-70% inhibition), and low (<40% inhibition; Hassimoto, 2005). An increasing trend in the free radical scavenging activity was observed as the portion of mango wine concentration in the blend increased. Resulting wine blends tested had intermediate antioxidant activities. 2M blend had the highest free radical scavenging activity with 45.26% inhibition followed by MPAPF. 2PA and 2PF had free radical scavenging activities of 42.67% and 41.27%, respectively, which were not significantly different from each other. In wine, the antioxidant activity is usually attributed to its phenolic content. However, components other than phenolics may also contribute to the antioxidant activity of wine. Vitamin C, vitamin E and carotene also have antioxidant power and are recognised as having the potential to reduce the risk for diseases such as cancer and heart disease. All three substrates used are rich sources of vitamin C. However, other than vitamin C, mango is also rich in polyphenolic compounds and is a good source of vitamin A due to its carotenoid compounds (Hui and Evranuz, 2012).

Sensory properties of fruit wine blend formulations

The sensory quality of wine depends on the harmony of the different tastes present in the wine. The basic flavour of a wine is formed from the balance of the sweet, sour and bitter taste. The mean scores for the sensory attributes of the blended wines are shown in Figure 1.

Based on the result, the wines were significantly different in terms of colour, aroma and overall acceptability. The samples were perceived to be near pale yellow in colour since mean scores obtained for this attribute were low. 2PF received the highest rating while 2M obtained the lightest yellow hue. Colour can be influenced by the type and variety of fruit, way of fermentation and aging, and by pH. Clarity of the wine samples achieved high mean sensory scores ranging from 5.00 to 5.73, an indication that the clarification method employed was effective. The appearance of the wine can be an indicator of the wine's condition. According to Amerine *et al*. (1967), a silky sheen in a hazy or cloudy wine

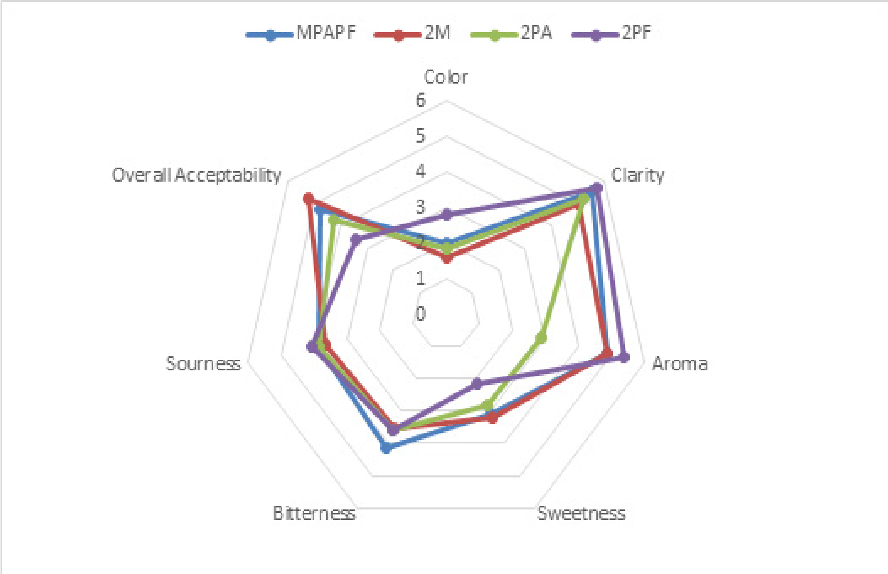

Figure 1. Spider plot of the mean sensory scores of the different formulations of blended fruit wines.

Sensory Scores: Colour = 1 - pale yellow, 7 - dark yellow; Clarity = 1 - very turbid, 7 - very clear; Aroma = 1 - weak, 7 - very strong; Sweetness = 1 - dry, 4 - just right, 7 - very sweet; Bitterness = 1 - bland, 4 - just right, 7 - very bitter; Sourness = 1 - bland, 4 - just right, 7 - very sour; General Acceptability =1 - not acceptable, 4 - acceptable, 7 - very acceptable

accompanied by a characteristic odour is a sign of bacterial spoilage. Suspended bacteria and yeast cells can cause hazy white appearance.

Among the features of wine, aroma is one of the main determinants of its quality. Volatile aroma compounds are present in fruit juices and during alcoholic fermentation; many are formed by *Saccharomyces* as secondary metabolites. Result showed that 2PF which had the greatest portion of passion fruit wine had the strongest aroma while 2PA rated significantly lower compared to the rest of the treatments. Typically, yellow passion fruit, has a distinctive floral, fruity and estery aroma with an exotic tropical sulphury note (Werkhoff *et al*., 1998). The flavour and aroma of passion fruit are due to the thiol compounds 3-sulphanylhexanol and acetic acid 3-sulphanylhexyl (Srisamatthakarn *et al*., 2012). They also cited that linalool, octanol, hexanoic acid ethyl ester and butanoic acidethyl ester are the most abundant in yellow passion fruit. Like passion fruit, mango is naturally rich in flavour and aroma. Pino and Queries (2011) investigated the aroma active compounds in mango wine and found out aldehydes, esters and alcohols with ethyl butanoate and decanal as the most potent.

Sweetness is one of the basic tastes that contribute to the overall sensory quality of wine. Excessive sweetness can mask the acid taste in wine thus, it is important to have the sugar-acid balance. A wine that is too dry can become too sour or too bitter to the taste. Among the samples, 2M was perceived as the sweetest. A trend was observed that decreasing the mango wine concentration also decreased the perception of sweetness. Total sugar content and TSS were highest in mango wine. Alcohols and glycerol also said to contribute to wine sweetness.

The bitter taste in wine mainly comes from flavonoid phenolics, particularly catechins. But as the wine ages, bitterness is diminished due to polymerisation or precipitation of phenolics (Jackson, 2009). Other factors may also influence this taste sensation such as pH, level of ethanol and sweetness (Lesschaevel and Noble, 2005). The bitterness of the fruit wine blends turned out to be at intermediate levels. Lowest rating was observed in 2M while MPAPF was found to be most bitter.

The organic acids are primarily responsible for the sourness that is perceived in wine. Low acidity decreases flavour harmony while too much acidity increases perception of sourness (Moreno-Arribas and Polo, 2009). The pH of wine also contributes to the sour taste in wine. According to Jackson (2009), pH below 3.1 makes a wine sour while those above 3.7 are considered flat. Data revealed that the samples evaluated had sourness rating ranging from 3.4 to 3.93. MPAPF was perceived as most sour though this did not differ significantly among the blends.

The harmony of the different tastes in wine determines its overall sensory quality. One of these attributes should not mask another excessively. The sweetness should be able to balance the sourness and bitterness. Based on the result, all fruit wine blend formulations except 2PA were acceptable. 2M had the highest level of acceptability followed by MPAPF, both of which contained high portions of mango wine. Mango wine created a positive impact on the blend as indicated by the increasing level of acceptability as the concentration of mango wine increased. 2M was perceived to be the sweetest, least bitter, and least sour, and these created a balance resulting in a high overall acceptability.

Determination of carbonation temperature

Effervescence of a sparkling wine or any carbonated beverage depends mainly on the concentration of dissolved CO_2 and its ability to be transferred from the liquid phase to bubble formation (Descoins *et al*., 2006). There are certain factors that affect solubility of CO_2. According to Jackson (1994), the most significant factors are soluble solid content and temperature of the wine. Ethanol content also has a great influence.

Varying the carbonation temperature resulted in significant differences among wine samples in the amount of dissolved CO_2 as shown in Table 2. Three carbonation temperatures were used and carbonating the fruit wine blend at 4°C yielded 2.2 volumes of dissolved CO_2 while at -4°C, the dissolved CO_2 content was 3.0 volumes. The trend showed that decreasing the temperature yielded a significant increase in the mean level of dissolved CO_2 in the bottled wine blend. This is because as temperature increases, the kinetic energy also increases which leads to greater molecular motion of the carbon dioxide gas particles. As a result, CO_2 gas dissolved in the wine are more likely to escape to the gas phase and the existing gas particles are less likely to be dissolved.

Table 2. Carbon dioxide content of fruit wine blend at different temperatures.

Temperature (°C)	Dissolved CO_2 Content (volume)
4	2.2[a]
0	2.5[b]
-4	3.0[c]

[1]Means within column followed by the same letter(s) are not significantly different from each other at $p < 0.05$.

Determination of sweetness preference

Carbonating 2M wine blend at different TSS levels showed an inverse relationship between sugar concentration and CO_2 content (Table 3). Mean CO_2 contents were 2.90, 2.70 and 2.43 volumes for 6.5, 10 and 12°Brix, respectively. This showed that increasing the concentration of sugar increased the concentration of solutes. This changed the viscosity of the wine thus; the solubility of CO_2 was affected resulting in a decreased concentration in terms of volume.

Table 3. Carbon dioxide content of fruit wine blend at different TSS levels.

Total Soluble Solids (°Brix)	Dissolved CO_2 Content (volume)
6.5 (unadjusted)	2.90[a]
10.0	2.70[b]
12.0	2.43[c]

[1]Means within column followed by the same letter(s) are not significantly different from each other at $p < 0.05$.

Sensory analysis (Figure 2) of the carbonated wines indicated that effervescence, clarity, sweetness, sourness and overall acceptability differed significantly among samples. When a bottle of carbonated wine is opened, there is a great reduction

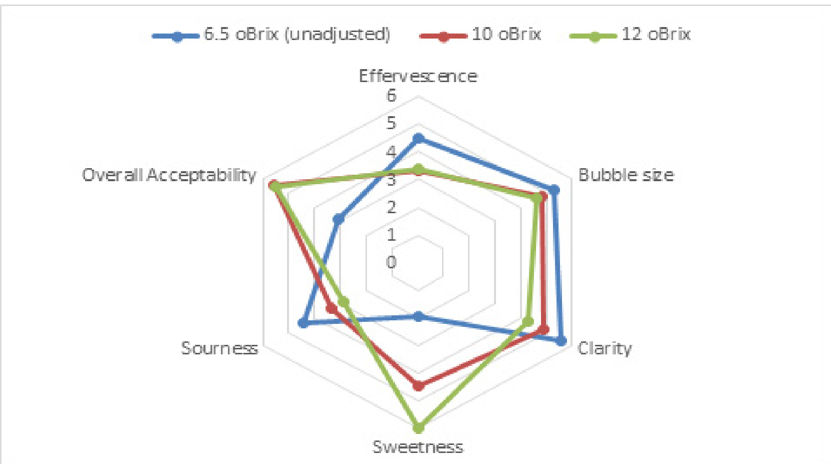

Figure 2. Spider plot of the mean sensory scores of carbonated fruit wine blends with different TSS levels.

Sensory Scores: Effervescence = 1 - very fast, 7 - very slow; Bubble Size = 1 - very large, 7 - very small; Clarity = 1 - very turbid, 7 - very clear Sweetness = 1 - dry, 4 - just right, 7 - very sweet; Sourness = 1 - bland, 4 - just right, 7 - very sour; General Acceptability = 1 - not acceptable, 4 - acceptable, 7 - very acceptable on the pressure of gas, initiating bubble formation and this process is called effervescence. Carbonated wine blend with TSS of 6.5°Brix scored highest in its effervescence. It was also in this treatment that the smallest bubbles were observed. This is because the said treatment had the highest volume of dissolved CO_2. Highest clarity rating was observed in wine with 6.5°Brix while wine sample adjusted to 12.0°Brix scored the lowest. As the total TSS increased, the clarity rating decreased.

Sweetness and sourness are two important attributes to achieve balance. An inverse relation in the sweetness and sourness were observed in the fruit wine blends. As the perception of sweetness intensified, the sourness weakened. The sweetness of the wine samples differed significantly from each other. Naturally, as the sugar concentration of the samples increased, sweetness rating also increased. Thus, wine adjusted to 12.0°Brix had the highest rating while unadjusted wine (6.5°Brix) had the lowest. Low sourness ratings were observed in wines with 10°Brix (3.33) and 12°Brix (2.87), and they did not differ significantly from each other, but both differed significantly from unadjusted sample (6.5°Brix). Among the treatments, wine sample with 10.0°Brix was the most preferred by the panellists. Wine with the lowest level of total soluble solids (6.5°Brix) scored least in terms of overall acceptability.

Conclusion

In conclusion, a carbonated fruit wine blend from locally available fruits of acceptable quality was developed. The optimised formulation consisting of 50% mango, 25% pineapple and 25% passion fruit wines was found to be of better quality in terms of antioxidant and sensory properties as compared to other formulations. The wine blend was most preferred when adjusted to 10°Brix TSS level. Carbonation temperature was identified at -4°C.

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