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Oxalate Free Averrhoa Carambola Fruit Juice and Wine Produced by a Modified Oxalate Route (Synthesis of Zinc Oxalate)

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Abstract

Carambola is highly valued for its fruits, especially the pulp which is used for a wide variety of domestic and industrial purposes. The Carambola fruit is a nutritious food containing acids, vitamins, phytosterols and other phytochemicals. The drinks (Fruit juice and fruit wine) from *Averrhoa Carambola L.* are specific due to their taste and flavor. However, they contain oxalate at concentrations which are not health promoting when consumed. We are taking this advantage to explore wine fermentation from Carambola fruit pulp by focusing on the effect of different parameters such as time of treatment for juice extraction, yeast inoculate for wine fermentation and secondary fermentation to wine quality, and to precipitated the oxalate ions from the juice and wine using metal zinc ions as a fruit metal. The main fermentation was done at 25^{o} C in 13 days, and 1 week of secondary fermentation in dark bottle at 25^{o} C. The wine produced was characterized for alcoholic content, pH, titratable acidity, Brix, and consumer acceptability while the precipitate (Zinc Oxalate) was characterized by FTIR and PXRD. The carambola fruit pulp juice and wine produced were of acceptable quality (clear, sweet, sparkling, smooth and flavoured) following the olfactory test. The drink had a pH of 4.02 and 11.2 %, alcohol content while the FTIR and PXRD results revealed that oxalate ions were precipitated from the juice and wine by zinc ions, and after precipitation of the juice and wine, the oxalate concentration in the drinks were reduced to health promoting levels. Furthermore, the precipitation process did not affect the acceptability of the wine as the olfactory parameter were not actually affected.

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Keywords: Carambola L; Fruit Juice; Fruit Wine; renal problems; Oxalate Precipitation .

1. Introduction

Fruit Juice and Wine are beverages normally prepared from fresh and /or fermented fruit juice. Traditionally from the days of the early man, fruits have served as vital components of a balanced diet as they contain essential components such as water, sugars (carbohydrates), Fats, proteins, fiber and vitamins. Furthermore, Fruits like vegetables and herbs offer great medicinal benefits through their abundant quantities of nutrients and biologically active phytochemical constituents such as phenolic and polyphenolics, flavonoids, carotenoids, ascorbic acid and glutathione. *Averrhoa carambola L.* is one of such easily grown tropical fruit plants that has recently attracted some interest as a fruit for wine production. The skin is smooth with a very thin waxy cuticle. When fresh the fruits are yellow when ripe and green when unripe, sweet smelling, crispy and very juicy, without fiber which makes the fruit easily pureed to produce a yellow juice with moderate acidity. The ripe fruits are popularly processed into fermented or unfermented drinks, jam or jelly, or eaten fresh as dessert [1]. The optimized parameters of star fruit juice have been identified as appropriate for the commercial production of wine from star fruit and sugar is an effective carbohydrate (energy) source for the growth and development of yeast during the wine processing with better retention of nutrient and sensory parameters **[2]** However, the wines contained significant amount of minerals as well as below permissible limit of microbial loads after six months of aging in dark place at ambient temperature. The sensory characteristics (color, flavor, sweetness, taste and overall acceptability) of wines do not vary with the age of the wine. Furthermore, a significant amount of minerals such as calcium, phosphorus, potassium, magnesium, iron, zinc and copper are found in Carambola wine and their concentrations do not vary much with aging of the wine. Star fruit juice is more solicited for the preparation of wine than its pulp. The predominant sugar present in both ripened and unripen fruit juice is Sucrose but the total sugar content and pH are very low which accounts for the use of extra sugar during the processing of wine. In the production of wine from carambola fruit juice, sugar and yeast starter culture help in increasing the alcohol content and sensory evaluation rates, and the Carambola wine is quite acceptable as an alcoholic beverage [3]. At initial conditions of: 23–25 Brix; pH = 4.8–5.0 and 1.6–2.5 g L^{-1} of yeast concentration, a fermented drink, from star fruit, with: an alcohol content of 11.15 GL, pH from 4.13 to 4.22, a final yeast concentration of 89 g L^{-1} , volumetric fermentation yield from 82 to 94 % (v/v), total acidity from 42 to 52 meq L^{-1} , a volatile acidity of 5 meq L^{-1} and fixed acidities of 37–47 meq L^{-1} [4]. It is unfortunate that excessive uptake of *A. carambola* could lead to acute kidney injury, particularly on an empty stomach, or dehydration state, and the use of *A. carambola* as therapy for an elderly patient is not recommended on an empty stomach. In literature, [5 and 6] reported acute oxalate nephropathy with tubulointerstitial nephritis or tubular necrosis in patients with pre-renal history (neurotoxicity performances ranged from hiccups to status epilepticus). High dose of the different *A. carambola* extracts showed different degrees of toxicity or adverse reactions and overdose intake of the fruit could result in nephrotoxicity and neurotoxicity in some individuals. Consumption of *A. carambola* could cause fatality in patients suffering from uremia, with oxalate identified as being a significant element in the toxicity of the fruit [7]. Recent investigations revealed that the nephrotoxic effect of *A. carambola* stems primarily from oxalate deposition in renal tubules, causing interstitial nephritis and acute tubular necrosis [8]. These results indicate that excessive and long-term consumption of *A. carambola* fruit juice, Jam or wine could be nephrotoxic and hepatotoxic. The situation is compounded by the fact that intake dose as well as effective and safe dose have not been sufficiently reported. Huynh and Nguyen (2017) [9], reported that alcohol fermentation technologies efficaciously reduced oxalate contents in *A. carambola*, preventing the risk of kidney stone formation. However, the works on the dose and the bioavailability of oxalate after ingestion are still insufficient, and more comprehensive studies to evaluate the dose of Oxalates of star fruit juice and its pharmacokinetic parameters in healthy individuals and patients with renal health problems are yet to be established. It is reported that *A. carambola* induced oxalate nephropathy remains an under-recognized cause for both acute and chronic kidney disease and the public must be well educated on the benefits as well as the hazardous effects of star fruits related foods and drinks [10]. Therefore, added to more public awareness about oxalate poisoning on uremic patients, there is the need to seek for ways of reducing or eliminating the concentration of the Oxalates in *A. carambola* based drinks as this will help to avoid adverse reactions to star fruits in high uremic patients. This is also very necessary because up to date, no acceptable dose has been reported. *Averrhoa Carambola* fruit juice has been used as a precipitating agent in green synthesis of nanomaterials by modified oxalate route [11]**.** Therefore in this study we are reporting the reduction of oxalate concentration in wine and fruit juice by precipitation with Zn ions (one of the metal fruit ions) which itself is required by the body for proper functioning.

2. Experimental (Materials and Methods)

2.1 Materials

Zinc Chloride, Calcium carbonate, commercial oxalic acid, and potassium permanganate were of analytical grade and were used without further purification. Sugar, yeast (*Saccharomyces cerevisiae*), *A. carambola* fruit juice and wine (source of the precipitating agent) were also used.

2.2 Methods

2.2.1 Preparation of the Star Fruit Pulp and Must

Ripe and unripe Carambola fruits were harvested from trees at Mile 15 Buea, Limbe road. The fruits were washed under running tap water, selected fruits with qualities (good appearance, smell and texture) were cut and blitzed in a blender until a pulp consistency was achieved. The pulp was filtered with the help of a cheese cloth, in order to remove the insoluble solids. 500ml of the raw juice was diluted up to 1000 ml with distilled water to make a solution of 50%. The extract was termed as the carambola fruit juice, coded (JJ) which was used for sampling and wine preparation. The extract was pasteurized, transferred into plastic bottles (labelled in two groups, JJ for juice and JW for samples used for wine production) preserved in the refrigerator for further use. To prepare the must for the fermentation, the preserved sample JW was brought out of the refrigerator, allowed to stabilize at room temperature (25 ^{o}C) and mixed with sugar to adjust the concentration of soluble solids. Table 1 shows the formulation for the Must used for fermentation while Figure 1 shows: a) Fresh unripen fruits on the tree, (b) an unripen fruit and (c) a ripe fruit.

Figure 2.1: showing a) Fresh unripen fruits on the tree, b) an unripen fruit, c) a ripe fruit

Table 2. 1: Formulation and *A. carambola* fruit juice solutions

Sample Code	Volume of juice/mL	Volume of water/mL	Mass of Sugar added/mg	Mass of yeast	Fermentation period/days
JW_1	500	500	250	10	7
JW_2	500	500	250	10	9
JW_3	500	500	250	10	14
JW_4	500	500	250	10	21

2.2.2 Characterization of Carambola Juice

The pH, moisture content, Total Solids, Total Sugars, Reducing Sugars and Oxalate content of the fruit juice were determined. The pH was determined using a pH meter while the moisture content was determined by placing 1gm of the cut fruit in a pre-weighed crucible. The crucible was placed in a hot oven and dried till a constant weight was obtained. The total solids were determined by filtering 10 ml of the juice through a pre-weighed Whitman filter paper No. 1. The filtrate was taken in a pre-weighed crucible. The crucible and the filter paper were dried in a hot air oven till a constancy of weight was reached.

2.2.3 Alcoholic Fermentation

In this study, carambola fruit juice was used to produce wine through a fermentation process for three weeks by inoculating *S. cerevisiae* (yeast) as starter. The wine production was based on one formulation: fruit juice 500ml and sugar 250 g in 500mL of water. The experiment was repeated four times and Sample JW was used for the alcoholic fermentation process. The yeast was dissolved with a small portion of Sample JW and thereafter added to the total sample volume (1.0 L). All fermentations were conducted at room temperature 298K without agitation in Clay pots covered with white cotton cloths, which allowed the elimination of the carbon dioxide produced during the fermentation process. The four series of fermentation lasted 7days, 9 days, 14 days and 21days. The Oxalate and alcohol concentration, as well as pH, were measured after 7days, 9days, 14days and

21days. By the end of the fermentation process, the alcoholic content, the total, fixed and volatile acidities were also analyzed for each wine obtained.

2.2.4 Determination of Oxalate Ion Concentration.

The concentration of oxalate ions in the fruit juice and wine were determined by titration with permanganate solution. The permanganate solution was standardized using a standard solution of commercial oxalic acid.

2.2.5 Analytical Methods

The soluble solids (S) (Brix = g 100 g^{-1}) were determined from a portable refractometer (Instrutemp, model ITREF 25). Knowing the stoichiometry ratio of sugar consumption/ethanol production (1: 4 mol of sugar per mol of ethanol), the alcohol content was estimated as a function of the fermentation time. At the end of each fermentation, a sample was collected to measure the alcohol content. The fixed and total acidities (meq L^{-1}) were measured using titrimetric methods according to Adolfo Lutz Institute [12] the volatile acidity was derived by the difference between total and fixed acidities. The pH was measured directly with a bench pH meter (Bel Engineering, W3B model).

2.2.6 Precipitation of the Oxalate from the Fruit and Wine

The precipitation of the fruit juice and wine were obtained via two experimental steps. Solutions (0.04 M) of Zinc metal chlorides (Zn^{2+}) were prepared in 200 mL of distilled water. A single molecular Zinc oxalate was synthesized by precipitation in aqueous solution containing the metal chlorides using *A. carambola* fruit juice as the source of the precipitating agent. 100 ml portion of the juice or wine extract was poured into a 400 mL round bottom flask at 80^{o}C temperature. The metal ion solutions 200ml were slowly and simultaneously added to the juice or wine while stirring with a magnetic stirrer. The mixture in each case was further stirred for 5 hours to ensure maximum precipitation of the Oxalate. The slurry obtained was aged for 8 hours. The precipitate obtained was filtered, washed successively with distilled water and ethanol, and dried in an oven at 80∘C while the Final Slurry (juice or wine) was preserved for Characterization.

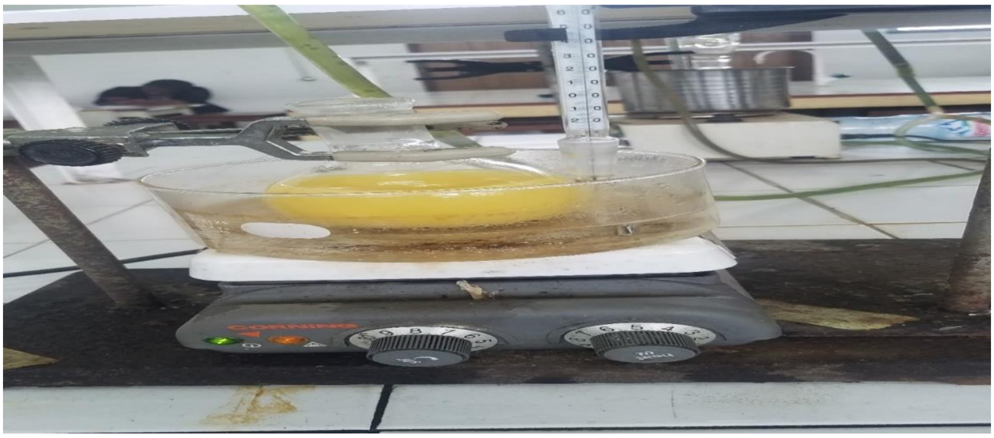

Figure 2.2: showing precipitation of JJ sample on a heating Magnetic stirrer

2.2.7 Characterization of the precipitate

The precipitate obtained from the precipitation of the juice and wine were characterized by FTIR and XRD. The FTIR spectra were recorded from 4000 to 400 cm^{-1} on a PerkinElmer Spectrum Two Universal Attenuated Total Reflectance Fourier Transform Infrared (UATR-FTIR) spectrometer while the XRD diffractograms of the precursors and the decomposition products were recorded on a Bruker D8 Advance X-ray diffractometer, using a Cu Kα radiation source (λ = 0.15406 nm, 40 kV, and 40 mA). Scans were taken over the 2θ range from 10∘ to 100∘ in steps of 0.01∘ at room temperature in open quartz sample holders. The phases were identified with the help of the Bruker DIFFRAC plus evaluation software in combination with the ICDD powder diffraction database (International Centre for Diffraction Data).

3. Results and discussions

3.1 The Physical Evaluation of the Carambola fruit

The Physical Evaluation of the Carambola fruit is presented on Table 3.1. The results show that the moisture content increases (from 85.97 % to 91 %) with maturity of the fruit and gets to maximum when the fruits ripen. These results are similar to those reported in literature: 88.6 to 91.7 [3]. The fresh and dry weight of the fruit also increase with maturity from 26.77g to 68.99g for fresh weight and from 1.77g to 2.78g for dry weight. These results may vary with the geographical zone (the climate, soil) and season during which the fruits were harvested.

Table 3.1 : weight of different components and physico-chemeical parameters of the carambola fruit pulp

	Parameter	Apparent maturity		
		Green	Half-ripe	Ripe
weight of different components of the carambola fruit	Weight of fruit (g)	60.24± 17.81	67.27± 16.36	78.68 ± 19.56
	Weight of edible portion (g)	59.42	66.66.55	78.22
	% weight of edible portion	98.64	99.10	99.42
	Dry weight (g)	1.28	1.68	2.57
physico-chemeical parameters of the carambola fruit pulp	pH	3.52	3.58	4.95
	Moisture content	85.97	88.93	91.29
	Soluble solids (^{o}brix)	6.21a ± 0.86	6.90a ± 1.01	10.23b ± 0.29
	Titratable acidity	6.25± 2.13	14.11 ± 2.99	31.21 ± 1.70

3.2 Total soluble solid

Table 3.1 summarizes the changes in the mean total soluble solid (TSS) / ^{o} Brix content of star fruit during growth. However, during wine processing, the TSS content of star fruit decreased gradually from 10.20 to 9.40 ^{o}Brix. This results are relatively low compared to literature values, 16.4 ^{o}Brix in wine prepared using sugar **[2]** but compares with 12-9.36 in papaya wine fermentation reported [13]. The TSS content of fruit wines was decreased with both fermentation and advancement in aging. The fact that the fruit juice was a blend of both ripen and unripen fruit gave the juice a greenish yellow coloration which probably created a strong barrier to oxidation reaction against light and temperature. Whereas the wine prepared using ripe fruits from literature produced slight golden yellow color, which did not affect their oxidation reaction and resulted in slightly lower changes in TSS content in the wine prepared from unripen and Semi- ripen as compared to the wine prepared from ripen fruits during the fermentation process. However the general trend which is the decreasing in TSS content is in line with other literature already mentioned.

3.3 pH and Titratable acidity

The variation of the pH over fermentation time is shown on Table 3.2 and Figure 3.1. The results revealed a drop in the final pH of the wine from 4.58 to 4.02. The results fall within similar limits to that reported [4]**,** (4.50 to 4.05) though [2] reported a drop from 3.26 to 3.07 which was lower than those observed in the present study. However, all the studies show a decrease in pH which is attributed to the oxidation of sugar molecules to organic acids. The decrease in pH was gentle and steady within the first 9 days of fermentation and then stabilizes by day 13 indicating that most of the substrate was actively used for ethanol production and cellular growth. The process profile is modified by the end of the second week with little or no formation of acid. Excessive production of acids would have been indicative of microbial contamination by acetic bacteria, excess oxygen in the fermentation medium or excessive fermentation time. The carambola juice with natural pH of around 4, makes the processing of the juice into wine more feasible even without stabilization of pH.

3.4 Alcohol content

The trend in alcohol content as shown on table 3.2 and figure 3.2, is the direct reverse of the pH trend. After 5 days of fermentation, the trend for stabilization is observed with low consumption of sugar and little ethanol production. Finally by the day 13 the level of alcohol becomes constant indicating that the fermentation process had come to an end. The maximum percentage alcohol observed was 11.2 which is similar to 10.45-11.15 reported in literature [4], 10.25-11.5 after four weeks [14] but greater than 8.3 % [15] and 0.2^{o}GL after 21days reported [3]. There is no distinct trend in the alcohol levels after fermentation but the variation could be attributed to the geographical zone and the season of the year during which the carambola fruits were harvested, the level of sugar in the must and other environmental factors . This result confirm the Suggestion from pH studies that fermentation is over by day 13 while the appreciable alcohol content observed in the present study could be attributed to the addition of sugar and yeast since the amount of reducible sugar in the natural juice was relatively low. The rapid drop in pH and increase in alcohol within the first 7days indicated that most of the fermentation actually occurs during the first week and ends around day 12 or 13 when the variation of both pH and percentage alcohol become stable.

Table 3.2: pH and alcohol content of fresh juice and various stages of wine fermentation

Parameter	JJ	JW1	JW2	JW3	JW4
pH	4.58	4.24	4.05	4.02	4.02
% alcohol content	negligible	8.1	**10.2**	**11.2**	**11.2**

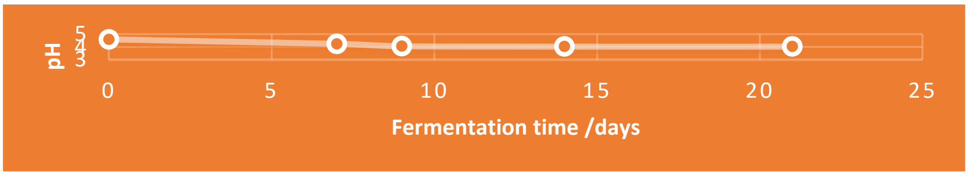

Figure 3.1: Change in pH with fermentation time

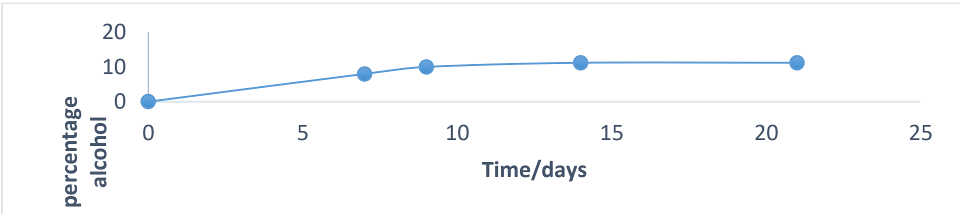

Figure 3.2: Change of % alcohol with fermentation time

3.5 Characterization of the sample precipitate JJ (fruit juice) and JW (wine)

3.5.1 FT.IR analysis

Figure 3.3 shows the FT-IR spectra of Zinc precipitates synthesized by co-precipitation using *A. Carambola* fruit juice (JJ) and wine (JW) as precipitating agents. The spectra of the Sample JJ and JW precipitates formed shows weak IR bands with a peak occurring at 3372cm^{-1} attributed to the stretching vibrations of the –OH group associated with the presence of water of crystallization. The notion of water of crystallization is confirmed by the H-O-H bending vibration observed at 739cm^{-1}. The Infrared bands observed below 1700 cm^{-1} are assigned to the oxalate vibrational modes (Ekane peter Etape *and his colleagues* 2017) (with the sharp intense peaks at 1622cm^{-1} attributed to the asymmetric stretching vibrations of the O-C-O group while those around 1450cm^{-1} and 1300cm^{-1} are attributed to the asymmetric (C=O) and symmetric (C-O) stretching vibrations in the O-C-O group). The appearance of bands, which are generated by M–O stretching vibrations at 507-490 cm^{-1} in the IR spectra of these samples, provides further support for the notion that the carbonyl oxygen is coordinated to the metal Zinc ions and are assigned to ($V_{o\text{-}c=o}$ +$\delta_{Zn\text{-}O}$). Furthermore, the fact that the free IR ($V_{C=O}$, vibration) expected at 1735-1705cm^{-1} is not observed suggests that the carboxylate group of the oxalate is completely engaged in the formation of the coordination compound and this results suggest that the precipitates formed with both the JJ and JW are actually the hydrated Zinc Oxalate.

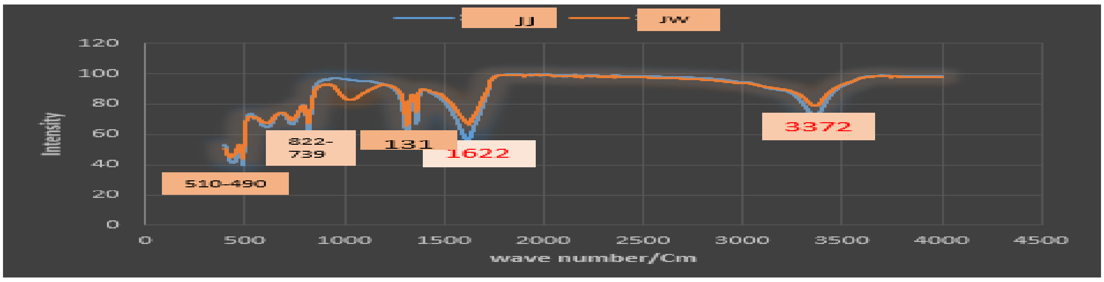

Figure 3.3: FTIR of Juice JJ and wine JW

3.5.2 PXRD Analysis

Figures 3.4 shows respectively the room temperature powder X-ray diffraction spectra for the Zinc precipitate of the fruit juice (JJ) and the wine (JW). All the reflections in the pattern could be indexed to the zinc oxalate, JCPDS card No. 25-1029 structure. The diffraction patterns for sample JJ and JW are virtually indifferent and matched perfectly with JCPDS card No.25-1029 for Zinc oxalate dehydrate as shown in Table 3.3. This results are in conformity with FT-IR results which suggested that the precipitates from JJ and JW were both hydrated Zinc Oxalates.

Table 3.3 : PXRD data for the Zinc precipitate of (JJ and JW) indexed with JCPDS card No. 25-1029 for zinc oxalate dihydrate

JCPDS No.25-1029 for Zinc Oxalate dehydrate			JJ and JW (Precipitate generated using fruit juice and Wine respectively		
hkl	d(Å)	intensity	2θ	d(Å)	intensity
-202	4.77	100	18.27	4.85	100
002	3.60	35	22.83	3.79	31
-113	2.65	35	33.38	2.72	16
021	2.55	25	35.12	2 53	24
022	2.22	30	40.53	2.25	21
221	2.03	18	43.67	2.05	16
023	1.88	25	48.43	1.89	19

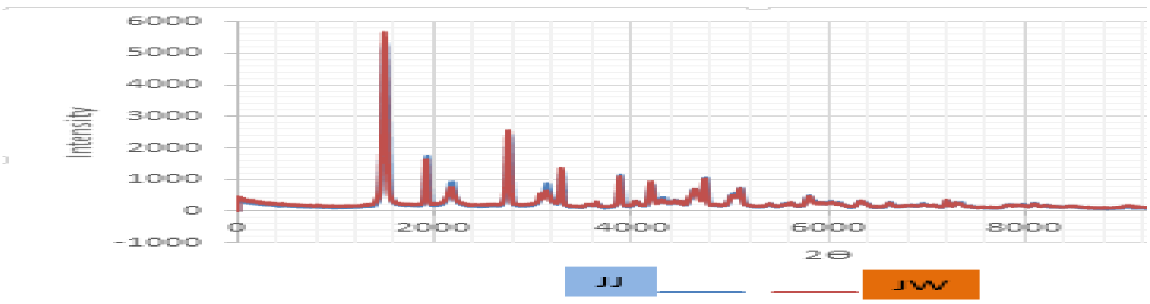

Figure 3.4: PXRD of the fruit juice JJ and the wine JW

The results from FTIR and PXRD show that the oxalate ions are actually precipitated by Zinc ions from both the Carambola fruit juice and the wine.

3.6 Control of oxalate concentrations in the juice and wine

Evaluation of Oxalate concentration in the juice JJ, and wine JW1, JW2, JW3 and JW4 before and after precipitation was done by wet chemical method (titration with acidified permanganate solution) and the results are presented on Table 3.4. The results indicate that the level of oxalate in the wine reduces with increase in fermentation time but the concentration (4.612-5.558 mg/mL) is still substantial to cause health effects in patients with renal problems and the summative effect in normal humans can still be dangerous if not addressed. Commercial carambola juices are usually prepared by placing the fruit in brine. The dilution process considerably reduces the oxalate content, while the pure juice obtained from the fresh fruit and used in traditional medicine is slightly diluted after the brine and contains high amounts of oxalate. An empty stomach and a state of dehydration could pose an additional risk for kidney damage. To avoid toxicity, neither pure carambola juice nor the undiluted juice should be consumed in large quantities, especially on an empty stomach or in a state of kidney failure (Ferrara, 2018). Lethal doses of carambola juice are rendered harmless by the oxalate removal procedure. Therefore Oxalate is a main constituent of carambola neurotoxicity [16]. Currently, scientists are focusing their research on methods to lower the concentration of toxic substances in the fruit, such as the precipitation of oxalic acid from the juice. This study has shown that the concentration of the oxalate in fruit Juice and wine after precipitation reduced considerably (99 % in wine and 86.92% in juice). This reduction is highly considerable to ensure safety in both healthy persons and patients with renal health problems.

Table 3.4: Evaluation of Oxalate concentration in the juice JJ, and wine JW1, JW2, JW3and JW4 before and after precipitation

Sample	Oxalate conc. before fermentation	Oxalate conc. after fermentation	Oxalate conc. after precipitation of fermented wine
JJ	9.250 mg/mL	NA	1.21 mg/mL
JW1	9.250 mg/mL	5.558 mg/mL	0.889 mg/mL
JW2	9.250 mg/mL	5.498 mg/mL	0.887 mg/mL
JW3	9.250 mg/mL	5.322 mg/mL	0.887 mg/mL
JW4	9.250 mg/mL	4,612 mg/mL	0.887 mg/mL

3.7 Organoleptic Characterization

Acceptability of star fruit wines by the consumers is a function of its sensory attributes, such as colour, flavor, sweetness, taste and overall acceptability of wines. These characteristics were evaluated by a team of 8 semi-trained judges' representative of the consumer sample population, with 10 points scale and they are presented on Table 6. Initially, the sensory scores on color, flavor, sweetness, taste and overall acceptability of both wines were similar (10/10). At the end of 21 days of fermentation, there was variability in almost all the characteristics except flavor. The members of the team preferred the flashy brown golden yellow color. However all the wine was generally accepted and precipitation of the wine did not affect the acceptability of the wine.

4. Conclusion

Health promoting fruit juice and Fruit wine have been produced from *A. carambola*, by a modified oxalate route for inorganic Synthesis. The drinks have won acceptability following the olfactory test. The drinks have a pH of 4.05 and % alcohol of 11.2%, with oxalate concentration of 0.887 mg/mL.

5. Data Availability

The datasets generated during and/or analyzed during the current study that are not included in this article are available from the corresponding author upon reasonable request.

6. Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

7. Conflicts of Interest

The authors declare no conflict of interest.

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