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Production, characterization and safety of wine obtained from a blend of tomato, almond, orange, lemon and African star apple extract

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ABSTRACT

The production and characterization of wine from a blend of five fruit extracts were carried out in this study. Physico-chemical parameters studied include total, fixed / volatile acidities, specific gravity and total dissolved solids. Acceptability of the wine was determined by organoleptic evaluation. Biological studies to evaluate the safety of the wine were also carried out on seventy male and female albino Wistar rats. The rats were randomly divided on the basis of body weight into seven study groups of ten animals per group (n=10). Group i served as the normal control and received normal diet and distilled water, group ii received low dose of the multi-fruit wine with additives (MFWA), group iii, MFWA given at high dose, group iv, multi-fruit wine-plain (MFWP) in low dose, group v, MFWP in high dose, group vi, standard 1 (red wine) and group vii, standard 2 (cashew wine) were all administered. The standards were administered in low doses only. Weight changes were monitored and recorded. At the expiration of the administration which lasted for 2 weeks, the animals were sacrificed and liver sample obtained for hepatic superoxide dismutase (SOD) antioxidant enzyme assay. Results of the investigation identified glycosides, reducing compounds and polyphenols in all the wine samples, with tannins present in the mixed fruit wine sample but not in the standard wines. Organoleptic and physico-chemical attributes of the wines compared well with those of the standard. There was significant ($p<0.05$) increase in SOD activity of the liver tissue of experimental animals upon administration of the mixed fruit wine indicating reduced free radical activity hence the wine is safe for human consumption.

Key words: Multi-fruit wine, superoxide dismutase, phytochemicals.

INTRODUCTION

Wine is a term associated mainly with alcoholic beverage made from the fermentation of grape juice. The commercial use of this English word and its equivalent in other languages is backed by law; therefore the term is only applicable to the alcoholic fermentation of the grape juice [1].

However, wine in general is the juice of fruits, tubers, leaves of plant etc that has been subjected to alcoholic fermentation [2]. Such wines are always classified. In other words, wines made from fruits other than grape are classified as fruit wines [3].

Over the years, the primitive procedures for wine production have been replaced by improved science and technology, to reduce costs and make more uniform products. Generally, the quality of the product is largely dependent on fruit, soil and sun resulting in a variation in flavour, bouquet and aroma.

The Nigerian wine industry is still young as the processing and bottling of palm wine is only recent despite the fact that production of palm wines locally was achieved several decades ago.

Inyang [4] reported that a major breakthrough in wine making technology by Nigerians which received patent right was the production of cocoa wine in 1983.

Although, there has been tremendous efforts at exploring different forms of fruits from which wine is made such that wines like coconut wine, kolanut wine [5] pineapple wine [6] cashew-apple wine[7] and star apple wine[8] have been produced, yet the large scale production of most of these wines remain abysmal.

South Africa is among the top ten world producers and exporters of wine [9]. This is not so for Nigeria as most of the wines consumed are imported. There is high preference for imported wines made from grapes than those produced in the country [10]. People tend to have a preference also for beer than bottled wine and see it as a ‘class thing’ or something expensive. There is need for re-orientation by value assessment and presentation as a way of solving amongst others the problems facing the Nigerian wine industry.

Recent research has demonstrated that compared to other alcoholic beverages, wines generally have tremendous health benefits with red wine having significant beneficial effects on the heart [11-14].

Wine has enormous health benefits similar to those of fruits from which they are derived [3]. A number of these effects have been documented in recent times. For instance, almonds have been found to be more effective in reducing blood levels of low density lipoprotein cholesterol (LDL-C) when combined with other foods known to independently lower cholesterol [15].

The consumption of citrus fruits like orange and lemon singly and especially when combined offer significant protection against various cancers, diabetes, Parkinson’s disease and inflammatory bowel disease [16].

Sesso *et al* [17] mentioned the cardiovascular benefits of tomato consumption. Also, it is found to be protective against various forms of cancer including pancreatic cancer [18] though its effectiveness is more in combination with fat-rich foods, such as avocado, olive oil or nuts [19].

African star apple, with high content of vitamin C 100 times that of orange [20] may be effective against peptic ulceration and stomach cancer as well as kidney stones [21-22].
Wines have been known for their medicinal effect and Physicians believe that wine consumption can aid digestion and help relief tension [23]. The Consumption of alcoholic beverages has been found to be inversely associated with the risk of cholecystectomy [24].

A derivative of guanosine, 8 – hydroxydeoxyguanosine, the primary marker for cancer development is found to be higher in individuals that drink alcohol every day, but less in those that do not drink every day compared to those who do not consume alcohol at all [25].

The consumption of red wine is known to have a remarkable protective effect against oxidative stress by decreasing 8-hydroxydeoxyguanosine levels in blood plasma [26]

Epidemiologic studies indicate that alcoholic wine consumption is associated with improved insulin sensitivity, which is masked in overweight or obese people [27-28]). The delay of tumorigenesis is prominent in Red wine consumption due to the presence of specific dietary polyphenols, such as catechins [29].

The risk of kidney stones is reduced significantly when more wine is consumed than beer [11] [12].

Wine consumption but not liquor reduces risk of prostate cancer [13] which is pronounced in red wine due to resveratrol (abundant in the skins of grapes). Resveratrol has been shown to exert both cardio protective as well as chemo protective mechanisms in animal studies [14]. Wines, both red and white are effective antibacterial agents against strains of streptococcus [30] and entheropathogens like shiggella and salmonella, protecting against bacterial diarrhea in a similar way like bismuth salicylate [31].

The presence of polyphenols, flavonoids and antioxidants in wines has an overall beneficial effect on health, even in the prevention of cancer, ameliorating the effects of alcohol when it acts adversely [32].

Most people consume alcoholic beverages either for relaxative effect or to aid digestion without necessarily considering their health benefits. There is still a lot that is not known about the combined activity of fruits when processed into wine.

The overall health benefit of wine differs and in this research, wine produced from a combination of several local fruits is expected to have a positive cumulative health benefit than single fruit wine. There is no evidence or documentation that explores this combination. Therefore, it is pertinent to investigate the various ways in which this mixed fruit wine will influence certain health parameters.

This study therefore aims to assess the characteristics of wine processed from a combination of locally available fruits in Nigeria and the health benefits when administered to Wistar rats.

MATERIALS AND METHODS

This research was carried out in two phases. The first phase involved sample collection and the production of mixed fruit wine from a blend of tomato, almond, orange, lemon and African star apple juice. This was carried out at the Cocoa Research Institute of Nigeria (CRIN), Ibadan. The

wine was characterized - screened for glycosides and reducing constituents and its organoleptic attributes evaluated. The second phase was the animal experiment and determination of some biochemical indices and physiological changes.

Source of fruits

Five tropical fruits, namely, *Lycopersicon esculentum* (tomato), *Citrus sinensis* (orange), *Citrus lemon* (lemon), *Chrysophyllum africanum* (African star apple) and *Prunus amygdalus var dulcis* (almond) were used in this study. The almond fruit was collected at Forcados Terminal, Burutu Local Government Area of Delta State. The fresh and ripe fruits of almond so collected, were washed clean of debris and sun-dried for a week. They were then cracked to extract the nuts. The extracted nuts weighing 200g were stored in a bottle, properly corked and kept in a cool dry place at about 25˚C until when used for wine production.

The *Lycopersicon esculentum* (tomato), *Chrysophyllum africanum* (African star apple), orange (*Citrus sinesis)* and *Citrus lemon* (lemon) were purchased fresh from a local market at Ibadan, Oyo State, Nigeria and identified by Mrs. Susan Onoride and Mrs. Christiana Jayeola of CRIN, Ibadan.

Sample treatment and production of immature wine

The samples so collected were processed for the production of the multi-fruit wine based on Berry's [33] method of wine production. The fermentation media was obtained and must prepared. Briefly, 20 balls of tomato weighing 1kg were thoroughly washed with clean water and ground with a blender, 70g of the almond nut was weighed and also ground with a blender, while 20 balls of orange weighing 3kg, 10 balls of lemon weighing 800g and 20 balls of African star apple weighing 2kg were all squeezed out manually to obtain their respective juice and then covered in pre-sterilized containers.

The respective fruit blends of tomato, orange, almond, African star apple and lemon were all mixed together in a 10 litre fermentation jar which was filled to the mark with distilled water. Seven (7g) of instant baker's yeast, 3.06g of sodium metabisulphite, 3.45g of ammonium sulphate were all added and finally, 1.90kg of granulated sugar was also added to induce fermentation by the baker's yeast.

The specific gravity of the resultant mixture in the fermentation jar containing the above mixture of the must was taken at 1.085 at the start of the experiment. On the sixth day of the experiment, the primary fermentation stopped when the specific gravity dropped to 1.000 and remained unchanged.

The young fruit-wine was racked (decanted) every week after the primary fermentation stopped, to separate sediments of the wine in order to achieve clarity.

After two (2) months, the wine was further left for 3 months to age. About 8 litres of the wine was produced and collected into clean white plastic containers and carried in black plastic bags to prevent interaction with sunrays. It was later stored in the refrigerator at 10-15^{o}C until when required for use.

Wine analysis

The wine obtained was analysed by determination of physico-chemical, microbial load, organoleptic and phytochemical indices.

The physico-chemical properties determined included appearance, colour, odour, taste, the condition on opening, pH, specific gravity, total acidity, fixed acidity, volatile acidity, total dissolved solid, ethanol content and residual sugar. Determination of appearance and colour were done by visual inspection by the method reported by Amadi *et al* [34] while the odour and taste were both determined by sensory evaluation of smell and taste with the palate respectively as described by Amadi et al [34] and the condition on opening was determined by the method of FDA [35]

The pH of the multi-fruit wine was determined using a pH-meter (model: Jenway 3305, U.K).

The specific gravity, total acidity (T.A), fixed acidity (F.A), volatile acidity (V.A), total dissolved solid (TDS) and residual sugar were determined by the method of FDA [35]

The alcohol content was determined using the specific gravity method and confirmed by specific gravity method for ethanol. The specific gravity was measured at the initial process or start of fermentation and then at the final process or the stop of fermentation using a hydrometer. The difference between the initial and final reading was calculated and then divided by a factor of 7.04 corresponding to the initial reading only. The value then gave the alcohol content and expressed as percentage alcohol by volume [33, 36]

Microbial load was determined using the spreadsheet method of FDA [35]

The organoleptic properties of the wine were determined using human senses as described by Amadi et al [34].

The four (4) wine samples were screened to establish their phytochemicals composition.

The following phytochemicals were assayed for: alkaloids, saponins [37] glycosides, tannins and phlobatanin [38] flavonoids, reducing compounds, polyphenols anthraquinones and hydroxymethylanthraquinones [39]. After analysis to determine the physico-chemical properties, phytochemical composition of the wine and its microbial load, the wine was divided into two halves. To 4 litres of one portion of the wine were added 0.4% citric acid, 6% caramel, 0.003% potassium metabisulphite and 3% sugar. The wine was then filtered (using a Whatman filter paper) and bottled to obtain a standard wine referred to as ‘wine with additives’. The other not treated with additives was left as plain wine.

Thus two different wines were then obtained: the one with the above additives and the plain wine. Further phytochemical analysis was carried out for the wine with additives as well as for the two other wines used as control standards (cashew wine and red wine obtained from CRIN, Ibadan and Rabana Supermarket Calabar respectively).

All chemicals and reagents used in this work were of analytical grade. Superoxide dismutase activities were estimated using OXISResearch Kits SOD-525 based on the method of Nebot *et al* [40]

Animal Studies

The second phase of the research involved animal experimentation and treatment with the mixed fruit wine to determine some biochemical and physiological changes.

Animal grouping and wine administration

A total of seventy albino Wistar rats were obtained from the animal house of the Faculty of Agriculture, University of Calabar, Calabar with permission from the animal house committee of the University and used for the study. The animals were reared with a commercial stock diet – guinea feeds rat chow (Guinea Feeds Nigeria Ltd, Benin) until they weighed 100 – 220g when they were used for the experiment.

The animals were housed in wooden box cages (size 1m x 0.5m x 0.2m). The cages had a stainless steel mesh top to provide for adequate ventilation and the floor was filled with sawdust as beddings to trap urine and faeces. The beddings were changed regularly. The animals were kept in the Department of Biochemistry animal house under adequate ventilation with a temperature and relative humidity of 26 ± 2^{o}C and 46% respectively to acclimatize. Feed and water were provided *ad libitum*. The 70 rats were randomly assigned on the basis of weight into 7 study groups of 10 rats per group, and treated according to the doses schedule in Table 1

The overall dose administration was on the basis of body weight of animals. The animals were administered low and high doses of wine samples 1 and 2, while low doses only of the standard wines 1 (red wine) and 2 (cashew wine) were given to their respective groups. The control was given placebo treatment. The dose level used was based on the AGDHA on 'Standard drinks guide' for levels considered to be low-risk [41].

The sample administration was carried out for 2 weeks. The animal grouping, doses of wine samples administered is summarized in Table 1

The dose administration of the wine samples were by oral intubations for groups II, III, IV, V, VI and VII only, between the hours of 8am and 12 noon. All the experimental animals groups in the respective groups were allowed free access to rat chow and tap water *ad libitum*. The body weights were monitored every two days throughout the time of the experiment and penultimate to the day of sacrifice, all the experimental animals were finally weighed and denied access to feed for 24 hours before they were sacrificed under chloroform anaesthesia.

All the experimental animals were anaesthetized using chloroform vapour, 24 hours after the last sample administration. Liver samples were also collected and stored frozen for superoxide dismutase (SOD) activity determination using OXISResearch Kits SOD-525 based on method of Nebot *et al* [40].

TABLE 1: Animal grouping and dose administration

Group	Treatment schedule	Quantity of wine administered (ml /Kg body weight)	Number of rats
I	Normal diet + water	Nil	10
II	Normal diet + water + low dose of wine produced with additives (sample 1)	1.0	10
III	Normal diet + water + high dose of wine produced with additives (sample 1)	1.5	10
IV	Normal diet + water + low dose of wine produced without additives (sample 2)	0.8	10
V	Normal diet + water + high dose of wine produced without additives (sample 2)	1.3	10
VI	Normal diet + water + low dose of standard wine 1 (STD 1)	0.9	10
VII	Normal diet + water + low dose of wine standard wine 2 (STD 2)	0.7	10

Statistical analysis of the results
Data obtained were subjected to statistical analysis using standard computerized Statistical Package for Social Science (SPSS) version 11. ANOVA, post hoc (least standard deviation multiple comparison) test were carried out and values expressed as mean±SEM of which samples for $p<0.05$ were considered significant.

RESULTS

Physicochemical properties:
Table 2 shows the physicochemical properties of the mixed fruit wine. The physical properties of the wine samples 1 and 2 were almost the same except for their colour. Thus their appearance, taste, odour and condition on opening were clear, dry, vinous and still for both samples 1 and 2, and the colour was light caramel for sample 1 and light golden yellow for sample 2.

The physico-chemical properties indicated a pH of 3.5 and 4.1 for samples1 and 2 respectively, while the total, fixed and volatile acidities were 0.4%, 0.3% and 0.1% respectively for sample 1 and 0.3%, 0.2% and 0.1% respectively for sample 2. The TDS were 1.81 and 1.61% for sample 1 and 2 respectively, while the residual sugar was 3.6 and 2.2 ^{o}Bx respectively. The specific gravity for sample 1 and 2 were almost the same and taken as 0.998 and 0.995 respectively.

The organoleptic evaluation of the mixed fruit wine produced in comparison with standard wines is shown in Table 3. The results show no significant difference ($p>0.05$) between the multi-fruit wines and the standard control in odour, taste, sweetness and general acceptability of the multi-fruit wines (plain versus wine with additives) but there was significant difference between samples 1 and 2 and the standards in taste and general acceptability, however there was no significant difference in sweetness and acidity balance only between sample 1 and red wine.

Phytochemical screening:
The results of phytochemical screening of wine samples of the multi-fruit wine produced (wine with additives, sample 1 and the plain, sample2), the standard wine control samples 1 and 2 (STD1-red wine and STD2-cashew wine) are presented in Table 4

The results indicated the presence of glycosides, reducing compounds and polyphenols in all the wine samples, while alkaloids were only present in STD2. Saponins were present in all except sample 2, but tannin was present only in sample 1 and 2. Anthraquinones were conspicuously absent in all the wine samples with phlobatanins and hydroxymethyl anthraquinones present only in STD1.

Effect of wine administration on hepatic superoxide dismutase (SOD):
The effect of wine administration on hepatic superoxide dismutase (SOD) of albino Wistar rat is presented in Table 5. The SOD activities of the experimental animals were significantly ($p<0.05$) different only for groups iii and vi, which maintained high and reduced liver tissue SOD activities respectively when compared with the normal control. When compared with the standards, group iii showed significantly ($p<0.05$) higher liver SOD activity compared with STD1 but not with STD 2. Groups ii, iv and v compared well with STD1 but group ii and iv were significantly ($p<0.05$) lower than STD 2.

Taken together, MFWA at high dose produced higher SOD activity compared with the normal control and compared well with STD 2.

TABLE 2: Physico-chemical properties of multi-fruit wine

Parameters \ Wine type	Wine sample 1 (with additives)	Wine sample 2 (plain)
Appearance	Clear	Clear
Colour	Light caramel	Light golden yellow
Taste	Dry	Dry
Odour	Vinous	Vinous
Condition on opening	Still	Still
pH	3.5	4.1
%Total acidity (T.A)	0.4	0.3
%Fixed acidity (F.A)	0.3	0.2
%Volatile acidity (V.A)	0.1	0.1
Alcohol content (% by volume)	12	12
Total dissolved solids (TDS)	1.81	1.61
Residual sugar (^{o}Bx)	3.6	2.2
Specific gravity	0.998	0.995

TABLE 3: Organoleptic attributes of multi-fruit wines and standards

Attributes / Wine type	Odour	Taste	Sweetness/acidity balance	General acceptability
Sample 1 (MFWA)	2.60±0.40	3.20±0.42 $^{+y}$	2.90±0.41 ^{y}	3.00±0.42^{+y}
Sample 2 (MFWP)	2.70±0.40	3.80±0.36 $^{+y}$	3.30±0.40 $^{+y}$	3.50±0.43^{+y}
STD 1 (Red wine)	1.80±0.25	1.80±0.20	2.00±0.21	1.90±0.23
STD 2 (Cashew wine)	1.80±0.33	1.80±0.39	1.80±0.33	1.90±0.31

MFWA = multi-fruit wine with additives, MFWP = multi-fruit wine-plain
Values are mean ± SEM of 10 determinations
+ = p< 0.05 for respective samples versus standard 1 (STD 1- red wine),
y = p< 0.05 for respective samples versus standard 2 (STD 2) - cashew wine

TABLE 4: Phytochemical composition of wine samples

Phytochemical \ Wine type	Sample 1	Sample 2	STD1 (red wine)	STD2 (cashew wine)
Alkaloids	-	-	-	+
Glycosides	+	+	++	++
Saponins	+	-	++	+
Tannins	+	+	-	-
Flavonoids	+	++	++	+++
Reducing compounds	++	++	+++	++
Polyphenols	++	++	+++	++
Phlobatanins	-	-	+	-
Anthraquinones	-	-	-	-
Hydroxyl anthraquinones	-	-	+	-

Slight presence	+
Strong presence	++
Very strong presence	+++
Absent	-

TABLE 5: Effect of multi-fruit wine administration on superoxide dismutase activity of albino Wistar rat

Parameter / Group	SOD (U/g)
Group i (Normal Control)	2299.36 ± 87.2
Group ii (Low dose, MFWA)	1945.82 ± 170.11 ^{y}
Group iii (High dose, MFWA)	2822.28 ± 125.29* $^{+}$
Group iv (Low dose, MFWP)	1924.37 ± 59.9 ^{y}
Group v (High dose, MFWP)	1994.53 ± 155.77
Group vi (STD 1; red wine)	1866.31 ± 92.8*
Group vii (STD 2; cashew wine)	2412.50 ± 266.4

MFWA = Multi-fruit wine with additives, MFWP = Multi-fruit wine-plain
Values are mean ± SEM of 10 determinations
** = p < 0.05 for respective samples versus normal control,*
+ = p< 0.05 for respective samples versus standard 1 (STD 1- red wine),
y = p< 0.05 for respective samples versus standard 2 (STD 2) - cashew wine

DISCUSSION

The consumption of wine dates back to 8000 years ago when it was consumed mainly for relaxative and digestive purposes [23]. Today wine is known to have medicinal benefits. This has become increasingly clear in the 21st century with increasing research in phytochemistry [42] [43] [44] [45].

The action of alcohol on biological systems has both positive (when taken moderately) and negative (when taken in excess) effects. In humans, these negative effects are seen in increased tolerance to alcohol eventually leading to chronic diseases like liver cirrhosis [46] and certain kinds of cancer [47] while the positive aspects of alcohol can be seen in improved cardiovascular system, reduced incidence of stroke, increased cognitive performance, improved insulin sensitivity among other benefits [48-49, 27-29].

The consumption and interaction of phytochemical component of wine reduces the negative effects produced by the alcoholic component and increases its health benefit compared to other alcoholic beverages [12-13] which is due predominantly to the presence of phytochemicals [14, 32, 50, 18].

The consumption on average of one to two standard drinks per day has been considered to be moderate, even from a biochemical standpoint [51]. Therefore the application of four (4) standards and above should express certain levels of toxicity which may vary with the type of alcoholic beverage. Wine has been produced from multiple fruits such as tomato, almond, orange, African star apple and lemon with excellent wine characteristics judged by the specific gravity, fixed and volatile acidities, residual sugars, total dissolved solids and other organoleptic parameters compared with standard wines.

In this study mixed fruit wine was produced and characterized by measurement of such parameters as physico-chemical properties, organoleptic attributes and phytochemical screening as well as enzyme assay of liver tissue SOD (hence biochemical and physiological indices of toxicity). The wine produced compared well with standard wines with respect to organoleptic and physico-chemical attributes.

SOD activities were elevated following MFWA administration at high doses. There have been no previous reports on the effect of multi-fruit wine on SOD activity. The probable biochemical basis for the observed effect on SOD activity may be due largely to the interactive effect of the phytochemicals present in the wine sample with the liver tissue. Phytochemicals such as flavonoids have antioxidant activity and were able to mop up free radicals and plays a compensatory role in balancing and ameliorating the negative effects of alcohol. These taken together produced an increase in the positive benefits of the wine.

High levels of tissue SOD means that antioxidative activity has been minimized. This could be due to the fact that phytochemicals or secondary metabolites in the wine which have antioxidant ability have played modulatory role of mopping up free radicals hence sparing tissue SOD activity [14, 32, 52-57].

Alcohol metabolism releases free radicals which must be neutralized for healthy tissue integrity. The secondary metabolites may play three major roles; to counteract the adverse effects of excess alcohol consumption in three ways viz: by neutralizing the free radicals produced during metabolism of alcohol, to neutralize free radicals produced by normal tissue activities and thirdly to play a support role in tissue building [58, 44].

CONCLUSION

In conclusion, multi-fruit wine of high quality (judged by physico-chemical and organoleptic properties) from locally available fruits have been produced. The assessment revealed it compared well with standard wines in respect to organoleptic and physico-chemical attributes. It also revealed that it is safe with attendant health benefits as superoxide dismutase (SOD) activities in liver tissues were high suggesting that the wine did not produce free radicals, and those produced from normal tissue metabolism were mopped up by the rich phytochemicals present in the wine. It also compared well with known standard wines. Therefore the consumption of multi-fruit wines from a combination of tomato, orange, almond, African star apple and lemon juice extract should be encouraged as this may present better health benefits than red wine.

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