

TOXICOLOGICAL ASSESSMENT OF LOCALLY PRODUCED CASHEW WINE

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ABSTRACT

Background: Wines are normally produced by alcoholic fermentation of the juice of ripe grapes by yeast; however sugar-rich fruits can be used. Nigeria is blessed with a vast array of seasonal fruits, which are rich in sugars. Large quantities of these fruits are wasted because they are produced in quantities that are in excess of consumption, and storage facilities are not available or poor. One of such seasonal sugar-rich fruits is cashew; the nutrients otherwise lost due to poor storage can be harnessed by producing wine from the cashew.

Method: Cashew wine was produced by fermenting 30% sucrose-supplemented juice of Cashew using *Saccharomyces cerevisiae* SIL 59703. The physicochemical properties, proximate composition and microbiological quality of the wine were assessed. Varying ethanol concentrations (5, 7 and 10%) of the wine were orally administered to groups of experimental animals at 1ml/160g body weight over 18 days and the toxicity assessed based on hepato-histology of the experimental animals.

Results: The wine produced had pH of 3.8; Titratable acidity of 0.25, Vitamin C content was 70mg/ 100ml, and 10.1% alcohol content. It was free of sugar and microorganisms. Histological examination revealed that liver damage occurred at 7.5% alcohol (ethanol) level and above.

Conclusion: Wine that compared favourably with imported wine was successfully produced from Cashew. The wine was rich in Vitamin C, free of fat and crude fibre and safe for consumption at 5% alcohol content. At 7% alcohol content and above it caused distortion to the liver architecture indicative of toxicity.

Keyword: *Cashew, wine, alcoholic fermentation, ethanol, liver.*

1. INTRODUCTION

Wine is an alcoholic beverage; produced by normal alcoholic fermentation of the juice of ripe grapes by the natural yeasts found on the skin of the fruit or by using selected yeast such as *Saccharomyces cerevisiae* (1). Apples, berries and blackcurrants are sometime also fermented for wine production (2). Wine was a very social drink in Ancient Egypt and great importance was given to its production and consumption. Although the Egyptians were not the first to brew wine; they were the first to record the process of wine making and celebrate its values (3). France, Italy and Germany produced over half the total world output of wine (4 and 5).

Wines are categorized using a number of different criteria, this include grape variety, region of origin, by colour, by name of the wine maker or viculturalist, or by production technique. However, three basic groups of wines are most easily distinguishable for the consumer: table wines, sparkling wines and fortified wines (6). Red wine protects against heart attack by interfering with production of a body chemical: endothelin-1 (ET-1) which clogs up arteries (7). It has been suggested (8) that alcohol may benefit many bodily organs, including the heart and the brain. However, the benefits are available only when wine is taken in moderation as over consumption of alcohol including wine can cause some diseases including cirrhosis of the liver and alcoholism.

Cashew (*Anacardium occidentale L.*) is one of the important nut crops, ranking third in the international trade. The cashew tree is believed to be a native of Brazil, from where it is dispersed to many of the tropical areas (9). It grows every well in the Guenea savannah areas of Nigeria. The apple is not a true fruit but a swollen peduncle to which the cashew nut (fruit) is attached (10). It is soft, fibrous and juicy possessing exotic flavor characteristics. There are two varieties based on external color of the fruit: red and yellow. Cashew has a harsh and acid taste; attributed to its tannins and an oily substance present in the fruit, although the nature of the oily substance is not identified.

Nigeria is blessed with a vast array of seasonal fruits, which are rich in sugars. Like many other fruits, cashew is produced yearly in quantities that are in excess of their consumption. Large quantities of those fruits are disposed off yearly due to non- availability of, or poor, storage facilities. This results in loss of the vital nutrients (Vitamins) that are associated with them and loss of potential revenue source. If the fruits could be put to other use such as wine production, the nutrients that are so lost can be harnessed and made available all year round in addition to generating revenue. This study is aimed at producing wine from cashew fruits and evaluating its toxicological effect

2. METHODS

Fermenting Organism:

Pure culture of *Saccharomyces cerevisiae* SIL 59703 was purchased and used to ferment the must.

Preparation of Cashew Wine

Ripened, fresh and healthy cashew apple were weighed (9.8kg) and surface sterilized with sodium metabisulphate solution to remove microbial contaminants. The apple was cut to bits and crushed with disinfected blender to produce 16 litres of juice, which was made up to 24 litres with warm water (45°C) to give the 'must' needed for wine production. Standardized campden tablet, sucrose (30%) and yeast nutrient were added and allowed to stay for 24 hours after which yeast was added (2).

Aerobic fermentation

Standardized amount of yeast was added to 24 litres of must in a fermenting jar by sprinkling it over the surface of the juice. The inoculated must was covered with muslin cloth and incubated at room temperature (29±2°C); it was aerated daily by stirring twice to encourage yeast multiplication (2). Aerobic fermentation was terminated after 6 days and the must was sieved to remove the shaft and debris of the crushed fruits.

Anaerobic phase of fermentation

The filtrate obtained after sieving the must was transferred into anaerobic fermentation jar and incubated at room temperature. An air trap was fixed to the fermenting jar. Campden tablet was added to the filtrate to supply sulfur dioxide gas. Fermentation was terminated after six weeks; the wine was then stored to allow the yeast to flocculate. The resulting wine was racked monthly for three months and then aged for 6 months. The aged wine was then filtered using pressurized filtering kit, decanted into sterile bottles and corked.

Fermenting organism monitoring

The population of yeast in the fermenting must during aerobic and anaerobic phases was monitored by microscopic counting using Haemocytometer (11).

Enumeration of total heterotrophic bacterial count in the wine

Populations of bacteria in the wine were assessed by standard pour plate method using nutrient agar. Tenfold serial dilution of the wine were made and 1ml of desired dilution plated using pour plate technique. The plate were incubated at 37°C for 48 hours (12).

Determination of Physicochemical Properties

The pH was measured with a pH meter (Philips PW 9418), the titratable acidity was determined using wine maker's acid kit (13). Total sugar was determined and the specific gravity was determined using a wine hydrometer (2). The alcohol content of the must was determined using Triple scale Hydrometer for Beer and wine (Model HY110).

Experimental Animals

A total of (42) forty two albino rats (*Rattus norvegicus*) weighing an average of 160g were obtained from the small animal unit of the Department of Zoology, University of Ilorin, Ilorin, Nigeria. The animals were acclimatized in metabolic cages for a week, they were maintained on rat pellet and clean drinking water *ad libitum* throughout the period of wine administration. The rats were randomly divided into three experimental groups: Group A consisted of six rats and served as the control only distilled water was administered orally. Group B consisted of 18 rats in 3 subgroups of 6 rats each and served as positive control. Red wine (10%, 7.5% and 5%) was administered to them orally at 1ml/160g body weight using canular (Silkard, 2007). Group C also consisted of 18 rats in 3 subgroups and cashew wine (10%, 7.5% and 5%) was administered to them orally at 1ml/160g body weight using canular. At the end of eighteen days the rats were anaesthetized, dissected and the liver extracted and transferred into cooled formal saline solution.

Histopathology of liver

The tissue was fixed, dehydrated, cleared, infiltrated, embedded, sectioned and stained, using haematoxylin (14). After which the mounting was done using canada balsam as the mountant after which the slides were examined using Leitz microscope and their photomicrographs taken and examined.

3. RESULTS

Generally, there was a decline in the pH from 3.8 to 3.2; while there was increase in titratable acidity from initial volume of 0.15 to 0.45. The sugar content and specific gravity of the must dropped from 16% to 4% and from 1064 sp gr to 996 sp.gr respectively. Yeast counts increased from 0 to 3.87×10^6 cells/mls while alcohol content increased from 0 to 8.5%. The total heterotrophic bacterial counts ranged from 12 to 40cfu/ml. During the anaerobic fermentation stage; pH increased from 3.3 to 3.7, while titratable acidity decreased from 0.34 to 0.25s. SG dropped from 992 to 990 sp gr; no sugar detected and final alcohol content was 10.1%.

The liver architecture was well preserved in the liver of rats to which cashew wine was administration at 5.0% alcohol content, while the architecture of the liver of rats to which cashew wine was administration at 7.5% and 10% alcohol content was significantly affected. A similar effect was observed with the Red wine. The photomicrographs show well preserved hepatocytes in the liver from control rats and those to which wines were administration at 5.0% alcohol content (Figures 1, 2 and 3). The photomicrographs also show mild hyperplasia, sever hyperplasia, distorted hepatocytes, and areas of fatty acid degeneration in the liver from rats to which wines were administration at 7.5% and 10% alcohol content (Figures 1, 2 and 3). The cashew wine produced to was acceptable to human volunteers with 64% acceptance against 86% acceptance of Red wine.

4. DISCUSSION

The drop in pH and correspond increase in titratable acidity of must during the aerobic fermentation and anaerobic fermentation stages are attributable to yeast metabolism. Acidity plays a vital role in determining wine quality by aiding the fermentation process and enhancing the overall characteristic and balance of the wine. Lack of acidity will mean a poor fermentation (2). The pH obtained for the final products fall within the acidity level of sweet and dry wines. Usually, acidity of wines lies between pH 3 and 7; higher acidities are sometime encountered with fortified and sparkling wines (6).

The decrease in sugar content during the aerobic fermentation and absence during the anaerobic fermentation stages suggest utilization of the fermentable sucrose by the yeast. The final SG of the fermenting medium (990sp gr.) falls within the 1000 and 990 sp gr. S.G range of wine (15). The alcohol content confirmed the conversion of the sugar into alcohol. As much as 91.1% of the alcohol was produced during the aerobic fermentation stage, which is consistent with previous submission (13) that about 70% of fermentation activity occurred during aerobic fermentation stage. The final alcohol content of the wine (10.1%) ranks it among good table wines; based on Bisson's submission (6) that a good Table wine must have alcohol content between 10% and 14%.

The increase in the total yeast count during aerobic fermentation can be attributed to the presence of utilizable sugar (sucrose) and yeast nutrient. Daily aeration aided rapid multiplication of the yeast cells (2). During anaerobic growth, the yeast utilizes intermediate product like acetaldehydes as hydrogen acceptors and alcohol production (1 and 16). A very important desirable property of *S. cerevisiae* SIL 59703 is that it produces alcohol efficiently and tolerates higher levels of ethanol than other fungi. The three bacterial species were identified: *Lactobacillus plantarum*, *L. brevis* and *Pediococcus pentosaceus* are non pathogenic bacterial and therefore do not constitute any threat to health. They are associated with fruits and locally fermented drink (1).

Histopathological change in a tissue is a late manifestation of a chemical, physical, mechanical or inflammatory assault on the tissue and usually complements enzyme studies. Administration of Red wine and cashew wine at 5% alcohol content showed minimal effect on the liver histology. There were no apparent disruptions of the normal liver architecture. However, administration at 7.5% and 10% alcohol content caused mild and serve hyperplasia (Figures 2 and 3). These suggest that administration of the wines at 7.5% and 10% alcohol contents could cause major histopathological changes.

Liver damage by alcohol has been attributed to metabolism of alcohol and the by-product of the metabolism such as acetaldehyde and highly reactive molecules -free radicals (17). Similar effects were observed with the red wine and cashew wine produced, this indicates that the wine comparable in terms of histological effects on the liver, hence the quality of the wines can be ascertained. It is concluded that the wine produce was of microbiological standard and its quality attributes compared favourably with the imported equivalents.

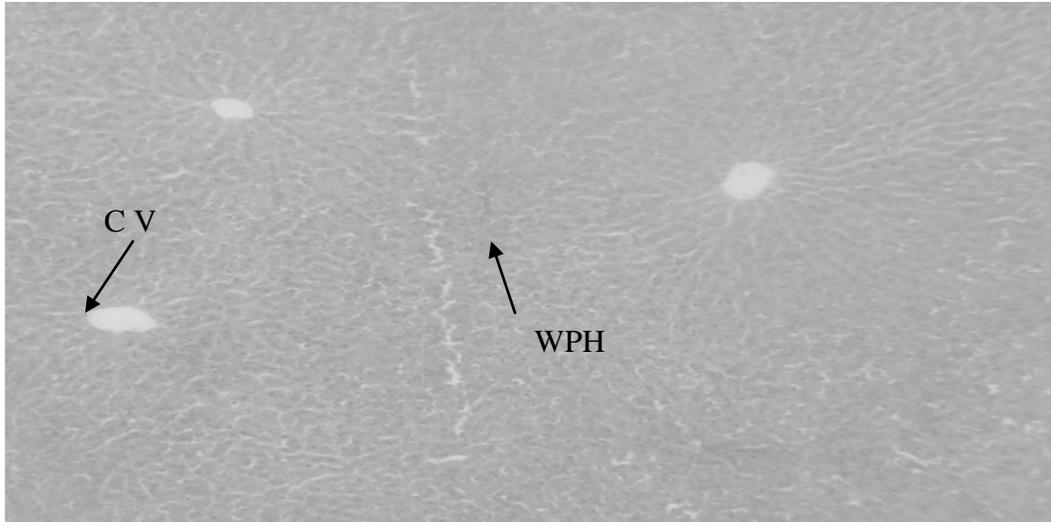


Plate 1. Photomicrograph (X120) showing well preserved hepatocytes (WPH) and central vein (CV) in the liver of control (distilled water) rats (XH stain)

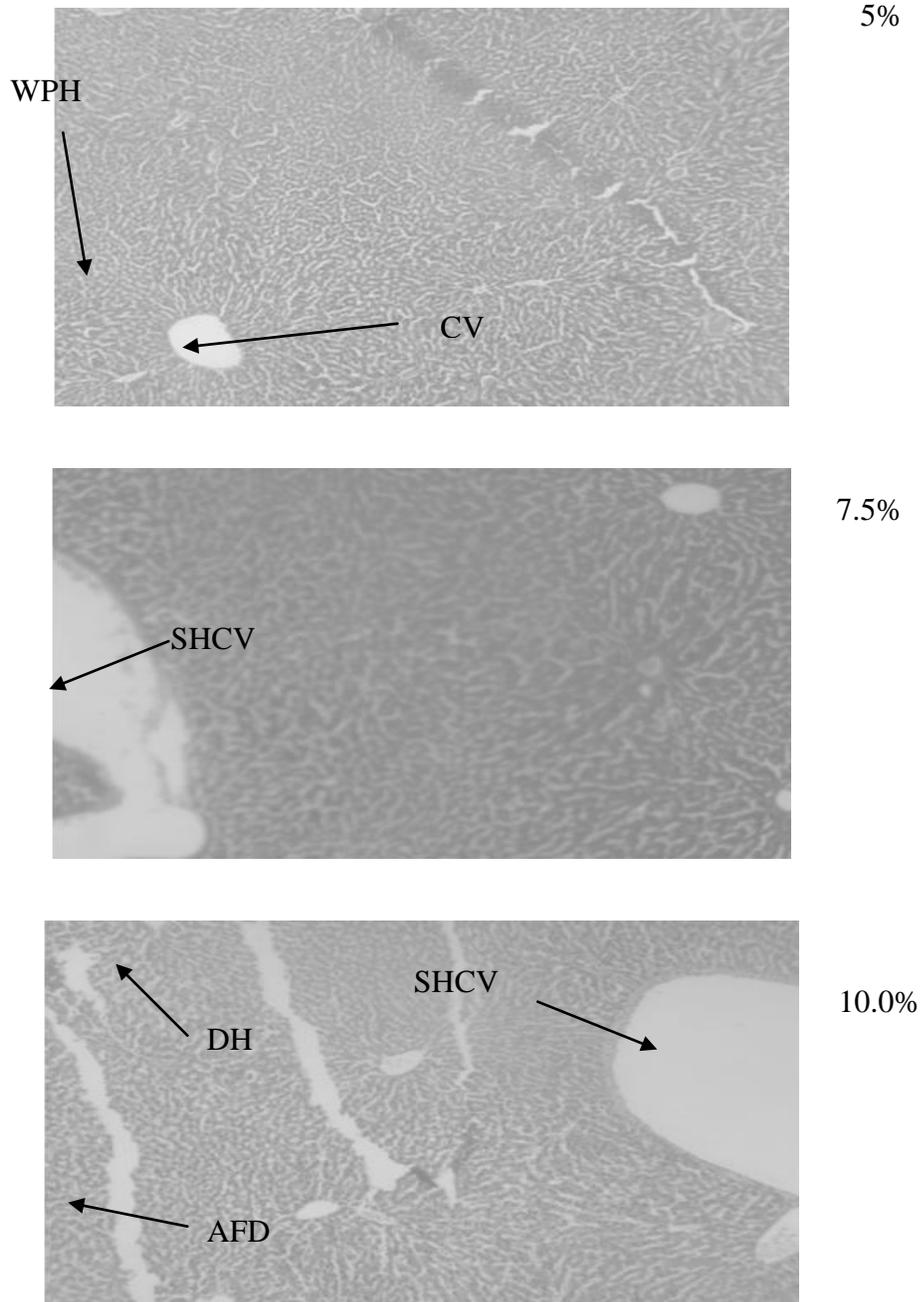


Plate 2. Photomicrograph (X120) showing Well preserved hepatocytes (WPH), Sever hyperplasia central vein (SHCV), Fatty acid degeneration (AFD), Distorted Hepatocyte (DH) and Central vein (CV) in the liver of rat to which cashew wine was administered.

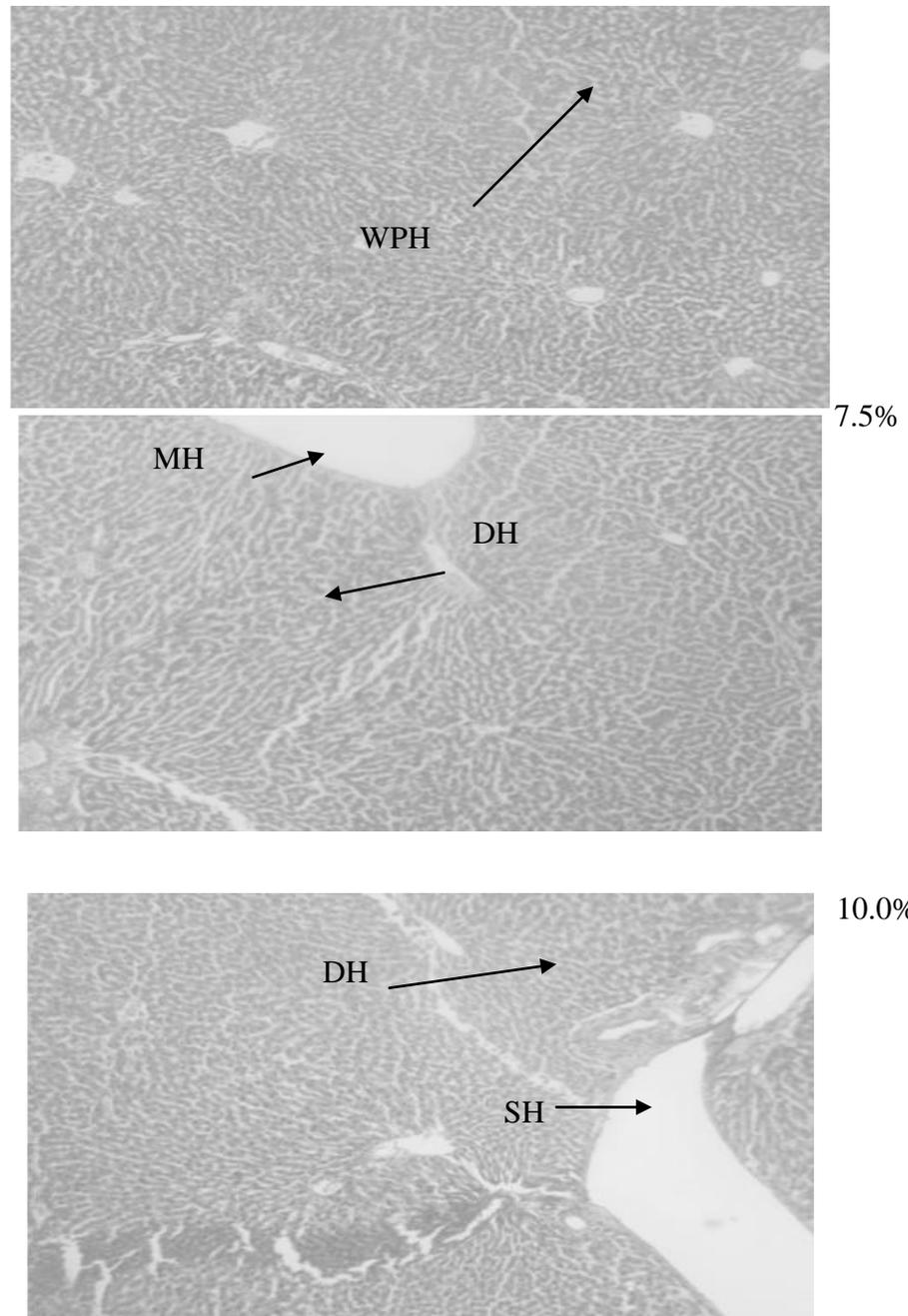


Plate 3. Photomicrograph (X120) showing well preserved hepatocytes (WPH), Mild hyperplasia (MH), Distorted Hepatocyte (DH) and sever hyperplasia (SH) in the liver of rat to which Red wine was administered

5. REFERENCES

- [1]. Okafor, N. (1987). Industrial Microbiology. University of Ife Press Ltd. Pp. 413.
- [2]. Berry, C. J. J. (2000). First steps in wine making. Published by G. W. Kent, Inc. 3667 Morgan Road, Ann Arbor MI 48108. pp 235.
- [3]. Michael, P (2000). Foods of the Gods: part 1 – Wine in Ancient Egypt
- [4]. Fessler, H. J. (1988). Guidelines to Practical Wine making. Oregon Speciality Colins 7024 NE Glisem ST. Portland or 97213 pp 1-117.
- [5]. Lau, C. K. and Jaworski, J. F. (2003) Industrial Sustainability through Biotechnology ASM News Vol. 69:110-111.
- [6]. Bisson, L. F. and Butzkc, C. E. (2007). History of Wine making. In WINE, Microsoft R Encarta.
- [7]. Slinkard Stacy (2007) Wine: Red for health – The Guardian (life) vol 2 No 14 pp 10-11.
- [8]. Jordan, D (2002). An Offering of Wine, Doctoral Thesis, The department of SemiticStudiesUniversityofSydney.([http://ses.library.usyd.edu.au/bitstream/2123/482/1/dat-NU20031211.15583702 whole .pdf](http://ses.library.usyd.edu.au/bitstream/2123/482/1/dat-NU20031211.15583702%20whole.pdf))
- [9]. Nambiar, M.C and Pillai, P.K.T (1985). Cashew. Fruits of India; Tropical and sub tropical (T.K. Bose, ed.). Naya Prokash, Caleutta. P 409.
- [10]. Morales, R. and Landires, M. C. (1988). Biote Chrology in Cashew (*Anacardium Occidentale L.*) Processing, Ciencia Agropecuaria 5: 51.
- [11]. Fawole M. O. and Oso B. A. (2004). Laboratory Manual of Microbiology. Spectrum Books Limited Spectrum House, Ring Road, Ibadan, Nigeria. Pp 1-48.
- [12]. Collins E H., Lyne PM, Greange, JM (1989). Microbiological Methods. 6th edition. Butterworth and Co. Ltd., London. 102-106.
- [13]. Kraus, E. C. (2005). Fermentation: Wine Making Articles [http://www. Eckrauso corp](http://www.Eckrauso corp).
- [14]. Baker, F. J. and Silverton, R. E. and Luckcock E. D. (1972). Haematological Indices in: introduction to Medical Laboratory Technology. Fourth Ed. Butterworth and Co. Ltd. Pp. 558-562.
- [15]. Keller, J. B. (2007). “Wine Making. [http://www .Jackkeller.net](http://www.Jackkeller.net).
- [16]. Prescott, Harley and Klein’s (2008) microbiology. 7th edition. Mc Graw-Hill Companies, Inc., 1221 Avenue of the Americas, New York, NY 10020.
- [17]. Jacqueln, J and Maher, M.D (1997). Exploring Alcohol’s Effect on Liver Function. *Alcohol Health and Research Word* Vol. 21 (1)