

Comparative Studies of Wine Produced by Spontaneous and Controlled Fermentation of Preserved Cashew (*Anacardium occidentale*) Juice

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Abstract: Cashew (*Anacardium occidentals*) wine was produced from preserved cashew juice using spontaneous and controlled fermentations. The microorganisms involved in the spontaneous fermentation were *Saccharomyces cerevisiae*, *S. cerevisiae* var. *ellipsoideus*, *Rhodotorula* sp., *Candida* sp., *Lactobacillus brevis*, *Gluconobacter oxydans* and *Klebsiella aerogenes*. In the controlled fermentation, the ameliorated juice was pitched with *S. cerevisiae* var. *ellipsoideus*. Chemical monitoring of the fermentation processes showed that the sugar content of the juice reduced from 7.5-1.7% in the spontaneous fermentation and from 15.0-3.0% in the controlled type. There was also a steady rise in alcohol content of the juice from 1.77-6.72% in the former and from 2.04-7.70% in the latter. Analysis of both wine showed that they contain protein, vitamin A and C malic, citric and lactic acid and reducing sugar. Result of sensory evaluation showed that the controlled wine was generally superior to the spontaneous wine in organoleptics and keeping qualities. Finally, the alcohol and sugar content of the controlled wine, 8.30 and 3.0% (15 g L⁻¹), respectively met the standard specifications for table wines.

Key words: Cashew, wine, fermentation, yeast, bacteria, organoleptic

INTRODUCTION

Wines are alcoholic beverages made from a variety of fruit juices by the fermentative action of selected yeast adapted to a particular type of wine followed by an ageing process. Traditionally, wine is produced from apples, pear, grape, berry and pome fruits. The wine from these fruits are products of Europe, far East, middle East, America and North Africa (Kunkee and Goswell, 1972) but not in tropical countries like Nigeria where these crops do not thrive. Thus, leading to wine importation which result in loss of foreign exchange.

Due to the poor economic situation of Nigeria, researchers must find alternative sources other than the traditional raw materials for wine making. This necessitated the production of wine from carrot by Izuagbe (1982), pineapple by Gardner *et al.* (1989), plantain by Akhimien *et al.* (1987) and from cashew (present studies).

The cashew plant *Anacardium occidentaie* is a tree crop generally considered to be native of northern part of South America and it is now found in many tropical countries including Nigeria (Ohler, 1988; Cormier, 2008). Cashew fruit consist of a hard nut and soft succulent apple. The nut is kidney shaped and it is composed of the shell and kernel while the apple is heart-shaped. Human interest on cashew is centered on the kernel which is edible and the Cashew Nut Shell Liquid (CNSL). The

CNSL has useful applications in the textile and allied industries (Ohler, 1988). The cashew apple is of less economic value than the nut and a vast quantity of it is wasted annually. When ripe, the apples appear red or yellow or colors in-between. The ripe apple is very juicy and rich in vitamins A, B and C; sugars, tannins, proteins and minerals (Akinwale, 2000).

Apart from the use of the apples in the manufacture of jams, jellies, fruit juice and candied fruits, it had also been converted into local wine in Brazil and other topical countries (Shuklajasha *et al.*, 2005). This study is therefore aimed at producing cashew wine in the laboratory using spontaneous and controlled fermentations with a view to upgrading the quality and acceptability of the local products.

MATERIALS AND METHODS

Ripe cashew apples of the yellow and red varieties were obtained fresh from orchards in Jattu and Afashio in Etsako council of Edo state. The apples were washed clean in distilled water, surface sterilized in 1.0% sodium hypochlorite solution and the juice extracted with a mechanical grinder. The preserved extracted juice was converted to wine by fermentation as in Fig. 1a, b. Fermentation of juice was carried out in sterile 5 L aspirator bottles containing 2 L of the preserved juice. The juice was pitched with 5% v/v culture of

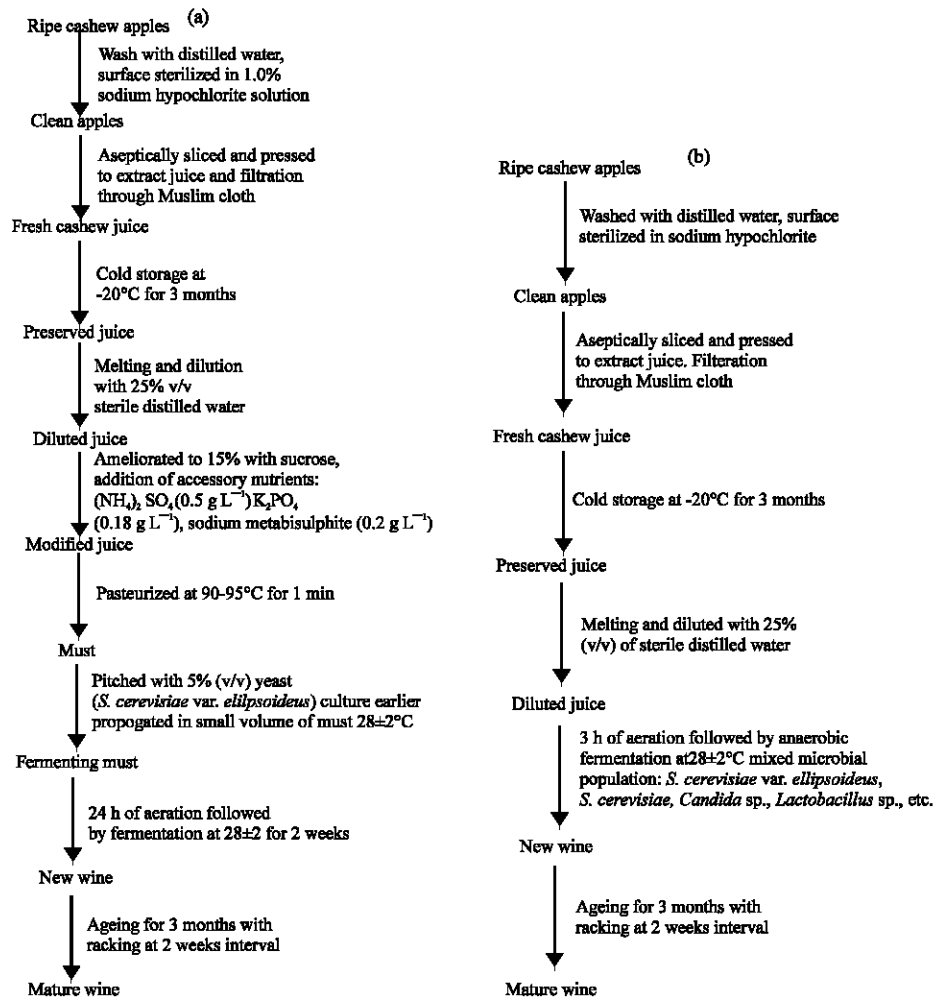


Fig. 1: a) Flow diagram for the production of cashew wine by controlled fermentation and b) flow diagram for the production of cashew wine by spontaneous fermentation

Saccharomyces cerevisiae var. *ellipsoideus* earlier propagated in small volume of must (Prescott *et al.*, 1999). The process was monitored by chemical and microbiological analysis of the fermenting must every 24 and 48 h, respectively.

Finally, the juice and matured wine produced were subjected to chemical and microbiological analysis as well as sensory evaluation.

Chemical analysis: Acidity (total, volatile and fixed), specific gravity, methanol and vitamin C content were determined by methods of Pearson (1976). Total sugar, reducing and non-reducing sugar as well as protein were determined according to AOAC (2000)'s methods. pH was measured using pH meter (Kent electronics, Ltd; Model 7065). Alcohol was measured using the specific gravity method. Organic acids were detected with paper

chromatography as described by Kunkel and Goswell (1972). Vitamin A was determined by the method of British pharmacopoeia.

Microbiological analysis: Enumeration of bacteria was by aerobic plate count technique of Gilliland *et al.* (1976). Yeast and mould were enumerated by methods outlined by Koburger (1976).

Characterization of isolates was by colonial and morphological characteristics as well as biochemical tests (Collins and Lyne, 1984). Final identification was achieved by comparing characteristics of isolates with those of known taxa.

Sensory evaluation: Organoleptic assessment of wine samples by 10 persons were made with reference to the standards of Roycroft *et al.* (1979). The parameter of wine

examined for are clarity, aroma, flavour, sweetness, astringency, colour and general quality. The maximum score for each parameter is 3 points and the minimum is 0.

RESULTS AND DISCUSSION

Results of the analysis of fresh and preserved juice showed that the former retained its organoleptic and chemical properties as well as its yeast flora after a period of 3 months cold storage. There was however, a reduction in its bacterial flora, i.e., freezing destroyed *Leuconostoc* sp. and *Pediococcus* sp. (Table 1). This implies that *Lactobacillus brevis*, *Gluconobacter oxydans*, *Klebsiella aerogenes*, *Saccharomyces cerevisiae* var. *elliipsoideus*, *S. cerevisiae*, *Candida* sp. and *Rhodotorula* sp. were associated with the spontaneous fermentation of the preserved juice.

The presence of few microbial types could be attributed to the sterilization of apples as well as its high acidic and tannin content which are inhibitory to microbial growth (Ohler, 1988). Chemical and microbiological monitoring of spontaneous and controlled fermentation of cashew juice (Table 2-4) showed that there was a general decrease in pH, total sugar and specific gravity as well as increase in alcohol, yeast count and titratable acidity of the fermenting must with increasing incubation time. This is an affirmation that sugar was utilized by yeasts, thus producing alcohol and acidic metabolites (Adeoye *et al.*, 1991; Ikenebomeh and Madagwu, 2001).

Result of Table 2 also showed that the changes in chemical parameters such as pH and titratable acidity were comparatively more uniform in the controlled fermentation.

This may be due to other organisms competing with the yeast for the limited nutrients available and the absence of limiting and non-limiting factors in the spontaneous fermentation. Ammonium sulphate and added sugar were the limiting and non-limiting factors, respectively in the controlled fermentation (Phaff and Amerine, 1979).

Result of Table 4 showed that the yeast cells were more active in the controlled fermentation. This was attested to by the relatively short time (<24 h) before vigorous evolution of gas occur. This may be attributed to the fact that the juice was pitched with a higher concentration of actively growing yeast (Gliigbo, 1963). Aeration for about 24 h may also have contributed to the growth of the yeast because oxygen speeds up the synthesis of membrane and sterols needed for growth (Maldonado *et al.*, 1975). Table 5 showed that the sugar content of juice (7.5%) was reduced to 1.7% in the spontaneous wine while it was reduced from 15-3.0% in

Table 1: Microorganisms in cashew Juice and wine

Micro-organisms	Fresh juice	Preserved juice	Wine (CF)	Wine (SF)
Bacteria	<i>Lactobacillus</i>	<i>L. brevis</i>	<i>L. brevis</i>	-
	<i>L. brevis</i>			
	<i>Gluconobacter</i>	<i>G. oxydans</i>	<i>G. oxydans</i>	
	<i>Oxydans</i>			
	<i>Pediococcus</i> sp.	<i>K. aerogenes</i>		
	<i>Leuconostoc</i> sp.			
Yeast	<i>Saccharomyces</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae</i>
	<i>Cerevisiae</i>		Var. <i>cerevisiae</i>	
	<i>Candida</i> sp.	<i>Candida</i> sp.	<i>S. cerevisiae</i> Var.	
	<i>Rhodotorula</i> sp.	<i>Rhodotorula</i> sp.	<i>Rhodotorula</i> sp.	
		<i>S. cerevisiae</i>		
Mould	<i>Aspergillus</i> sp.	-	-	-

CF = Controlled Fermentation; SF = Spontaneous Fermentation

Table 2: Specific gravity, total sugar and alcohol content of juice during fermentation

Time (days)	*Specific gravity		*Total sugar		*Alcohol content	
	SF	CF	SF	CF	SF	CF
0	1.050	1.060	7.50	15.00	-	-
1	1.040	1.045	6.20	13.60	1.77	2.04
2	1.027	1.033	5.10	11.40	3.40	3.67
3	1.020	1.018	4.20	10.00	3.53	5.71
4	1.018	1.008	3.60	9.00	3.94	7.07
5	1.015	1.005	3.30	7.20	4.76	7.47
6	1.012	1.003	2.80	6.00	5.43	8.02
7	1.007	1.001	2.50	5.20	6.11	8.16
8	1.002	1.000	2.10	4.80	6.27	8.22
9	0.098	0.099	1.70	3.00	6.72	7.70
10	0.098	0.099	1.70	3.00	6.72	7.70

*Mean of 3 determinations; CF = Controlled Fermentation; SF = Spontaneous Fermentation

Table 3: pH and titratable acidity of juice during fermentation

Time (days)	pH*		Titratable acidity	
	SF	CF	SF	CF
0	4.80	4.68	0.396	0.410
1	4.69	3.40	0.411	0.422
2	4.24	4.32	0.518	0.520
3	4.21	4.28	0.572	0.590
4	4.25	4.21	0.620	0.630
5	4.28	4.10	0.650	0.680
6	4.00	4.02	0.680	0.700
7	3.98	3.95	0.720	0.740
8	3.95	3.84	0.760	0.770
9	3.94	3.80	0.780	0.830
10	3.94	3.80	0.780	0.830

*Mean of 3 determinations; CF = Controlled Fermentation; SF = Spontaneous Fermentation

the controlled wine. This reduction of sugar content in the spontaneous (77.8%) and controlled (80%) wine is an indication that the organisms have utilized a major proportion of the sugar present in the juice. The sugars identified in the juice were of the reducing and non-reducing type and those detected as residual sugar in the two wines were of the reducing type only. This showed that there was selective utilization of sugars by wine yeast (Phaff and Amerine, 1979). The organic acid types in the juice and wines were lactic, malic and citric

Table 4: Total yeast and bacterial count of must during controlled and spontaneous fermentations

Time (days)	Yeast count* (cfu mL ⁻¹)		Bacterial count* (cfu mL ⁻¹)	
	SF	CF	SF	CF
0	6.1×10 ⁵	5.0×10 ⁶	2.4×10 ⁵	3.0×10 ¹
2	4.2×10 ⁶	8.5×10 ⁷	2.1×10 ⁷	0
4	6.0×10 ⁶	4.0×10 ⁸	4.0×10 ⁷	0
6	1.3×10 ⁷	7.0×10 ⁸	1.0×10 ⁷	0
8	8.0×10 ⁵	6.2×10 ⁶	6.5×10 ⁵	0
10	2.2×10 ⁵	1.7×10 ⁶	7.1×10 ⁴	0

*Mean of 3 determinations; CF = Controlled Fermentation; SF = Spontaneous Fermentation

Table 5: Chemical analysis of cashew juice and wines

Contents	Parameters*		
	Juice	Wine (SF)	Wine (CF)
Total sugar (%)	7.50	0.30	1.50
Reducing sugar	Present	Present	Present
Non-reducing sugar	Present	Absent	Absent
Lactic acid	Present	Present	Present
Citric acid	Present	Present	Present
Malic acid	Present	Present	Present
Protein content (%)	3.1	0.5	0.6
Vitamin A	Present	Present	Present
Vitamin C (mg mL ⁻¹)	9.00	6.00	2.00
pH	4.80	3.94	3.80
Titrateable acidity	0.590	0.800	0.860
Volatile acidity	-	0.160	0.180
Fixed acidity	-	0.640	0.680
Alcohol (%)	-	7.08	8.30
Methanol	Present	Present	Absent

Mean of three determinations; CF = Controlled Fermentation; SF = Spontaneous Fermentation

acids. This is an indication that malolactic fermentation may not have occurred in the wines since malic acid present in the wine may have originated from the juice. The vitamin C content of the spontaneous and controlled wines were 0.6 and 0.2 mg mL⁻¹, respectively. The comparatively low vitamin C content of the controlled wine may be due to the effect of pasteurization. It is structurally destroyed in the presence of heat and oxygen. Reduction of vitamin C could also be due to its participation in caramel formation in the presence of invert sugar. The yeast used for the fermentation may probably have metabolized some of the vitamin C in the juice.

The titrateable acidity of the juice, spontaneous and controlled wine were 0.590, 0.800 and 0.860, respectively. This is in consonance with findings of Adeoye *et al.* (1991) who obtained 0.530 titrateable acidity in cashew juice. The relatively low titrateable acidity of the spontaneous wine may be due to mixed fermentation by yeast and bacteria.

Also, it could be that the organic acids were held up in neutral product such as esters and ketenes formed from the conjugation of acids and other microbial metabolites (Phaff and Amerine, 1979). The protein content of juice, spontaneous and controlled wine were 3.10, 0.5 and 0.6%, respectively. This may be due to the utilization of protein

Table 6: Sensory attributes of wines from spontaneous and controlled fermentations

Parameters	Average score	
	Wine (SF)	Wine (CF)
Colour	2.0	3.0
Clarity	1.0	2.0
Aroma	2.0	1.5
Flavour	1.0	3.0
Sweetness	1.0	2.0
Astringency	1.0	1.0
General quality	1.5	2.0
Total points	9.5	14.5

Outstanding wine = 17-20 points; Standard wine = 13-16 points; Commercial wine with noticeable defects = 9-12 points; Below commercial acceptability = 5-8 points; Completely spoiled wine = 1-4 points (Roycroft *et al.*, 1979)

by yeast (bacteria) for growth (Peynaud and Ribereau-Gayon, 1971). The protein content of the controlled wine was high enough to fall within the limit of standards for American wines but not enough to cause haziness in the wine (Prescott and Dunn, 1959).

Methanol was detected in the juice and spontaneous wine but not in the controlled wine. This is in agreement with findings of Reed and Pepler (1973) that small quantities of methanol were usually formed in fruit juices as the fruit pectin undergoes demethylation by pectin methylesterase. The absence of methanol in the controlled wine may be due to its loss by evaporation during pasteurization (The boiling point of methanol is 65°C). Results of sensory evaluation (Table 6) showed that the controlled wine with 14.5% points was generally better. It record more points in all the parameters tested for except in aroma where the spontaneous wine was superior. The better aroma of the spontaneous wine could be attributed to the presence of methanol which had been shown to contribute significantly to the aroma of wine (Reed and Pepler, 1973).

Finally, there was no microbial deterioration of controlled wine whereas, a noticeable spoilage (slime formation) of the spontaneous wine occurred after ageing for 3 months. The superior keeping quality of the controlled wine result from the addition of sodium metabisulphite and pasteurization of juice before fermentation. Sulphur trioxide released from sodium metabisulphite acts as an antioxidant, antiseptic and a stabilizer (Phaff and Amerine, 1979).

CONCLUSION

The seasonality of cashew fruits as well as the poor keeping qualities of the apples and the absence of industries utilizing the apples as raw material resulted in the annual economic stage of the apples in Nigeria. Measures put in place to reduce this wastage include the

extraction and preservation of cashew juice and conversion of the juice to wine by fermentation. These measures have greatly enhanced the keeping qualities of cashew juice, thus reducing wastage.

The suitability of the juice for yeast growth and acidity of the product are advantages to the wine maker, since little effort is needed to propagate the yeast cells and the acidity discourages the growth of undesirable spoilage microorganisms. Cashew wine is a nutritious, palatable and nourishing drink rich in vitamins A and C, hence can help solve vitamin deficiencies in man.

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