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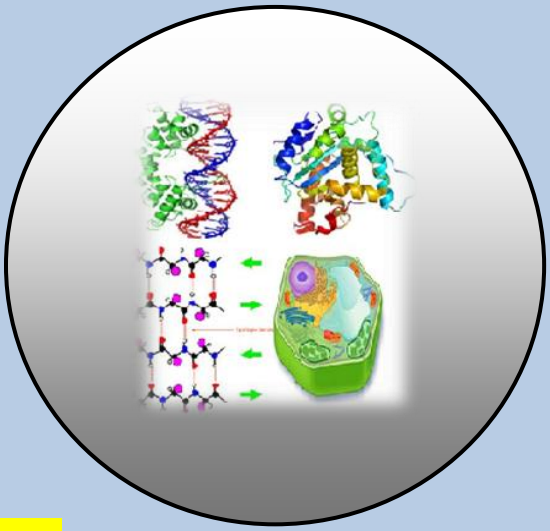

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Palm Wine Preservation Using Bitter Leaf (*Vernonia amygdalina*)

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ABSTRACT

This study evaluated the bio-preservative effect of leaves of Vernonia amygdalina on palm wine by analysing the alcohol content and the rate of yeast growth of the control and treated samples of palm wine. The duration of the study was for seven days (168 hours).The results of the alcohol content determination showed that the average increase in alcohol content of the control sample of the palm wine was 3.79 ± 3.11%, while that of the treated sample of the palm wine was 0.55 ± 0.16%. The mean alcohol content values of the control and the treated samples were 14.61± 6.62% and 6.41 ± 0.91% respectively. The results of the alcohol content therefore showed significant ($p<0.05$) increase in the alcohol content of the control sample compared to the treated sample of the palm wine. The results of the yeast count of the two palm wine samples indicated that the mean yeast count values of the control and treated samples were $(3.16 \pm 4.16) \times 10^8$ and $(1.42 \pm 0.39) \times 10^8$ CFU ml^{-1} respectively while the average growth rate of yeast was $(2.85 \pm 4.48) \times 10^8$ CFU ml^{-1} for the control sample of the palm wine and $(2.83 \pm 2.86) \times 10^7$ CFU $ml^{-1}$for the treated sample of the palm wine. The results of the yeast content showed that Vernonia amygdalina hindered the growth of yeast in the treated sample of palm wine and at the same time reducing significantly the alcohol content of palm wine and so Vernonia amygdalina can be actively used to preserve palm wine.
Keywords: Palm wine, Vernonia amygdalina, Yeast and Alcohol.

INTRODUCTION

Palm wine is the fermented sap of various palm trees especially Palmyra, silver date palm and coconut palms. Palm wine is an alcoholic beverage that is made by fermenting the sugary sap from various palm plants. It is collected by tapping the top of the trunk by felling the palm tree and boring a hole into the trunk. It is a cloudy whitish beverage with a sweet alcoholic taste and a very short shelf life of only one day. The wine is consumed in a variety of flavours, varying from sweet-unfermented to sour-fermented and vinegar

(Chandrasekhar *et al.,* 2012). The wine is an excellent substrate for microbial growth and fermentation starts soon after the sap is collected and within an hour or two becomes reasonably high in alcohol (up to 4%); if allowed to continue to ferment for more than a day, it starts turning into vinegar (Onwuakor and Ukaegbu-Obi, 2014).

The unfermented sap is clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose (Okafor, 2012). Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products (Obire, 2009) whereas, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organisms (Ogbulie *et al.,* 2007).

Vernonia amygdalina, commonly called bitter leaf, is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs. It belongs to the family Astaraceae. It is popular in most West African countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic. V. amygdalina contains antioxidants that have beneficial effect on the body. It is particularly rich in flavonoids, alkaloids and tannins, and contains saponins, anthraquinones, phenols, terpenes and cardiac glycosides in small and moderate concentrations (Imaga and Bamigbetan, 2013).

MATERIAL AND METHODS

Procurement of Samples

The fresh palm wine used for the experiment was tapped from an *Elaeis guineensis* tree in Aguleri, Anambra State, Nigeria, at about 7:30am on the morning of the experiment, while the leaves of *Vernonia amygdalina* were freshly plucked from a garden in the same town and on the same day. The samples were then quickly transported to the laboratory within one hour of collection. The samples were authenticated by the appropriate scientific authority.

Sample Treatment

First, the leaves of *Vernonia amygdalina* were washed with distilled water and dried for fifteen minutes.Two bottles for the palm wine samples were labelled thus; Control **(C)** and Treated **(T).**Two litres each of the palm wine samples were transferred into the respective labelled bottles. The bottle labelled (T) was treated with 0.67g of *Vernonia amygdalina* while the bottle labelled (C) was left without any form of treatment (no *Vernonia amygdalina* added to it) as control. The sample was analysed for alcohol content and yeast count before the sample was divided into control and treated groups. Then the alcohol content and yeast count of the two groups were determined at 24 hours interval for 72 hours and after which the study was carried out finally on the seventh day.

Reagents

All reagents used were of analytical grade.

Methods

Alcohol content Determination

The method of Pearson (1974) was used to determine the alcohol content of the palm wine samples.

Yeast count (Saccharomyces cerevisiae)

The method of Cheesbrough (1994) was used to determine the yeast count of the palm wine samples.

RESULTS

The results of the yeast count for both the control (C) and treated (T) samples of the palm wine are shown in table 1.

Table 1. Yeast Count of Both Control and Treated Samples.

Duration (Hours)	Control	Treated
0	8.5×10^{7}	8.5×10^{7}
24	1.02×10^{8}	1.46×10^{8}
48	1.38×10^{8}	1.62×10^{8}
72	9.4×10^{8}	1.70×10^{8}
168	Nil	Nil

Key: Nil = No yeast growth. All values are in CFU ml^{-1}.

The results of the alcohol content determination for both the control (C) and treated (T) samples of the palm wine are shown in table 2.

Table 2. Alcohol Content Determination of Both Control and Treated Samples.

Duration (Hours)	Control	Treated
0	5.26%	5.26%
24	10.14%	5.78%
48	17.82%	6.47%
72	19.44%	7.11%
168	20.41%	7.45%
Mean	14.61±6.62%	6.41±0.91%

DISCUSSION

The results of the yeast count of the palm wine samples for the control were 8.5×10^{7}CFU/ml, 1.02×10^{8} CFU/ml, 1.38×10^{8} CFU/ml, 9.4×10^{8} CFU/ml, and nil for the zeroth hour, 24^{th} hour, 48^{th} hour, 72^{nd} hour and 168^{th} hour respectively while the results of the yeast count for the treated samples of the palm wine were 8.5×10^{7} CFU/ml, 1.46×10^{8} CFU/ml, 1.62×10^{8} CFU/ml, 1.70×10^{8} CFU/ml, and nil for the zeroth hour, 24^{th} hour, 48^{th} hour, 72^{nd} hour and 168^{th} hour respectively. The yeast count in the control sample of the palm wine increased by a factor of 1.7×10^{7} CFU/ml after the initial 24 hours, 3.6×10^{7} CFU/ml from the 24^{th} hour to the 48^{th} hour, 8.02×10^{8} CFU/ml from the 48^{th} hour to the 72^{nd} hour and nil on the 168^{th} hour while the yeast count in the treated sample of the palm wine increased by a factor of 6.1×10^{7} CFU/ml after the initial 24 hours, 1.6×10^{7} CFU/ml from the 24^{th} hour to the 48^{th} hour, 8×10^{6} CFU/ml from the 48^{th} hour to the 72^{nd} hour and nil on the 168^{th} hour. The average growth rate of yeast was $(2.85 \pm 4.48) \times 10^{8}$ CFU/ml for the control sample of the palm wine and $(2.83 \pm 2.86) \times 10^{7}$ CFU/ml for the treated sample of the palm wine, while the mean yeast count values of the control and treated samples were $(3.16 \pm 4.16) \times 10^{8}$ CFU/ml and $(1.42 \pm 0.39) \times 10^{8}$ CFU/ml respectively. Therefore, the average rate of growth and the mean yeast count for the control group were much greater than those of the treated group.

The results of the alcohol content determination of the palm wine samples for the control were 5.26%, 10.14%, 17.82%, 19.44%, 20.41% for the zeroth hour, 24th hour, 48th hour, 72nd hour and 168th hour respectively while the results of the alcohol content determination for the treated samples of the palm wine were 5.26%, 5.78%, 6.47%, 7.11%, 7.45% for the zeroth hour, 24th hour, 48th hour, 72nd hour and 168th hour respectively. The alcohol content of the control sample of the palm wine increased by a factor of 4.88% after the initial 24 hours, 7.68% from the 24th hour to the 48th hour, 1.62% from the 48th hour to the 72nd hour and 0.97% from the 72nd hour to the 168th hour while the alcohol content of the treated sample of the palm wine increased by a factor of 0.52% after the initial 24 hours, 0.69% from the 24th hour to the 48th hour, 0.64% from the 48th hour to the 72nd hour and 0.34% from the 72nd hour to the 168th hour. The average increase in alcohol content for the control sample of the palm wine was 3.79 ± 3.11%, while for the treated sample of the palm wine was 0.55 ± 0.16%, while the mean alcohol content values of the control and the treated samples were 14.61% ± 6.62 and 6.41% ± 0.91 respectively.The difference between the mean alcohol contents of the control and treated samples was statistically significant ($p<0.05$).

The results of this study on yeast count within the first 24 hours were 1.02×10^8 CFU ml^{-1} for the control sample and 1.46×10^8 CFU ml^{-1} for the treated sample agree with the results of the study by Amoa-Awua *et al.* (2007) on yeast count of palm wine. Amoa-Awua *et al.* (2007) reported between 3.5×10^7 and 1.5×10^8 CFU ml^{-1} within 24 hours after tapping.

Vernonia amygdalina is rich in phytochemicals like alkaloids, flavonoids, cardiac glycosides, saponins, tannins and terpenes, which, according to Patel *et al.* (2014), has intense antifungal activity. The medicinal plant *Vernonia amygdalina* showed good antifungal activity against *Saccharomyces cerevisiae* (Patel *et al.,* 2014).

Swee *et al.* (2010) reported that "because *Saccharomyces cerevisiae* is the dominant yeast species and the main microorganism responsible for alcoholic fermentation of palm wine due to its ability to grow on glucose, sucrose and fructose, and due to the presence of pyruvate decarboxylase in the yeast species, any reduction or inhibition its activity leads to a reduction in the level of alcoholic fermentation of glucose and other sugars present in palm wine". The gradual loss of fungal viability observed in the control sample of the palm wine could be attributed to the gradual depletion of fermentable sugar, production of organic acids and consequent reduction of pH. The gradual reduction of fungal viability in palm wine treated with *Vernonia amygdalina* could also be attributed to the presence of bioactive phytochemicals present in the plant (Onwuakor and Ukaegbu-Obi, 2014).

CONCLUSION

Palm wine is a rapidly fermenting beverage. The results of this study showed that the leaves of *Vernonia amygdalina* is useful in reducing the rate of fermentation in palm wine, hence reducing the rate of alcohol formation and extending the shelf life of palm wine without affecting its taste and acceptability.

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