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Bio-preservation of atan, a wine extracted from palm trees in Benin, using orange peelings

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ABSTRACT: Palm wine, known as atan in Benin, is a sweet beverage extracted from the oil palm (*Elaeis guineens*). Highly prized by the population for its high nutritional value, its marketing is very limited due to a lack of or inappropriate means of preservation. In the search for palliatives, the present study aims at the Bio-preservation of palm wine by using orange peels to extend its shelf life. To achieve this objective, the preservative effect of orange peels was evaluated, and the stabilized wine was characterized in physicochemical, nutritional, microbiological and organoleptic terms. Palm wine samples graded A, B and C received 10g, 15g and 20g of orange peel respectively. During the storage period, the bottles that received the peelings in this order burst after one, two, three and four weeks respectively. The flasks that burst early were those with a low peel incorporation rate. The 33cL bottles containing palm wine and 25g orange peel resisted bursting up to 4 weeks of storage. Analysis of physicochemical and nutritional parameters showed that stabilized palm wine was richer in potassium, magnesium, sodium and calcium than the unstabilized control wine. Microbiological analysis of the stabilized palm wine showed that the yeast, E*scherichia coli* and Staphylococcus loads were lower than in the unstabilized wine. In terms of sensory quality, the study showed that consumers rated the consistency, color and taste of stabilized wines higher than those of unstabilized (control) wines.

KEYWORDS: palm wine, orange peel, stabilization, sensory quality, nutritional parameters.

1 INTRODUCTION

The oil palm known as *Elaeis guineensis* is widely cultivated in southern Benin for its oil-rich nuts for food and industrial use [1], [2], [3]. The sap from the trunk is also consumed either directly or fermented to produce palm wine called "atan" or "déhan" in Fongbé. Oil palm sap can be collected daily. It is very rich in sugar, vitamins and amino acids [4], [5]. Palm wine is a popular and widely consumed beverage in most African tropical regions [6]. In Benin, palm wine is mainly derived from the *Elaeis guineesis* and *Raphia bookeri* palms [7]. It is a whitish effervescent beverage obtained by spontaneous fermentation under the action of microorganisms from the sweet transparent sap of palm trees [8]. Palm wine is a highly unstable beverage due to the rapid fermentation it undergoes, which can destroy its organoleptic, nutritional and market qualities. This makes it difficult to preserve over the long term. The preservation of this beverage is very difficult because it is the seat of biochemical reactions linked to significant microorganism activity [9]. It therefore seems important to help make it available by implementing appropriate preservation methods to preserve its nutritional and commercial qualities.

Chemical preservatives are commonly used to stabilize foods in general and beverages in particular [10]. In view of the growing number of voices speaking out against the use of these types of preservatives and considering the harmful effects they have on human and animal health, the use of extracts of plant origin is proving to be a credible alternative. In fact, the use of bioconservatives to extend food shelf-life has two main advantages. They are of plant origin, and therefore virtually non-toxic, and do not encourage the development of resistance by pests. Among bioconservatives, orange peel essences, which are plant extracts, have microbicidal and microbiostatic effects on food spoilage germs [11]. However, orange peel is often considered as waste, and rejected as a food by-product. It is therefore necessary to consider their valorization through the stabilization of palm wine. This justifies the present title.

2 MATERIALS AND METHODS

2.1 MATERIALS

- **Plant material**

It is made from palm wine harvested at Zoundja in the Abomey-Calavi commune in the Atlantic department, and the orange peelings were collected after peeling a few samples of orange bought from vendors in the same commune.

Fig. 1. Palm wine extracted from Elaeis guineensis in Benin

- **Production equipment**

The following equipment was essential for stabilizing palm wine. It includes: a gas burner and a pan for sterilizing bottles and capsules; 33 cl bottles for packaging the wine; a beaker and a funnel for bottling; a filter cloth for filtering and clarifying the palm wine; a precision balance for weighing.

- **Laboratory equipment**

The laboratory equipment consists of standard laboratory equipment for physicochemical and microbiological analysis [11].

2.2 METHODS

- **Evaluation of the preservative effect of orange peel essences on palm wine stabilization**

After harvesting, the filtered wine was poured into sterilized 33cL bottles. In each bottle containing palm wine, increasing quantities of orange peel (10g; 15g; 20g; 25g; 30g; 35g; 40g) were introduced (figure 2). A control without orange peel was set up. The filled bottles were stored on the bench at room temperature (25-30°C). Every week for two months, the bottle contents of each dose were removed and filtered. Nutritional, physicochemical and organoleptic parameters were assessed. The technological diagram in figure 3 summarizes the method described above. It shows all the unit operations involved in stabilizing palm wine.

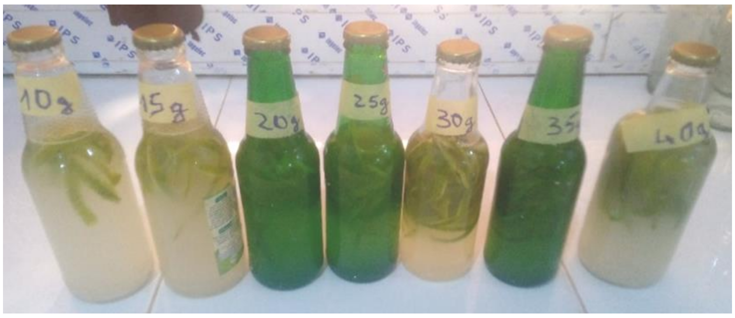

Fig. 2. ***Palm wine stabilization with orange peel maceration***

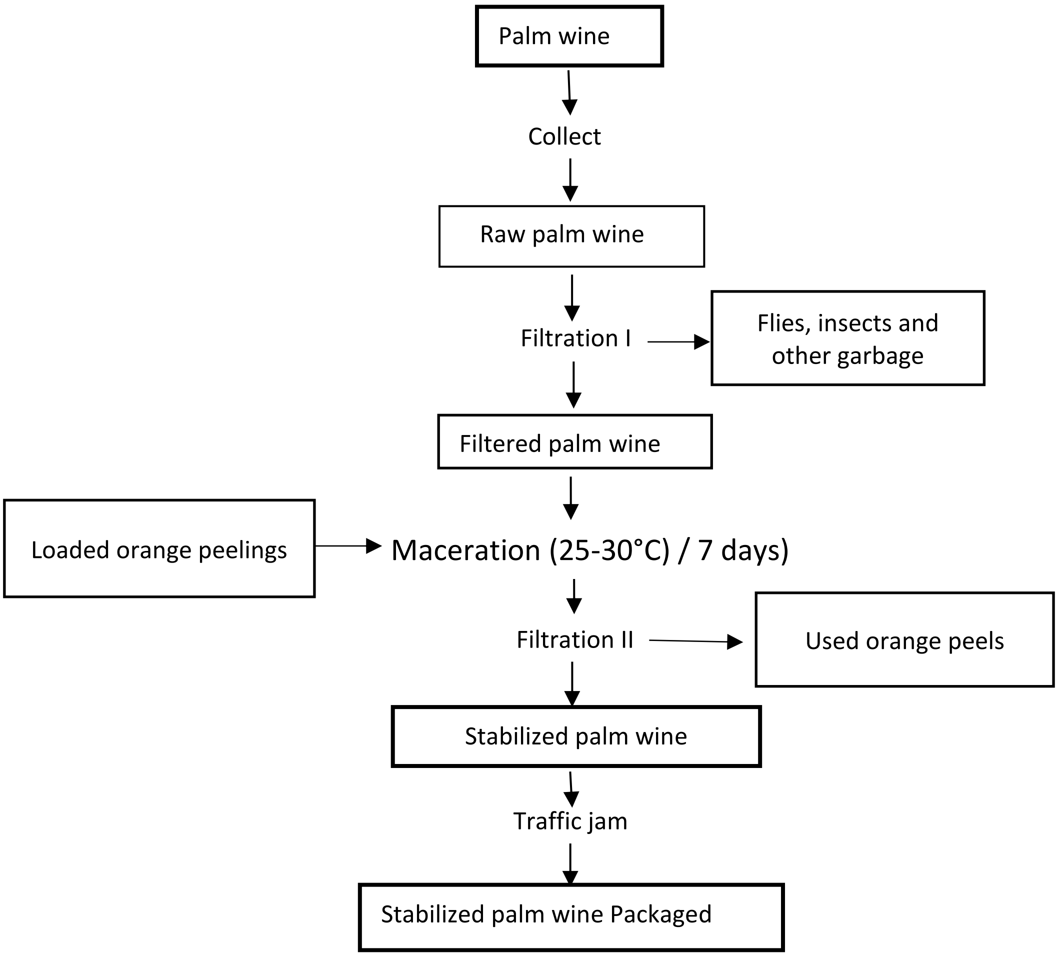

Fig. 3. ***Technological diagram for the production of stabilized palm wine***

- **Evaluation of microbiological quality**

Microbiological analysis was carried out by isolating, identifying and counting colonies. The microbiological parameters investigated in the course of this work were Aerobic Mesophilic Germs (AMG), lactic acid bacteria, Enterobacteriaceae, *Escherichia coli* and Staphylococci [11].

- **Preparation of decimal dilutions (NF V08-051, 1999)**

A 1mL volume of each sample was taken and placed in a tube containing 9 mL of sterile Tryptone Sel (TS) broth. The whole was homogenized. The resulting solution was 10^{-1}. A volume of 1mL of the 10^{-1} dilution was taken and placed in a tube containing 9mL of sterile TS broth. The whole was homogenized. The resulting solution is 10^{-2}. We proceeded in the same way for successive decimal dilutions [11].

- **Enumeration of Mesophilic Aerobic Germs (ISO 4833, 2013)**

For each sample, two successive decimal dilutions (10^{-2} and 10^{-3}) were inoculated by incorporation into Plate Count Agar (PCA). A 1mL of each dilution was introduced into a sterile Petri dish. Approximately 15mL of PCA agar was poured onto the inoculum. The whole was homogenized and allowed to solidify. After solidification, a second layer of approximately 5mL of PCA agar was applied. After solidification, both plates were incubated at 30°C for 72 h ± 2 h.

- **Enumeration of thermotolerant coliforms (NF V08-060, 2009)**

Dilutions of 10^{-1} and 10^{-2} were selected. A volume of 1 mL of each dilution was introduced into a sterile Petri dish. Approximately 15 mL of neutral red crystal violet lactose agar (VRBL) was poured onto the inoculum. The whole was homogenized and allowed to solidify. After solidification, a second layer of approximately 5 mL VRBL was poured onto the inoculum. After solidification, both plates were incubated at 44°C for 24 ± 2h.

- **E. coli enumeration (ISO 16649-2, 2001)**

One milliliter of each sample and 1mL of the 10^{-1} dilution were used to enumerate *Escherichia coli*. A volume of 1 mL of each dilution was introduced into a sterile Petri dish. Approximately 20 mL of Tryptone Bile X (TBX) agar was poured onto the inoculum. The whole was homogenized and left to solidify. After solidification, both plates were incubated at 44°C for 24 h ± 2 h [11].

- **Physicochemical quality**

- **Measurement of titratable acidity**

Titratable acidity is measured by neutralizing the acid with a NaOH solution. Neutralization is monitored using a colored indicator (phenolphthalein). Dosing is stopped when the indicator turns pink. The decimolar soda solution is poured into a 10mL test sample of palm wine until the indicator turns pink and remains so for 10 seconds [11].

- **Determination of Brix by refractometer**

The Brix degree is determined using the method reported by [12]. A drop of moist sample is placed on the refractometer lens and read directly after exposure to light.

- **Determination of minerals**

The determination of microelements (calcium, magnesium, zinc, potassium, phosphate, sodium) in palm wine was carried out by atomic absorption spectrophotometry following the NF 14082 method described by [13]. 3 g of wine were weighed into a porcelain crucible. This sample was incinerated in an oven at 450°C / 24h. The resulting ash was dissolved in 5 mL of aqueous hydrochloric acid solution (2 N), which was evaporated on a hot plate at 125°C. The viscous residue obtained was again dissolved and recovered using a $10^{-1}10^{-1}$ mol/L nitric acid solution in a 100 mL flask. The resulting solution was used for metal determination by atomic absorption spectrophotometry.

- **Evaluation of the organoleptic quality of palm wine**

Organoleptic characteristics play an important role in determining the marketability of a product. Various characteristics are therefore assessed by tasters on all samples after production and storage.

Sensory evaluation was carried out to assess the various organoleptic characteristics of palm wine. The characteristics assessed were: taste, color, consistency, aroma and overall acceptability. These characteristics were assessed on a scale of 1 to 9, with 1 = extremely inferior, 2 = very inferior, 3 = moderately inferior, 4 = slightly inferior, 5 = identical to the reference sample, 6= slightly better, 7= moderately better, 8 = better and 9 = extremely better [11]. The tasting panel was made up of 30 randomly selected, specially trained people. A sample of each bottle of preserved palm wine was evaluated.

3 Results

3.1 Technological Aspects Of Palm Wine Stabilization

Figure 4 shows the appearance of palm wine before stabilization (T) and 4 weeks after stabilization (A, B and C). Samples A, B and C received 10g, 15g and 20g of orange peel respectively. Observation of this figure shows that the wine takes on more yellow coloring as the amount of peel added increases. During storage, it was found that the lower the rate of orange peel incorporation, the earlier the bottles expanded and burst. Bottles containing 10g, 15g or 20g of orange peel burst within one, two and four weeks respectively. Only bottles with at least 25g of orange peel per liter of wine were recovered after 4 weeks of storage.

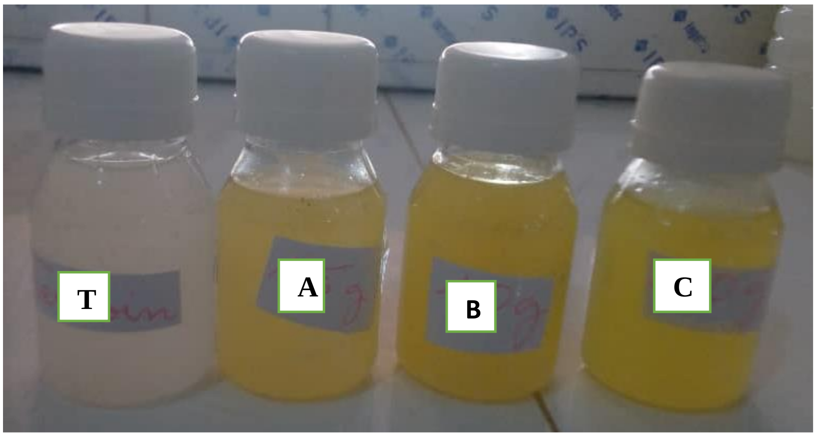

Fig. 4. Visual appearance of wine before and after stabilization

T= control (0g/L); A= stabilized wine (10g/L); B= stabilized wine (15g/L) and C= stabilized wine (20g/L)

3.2 Nutritional And Physicochemical Characteristics

Table 1 shows the results of nutritional analyses carried out on stabilized palm wine and untreated control palm wine. The table shows that palm wine contains minerals such as nitrogen, potassium, magnesium, sodium and calcium. The level of these minerals is higher in stabilized palm wine than in the control. Furthermore, the control palm wine is more acidic (low pH) and less sweet (low Brix) than the stabilized palm wine.

Table 1. Nutritional and physicochemical quality of stabilized palm wine and control palm wine

References	VP1 control	VP2 treated
Nitrogen (N) %	0.0224	0.0532
K mg/kg	1480.225	1752.379
Ca mg/kg	14.822	20.933
Mg mg/kg	35.960	40.334
Na mg/kg	77.657	104.426
pH	3.46	3.78
Titrable acidity	6.58	7.81
Brix degree	9.47	14.93

3.3 MICROBIOLOGICAL CHARACTERISTICS OF DIFFERENT PALM WINES

Table 2 shows the results of the Student Newman Keuls test on the microbiological characteristics of the different palm wine samples. Analysis of this table reveals that there was no significant difference in the rate of contamination by GAM, lactic acid bacteria, enterobacteria, E coli and staphylococci in stabilized and unstabilized wines at the 5% threshold ($p > 0.05$). On the other hand, the unstabilized palm wine (control) had a significantly higher microbial load of yeasts, and was very significantly higher in Escherichia coli and Staphylococcus than the stabilized palm wine at the 5% threshold. No traces of salmonella were detected in the palm wines.

Table 2. Microbiological characteristics of stabilized and control palm wines

Microorganisms of interest	Number of colonies (CFU / ml)		F-Value	P
	Control Palm Wine	Stabilized palm wine		
GAM	$6.01.10^7 \pm 3.00.10^3$ a	$5.70.10^6 \pm 2.81.10^2$ a	**1.01 ns**	**0.3727**
Lactic Bacteria	$3.40.10^2 \pm 1.4.10^1$ a	173 ± 29 a	**1.35 ns**	**0.3099**
Enterobacteria	$2.40.10^3 \pm 9.16.10^2$ a	$2.1.10^3 \pm 2.6.10^2$ a	**0.7689 ns**	**0.1**
Escherichia coli	31 ± 14 a	1.33 ± 0.33 b	**0.37150****	**0.001**
Staphylococcus	46 ± 17 a	10 ± 4 b	**0.3243****	**0.002**
Yeasts	$5.17.10^4 \pm 4.70.10^2$ a	$2.47.10^4 \pm 8.21.10^2$ b	**8.14***	**0.0463**
Molds	< 10	< 10	**-**	**-**
Salmonellae	Absent	Absent	**-**	**-**

Mean values bearing the same letter in the same row are not significantly different at the 5% threshold (p<0.05) (=significant difference; **= very significant difference; ns = non-significant difference).*

3.4 SENSORY CHARACTERISTICS OF STABILIZED PALM WINE

Table 3 shows the results of sensory analyses carried out on stabilized and control palm wines. Analysis of this table shows that stabilized palm wines have a better aroma than control palm wines. In terms of overall acceptability, there was no significant difference, but consistency, color and taste were more acceptable in stabilized wines than in unstabilized (control) wines. Consumers therefore appreciated color, consistency and aroma much more. Stabilization did not alter the taste of the palm wine according to consumers.

Table 3. Sensory quality of stabilized palm wine and control

Palm wine stabilized with :	Organoleptic parameters				
	Color	Consistency	Taste	Aroma	Overall acceptability
0 g of OP (Control)	5.0±0.00 b	5.0±0.00b	5.0±0.00a	5.0±00.00b	5.0±0.00a
15g of OP	6.80±0.84 ab	6.60±0.62ab	4.20±0.73a	6.20±0.81ab	5.90±0.78a
20g of OP	6.70±0.62 ab	6.00±0.52ab	3.70±0.67a	5.80±0.61ab	4.60±0.73a
25g of OP	7.60±0.40 a	7.20±0.49a	4.50±0.83a	7.70±0.42a	6.10±0.81a
30g of OP	7.70±0.65 a	7.20±0.53a	3.40±0.78a	7.30±0.47ab	5.40±0.93a
35g of OP	7.80±0.71 a	7.40±0.58a	3.50±0.65a	6.70±0.47ab	5.50±0.81a
40g of OP	7.40±0.72 a	7.50±0.58a	5.70±0.90a	6.90±0.81ab	6.60±0.62a
F-value	**2.29***	**2.93***	**1.40ns**	**2.42***	**0.84ns**
Probability	**0.0461**	**0.0139**	**0.2307**	**0.0365**	**0.5433**

*ns = not significant; * = significant; Average rates bearing the same letter are not significantly different at the 5% level; Values bearing different letters are significantly or very significantly different at the 5% level; OP = Orange Peel.*

4 Discussion

4.1 Nutritional And Physicochemical Characteristics

As far as nutritional parameters are concerned, stabilized palm wine was more concentrated in mineral salts, namely potassium, magnesium, sodium and calcium, than the unstabilized control wine. This could be explained by the fact that the orange peel used for stabilization is a vegetable material that is highly rich in mineral salts, which it would have added to the wine during the stabilization process. On the other hand, we note that the control palm wine is more acidic (low pH) and less sweet (low Brix) than the stabilized palm wine. Orange peel essences would have prevented sugar degradation by palm wine microorganisms during stabilization. The values are still higher than those obtained on palm wine from Beni in DR Congo (Brix degree: 7.64%; pH = 3.7) [14], but the pH is close to that (between 3.5 and 4.5) found for wine from Ghana [15]. [16] had explained that the acidity of the different beverages could be explained by the fact that they ferment during the manufacturing process.

4.2 Microbiological Characteristics Of Different Palm Wines

Evaluation of the microbiological parameters of palm wine showed that, after stabilization, loads of microorganisms such as yeasts, Escherichia coli and Staphylococci were significantly lower than in unstabilized wine. Orange peel essences are said to have microbiostatic or microbicidal effects on the microbial flora of palm wine. Indeed, we have shown in previous study that orange peel essential oil exerts a microbicidal effect on microorganisms, in this case molds in cowpea and corn stocks [10]. The microbial load of yeasts and molds obtained in the samples can be explained by the inappropriate storage of wines as indicated by [17]. The latter showed that the abundance of yeasts in the juices could be explained by failure to observe hygiene rules during extraction. Furthermore, the sharp increase in yeast load could be linked to the physicochemical composition of the palm wine [18]. In addition, no salmonella microbial load was observed in any of the wines sampled, which testifies to the operator’s compliance with hygiene rules during extraction, as highlighted by [19]. The presence of these germs in the samples indicates contamination of faecal origin (contamination from the environment) [20] The wine samples analyzed contained higher microbial loads (6.01.107 CFU/mL) of aerobic mesophilic germs (AMG) in the oil palm wine; flora higher than that set by the standard (106 CFU/mL). This may be explained by the lack of hygiene in the production environment, as pointed out by [16]. These results differ from those obtained previously by carrying microbiological analyses on Djrèrègbé’s (Southest Benin) palm wine, with microbial loads of 3,106 CFU/ml for this germ [21].

4.3 Sensory Characteristics Of Stabilite Palm Wine

Consistency, color and taste are more appreciated by consumers in stabilized wines than in unstabilized (control) wines. This suggests that orange peel essences improve these wine parameters during stabilization. In fact, by inhibiting microbial growth in palm wine, orange peel essences would help preserve the wine’s organoleptic quality.

5 Conclusion

The present study has enabled us to work on the stabilization of palm wine using orange peels. Through our research and the work we have carried out, we can see that the field of agricultural product processing has evolved in terms of the valorization of agricultural products in Benin’s agri-food processing companies. That’s why we were interested in the valorization of palm wine and orange by-products such as orange peels. Our aim is to help palm wine producers extend the post-harvest shelf life of their product. Our results show that 25 g of orange peel preserves the nutritional, physicochemical and microbiological quality of a liter of palm wine for at least 4 weeks. Palm wine stabilized in this way has a better organoleptic quality and is appreciated by consumers.

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