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Diagnosis and Optimization of the Production of "*Mbite*" Drink Based on *Sclerocarya birrea* (A. Rich) Hostcht

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Abstract

Today, various traditional country foods are increasingly being neglected as a result of modernisation. Consequently, the knowledge and know-how necessary to prepare those foods are strongly threatened. To safeguard and foster appreciation of traditional knowledge, this study aims, on the one hand, to describe the manufacturing process of an alcoholic drink called "*Mbite*". The latter is traditionally prepared with the fruits of *Sclerocarya birrea* (A. Rich) Hostcht in Senegal. On the other hand, various controlled fermentations have been tested for better control of their hygienic and sanitary qualities. The manufacturing of "*Mbite*" drink begins with a manual extraction of the juice using stems of *Guiera senegalensis* or *Combretum glutinosum* to facilitate the separation of the pericarp from the almond. Subsequently, the extracts are filtered and/or mixed according to the producers. Finally, a 2-day spontaneous fermentation by endogenous yeasts of the fruit makes it possible to obtain the alcoholic drink within 3 days. "*Mbite*" is a low acid drink with a pH ranging from 3.82 to 3.97 and its ethanol content varies from 2 to 4% (v/v). Polyphenols vary between 124.92 and 158.25 mg/100 mL. However, microbiological analyses have shown a high number of lactic acid bacteria involved in the formation of volatile acids. The controlled fermentation trials have resulted in a unique alcoholic fermentation of *Sclerocarya birrea* juices by selected strains of *Saccharomyces cerevisae*. This has the advantage of guaranteeing sanitary qualities and reducing the fermentation time from three days to one.

Keywords

Traditional Drinks, Fermentation, "*Mbite*", *Sclerocarya birrea*

1. Introduction

Traditional fermented beverages can be defined as fermented extracts, prepared by indigenous populations, brewed or not according to ancestral processes specific to each locality. African fermented drinks are known to be an integral part of the traditions of hospitality and friendliness of the locals [1]. In many African countries, fermented drinks are prepared from plant extracts and sweet substances by indigenous populations according to ancestral processes which are specific to each locality (civilizations, terroirs) [2]-[4]. Among these extracts, those fermented from the fruits of *Sclerocarya birrea* are widely consumed. The juice of *Sclerocarya birrea* (A. Rich) Hostcht. called Marula is fermented to obtain alcoholic drinks called "*Umkumbi*" in Zimbabwe [5], "*Buganu*" in Swaziland [6]. It has been noticed that these wines are not only acidic linked to acetic fermentation, but also contain undesirable compounds such as esters [7]. In Senegal, the alcoholic drink called "*Mbite*" is traditionally prepared by the Seereer Sine. However, data on the manufacturing and characterization of this drink are almost non-existent. The traditional production method of "*Mbite*" resulting from spontaneous fermentation presents high health risks because the germs present are not controlled. Therefore, for safeguarding and developing traditional knowledge, this study aims, on the one hand, to describe the "*Mbite*" making process. On the other hand, various controlled fermentations have been tested for better control of their hygienic and sanitary qualities.

2. Materials and Methods

2.1. Diagnosis of Production Processes

The description of the processes was carried out twice at two production sites Loul-séssène and Boyard in the Fatick region (Senegal) in May 2019. Each unit operation was identified and described. Moreover, material flows and physical parameters were identified and recorded. This allowed establishing the synthetic diagram to obtain the "*Mbite*" drink which is based on the fruits of *Sclerocarya birrea*.

2.2. Samples of "*Mbite*"

The various samples collected during the description of the processes were analyzed to assess both the effect of the involved factors on the quality of the "*Mbite*" drink and the overall performance of the production process. Two samples per site are sent to the laboratory for the purposes of biochemical and microbiological analyses.

2.3. Physicochemical and Biochemical Methods

Classical physicochemical analysis methods were used to characterize the fruits of *Sclerocarya birrea* and the "*Mbite*" drinks. These are pH, titratable acidity, volatile acidity, ethanol content, polyphenols, tannins, antioxidant activity, color indices, reducing and total sugars according to standards AFNOR [8]. All analyses were done in duplicate.

2.4. Microbiological Methods

Lactic acid bacteria, yeasts and moulds were counted in accordance with French standards [9]. The standards and culture media used are presented in Table 1.

Table 1. Standards and culture media used for microbiological analyses.

Germs	References	Culture media	Temperature and incubation time
Lactic acid bacteria	NF V 04-503	Man, Rogosa, Sharpe (MRS)	30˚C/72h
Yeats and Molds	NF EN ISO 7954	Sabouraud with chloramphenicol	30˚C/72h

2.5. Statistical Analyzes

The analysis of variance (ANOVA) makes it possible to define the interdependence relationships, for example between the analyzed samples and the physicochemical parameters. The statistical differences are then compared with a threshold of 5%.

2.6. Maturation Study of "*Mbite*" Drinks

The "*Mbite*" drinks collected at the production sites were analyzed during storage at 25˚C (ambient temperature). The physicochemical and biochemical parameters had been studied every day for one month.

2.7. Optimization of Fermentation

The Optimization of fermentation was carried out with two strains of yeast: Sac*charomyces cerevisiae* and *Saccharomyces boulardii*. The biological conditions were linked to the optimal parameters of the strains' activity while taking into account the physicochemical characteristics of the raw (unfermented) "*Mbite*" drinks. The latter were thus incubated at 20˚C in a thermostatically controlled refrigerator. Monitoring with a frequency of 6 hours made it possible to describe the evolution of the fermentation by the strains.

3. Results and Discussion

3.1. Production Process for the Traditional Drink "*Mbite*"

The alcoholic drink "*Mbite*" is traditionally prepared, and the diagram is

composed of two main stages: extraction of the marula juice and fermentation (Figure 1).

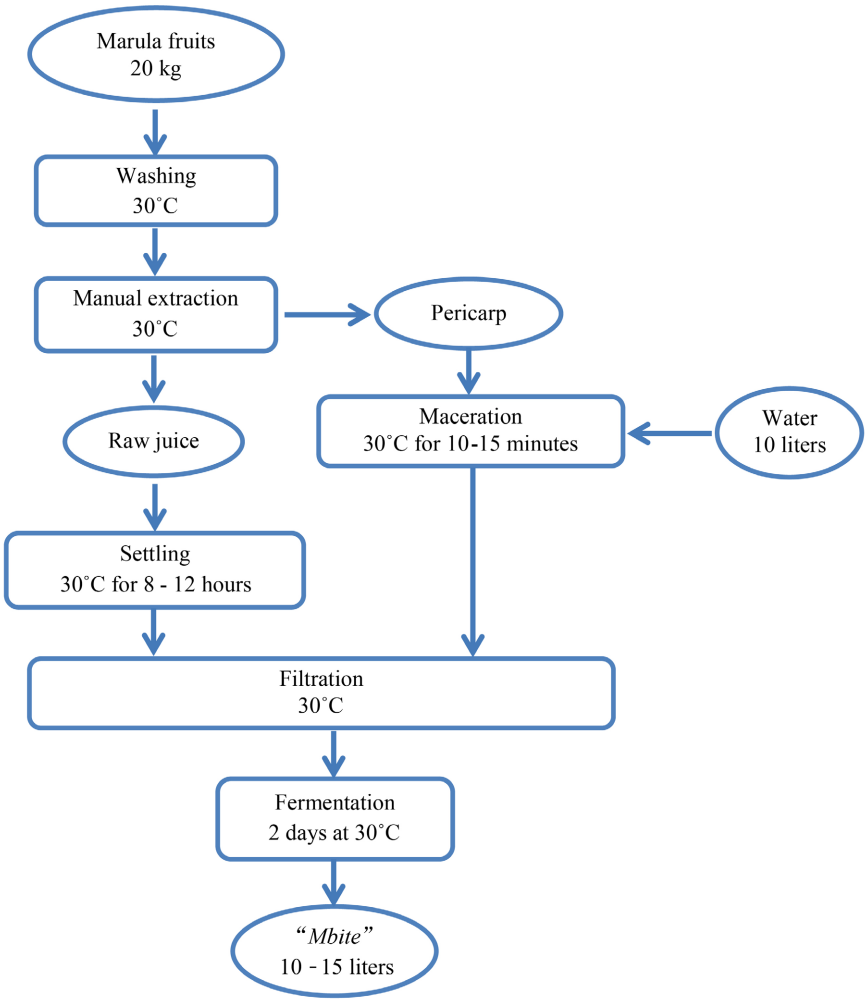

Figure 1. Production diagram of "*Mbite*" drink.

The manufacturing of the "*Mbite*" begins with manual extraction of the juice. This is a difficult step which involves using stems of *Guiera senegalensis* or *Combretum glutinosum* to facilitate the separation of the pericarp from the almond. As a result, two types of extracts are obtained: the pure juice called "*O gone*" and the pericarp macerate called "*A thiamba*" in the local language (Seerer, Senegal). The extracts are then filtered and mixed according to the producers. Finally, a 2-day natural fermentation produces the alcoholic fermented drink made from *Sclerocarya birrea* (Figure 2).

Marula juice has traditionally been used to make two types of drinks. The tangy and nutritious juice is reserved for children and the alcoholic drink is exclusively for adults [10]. In Zimbabwe, the local drink "*Umkumbi*" is prepared after extracting marula juice using tapered goat horns. The must undergoes a first natural fermentation of 24 hours and is then clarified using a basket of woven palm leaves

[11]. The manufacturing process of "*Buganu*" drink from Swaziland has the particularity of extracting the marula juice by grinding in a wooden mortar [6]. In the case of "*Mbite*" drink, clarification by decantation takes place prior to the fermentation to eliminate as much mucilage as possible. Filtration of the must is then done using millet straws (*Pennisetum glaucum* L.) as support.

Figure 2. "*Mbite*" drink.

In the "*Mbite*" manufacturing, no differentiated step of adding leaven has been detected. In fact, it is a spontaneous fermentation linked to the endogenous yeasts in *S. birrea* fruits [5] [11]. This is well confirmed by the microbiological analyses on the fermented extracts. The yeasts and lactic acid bacteria were counted at respective values of 10^6 and 10^4 CFU/ml (**Table 2**).

Table 2. Microorganisms counted in "*Mbite*" drinks.

Microorganisms	"*Mbite*"1	"*Mbite*" 2	"*Mbite*" 3
Lactic acid bacteria (CFU/ml)	6×10^5	3×10^4	1×10^5
Yeats (CFU/ml)	4×10^6	1.6×10^6	4×10^6
Molds (CFU/ml)	0	0	0

3.2. Physicochemical and Biochemical Characteristics of "*Mbite*" Drink

The results of the analysis of *S. birrea* drinks correspond to samples from the three manufacturing sites (**Table 3**). "*Mbite*" are not very acidic with a pH of 3.82 - 3.97. They are also very rich in polyphenols at respective concentrations of 158.25; 151.10 and 124.92 mg GA/100 ml. However, there is a significant difference in the obtained ethanol contents (4.09; 4.04 and 1.85 ml/100 ml) in "*Mbite*".

Table 3. Physicochemical and biochemical characteristics of "*Mbite*".

Analyzes	"*Mbite*"1	"*Mbite*" 2	"*Mbite*" 3
pH	3.85 ± 0.02^a	3.82 ± 0.00^a	3.97 ± 0.00^b
Titratable acidity (mEq/100 ml)	11.40 ± 0.42^a	12.85 ± 0.35^b	16.00 ± 0.14^c

Continued

Soluble dry matter (g/100 ml)	4.10 ± 0.00[a]	4.60 ± 0.00[b]	4.25 ± 0.01[c]
Ethanol (ml/100 ml)	4.09 ± 0.02[a]	4.04 ± 0.14[a]	1.85 ± 0.25[b]
Polyphenols (mg gallic acid/100 ml)	152.56 ± 7.15[a]	154.96 ± 7.15[b]	142.23 ± 13.75[c]
Tannins (mg tannic acid/100 ml)	10.35 ± 0.57[a]	9.36 ± 0.02[b]	9.32 ± 0.10[a.b]
Reducing sugars (g/100 ml)	0.77 ± 0.00[a]	0.19 ± 0.00[b]	0.24 ± 0.07[b]
Total sugars (g/100 ml)	0.77 ± 0.00[a]	0.72 ± 0.07[a]	2.30 ± 0.41[b]
Browning index	30.65 ± 0.01[a]	102.51 ± 9.65[b]	70.61 ± 1.46[c]
Yellow index	35.07 ± 0.01[a]	64.50 ± 2.28[b]	54.79 ± 1.46[c]

3.3. Maturation Effects of "*Mbite*" Drink

The ethanol content was monitored in the beverages prepared during the processes' descriptions (Figure 3). These samples stored at 25˚C (ambient temperature) reach a limit alcohol concentration of 5% (v/v) after 3 days. Furthermore, the pH and titratable acidity are 3.57 - 3.80 and 12.65 - 15.43 mEq/100 ml, respectively.

The concentrations of tannins (8.04 - 9.28 mg/100 ml) and polyphenols (124.92 - 158.25 mg/100 ml) as well as the color indices reflect good stability of the beverages during storage.

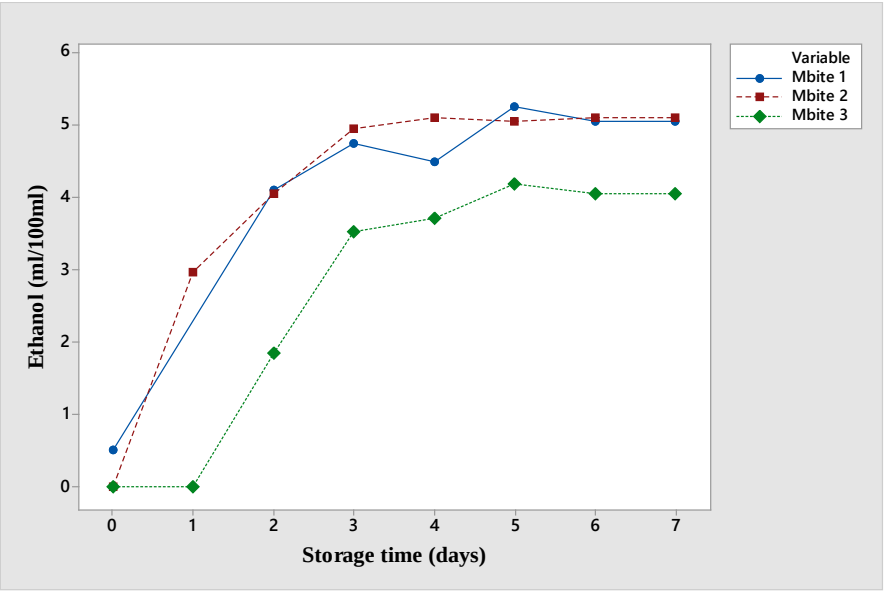

Figure 3. Evolution of the ethanol content during the maturation of "*Mbite*".

The maturation of fermented drinks is the phase corresponding to the peak of the ethanol concentration, but mostly to the development of aromatic and taste compounds. It depends on the duration of the various fermentations (main or secondary) which condition the sanitary and market qualities. This maturation period is 2 days for the Zimbabwean drink "*Umkumbi*" [11]; and 3 days for "*Buganu*" from Swaziland at ambient temperature (25 - 30˚C) [6].

The volatile acidities found in "*Mbite*" drinks respectively 1.6; 2.4 and 2.6 g of acetic acid/l are well above the tolerated limit (1.2 g/l) in wines [12]. It would be tempting to deduce that secondary fermentation leads to the production of organic acids, diacetyls and volatile esters [11] [13]. The same conclusions have been obtained in several traditional African drinks made from Marula [5] [6] [11]. "Mbite" has an alcoholic strength of 2 to 4% (v/v) which is quite similar to those of "*Umkumbi*" and "*Buganu*" varying from 2 to 5% (v/v) [5] [6].

Various genomic studies have shown the preponderance of strains of *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Salmonella* and *Shigella* in local marula-based drinks [5] [11].

These germs naturally present in the marula fruit are involved in the fermentation process and in many cases of food poisoning [14]. However, the high acidity of fermented drinks made from *Sclerocarya birrea* participate in the inhibition of pathogenic microorganisms such as *Escherichia coli* [15]. Nevertheless, it would be advisable to test controlled fermentations for better control of sanitary and hygienic quality.

3.4. Controlled Fermentation Trials of "*Mbite*"

Traditional marula-based drinks are considered to be fairly stable given their acidic pH (3.5 - 4.0) and alcoholic strength of 2 to 5% (v/v) [5] [6]. The fact remains that they present high health risks due to the spontaneous nature of the fermentation involving the native flora of the juice [11] [16]. In this sense, controlled fermentation trials will make it possible to control the process, which will condition both the hygienic and organoleptic qualities.

3.4.1. Physicochemical Characteristics of the Juice of *S. birrea*

The samples analyzed were the *S. birrea* juice obtained by manual extraction and filtered with a filter bag of 0.45 µm porosity. The extracts were then autoclaved at the scale of 120˚C/15 minutes. **Table 4** presents the physicochemical and biochemical characteristics of both the sterilized and unsterilized juices.

Table 4. Physicochemical and biochemical characteristics of sterilized and unsterilized juices of *Sclerocarya birrea*.

Analyzes	Unsterilized juice	Sterilized juice
pH	4.82 ± 0.01^{a}	4.90 ± 0.01^{b}
Titratable acidity (mEq/100 ml)	4.60 ± 0.00^{a}	4.75 ± 0.92^{a}
Soluble dry matter (g/100 ml)	16.90 ± 0.00^{a}	16.85 ± 0.07^{a}
Polyphenols (mg gallic acid/100 ml)	236.40 ± 1.00^{a}	221.98 ± 10.54^{a}
Tannins (mg tannic acid/100 ml)	18.65 ± 0.27^{a}	19.15 ± 1.05^{a}
Reducing sugars (g/100 ml)	5.28 ± 0.14^{a}	5.88 ± 0.14^{a}
Total sugars (g/100 ml)	8.86 ± 0.15^{a}	11.69 ± 0.15^{b}
Browning index	37.43 ± 0.64^{a}	67.73 ± 0.14^{b}
Yellow index	43.45 ± 0.16^{a}	73.68 ± 0.03^{b}

Unsterilized and sterilized *S. birrea* juices are not acidic with pH of 4.82 and 4.90, respectively. This translates into titratable acidities of 4.60 and 4.75 mEq/100 ml, respectively. These results are quite different from the samples from the Ferlo zone (Senegal) with a pH of 3.88 - 3.95 and are highly acidic (150 - 199 mEq/l) [17]. These parameters are closely related to the maturity state of the plant and to pedoclimatic conditions. However, the juices of the fruit of *Sclerocarya birrea* have high contents of antioxidant compounds (polyphenols: 221 - 236 mg/100 ml and tannins: 18 - 19 mg/100 ml), of reducing sugars (5.28 - 5.88 g/100 ml). and total sugars (8.86 - 11.69 g/100 ml).

The heat treatment in an autoclave did not induce any major changes in the physicochemical and biochemical characteristics. Only the color indices have undergone a significant modification. Unsterilized and sterilized juices are statistically similar for both nutritional and energetic compounds. Indeed, the objective of sterilization is to eliminate all pathogenic and spoilage microorganisms while preserving the nutritional and sensory qualities of the product. Concerning the marula juice, sterilization had to eliminate the native flora composed of pathogens such as *Salmonella* and *Shigella* [11] and *Lactobacillus* which are responsible for secondary fermentation. Also, the noted color indices prove that there were no major browning reactions.

3.4.2. Monitoring of Ethanol Content during Fermentation

The sterilized juices of *S. birrea* were inoculated with *Saccharomyces cerevisae* strain: MC1 and MC2; and *Saccharomyces boularii* strain: MB1 and MB2. Monitoring the ethanol content established that the fermentation was optimal at 24 hours for MC1 and MC2 drinks and at 30 hours for MB1 and MB2 drinks. Alcohol production is slow in the early hours of fermentation and tends to speed up after 12 hours of storage (**Figure 4**).

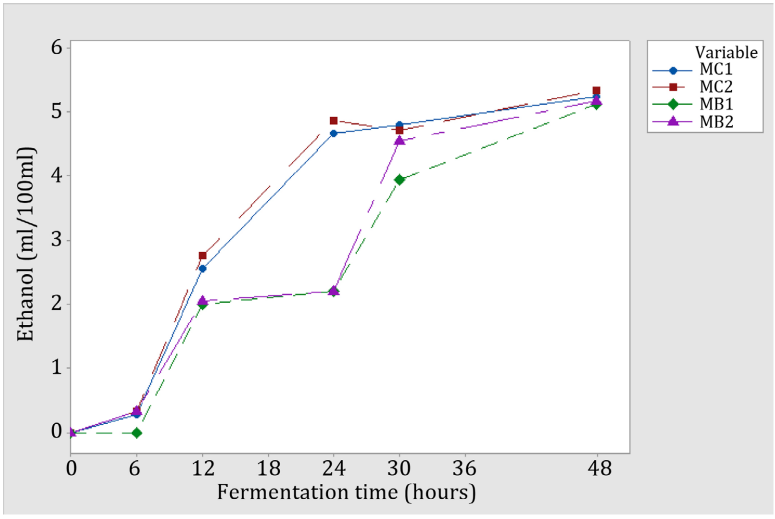

Figure 4. Monitoring of ethanol content during fermentation.

Alcoholic fermentation corresponds to the breakdown of sugars into ethanol

and carbon dioxide by yeasts. These fungi are responsible for producing the energy necessary for their growth and development. In the case of industrial beer such as wine, *Saccharomyces cerevisae* remains the used species par excellence. Regarding *Saccharomyces boulardii*, it is a probiotic used as an anti-diarrhea. It participates in the maintenance and restoration of the intestinal flora of both the large and small intestines. Traditional fermented drinks are characterized by the fact that multiple fermentations can occur in them [5] [18]. In previous works, fermentation germs belonging to the genus *Saccharomyces* and *Lactobacillus* as well as some pathogens were discovered [5] [16].

Sterilization ensured better control of both fermentation and hygienic quality. Contrary to the traditional method, the unique alcoholic fermentation triggered by the *Saccharomyces* strains resulted in a substantial reduction in the fermentation time from 3 days to 24 hours. Furthermore, the incubation temperature is beneficial for the preservation of antioxidant molecules. Studies have shown that fermentation of *S. birrea* juice at a temperature below 30˚C allows more than 90% of the polyphenols to be retained [16]. This is a clear advantage for industrial production.

4. Conclusion

The production process of "*Mbite*" showed a spontaneous fermentation step which resulted in an ethanol content of 2% to 4% (v/v). In addition, the volatile acidities were 1.6 - 2.4 g of acetic acid/l which are well above the tolerated limit in wines. The controlled fermentation trials have not only ensured the hygienic quality of the fermentation, but also significantly reduced its duration from 3 days to 24 hours compared to the traditional method. This work provides a better knowledge of the traditional drink "*Mbite*" and offers perspectives on popularization.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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