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Research Article

Physicochemical and Microbiological Analysis of Wines from Fresh Cocoa Bean (*Theobroma cacao* L.) Mucilage Pulp

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Abstract

Cocoa (*Theobroma cacao* L.) pulp, a by-product of cocoa beans processing, constitute about 10% of total cocoa beans, which is rich in micronutrients and macronutrients mostly sugars. Saccharomyces cerevisiae was used for wine fermentation which last for 15 days. One batch of wine was fermented with *Monodora myristica* essential oil as additive and another without additive. Fresh extracted juice was taken as control. The physicochemical, microbiological, antioxidant and sensorial properties of wines produced from fresh cocoa pulp juice were assessed. The fermented beverages were found to have an alcoholic percentage of 7.16±0.06 for the wine produced without additive (WPA) and

8.01±0.05 for the wine sample fermented in the presence of essential oil (WFE). The amount of total soluble sugars was found to decrease after the juice fermentation. The total phenolic compounds of both wines were estimated as 248.83±1.24 and 271.53±1.71mg EGA/L respectively for WPA and WPE. Positive correlations were recorded between the total phenolic content and the antioxidant activity. The produced wines exhibited radical scavenging activities of 50.81±0.87% for WPA and 58.82±0.62% for WFE. With the ABTS method, the antioxidant activities recorded were 80.90±0.77 and 87.80 ± 1.77% respectively for WPA and WPE. After sensorial analysis, the overall acceptability of the WPA was greater compared to WPE. Results showed that both produced wines were contaminated but having a microbial load within the norm of acceptability. Harmful microorganisms such as salmonella sp. were totally absent.

Abbreviations

ABTS: 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
DNS: 3,5-Dinitrosalicylic acid
DPPH: 2,2-Diphenyl-1-Picrylhydrazyl
EGA: Gallic Acid Equivalent
FJ: Fresh cocoa Juice
TA: Titratable Acidity
TPC: Total Phenolic Content
TMAF: Total Mesophilic Aerobic Flora
WFE: Wine sample fermented in the presence of essential oil
WPA: Wine produced without additive

Introduction

Wine making or vinification is a good example of the biotechnological evolution in beverage production, which starts with the selection of the fruit and its fermentation into an alcoholic beverage [1]. Although grapes are generally preferred for wine production because of their natural chemical balance which permits the fermentation process to go on without chaptalization, adding acids or other nutrients, some fruits have also been used for wine production such as banana, pineapple, orange etc., [2,3]. In countries where grapes cannot be grown easily due to climatic condition and or different reasons, other fruits such as apple, banana, cucumber, watermelon, pineapple, oranges, guava, are used for wine production. *Saccharomyces* is the specie of yeast generally used for fermentation during which, fruit juice sugars are converted into alcohol and organic acids [2].

Cocoa, (*Theobroma cacao* L.), also as known as cocoa, is a tropical evergreen tree (family Malvaceae) grown for its edible seeds. Cocoa fruit contains the pod shell or husk (67-76 %), bean (21-23%), and the mucilage or pulp (8.7-9.9 %). Cocoa mucilage is rich in carbohydrates, minerals such as, phosphorus, calcium, magnesium, sodium, potassium, vitamin such as C, and proteins [4]. Cocoa pulp juice has been successfully used for beer, vinegar and jelly fabrication and also, the removal of the pulp from the beans is a way of improving the quality of the seeds by reducing its acidity [5]. The cocoa pulp removed is essentially considered as

agroindustry waste and disposed in the environment [6], whereas can be exploited to produce different products. Therefore, the main objective of this study is to valorize Cameroonian cocoa pulp (mucilage) by producing and characterizing wine from its juice. This involves the extraction of the mucilage from cocoa beans, production of wine from the mucilage using *Saccharomyces cerevisiae* as starter, evaluating the microbiological, physicochemical and sensory properties of the produced wines.

Materials and Methods

Materials

Cocoa fresh fruits were harvested during the rainy season, in the month of December 2021, from a farm in Mbanga situated in Moungo division, Littoral region of Cameroon.

Saccharomyces cerevisiae used as yeast strain, was from the laboratory of Microbiology and Food Biotechnology of the Department of Food Science and Nutrition, National School of Agro-Industrial Sciences, Ngaoundere University, Cameroon. All analytical chemicals used were of analytic grade.

Methods

Must Preparation

All utensils where thoroughly washed and sterilized before use. The cleaned cocoa fruits were sorted to remove microbial attacked fruits and broken up manually with the help of knife. This mechanical process was done in order to extract mucilage from the seeds using distilled water. The extraction process was done by washing the beans till the sticky flesh (mucilage) found on the beans is removed. The washing process was done twice; firstly without water, secondly with 4L of water. At the end of each step, the liquid obtained was successfully filtered using a 400µm sieve and muslin cloth. The juice obtained was pasteurized at 70°C for 15mins.

Yeast Activation

In order to fasten the fermentation rate, the yeast *Saccharomyces cerevisiae* was activated according to the modify method described by [7], before being added into the cocoa juice. For this, 50g of sugar and 50g of dry yeast were mixed with 0.5L of pasteurized must in 1L volumetric flask. After the complete dissolution of the suspended particles, the final solution was added to the must as starter to boost the fermentation process.

Alcoholic Fermentation

The must to be fermented was divided into two samples namely A and B whereas the unfermented fresh must denoted sample C served as control sample. In one of the samples (sample A), 6.4mL of *Monodora myristica* essential oil was added prior to the fermentation process. Both samples were then introduced into the fermentation vessels and sealed using aluminum foil. At each stage of the fermentation period, which lasted for 15 days at room temperature (24-25°C), the fermentation vessels were slightly opened to remove part of the produced CO_2 during the fermentation process.

Chemical and Microbiological Analysis

The produced wines and fresh cocoa must were analyzed in different aspect. Analysis performed were total coliforms count, yeasts and molds, salmonella sp., total mesophilic aerobic flora, clostridium perfrigens, pH, antioxidant activity, total phenolic content, total soluble sugar, alcohol content, titratable acidity and specific gravity.

Total Coliforms Count

The total coliforms count was done according to the ISO 4832 norm [8]. 1mL of different filtered and diluted samples (dilution factor 10) was placed on a sterilize plate agar (on VRBLA Agar). It was then incubated for 24 hours at a temperature of 37°C.

Yeasts and Molds

The yeast and mold were identified by placing 1mL of different dilutions samples on the surface of a sterilize plate of an acidified Potato Glucose Agar. Thereafter the plate was incubated at 25°C for 3 to 5 days, following the ISO 21527-2 norm [9].

Salmonella Species

The presence of Salmonella was evaluated at three stages as recommended by the ISO 6579-1 norm [10]. 25mL of wine were diluted in buffered peptone water and the culture incubated at 37°C for 16 h at the pre-enrichment stage. At the enrichment stage, 1mL of the culture was introduced in a 10mL sterilized test tube containing selenite broth. The tube was incubated at 37°C for 24 h. In the last stage, the culture previously enriched was isolated using Salmonella Shighella agar and then incubated at 37°C for 24 h. Red colonies with black centers appear in case of Salmonella contamination.

Total Mesophilic Aerobic Flora

The evaluation of the presence of mesophilic aerobic flora was done according to ISO 4832, norm [8]. 1mL of different diluted samples was introduced on a plate count agar to which 15 mL of the culture were added. Sample was maintained at the liquid state under cooling condition. After homogenization, the sample was left at ambient temperature (24-25°C) for jellification and later incubated at 30°C for 48 h.

Anaerobic Sulfite Reducers

Anaerobic sulfite reducer was checked according to the ISO 7937 norm [11]. 1mL of the diluted samples was introduced in a tube containing 15mL of Trypton Sulfite Neomycin Agar which was sterilized and maintained under cooling conditions. The mixture was homogenized, heated at 80°C for 10 min and cooled abruptly with tap water. After jellification, 1mL of sterile paraffin oil was introduced in the tube to create anaerobiosis and later incubated at 37°C for 48 h. Black colony units appearing in the tube taken as anaerobic sulfite reducer were counted.

Determination of pH

pH values of the wine samples were measured with a digital pH meter (Koolamo Digital pH Meter), pre-calibrated with buffer solutions at pH 4.0 and 7.0 following the method by [2].

Determination of Alcohol Content

The alcohol content of fermented wines was determined using specific gravity according to the method describe by [12].

Alcohol content by volume = (Original gravity-Final gravity) x 131.25.

Determination of Reducing Sugar by the DNS Method

Reducing sugar content was evaluated through Fisher and Stein method using glucose as standard as described by [13]. The 3,5-Dinitrosalicylic acid (DNS) reagent was prepared by mixing 5g of DNS in 250mL of distilled water at 80°C in a water bath. When this solution cooled down to room temperature, 100mL of NaOH 2 N and 150g of sodium potassium tartrate were introduced and the volume of the mixture completed to 500mL with distilled water. In the test tubes, 2mL of DNS reagent and 0.1mL of sample (must or wines), or distilled water (blank tube), were added to each of them. The tubes were plunged in a water bath at 100°C for 5 min and then cooled with cold water. After this step, 7.9mL of distilled water were introduced to each tube, which completed the final volume to 10mL and the absorbance was taken at 540nm.

Determination of Total Titratable Acidity (TA)

Titratable acidity was performed according to the standardized method described by [14], with 0.1 N sodium hydroxide (NaOH) in the presence of the phenolphthalein as indicator. Into a conical flask containing 10mL of juice, 50mL of distilled water and 0.1mL of phenolphthalein (0.05%) were respectively introduced. Titration was altered when the initial color changes to pink and persisting for at least 30s. The burette reading was noted. The titratable acidity (TA) was computed according to the formula:

$$A(\%) = \frac{V' \times 0.0067}{V(mL)} \times 100$$

Where:
V, is the volume of the sample during titration (mL)
V', is the volume of 0.1N Sodium hydroxide used (mL)
0.0067 is Conversion factor of titratable acidity as malic acid equivalent.

Total Phenolic Content Determination

The total phenolic content was evaluated according to the colorimetric method described by [15], using the Folin-Ciocalteu reagent and gallic acid as standard. 1mL of the appropriate diluted wines, 5mL of a normal solution of Folin-Ciocalteu reagent were mixed, agitated well and incubated for 5 min. After this period, 4mL of 7.5% (m/v) Na_2CO_3 solution were added, mixed thoroughly and kept in darkness at room temperature for an hour. The value of absorbance at 760nm was read and used in the calculation of the total phenolic content, expressed in milligrams of gallic acid equivalent per liter of wine.

Determination of Antioxidant Capacity

The Antioxidant capacity of produced wines and that of must were determined with two different methods.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of the different samples was determined according to the method reported by [16]. The stock solution of radical was made by dissolving 25mg DPPH in 100mL methanol. The working solution of DPPH was prepared by diluting the DPPH stock solution with methanol to obtain an optical density of 0.98 ±0.02 at 517nm [17].

In a test tubes, 3mL of DPPH working solution were mixed with 100µL of different samples to be analyzed and incubated in the dark for 30 min. After incubation, the absorbance was measured at 517nm. The percentage of scavenging activity was calculated using equation.

$$\%AA = \frac{Abscontrol - Abssample}{Abssample} \times 100$$

ABTS Radical Scavenging Activity

The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity of different samples was measured using the method reported by [18], with some modifications. The solution of ABTS radical prepared by reacting ABTS with potassium persulfate was kept overnight in the darkness at room temperature. The analyzed samples were prepared in methanol for a final concentration to range from 50 to 1250µg/mL. A specimen of 10µL were placed in a test tube and mixed with 3mL ABTS radical working solution. The optical density of the resulting mixture was taken at 734nm. The antioxidant activity of the sample was estimated as follows:

$$\%AA = \frac{Abscontrol - Abssample}{Abssample} \times 100$$

Determination of Specific Gravity

The specific gravity gives a density of a liquid, such as fermented must, compared with water. The specific gravity of the wine samples was evaluated according to the method descried by [19]. The pycnometer with 50mL capacity was thoroughly cleaned and dried. The mass of dried empty bottle (M_1) was taken then filled to the brim with distilled water. The bottle containing water was weighted and the mass recorded (M_2). The experiment was repeated after drying the bottle with wine samples substituting the water and the mass was taken (M_3).

The specific gravity (SG) of the wine samples was calculated as follow:

$$SG(g/cm3) = (M3 - M1)/(M2 + M1)$$

Sensory Analysis

The wines produced were compared for color, flavor, taste, sugar acid blend, appearance and overall acceptability using a 9-point hedonic scale. The final beverages were evaluated by 35 panelists, males and females. The panelists were asked to grade the different samples in the scale of 9-points with 1 = 'Dislike extremely'; 2 = Dislike very much'; 3 = 'Dislike moderately'; 4 = 'Dislike slightly'; 5 = 'Neither dislike no like'; 6 = 'Like slightly'; 7 = 'Like moderately'; 8 = 'Like very much'; 9 = 'Like extremely' [20,21]. Labelled samples marked with random numbers were served in a plastic cups. Mineral water was provided and acted as a neutralizer between the samples in order to avoid carryover effect.

Statistical Analysis

All values were represented as means ± SD. One-way ANOVA was used to evaluate the statistic differences of data with the help of Stagraphic Centurion version XVI.I, software package. Experimentation were carried out in triplicate and the differences were considered to be statistically significant when the probability was less than 0.05 ($p < 0.05$).

Result and Discussion

Result

Physicochemical Analysis

The physicochemical analysis of fresh cocoa juice (FJ), wine fermented with essential oil and wine fermented without additive (WFA) are presented in table 1. Out of the physicochemical parameters evaluated, a part of the specific gravity and the total sugar, the produced wines shown higher value compared to the fresh juice. The physicochemical values of the fresh cocoa juice vary significantly during the fermentation processes.

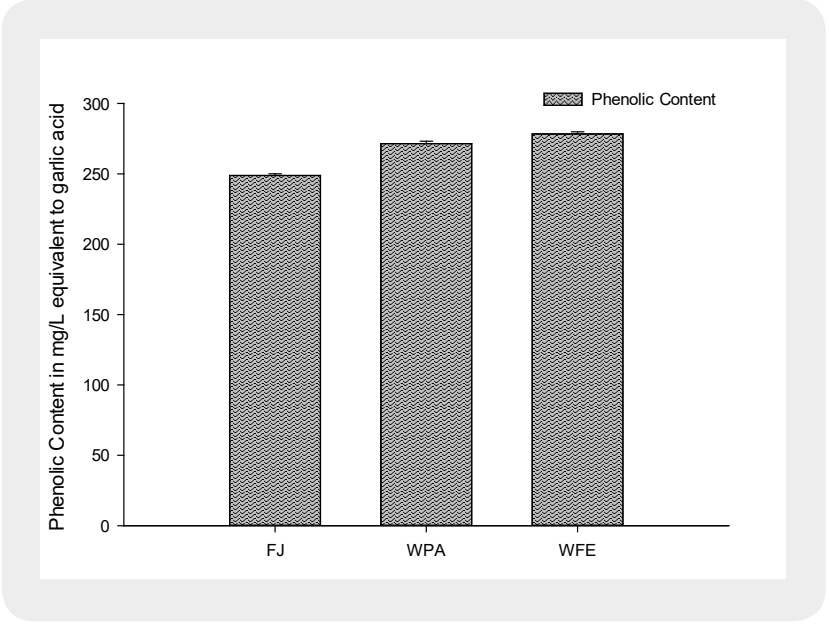
Table 1: *Proximal composition of fresh juice and wines.*

	Samples		
	FJ	**WFA**	**WFE**
pH	5.97±0.04[a]	5.06±.0.01[b]	4.88±0.30[b]
Specific gravity (g/cm^3)	1.87± 0.00[a]	1.02±0.00[b]	1.03± 0.00[c]
Alcohol (%)	0.00[a]	7.16±0.06[b]	8.01±0.05[c]
TA (mEq L^{-1} in malic acid)	0.68±0.01[a]	1.47±0.03[b]	1.48± 0.01[b]
Total sugar (mg/mL)	13.99±0.39[a]	7.12±0.76[b]	8.47±0.45[c]

The values having the same superscript lowercase letters (a, b, c) in the same row are not significantly different ($p<0.05$).

Total Phenolic Content

The total phenolic content (TPC) values of both fresh juice and the produced wines were estimated in terms of gallic acid equivalent, as presented in figure 1. Generally, the total phenolic content of the fresh juice and produced wines range from 248.43 to 278.38mg/L EGA. There was a significant ($p<0.05$) increase in TPC during fermentation processes.

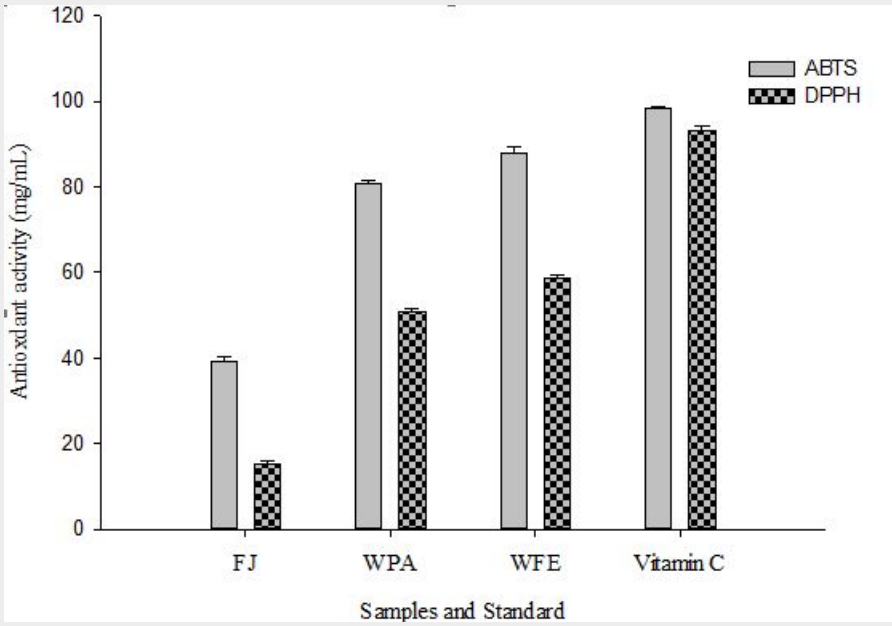


Fresh Juice (FJ); Wine fermented with essential oil (WFE); Wine produced with no additive (WPA).
The histogram bar carrying the same lowercase letters (a, b, c) are not significantly different ($p < 0.05$).

Figure 1: *Phenolic content in cocoa fresh juice and wines*

Antioxidant Activity

The antioxidant activity in food engineering depends on the method used for evaluation [22]. It cannot be fully evaluated by one single method due to many potential parameters [23,24]. In this line, the DPPH (1, 1-diphenyl-2-picrylhydrazyl) and ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) scavenging abilities were used to test the antioxidant activities of the wines and the results are shown in figure 2. Irrespective of the method used for the antioxidant evaluation, the result obtained for both wines was lower compared to that of vitamin C. Also, the WPE shown greater antioxidant activity than WPA for both investigated methods.



Fresh Juice (FJ); Wine fermented with essential oil (WFE); Wine produced with no additive (WPA).
The histogram bar carrying the same lowercase letters a, b, c, d or $a^{\dagger}$, $b^{\dagger}$, $c^{\dagger}$, $d^{\dagger}$ respectively for the ABTS and DPPH methods are not significantly different ($p < 0.05$).

Figure 2: *Antioxidant activity of different samples and standard*

Microbiological Analysis

The microbial loads of produced wines is presented in table 2. The wines produced were all contaminated at the end of the process. The concentration of the different microorganism detected was lower compared to the recommended norms. Harmful microorganism such as salmonella were completely absent.

Table 2: *Microbial load (Log CFU/mL) of different samples analyzed*

Parameters	Samples			Norms
	FJ	WPA	WFE	
Total mesophilic aerobic flora	$1.34 \pm 0.12 \times 10^{4c}$	$5.60 \pm 0.56 \times 10^{4a}$	$4.75 \pm 0.49 \times 10^{6b}$	10^6
Total coliform count	0^a	32.5 ± 0.21^b	87.5 ± 0.77^c	10^2
Anaerobic sulphite reducers	$7.55 \pm 0{,}49^b$	9.30 ± 0.56^a	4.70 ± 0.42^a	-
Yeast and molds	$9.35 \pm 0.49 \times 10^{2b}$	$6.00 \pm 0.42 \times 10^{2a}$	$3.90 \pm 0.56 \times 10^{2a}$	10^2
Salmonella species	-	-	-	-

Values are expressed as mean ± standard deviation. The values having the same lowercase letters (a, b, c) in the same row are not significantly different ($p<0.05$).
Fresh Juice (FJ); wine fermented with essential oil (WFE); wine produced with no additive (WPA).

Sensorial Analysis

A 9-point hedonic scale analysis was used to evaluate the color, flavor, appearance, texture, acid taste and the acceptability of the wine made from cocoa fresh juice and results are presented in the radar plot (figure 3). As expected, the sensorial score vary from one panelist to the other, however the overall acceptability of the products was investigated. The produced wines have approximately the same color but generally WPA shown the higher score compared to WFE as far as other evaluated parameter are concerned.

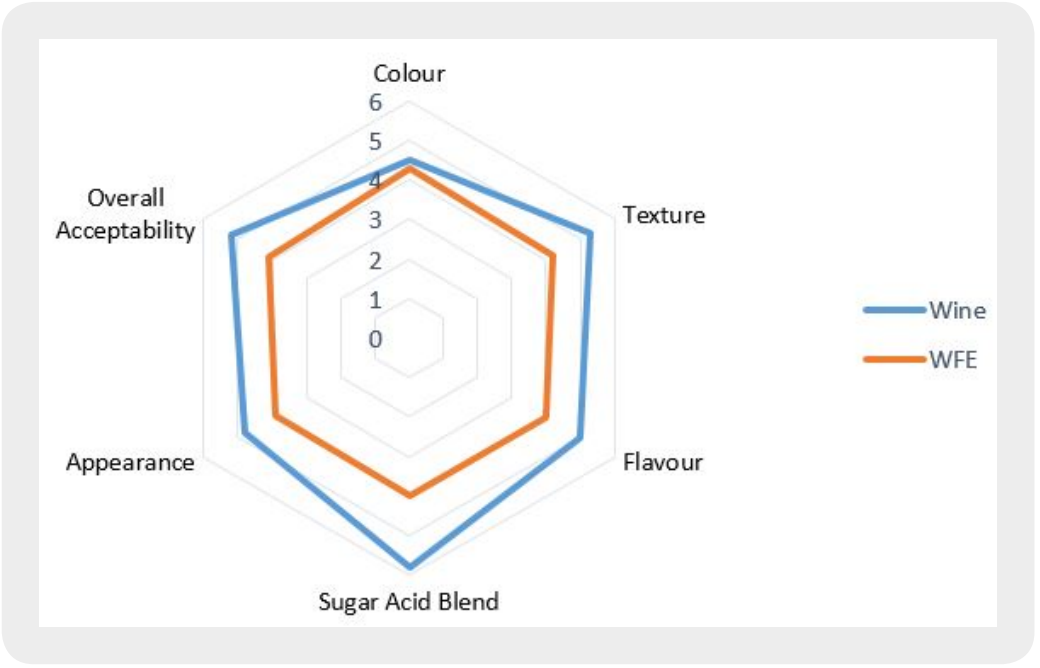


Figure 3: *Radar plot of the sensory evaluation of cocoa wines*

Discussion

pH Value of Fresh Juice and Wines

The pH of the worth was measured before (fresh juice) and after the fermentation (wines) process and the data were recorded (Table 1). The pH values decreased with fermentation processes and ranged from 5.97 ± 0.04 to 4.88 ± 0.3 for the sample fermented with essential oil and 5.97 ± 0.04 to 5.06 ± 0.01 for the sample fermented without essential oil. This can be due to the presence of lactic acid from the fermentation of sugar by yeast. It can also be explained by the proteolysis of some proteins present in the tegument. In fact, it was reported that, cocoa beans contain carboxypeptidase enzymes which release hydrophobic amino acids with optimal activity at a pH close to 5.8 [25]. These results are in agreement with the reports of [26], who found a decrease of pH values during the fermentation of banana juice and cocoa beans respectively.

Alcohol Content and Titratable Acids

The alcohol content of cocoa pulp wine as well as the titratable acids were evaluated and the results are presented in table 1.

The variation in alcohol content was inversely proportional to that of sugar during fermentation process. There was a significant increase in alcohol content percentage of wines compared to that of the must. An increase in alcohol content which ranged from 0 to 7.16 ± 0.06% (WFA) and 8.01 ± 0.05% (WFE), could be due to the metabolism of sugars by yeast in one hand or to other alcoholic compounds obtained from the metabolism of lactic acid bacteria or any other microorganism that could be found in the environment. These values are in line with that of [5], who found the final alcohol content of fruit wine within the range of 7-8%.

The taste and aroma of wines are greatly influenced by its titratable acidity content [5]. For this reason, the titratable acid values of produced wines were determined. From table 1, the result showed significant increase ($P < 0.05$) in titratable acidity value of wines compared to that of fresh juice. No significant difference was recorded between the titratable acid value of WFE and that of WFA. The changes in titratable acid values during the fermentation process could be due to the production of lactic acid and acetic acid during the process. According to [27] fresh pulp cocoa juice contains significant amount of citric acid which could justify its titratable acid value.

Variation of Solid Sugar Content and Specific Gravity during the Fermentation Processes

During the fermentation in wine making, sugar is the main raw material although other food macronutrients such as protein and fats can be metabolized by specific microorganism. Even with this highest importance, the concentration of sugar need to be checked before the process because, yeast can tolerate only certain level of sugar concentration [28]. The sugar content of the fresh sample as well as that of produced wines were evaluated and the result are shown in table 1. The sugar content values varied from 13.99 ± 0.39 to 7.12 ± 0.76mg/mL and 8.74 ± 0.45mg/mL respectively for the WFA and WFE. The presence of sugar in wines shows that the fermentation processes were not completed. A simple computation done indicated that 51 and 62% of sugar were metabolized during the fermentation respectively for WFA and WFE. The highest

conversion percentage of sugars suggested that, the presence of the essential oil inhibited or killed some microorganisms and increased the affinity of *Saccharomyces cerevisiae* for fermentable sugars. This result is similar to that of [29], in term of sugar content reduction during fermentation process but significantly different in term of the percentage of sugar converted. These authors reported that during the fermentation cherry, 19.18% of sugars are converted. The difference observed can be due to the variation of the fermentation conditions or environments.

The change in specific gravity (SG) value of cocoa pulp wines during fermentation is showed in table 1. Irrespective of the type of fermentation, the SG value of wines decreased compared to that of the must. The SG of the wines obtained from the fermentation of in the presence of yeast only (1.016 ± 0.001), decreased for 54% whereas that of the wine produced from the yeast with the addition of *Monodora myristica* essential oil (1.025 ± 0.002) decreased for 55%. The change in specific gravity can be justified by the microbial metabolism of available nutrients in line with the analysis of sugar content. This result is in conformity with that of [30].

Total Phenolic Content

The wine obtained from the fermentation in the presence *M. myritica* essential oil (WFE) exhibited the highest total phenolic content (278.38 ±1.55mg/L EGA) than that of wine produced from normal fermentation without additive (271.33 ± 1.71mg/L EGA) and the control (248.43 ±1.24mg/L EGA). This difference can be due to the phenolic compounds present in the *M. myritica* essential. In fact, [31-33], reported good phenolic content in *M. myritica*. The significant ($p < 0.05$) increase of the total phenolic content during fermentation could be attributed to the yeast or other microbial action. In fact, during the fermentation process of cocoa pulp, enzymes from microorganisms such as glucosidase and cellulose can destroyed the plant cell walls and ease the liberation of phenolic compounds [34]. In addition, some probiotic bacteria have the ability to increase the value of simple phenolic compounds by decomposing the flavan-3-ol molecules [35]. These values of phenolic content are higher compared to the highest value of 173.58mg GAE g^{-1} DW reported by [36], in cocoa beans. This could justify the fact that during the fermentation process, there are some biochemical (microorganism actions) which contributed to the increasing of the total phenolic content.

Antioxidant Activity

The fresh juice exhibited the lowest antioxidant activity with both methods and the highest was found with the wine obtained from the fermentation with *M. myristica* essential oil. There was a significant difference between the antioxidant of the produced wines with respect of the method used. This difference might be due to the antioxidant capacity of *M. myristica* essential oil present in the wine. The antioxidant capacity of different samples determined by the ABTS method was higher than the one obtained by the DPPH assay. This can be due to the fact that, DPPH radical reacts only with lipophilic antioxidants, while ABTS radical reacts with both hydrophilic and lipophilic antioxidants [37,38]. The estimated antioxidant activities both by ABTS and DPPH methods were lower compared to that of Vitamin C which can be justified by the fact that vitamin C used was a pure synthetic antioxidant molecule.

Several authors reported the influence of concentration of total phenolic compounds on the antioxidant activity of fruit wines [25,39]. In the same line, the correlation between the antioxidant activities (ABTS and DPPH) and the total phenolic content (TPC) was done. There was a strong significant positive correlation between the TPC and the antioxidant activity as evaluated by the ABTS (0.996) and DPPH (0.999) methods. The antioxidant activities of the fresh cocoa juice as well as that of produced wines can be attributed to the presence of phenolic compounds. This result is in conformity with the report of [40]. In many plant species, there exist a strong relationship between TPC and antioxidant activity [41].

Microbiological Analysis

From table 2, various sample had microbial loads varying from $1.34 \pm 0.12 \times 10^4$ to $4.75 \pm 0.49 \times 10^6$ CFU /mL. The load of total mesophilic aerobic flora (TMAF) found is higher than those reported by (Kántor *et al*., 2014). These authors found TMAF loads ranging from 4 to 3.48×10^2 CFU /mL in samples of red wine stored at room temperature (20-25˚C). These differences could be explained by post-production contamination of the wines on one hand and the nature of the wine on the other. In fact, during wine production, the last step which is to eliminate microorganisms via filtration is a very delicate step which can lead to contamination [42].

The high alcohol content in the wines produced and their acidic pH are likely to inhibit the growth of microorganisms [42].

Compared to the standard, the fruit juice (FJ) and the wine sample produced without additive (WPA) showed a TMAF load lower than the recommended value which is 10^6 CFU /mL [43] which indicates their good microbiological quality. However, the sanitary quality of a food does not only depend on the value of its TMAF load, but also on the loads of the groups of other microorganisms (pathogens and spoilage) present. In this line, pathogens providing information on the hygienic quality of the wines namely, coliforms, were analyzed and the results obtained are illustrated in table 2. FJ contains no coliforms despite numerous replications carried out. Wine produced without essential oil and wine produced with essential oil showed total coliform loads of 3.25 ± 0.21 and 8.75 ± 0.77 CFU /mL respectively. This result, though high, are lower than the standard which is 10^2 CFU /mL [8]. [44], pointed out that the presence of coliforms in food is the result of unhygienic conditions during the production process. Likewise, it has been reported that proper hygienic practices does eliminate unwanted bacteria from wine [42].

Salmonella is one of the dangerous food-borne pathogen and is used as a food safety index germ. The presence of any of its species should be presumed pathogenic for human being [43]. The microbiological analysis result indicated that no Salmonella sp. was found in any of the wines produced. This suggests that these wines can be considered as safe for consumption.

Yeasts and molds were found in all samples analyzed with microorganism loads of $9.35 \pm 0.49 \times 10^2$, $6.00 \pm 0.42 \times 10^2$ and $3.90 \pm 0.56 \times 10^2$ CFU/mL respectively in the samples FJ, wine without essential oil, (WPA) and wine produced with essential oil, WFE. These values are all close to the recommended value which is 10^2 CFU/mL. This yeast contamination could result from a deficit in the final filtration process wine at the end of the alcoholic fermentation. Indeed, the final filtration of wine eliminates the yeasts to

prevent fermentation from continuing and lead to the degradation of the wine [42]. These charges are slightly higher than the standard value which suggest a short shelf life of the produced wines. These results are similar to 9 - 13.8 × 10^2 CFU /mL reported by [45] in their study on the microbiological quality of red wines.

Sensorial Analysis

The sensory evaluation score differs significantly from one sample to another. The wine made without additive (WPA) received a mean score of 6.0 (like slightly) and the one fermented with essential oil (WFE) was attributed the lowest mean score of 4.3 (dislike slightly) which seemed to be the least preferred by the panelists. Both samples were not significantly different as far as color parameter is concerned. Generally, in food products, especially fruit based products, the consumers often assess the initial quality by their color and appearance; hence the perceived quality [46]. The overall acceptability of the samples, was found to be 6.0 and 4.3 for WPA and WFE respectively. The wine produced without addition of essential oil was the most preferred by the panelists.

Conclusion

In this study, two different wines were produced from cocoa mucilage (pulp) using *Saccharomyces cerevisiae* as yeast strain. The produced wines had good nutritional value and the overall acceptability revealed that, wine sample produced without *Monodora myristica* essential oil was the best sample though it has the lower alcohol content. The final wines were contaminated. Therefore paying attention to certain unit operation of the production process such as filtration will help improve the quality of the final product. As far as the shelf life of the produced wines, further research needs to be done.

Conflict of Interest

Authors declare no conflict of interest

Bibliography

1. Oguntokun, M. O., Oguntokun, E. A., Nwaokobia, K. & Abiodun, A. E. (2019). Analyzing and comparing the physico-chemical and mineral component of cocoa (*Theobroma cacao* L.) pod and bean. *International Journal of Agriculture, Forestry and Fisheries, 7*(2), 18-24.

2. Joy Ajit, E., Dominic, D., Farook, F., Promod, A., Kumar M.S., Blesy V.J., *et al*. (2018). Preparation of wine from fruits of *Musa accuminata* and *Ananas comosus*; its physico-chemical analyses and sensory evaluation. *Integrative Food, Nutrition and Metabolism, 5*(6), 1-5.

3. Tamrakar, K., Lama, A., Dhakal, B., Adhikari, L., Shrestha, M. & Amatya, J. (2020). Qualitative analysis of wine prepared from banana and orange. *International Journal of Food Science and Nutrition, 5*(1), 60-63.

4. Balladares, C., Garca, J., Chóez-Guaranda, I., Prez, S., Gonzlez, J., Sosa, D., *et al*. (2016). Physicochemical characterization of *Theobroma cacao* L. mucilage, in Ecuadorian coast. *Emirates Journal of Food and Agriculture, 28*(10), 741-745.

5. Izabel D. J. (2019). Cocoa's residual honey: physicochemical characterization and potential as a fermentative substrate by *Saccharomyces cerevisiae* AWRI726. *The Scientific World Journal, 2019,* 5698089.

6. Roini C., Limatahu N., Hartati T. & Sundari S. (2019). Characterization of cocoa pulp (*Theobroma cacao* L) from south halmahera as an alternative feedstock for bioethanol production. IOP Conf. Ser. Earth. Environ. Sci. 276, 012038.

7. Vallejo, B., Matallana, E. & Aranda, A. (2020). *Saccharomyces cerevisiae* nutrient signaling pathways show an unexpected early activation pattern during winemaking. *Microbial Cell Factories, 19*(124).

8. ISO 4832. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique. Edition 3, 2006: 6. 9.

9. ISO 21527-2. Microbiologie des aliments - Méthode horizontale pour le dénombrement des levures et moisissures - Partie 2: Technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95. Edition 1, 2008: 9.

10. ISO 6579-1. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. Edition 1, 2017, 50.

11. ISO 7937. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of *Clostridium perfringens*. Colony-count technique. Edition 3, 2004, 16.

12. Oba, D. O., Okunola, O. J., Oranusi, S. U. & Okagbue, H. I. (2018). Data on microbial and physicochemical assessment of mixed fruit wine produced from physically damaged fruits. *Data in Brief, 19,* 678-686.

13. Fombang, E. N. & Saa, R. W. (2016). Antihyperglycemic activity of *moringa oleifera* lam leaf functional tea in rat models and human subjects. *Food and Nutrition Sciences, 7*(11), 1021-1032.

14. Association Française de normalisation. Recueil de normes françaises des produits dérivés des fruits et légumes, jus de fruits. Paris: AFNOR; 1982.

15. Gao, X., Ohlander, M., Jeppsson, N., Björk, L. & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L) during maturation. *J Agric Food Chem., 48*(5), 1485-1490.

16. Waqas, M. K., Saqib, N., Rashid, S., Shah, P. A., Akhtar, N. & Murtaza, G. (2013). Screening of various botanical extracts for antioxidant activity using DPPH free radical method. *African Journal of Traditional, Complementary, and Alternative Medicines, 10*(6), 452-455.

17. Saeed, N., Khan, M. R. & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complementary and Alternative Medicine, 12*(1), 221.

18. Ratnavathi, C. V. & Komala, V. V. (2016). Sorghum Grain Quality. In: Ratnavathi CV, Patil JV, Chavan UD, editors. Sorghum Biochemistry. San Diego: Academic Press, 1-61.

19. Yousaf, A. A., Abbasi, K. S., Ahmad, A., Hassan, I., Sohail, A., Qayyum, A. & Akram, M. A. (2020). Physico-chemical and nutraceutical characterization of selected indigenous guava (*Psidium guajava* L.) cultivars. *Food Science and Technology, 41*(1), 47-58.

20. Mugob Bonyesih, K. M., Mezajoug Kenfack, L. B., Ngangoum, E. S., Djiazet, S. & Tchiégang, C. (2019). Formulation and production of Spiced Pepper (*Capsicum chinenses* Linn.) Cube. *J Food Stab., 2*(1), 1-20.

21. Kissiedu, K. O., Agbenorhevi, J. K. & Datsomor, D. N. (2020). Optimization of sensory acceptability of milk chocolate containing okra pectin as emulsifier. *International Journal of Food Properties, 23*(1), 1310-1323.

22. Munteanu, I. G. & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: a review. *International Journal of Molecular Sciences, 22*(7), 3380.

23. Dejian, H., Ou, B., Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *American Chemical Society, 53*(6), 1841-1856.

24. Villaño, D., Pachón, M. S., Troncoso, A. & Garcia-Parrilla, M. (2006). Influence of enological practices on the antioxidant activity of wines. *Food Chemistry, 95,* 394-404.

25. Di Lorenzo, C., Badea, M., Colombo, F., Orgiu, F., Frigerio, G., Pastor, R. F. & Restani, P. (2017). Antioxidant activity of wine assessed by different *in vitro* methods. Aurand J-M, editor. *BIO Web Conf., 9,* 04008.

26. Pauline, M., Alexandre, O., Andoseh, B. K., Abeline, M. T. S. & Agatha T. (2017). Production technique and sensory evaluation of traditional alcoholic beverage based maize and banana. *Int J Gastron Food Sci., 10,* 11-15.

27. Nunes, C. S. O., da Silva, M. L. C., Camilloto, G. P., Machado, B. A. S., Hodel, K. V. S., Koblitz, M. G. B., *et al*. (2020). Potential applicability of cocoa pulp (*Theobroma cacao* L) as an adjunct for beer production. *Sci World J.*, 3192585.

28. Nikhanj, P. & Kocher, G. (2015). Fermentative production of guava-wine (*Psidium guajava* L.) using S. cerevisiae MTCC 11815. *Curr Nutr Food Sci., 11*(1), 21-30.

29. Jia, C., Han, F., Miao, X., Yan, A., Wu, P. & Sun, Y. (2019). Study on the changes of sugar and acid in fermentation process of different varieties of cherry wine. *Journal of Food Science and Nutrition, 2*(1), 15-19.

30. Okafor, N. (2007). Modern industrial microbiology and biotechnology. Enfield, (NH): Science Publishers: 263-308.

31. Feyisayo, A. & Oluokun, O. O. (2014). Comparative analysis of phenolic profile of *Monodora myristica* and *Monodora tenuifolia*. *Afr. J. Agric. Res., 9*(16), 1296-1302.

32. Agiriga, A. & Siwela, M. (2018). Effects of thermal processing on the nutritional, antinutrient, and *in vitro* antioxidant profile of *monodora myristica* (Gaertn.) dunal seeds. *Preventive Nutrition and Food Science, 23*(3), 235-244.

33. Womeni, H. M., Djikeng, F. T., Tiencheu, B. & Linder, M. (2013). Antioxidant potential of methanolic extracts and powders of some Cameroonian spices during accelerated storage of soybean oil. *Adv Biol Chem., 3*(3), 304-313.

34. Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I. & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry, 160,* 346-356.

35. Li, S., Chen, L., Yang, T., Wu, Q., Lv, Z., Xie, B. & Sun, Z. (2013). Increasing antioxidant activity of procyanidin extracts from the pericarp of Litchi chinensis processing waste by two probiotic bacteria bioconversions. *Journal of Agricultural and Food Chemistry, 61*(10), 2506-2512.

36. Oracz, J. & Nebesny, E. (2016). Antioxidant properties of cocoa beans (*Theobroma cacao* L.): influence of cultivar and roasting conditions. *International Journal of Food Properties, 19*(6), 1242-1258.

37. Minnelli, C., Laudadio, E., Galeazzi, R., Rusciano, D., Armeni, T., Stipa, P., Cantarini, M. & Mobbili, G. (2019). Synthesis, characterization and antioxidant properties of a new lipophilic derivative of edaravone. *Antioxidants, 8*(8), 258.

38. Santos Sánchez, N., Salas-Coronado, R., Villanueva, C. & Hernandez-Carlos, B. (2019). Antioxidant Compounds and Their Antioxidant Mechanism.

39. Lorenzo, C. D., Mihaela, B., Francessa, C., Orgiu, F., Gianfranco, F., Raul, F. P. & Patrizia, R. (2017). Antioxidant activity of wine assessed by different *in vitro* methods. BIO Web Conf. 6.

40. Nagai, T., Inoue, R., Inoue, H. & Suzuki, N. (2003). Preparation and antioxidant properties of water extract of propolis. *Food Chemistry, 80*(1), 29-33.

41. Oktay, M., Gülçin, İ. & Küfrevioğlu, Ö. İ. (2003). Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT - Food Science and Technology, 36*(2), 263-271.

42. Bartowsky, E. J. (2019). Bacterial spoilage of wine and approaches to minimize it. *Lett Appl Microbiol., 48*(2), 149-156.

43. Arvanitoyannis, I., Tsitsika, E. & Panagiota, P. (2005). Implementation of quality control methods (Physico-Chemical, Microbiological and Sensory) in conjunction with multivariate analysis towards fish authenticity. *International Journal of Food Science & Technology, 40,* 237–263.

44. Dutta, M., Majumdar, P., Islam, M. R. U. & Saha, D. (2018). Bacterial and fungal population assessment in smoked fish during storage period. *Journal of Food: Microbiology, Safety & Hygiene, 3*(1).

45. Kántor, A., Kačániová, M., Petrová, J., Medo, J., Hleba, L. & Rovná, K. (2014). Identification of lactic acid bacteria isolated from red wine samples by RT-qPCR. *J. Microbiol. Biotech. Food Sci., 1*(3). 235-237.

46. Lawless, H., & Heymann, H. (1998). Sensory evaluation of food: principles and practices. *Food Technology, 23*(5), 108-110.