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Microbiological, Physicochemical and Nutritional Assessment Quality of Three Types of *Kombucha Drinks* Produced from Local Herbs and Lipton Tea in Benin

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Abstract The present study aims to develop high health safety and nutritional beverages from SCOBY (Symbiotic Culture Of Bacteria and Yeast), Lipton tea and local plants in Benin. To do this, three types of drinks, two of which are based on local plants which are fake *kinkeliba* (*Cassia occidentalis*) and lemongrass (*Cymbopogon citratus*) have been produced. The methodology of the work consisted in infusing for 24 hours on the one hand 500g of fresh leaves of each plant (fake *kinkeliba* and lemongrass) and on the other hand 20g of Lipton in 5L of boiling water containing 500g of sugar. Then 500g of SCOBY was added to the filtrate of each infusion and the mixtures were left to ferment. Then the analysis were carried out according to the standards of analysis in microbiology, physicochemistry and nutritional. Microbiological analysis revealed that the three types of drinks were free of pathogenic germs and indicators of good hygiene and manufacturing practices (GHPs and GMPs) such as *Escherichia coli*, *Staphylococcus aureus*, salmonella and Anaerobic bacteria Sulfito-Reducers. Physicochemical analysis showed that all drinks had a pH between 2.15 and 2.43. The fake kinkeliba drink was the sourest. From a nutritional point of view, all the drinks contained calcium, zinc, iron and vitamin C. However, the Kombucha drink with fake kinkeliba is the richest in mineral salts (calcium: 6.96 mg/100mL; zinc: 0.07 mg/100mL; iron: 0.31 mg/100mL) and vitamin C (0.064 mg/100mL). Beverages also contain phenolic compounds such as total flavonoids, total phenols and tannins. Sensory analysis revealed that the three drinks were accepted by tasters who liked the lemongrass Kombucha drink more. From these results, the Kombucha drink with fake kinkeliba can be recommended to consumers with a nutrient deficit, especially to vulnerable people such as pregnant women, the elderly, and children.

Keywords: *Kombucha, drink, SCOBY, fake kinkeliba, Physicochemical, health safety and nutritional, lemongrass, Lipton tea, Benin*

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1. Introduction

Beverage is a liquid of acceptable sanitary quality that is consumed. According to [1], beverages are by far the most popular functional food category due to convenience, ease of distribution, storage, ability to incorporate desirable nutrients and bioactive compounds. There are several categories of drinks. Among which are fermentable but unfermented sugary drinks and fermented drinks. In this last batch are strong alcoholic drinks and

weak alcoholic drinks. At the level of the first type of drinks mentioned we have natural fruit juices and synthetic sweetened drinks. Natural fruit juices are obtained from healthy and ripe fruits, fresh or preserved by cold [2]. According to the same source, synthetic sugary drinks are made up of syrup, artificial coloring and flavoring; there are energy drinks, carbonated drinks, etc. The fruits are known to be full of vitamins and mineral salts, which constitutes the richness of natural fruit juices. Calorie intake is an important benefit of other types of sugary drinks. But excessive consumption of these products has drawbacks such as the occurrence of certain diseases such as: diabetes. For example, a large study carried out in Europe demonstrated a close relationship between the consumption of sugary drinks and an increase in type 2 diabetes [3]. Highly alcoholic fermented beverages are above all responsible in whole or in part for diseases such as: infectious diseases, cancer, diabetes, neuropsychiatric diseases (including disorders related to alcohol consumption), cardiovascular diseases, liver, pancreas, even unintentional and intentional injuries [4]. As for low-alcoholic drinks, they represent an alternative means compared to other types of drinks, because due to their largely processed sugar content and their low alcohol content, they further limit the risks associated with the consumption of sugary and highly alcoholic drinks. Among the low alcohol drinks is Kombucha. Kombucha is defined as the fermentation of sweetened tea with a symbiotic culture of acetic acid bacteria and yeast [5]. According to the same source, it is consumed around the world for its refreshing and beneficial properties on human health. It can prevent various types of cancers and cardiovascular diseases promote liver functions and stimulate the immune system [5]. It contains iron, manganese, nickel, copper, zinc [6], vitamins B and C [7]. As its definition suggests, Kombucha is a product derived from tea. Tea originated in China. It is made by infusing the leaves of the tea tree in water. There are several types of tea because there are various forms of processing these leaves. Thus, we have green, yellow, black, and white teas [8]. In the world, there is an enormous variety of plant species allowing to have a diversity of origin of beneficial elements for the human organism. This means that the pharmacopoeia is very exploited in the world in general and in particular in Africa. On this continent, the art of healing with plants has been known and practiced for a long time, it is also in many african regions the most accessible and affordable health resource [9]. Beneficial innovation contributing to the progress of humanity has based our objective to work on the experimentation of plant species available in Africa, especially in Benin in the production of Kombucha drink. The plant species on which we have chosen are fake kinkeliba (*Cassia occidentalis*) and lemongrass (*Cymbopogon citratus*). Lemongrass is a perennial aromatic herb belonging to the Poaceae (Gramineae) family, traditionally used to cure various diseases [10]. Fake kinkeliba is an annual or perennial Ayurvedic plant that is used in several traditional medicines to treat various diseases [11]. These experiments will allow us to vary the conventional substrate for the production of Kombucha, which is the leaf of the tea tree, a tree that is not cultivated in Benin. By this fact, we intend to promote local plants and, in turn, reinforce the value of natural medicine. The main objective of this study is to develop high health safety and nutritional beverages based on "SCOBY", local plants and Lipton tea in Benin.

2. Materials and Methods

The plant material consisted of fresh leaves of fake kinkeliba (*Cassia occidentalis*) taken from the small vegetation of the municipality of Abomey-Calavi, fresh leaves of lemongrass (*Cymbopogon citratus*) purchased in the garden of the FSA of Abomey-Calavi, packaged Lipton brand tea leaves and commercially purchased sugar. Figure 1 illustrates the main plant materials in the production of Kombucha beverages. The biological material was a symbiotic strain of bacteria and yeast (SCOBY) from URSSA, was used for the formulations. Figure 2 shows the SCOBY that was used for the production of drinks.

Figure 1. Main plant materials in beverage production

Figure 2. SCOBY

2.1. Development of Three Types of Drinks with High Nutritional Values Based on "SCOBY", Local Plants and Lipton Tea

The production of the Kombucha drink was made according to the production diagram in Figure 3. The leaves of fake kinkeliba or lemongrass (approximately 500 g) were washed and then drained. They were infused in boiling water (5 L), put in plastic, where there was addition of 500 g of sugar. After cooling to 28°C-30°C, the leaves of fake kinkeliba or lemongrass were removed and then 500 g of the SCOBY was seeded. Once the container was closed, the solution was left to ferment for 5 days at 28°C-30°C. Then there was the withdrawal of the "SCOBY". It was followed by filtration and then pasteurization. This pasteurization consisted in heating the product up to 90°C for 2 min. Then the liquid was bottled hot in previously sterilized containers. The canning of the bottled products was done at 100°C for 15 minutes (min). Finally there was cooling at 28°C-30°C and storage at 28°C-30°C. The particularity of the Lipton tea drink is that 20g of the product was used. The rest of the procedure is identical.

2.2. Microbiological Analysis

The microbiological analysis focused not only on the search for pathogenic microorganisms but also on the telltale germs of good hygiene practices in the food industry [12]. The culture media were prepared according to the manufacturer's instructions and kept supercooled until the time of inoculation. To prepare the stock solution, 10 mL of each sample was taken with a sterile spatula into a sterile stomacher bag and 90 mL of tryptone salt broth was added. This mixture was homogenized in a sample mixer. Successive decimal dilutions were made from the stock solution. Total Mesophilic Aerobic Germs were counted on Plate Count Agar (PCA) after incubation at 30°C for 72 h ± 2 h [13]; thermo-tolerant coliforms on Violet Red Bile Lactose Agar (VRBLA) at 44°C for 24±2h [14]; *Staphylococcus aureus* on Baird Parker Agar (BPA) enriched with egg yolk and potassium tellurite were observed after incubation at 37°C for 48h±2h [15]; the search for β-glucuronidase of *Escherichia coli* was carried out using coliform dishes on Violet Red Bile Lactose Agar (VRBLA) by performing the Mac-Kenzie test (indole and oxidase) using reagents Kovacs and oxidase [16]. The enumeration of sulphite-reducing anaerobic bacteria was carried out on Tryptone Sulfite Neomycin Agar (TSNA) after incubation at 46°C for 20 h ±2 h [17]. Finally, the search for salmonella was carried out on *Salmonella Shigella* Agar (SSA) according to the ISO 6579-124 standard [18]. As for yeasts and molds, they were counted on glucose oxytetracycline agar (OGA) enriched after incubation at 25°C for 3-5 days [19]. The microbiological analysis were carried out in three repetitions on each sample. The number of germs was expressed in Colony Forming Units per gram (CFU g–1).

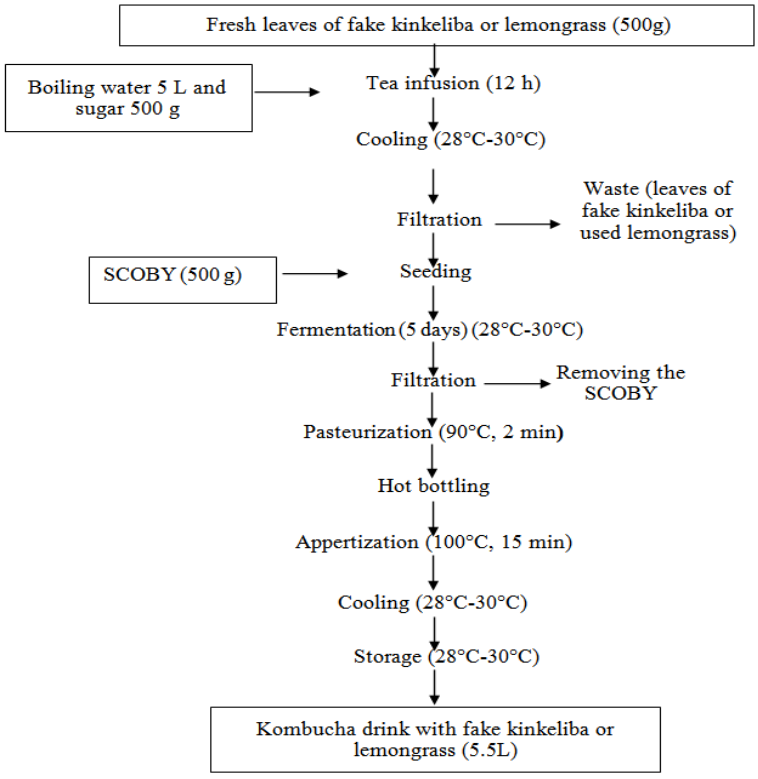

Figure 3. Production technology of Kombucha drink based on fake kinkeliba or lemongrass (Caption: SCOBY (Symbiotic Culture Of Bacteria and Yeast); L (Litre); g (gram); h (hour); min (minute); °C (Degrees Celsius))

2.3. Expressions of the Results of Microbiological Analysis

The results obtained during the research and counting of microbial germs were expressed in CFU/ml by the following formula:

$$\mathbf{N} = \frac{\Sigma \mathbf{C}}{V\left(\mathbf{n}1 + 0,1\mathbf{n}2\right) \times \mathbf{d}}$$

N = Number of germs in CFU per ml;
ΣC: Sum of colonies counted on the retained dishes of the two successive dilutions;
n1: Number of dishes retained at the first dilution;
n2: Number of dishes retained at the second dilution;
d: dilution rate corresponding to the first dilution (the 1st);
V = volume of inoculum applied to each dish.

The results obtained were used according to the standard [20].

2.4. Physicochemical and Nutritional Analysis

The pH was measured using an electronic probe pH meter that was previously calibrated with buffer solutions of pH7 and pH4 at 2°C. Titratable acidity, expressed as percentage citric acid, was determined by titration with NaOH (0.1N) in the presence of phenolphthalein. The soluble dry extract (°Brix) was determined using a portable refractometer (HI 96801 of 0-80° Brix). The dry matter was determined by drying in an oven at 105°C according to the recommended method [21]. As for the ashes, 5 grams of the sample were weighed into a porcelain crucible. The whole was carbonized and then incinerated in a muffle furnace at 550°C / 24 hours. The ash obtained was dissolved in 5 mL of hydrochloric acid (6N) then evaporated on a hot plate at 125°C. The resulting residue was dissolved and recovered with HNO_3 (0.1N) in a 100mL flask. This solution was used to obtain mineral content by atomic absorption spectrophotometer. The results are expressed in relation to dry matter [21]. Vitamin C was determined by high performance thin layer chromatography (HPTLC). Vitamin C was evaluated after derivation which consists of extracting the hydrazones. This derivation was applied to the samples as well as to the standard solution. It was determined carried out on an aliquot by measuring the absorbance at 360 nm with the Camag TLC Scanner III densitometer [22]. A standard sample of ascorbic acid was used as a reference and the results expressed in mg/L.

2.5. Search for Phenolic Compounds

√ **Research of total phenols**

The total phenol content was determined using the Folin-Ciocalteu colorimetric method with some modifications. This method consisted in using a mixture of phosphotungstic and phosphomolybdic acids, which were reduced during the oxidation of phenols to a mixture of blue oxide of tungsten and molybdenum. Finally, the absorbance was measured at 760 nm using a spectrophotometer and the total phenol contents are expressed in micrograms of gallic acid equivalence per mL (µgGAE/mL) [23,24,25,26].

√ **Research of flavonoids**

The aluminum trichloride (AlCl3) method was used to quantify total flavonoids. This technique was based on the formation of aluminum complex flavonoids. The absorbance was read at 415 nm using a spectrophotometer and the total flavonoid content is expressed in micrograms of quercetin equivalence per mL (µgQE/mL) [27].

√ **Search for condensed tannins**

The vanillin and hydrochloric acid method was used to determine the total content of condensed tannins. The absorbance was measured at 500 nm using a spectrophotometer and the tannin content was expressed in micrograms of catechin equivalent per mL (µgEC/mL) [28].

2.6. Sensory Analysis

The sensory evaluation was done to assess the different organoleptic characteristics of the drinks. The characteristics appreciated are: color, taste, aroma (smell), and overall acceptability on a scale of 1 to 9 with 1 = extremely inferior, 5 = identical to the reference sample and 9 = extremely better [29,30] and the preference test. This tasting panel was made up of 20 people.

2.7. Statistical Analysis of Data

The results were analyzed by the method of variance (ANOVA) using the PROC GLM procedure of SAS software (Statistical Analysis System) version 9.2. Multiple mean comparisons were performed with the Student Newman-Keuls test. The level of significance adopted was 5% ($p<0.05$). Principal component analysis were performed with MiniTab 2014.

3. Results

3.1. Production of Three Types of Drinks with High Nutritional Values Based on "SCOBY", Local Plants and Lipton Tea

Figure 4 presents the three types of drinks developed based on the exploitation of the technological diagram which was developed for this purpose (Figure 3). The production of 5.5L of the Kombucha drink required 5L of water, 500g of SCOBY and 500g of sugar to which was added 20g of the Lipton tea product, to obtain the Kombucha drink with Lipton tea. As for obtaining the lemongrass or fake kinkeliba Kombucha drink, 500g fresh lemongrass leaves were used. The unit operations that were followed in the production of each of the drinks remain almost the same from one drink to another. It mainly involved infusion, cooling, filtration, seeding, fermentation, pasteurization and canning.

Figure 4. Photos of the different drinks

3.2. Microbiological, Physicochemical and Nutritional Quality of the Beverages Produced

Table 1 and Table 2 present the microbiological characteristics of the different Kombucha drinks. The main germs sought and counted are mesophilic aerobic germs, thermotolerant coliforms, coagulase-positive staphylococci, sulphite-reducing anaerobes, yeasts and molds and salmonella. Analysis of this table reveals that without heat treatment, the microbial load (GAM) of fake kinkeliba and lemongrass drinks is significantly lower than that of Lipton tea at the 5% threshold. In general, beverages that have undergone heat treatment are less contaminated than those that have not undergone heat treatment (pasteurization and canning).

Table 1. Microbiological characteristics of the different Kombucha drinks according to the heat treatment undergone

BEVERAGE	HEAT TREATMENT	Microorganisms (CFU/mL)						
		GAM	CTT	*E Coli*	*S. aureus*	ASR	Yeasts and Molds	Salmonella
« Lipton » TEA	Untreated	20000 ± 1000^{a}	100 ± 20^{a}	<1	8.00 ± 2.0^{a}	<1	237.5 ± 42.5^{a}	Absent
	Treated	3900 ± 600^{b}	10±a	<1	$<1^{a}$	<1	Absent	Absent
	F-Value	818.03*	10.63^{ns}	-	6.63^{ns}	-	-	-
At fake kinkeliba	Untreated	8350 ± 1250^{a}	35 ± 25^{a}	<1	2.50 ± 1.50^{a}	<1	93 ± 18^{a}	Absent
	Treated	5300 ± 1200^{a}	10 ± 0^{a}	<1	$<1^{a}$	<1	Absent	Absent
	F-Value	0.78^{ns}	1.00^{ns}	-	1.00^{ns}	-	-	-
LEMONGRASS	Untreated	6200 ± 2700^{a}	15 ± 5^{a}	<1	1.50 ± 0.50^{a}	<1	58 ± 38^{a}	Absent
	Treated	6000 ± 1100^{a}	10 ± 0^{a}	<1	$<1^{a}$	<1	Absent	Absent
	F-Value	0.09ns	1.00ns	-	1.00ns	-	-	-

Caption: S. aureus = *Staphylococcus aureus*; CTT=Thermotolerant Coliforms; GAM=Mesophilic Aerobic Germs; ASR=Sulfite-Reducing Anaerobes; E. Coli = *Escherichia coli*.
*= significant; ns= not significant; Mean values with the same letter on the same line are not significantly different at the 5% level.
Untreated: drink that has not undergone heat treatment (pasteurization and canning); Treated: beverage that has undergone heat treatment.

Table 2. Comparison of the microbiological characteristics of the different types of kombucha drinks according to the plants used

Target microorganisms (CFU/mL)	Different types of kombucha drink			F-Value	Standards
	Lipton tea	At fake kinkeliba	Lemongrass		
GAM	3900 ± 600^{b}	5300 ± 1200^{a}	6000 ± 1100^{a}	9.07*	-
CTT	10 ± 0^{a}	10 ± 0^{a}	10 ± 0^{a}	4.70ns	10
E. Coli	<1	<1	<1	-	Absent
S. aureus	$<1^{a}$	$<1^{a}$	$<1^{a}$	4.84ns	-
ASR	<1	<1	<1	-	Absent
Yeasts and Molds	Absent	Absent	Absent	-	$10\text{-}10^{2}$
Salmonella	Absent	Absent	Absent	-	Absent

Caption: S. aureus = *Staphylococcus aureus*; CTT=Thermotolerant Coliforms; GAM=Mesophilic Aerobic Germs; ASR=Sulfite-Reducing Anaerobes; E. Coli = *Escherichia coli*.
*= significant; **= highly significant; ns= not significant; Mean values with the same letter in the same column are not significantly different at the 5% level.

3.3. Physicochemical Characteristics

The analysis of Table 3, relating to the physicochemical characteristics of the various beverages, reveals that the contents of total sugar, ash, dry matter and titratable acidity do not vary significantly from one beverage to another at the threshold by 5% ($p>0.05$). However, the pH of the Kombucha drink with Lipton tea is significantly lower compared to the pH values of the Kombucha drinks with fake kinkeliba and lemongrass ($p<0.05$).

3.4. Nutritional Characteristics

√ Composition of mineral salts

Table 4 presents the composition of Kombucha drinks in mineral salts. The minerals dosed in the different drinks are essentially calcium (Ca), zinc (Zn) and iron (Fe). The Kombucha drink with fake kinkeliba is richer in Ca (69.57 mg/L), Zn (0.68 mg/L) and Fe (3.09 mg/L) than the other two drinks. The contents of these minerals are Ca (10 mg/L), Zn (0.55 mg/L) and Fe (2.61 mg/L) for the Kombucha drink with Lipton tea. And for the lemongrass drink they are: Ca (36.42 mg/L), Zn (0.06 mg/L) and Fe (1.29 mg/L).

√ Vitamin C composition

Table 5 summarizes the vitamin C content of the different Kombucha drinks. Its analysis reveals that Kombucha tea with kinkeliba contains more vitamin C (about 0.64 mg in 1 liter of this drink) than Lipton tea (0.62 mg in 1 liter of drink) and Kombucha lemongrass drink (0.57 mg in 1 liter of drink).

3.5. Composition in Phenolic Compounds

Table 6 shows the contents of phenolic compounds of the different Kombucha drinks. Its analysis reveals that it is the Lipton tea drink which is the richest in total phenols and tannins, that with fake kinkeliba is the richest in total flavonoids.

3.6. Sensory Characteristics of Beverages Produced

Table 7 presents the results of the Student Newman Keuls test on the drinks taking into account their organoleptic characteristics. Its analysis reveals that sensory parameters such as color, aroma, taste and overall acceptability of the lemongrass Kombucha drink were very highly appreciated by consumers compared to the control ($p<0.0001$). On the other hand, the taste and overall acceptability of the Kombucha drink with fake kinkeliba showed no significant difference at the 5% level compared to the control. It was also noted that the color and aroma seem better for the fake kinkeliba drink compared to Lipton tea.

Table 3. Physicochemical characteristics of the different kombucha drinks

Parameters	DRINK Kombucha			Fisher's value
	Tea Lipton	At fake kinkeliba	Lemongrass	
pH	2.15±0.02[b]	2.43±0.01[a]	2.32±0.04[a]	33.73*
Brix degree	7.35±0.65[a]	5.95±0.95[a]	5.20±0.60[a]	0.97ns
Titratable acidity	24.96±2.88[a]	44.06±6.06[a]	33.12±9.12[a]	10.01ns
Ash content	0.28±0.12[a]	0.64±0.62[a]	0.14±0.06[a]	0.39ns
Dry Matter Content	8.91±1.97[a]	9.76±4.68[a]	4.50±0.92[a]	1.14ns

*= significant; ns= not significant; the mean values bearing the same letter on the same line are not significantly different at the 5% level.

Table 4. Calcium, zinc and iron compositions of Kombucha drinks

Mineral salts (mg/L)	DRINK Kombucha		
	At fake kinkeliba	At lemongrass	At Lipton tea
Calcium	69.58	36.43	10.00
Iron	3.09	1.30	2.61
Zinc	0.68	0.07	0.56

Table 5. Vitamin C content of Kombucha drinks

Vitamin C levels (mg/L)	Kombucha drink with fake kinkeliba	Lemongrass Kombucha Drink	Lipton Tea Kombucha Drink
	0.64	0.58	0.62

With this composition of mineral salts and vitamin C, the fake kinkeliba kombucha drink is the most nutritional followed by the Lipton tea kombucha drink and lastly the lemongrass Kombucha drink.

Table 6. Contents of phenolic compounds in Kombucha drinks

	Contents of phenolic compound		
Kombucha drinks	**Total phenols in mg EAG/mL**	**Total flavonoids in µg EQ/mL**	**Tannins in mg EL/mL**
With Lipton tea	7.35	8.14	11.66
At fake kinkeliba	6.87	10.72	7.12
With lemongrass	3.89	4.69	4.23

Table 7. Sensory characteristics of the different types of Kombucha drinks

Sensory parameters	Kombucha drink			F-value	Probability
	Lemongrass	Fake kinkeliba	Lipton Tea		
Color	11.45±0.88[a]	9.80±0.91[a]	5.00±00[b]	21.12***	<0.0001
Aroma	11.10±0.70[a]	7.65±0.73[b]	5.00±00[c]	27.89***	<0.0001
Taste	8.95±0.67[a]	6.40±0.66[b]	5.00±00[b]	13.70***	<0.0001
Overall Acceptability	9.45±0.76[a]	6.30±0.68[b]	5.00±00[b]	15.18***	<0.0001

***= very highly significant; Mean values with the same letter on the same line are not significantly different at the 5% level.

4. Discussion

The main stages of our production were: infusion, canning and pasteurization. Our ratio of fake kinkeliba or lemongrass leaves to water is 1/10 on the one hand and on the other hand that of Lipton tea (packaged finished product taken from the market) compared to water is of 1/50. Which is much higher than that described by Jayabalan et al. [5] which is 1/200. The plant has the action of the heat source unlike the decoction which was made by the previous ones. So our drinks should be more concentrated in vegetable substances than the others. Our ratio of sugar to water is 1:10. While that reported by Jayabalan et al. [5] is 1/20. Our ratio of SCOBY to water is 1/10. While it is 1/42 for the previous ones. These levels allow our SCOBY to develop better and therefore to secrete more beneficial substances in our drinks.

The analysis of the microbiological quality of the drinks produced allows several observations to be made. The microbial load in Aerobic Mesophilic Germs (GAM) of the Kombucha drink with Lipton tea is lower than that of the other two drinks. This greater contamination of Kombucha drinks with lemongrass and fake kinkeliba, could highlight the environmental pollution of the living environments of the plants (fake kinkeliba and lemongrass) used in the production of these two types of drinks. This is because these plants have been harvested from their natural environments, while Lipton tea is a packaged product that has been acquired from the market. Tchekessi *et al* [31] indicated that the presence of germs is due to the fact that the environment is not controlled. The fake kinkeliba and lemongrass drinks were less contaminated overall than the Lipton tea drink, except in GAM. This would be due to the antimicrobial potential of the plants used (fake kinkeliba and lemongrass). Indeed the three plants that we used, are composed of antimicrobial substances, the study carried out by Singh *et al.* [32], on lemongrass showed that it contains flavonoids, phenolic compounds which constitute these antimicrobial elements, this plant is effective against yeasts and molds, staphylococci, streptococci, …. That carried out by Sadiq *et al.* [33] on *Cassia occidentalis*, a plant containing tannins, saponins and anthraquinones, terpenoids etc… reveals that it is antimicrobial against *salmonella, E. coli*, etc. Finally, a study by Reygaert [34] shows that green tea is composed of catechins. These compounds have antimicrobial effects against *S. aureus*, *E. coli*, etc. But the tea could not be used in our work in its natural state. The Lipton tea drink was more sensitive to heat treatments (pasteurization and canning) than the other drinks. Because the other drinks thanks to the antimicrobial effects of the plants were largely less contaminated. In the end, none of the treated drinks is contaminated with yeasts and molds, *E. coli*, ASR, *salmonella* and *Staphylococcus aureus*. It should also be noted that fermentation and the observation of good hygiene practices are also factors that make it possible to reduce the microbial load of food, and therefore have contributed to its results. The microbiological quality of these drinks is therefore acceptable. On the other hand, Canadian Food Inspection Agency [35] during research work on Kombucha drink in Canada, showed that the analyzed Kombucha drinks were free from pathogenic microorganisms.

After physicochemical and nutritional characterization, we note that the pH of all three drinks is around 2. Indeed for the tea drink, the pH is 2.15, for the fake kinkeliba drink it is 2.43 then 2.32 for the lemongrass drink. These pH values show that the drinks are acidic. This acidity of the different drinks could be explained by the fact that during their production, they were fermented. The pH found by Kayisoglu and Coskun [36], after fermentation are around 2 for most Kombucha drinks. Tchekessi *et al* [37], listed the pH values of Beninese and Nigerian beers in the range of 1.99 to 4.11. These various studies like this one have shown that fermented drinks have relatively low pH values. The titratable acidity of the fake kinkeliba drink is higher than that of lemongrass which is in turn stronger than that of Lipton tea. This reveals that the quantity of undissociated acids in the fake kinkeliba drink is greater than in the other two cases. The analysis of the ash content reveals that the fake kinkeliba drink is richer in mineral elements than the other two types of drinks. This reinforces the conclusion on the mineral elements resulting from the confrontation of the respective works of Czernicka *et al.* [38] on tea, Manikandaselvi *et al.* [39] on *Cassia occidentalis* (fake kinkeliba) and Ranade and Thiagarajan [40] on lemongrass, conclusion that the fake kinkeliba is the one that has more varieties of mineral elements. More specifically at the level of the three mineral salts sought, we note that for the drink with fake kinkeliba, the contents of which are Ca (69.57 mg/L) zinc (0.68 mg/L) and iron (3.09 mg/L) is the richest in these mineral salts than the others. Then the Ca contents in Kombucha drinks with Lipton tea and lemongrass are respectively 10 mg/L and 36.42 mg/L. This makes the Lemongrass Kombucha drink the richest in this mineral salt among the two. This is verified in the confrontation of the Ca contents of this mineral in the work [40] on lemongrass which is 39.5 mg/100g, higher than that found by Czernicka *et al.* [38], on tea for the same mineral (0.137 to 0.398 mg/100mL). As for the Zn content of Kombucha drinks, it is 0.55 mg/L for the Lipton drink and 0.07 mg/L for the lemongrass drink. Thus the Kombucha

drink with Lipton tea is the richest in this constituent. But the confrontation with the respective work on lemongrass [40], and on tea [38], shows that lemongrass is richer in Zn with a content of 121 mg/L for work on lemongrass and from 0.011 to 0.033 mg/L in the work on tea. This finding could be due to the variation in the composition of the place of production (soil, climate, etc.). From there, we note that the production environment can influence the nutritional values of the plants and, by extension, of the finished products. These explanations are also valid for the analysis of mineral contents in general found by Mousavi *et al.* [6] and Soto *et al.* [41]. As for the dry matter content, it also proves that it is the fake kinkeliba drink that is less hydrating than the other two.

The results of the vitamin C content analysis showed that the Kombucha drink with fake kinkeliba was the richest in this vitamin with an amount of 0.64 mg/L. Which probably makes this drink the richest in antioxidants to fight against free radicals. Our results are superior to those found by Loncar *et al.* [42], who demonstrated that the vitamin C content increases and varies with time and temperature but does not exceed 0.25 mg/L for a temperature of 35°C in 10 days. Because vitamin C (ascorbic acid) can be produced synthetically by fermentation.

Beverages also contain phenolic compounds such as total flavonoids, total phenols and tannins. Thus the fake kinkéliba drink is the richest in flavonoids. While that with Lipton tea is the richest in total phenols and tannins. Mousavi *et al.* [6] explained that the phenols present in Kombucha drink are compounds that can effectively scavenge free radicals of Reactive Oxygen Species (ROS), which makes Kombucha drink a powerful antioxidant.

Sensory analysis shows that the lemongrass drink is the one most accepted by the majority of tasters. Its color is appreciated almost in the same way as that of fake kinkeliba, but much more appreciated than that of tea. Its aroma is also more attractive than that of fake kinkeliba and that of tea. Its taste also seems more interesting according to the opinion of the large number of our tasters. Overall the lemongrass drink is the favorite of the majority of the tasting panel. This global acceptability of the lemongrass Kombucha drink by consumers is mainly based on the aroma of this plant.

5. Conclusion

The present study carried out on the different types of Kombucha drinks allows us to know that apart from the plant usually used in the manufacture of this drink which is tea, other plants such as fake kinkeliba and lemongrass can be used for the realization of this drink. Microbiological analysis reveals that all three beverages are of acceptable quality and that the fresh plant species are more microbicidal than the packaged Lipton tea product. From the analysis of the physicochemical and nutritional parameters, it results that the fake kinkeliba drink is the richest in nutritional values. Awareness of the population on the benefits of the Kombucha drink is therefore necessary.

Authors Contribution

TCKC and HMJB were assigned to the study, conducted the experiments and wrote the manuscript; KY, YP, KS & GJG analyzed the data; SP, BSBJ, ADCP, BYI & AC reviewed and corrected. All the authors approved the final manuscript.

Conflicts of Interest

This study does not present any conflicts of interest.

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References

[1] Wynberg, R., Access and Benefit Sharing: Key Points for Policy-Makers. The Food and Beverage Industry. Agriculture. The ABS Capacity Development Initiative: Eschborn, Germany. Technical Report, 2015. 13p.

[2] Vinci, G. and Maddaloni, L., Biogenic Amines in Alcohol-Free Beverages. Beverages, 2020. 6(1), 17. pp. 1-19.

[3] InterAct, C., et al., Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. Diabetologia. 2013. 56(7), pp.1520-1530.

[4] Rehm, J., The Risks Associated with Alcohol Use and Alcoholism. Alcohol Res Health, 2011. 34(2), pp. 135-143.

[5] Jayabalan R., et al., A Review on Kombucha Tea—Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. Comprehensive Reviews in Food Science and Food Safety, 2014. 13, pp. 538-550.

[6] Mousavi, S.M., et al., Recent Progress in Chemical Composition, Production, and Pharmaceutical Effects of Kombucha Beverage: A Complementary and Alternative Medicine. Evidence-Based Complementary and Alternative Medicine, 2020. Article ID 4397543, pp. 1-14.

[7] Mindani, I.W., et al., Health, Wellness, and Safety Aspects of the Consumption of Kombucha. Journal of chemistry, 2015. 11, pp. 1-11.

[8] Kumar, V., et al., Tea Processing. Beverages Processing & Technology, cha 2018. ISBN: 978-93-87869-36-3, chapter, 10, 192-209p.

[9] Mahomoodally, F., Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-based Complementary and Alternative Medicine 2013. Article ID 617459, pp. 1-14.

[10] Kouame, N. M., et al., Cymbopogon citratus (DC.) Stapf: ethnopharmacology, phytochemical, pharmacological activities and toxicology. Phytothérapie, 2015. Phytotherapie 14(6), pp. 1-9.

[11] Yadav, J.P., et al., Cassia occidentalis L.: a review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 2010. 81(4), pp. 223-230.

[12] Hadrya, F., et al., Evaluation of the microbiological quality of dairy products marketed in the region of Rabat-Sale-Zemmour-Zaer in Morocco. Nutr. Diet Notebooks, 2012. 47, pp. 303-307.

[13] ISO, 4833-1, Microbiology of the Food Chain — Horizontal Method for the Enumeration of Microorganisms — Part 1: Colony Count at 30°C by the Pour Plate Technique. 2013. [https://www.iso.org/standard/53728.html]

[14] NF V08-060, Microbiology of food and animal feeding stuffs-Enumeration of thermotolerant coliforms by colony-count technique at 44°C. 2009. [https://www.boutique.afnor.org/en-gb/standard/nf-v08060/microbiology-of-food-and-animal-feeding-stuffs-enumeration-of-

thermotoleran/fa160465/33001#AreasStoreProductsSummaryView].

[15] ISO 6888-1, Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Method using Baird-Parker agar medium. 2021. [https://www.iso.org/standard/76672.html].

[16] ISO 16649-2, Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive Escherichia coli-Part 2: Colony-Count Technique at 44 Degrees C using 5-Bromo-4-Chloro-3-Indolyl Beta-D-Glucuronide. International Standards Organization, Geneva, Switzerland. 2001.

[17] ISO 15213, International Organization for Standardization, Geneva, Switzerland. Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. 2003.

[18] ISO 6579-1, Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp. 2017. [https://www.iso.org/standard/56712.html]

[19] ISO 21527-1, International Organization for Standardization, Geneva, Switzerland. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of yeasts and moulds-Part 1: Colony count technique in products with water activity greater than 0,95. 2008.

[20] ISO 7218, Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations. 2007.

[21] AOAC., Official methods of analysis. 16th edition. Association of Official Analytical Chemists, Washington DC. 1999.

[22] Ball, G.F.M. Vitamins in foods: analysis, bioavailability, and stability. Taylor & Francis: Boca Raton, FL, 2006. Vol. 156. [https://www.alcpo.org.ly/wp-content/uploads/2017/06/Ball_Vitamins-in-Foods-Analysis-Bioavailability-and-Stability.pdf].

[23] Lupoae P., et al., Phytochemical Screening: Antioxidant and Antibacterial Properties of Potamogeton Species in Order to Obtain Valuable Feed Additives. Journal of Oleo Science, 2015. pp. 1-13.

[24] Koudoro, Y.A., et al., Phenolic profiles, antioxidant, antiquorum sensing, antibiofilm and enzyme inhibitory activities of selected Acacia species collected from Benin. Food Science and Technology, 2022. 171, pp. 114-162.

[25] Singleton, V.L., et al., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol, 1999. 299, pp. 152-178.

[26] Dieng, M.I.S., et al., Evaluation of the antioxidant activity of hydro-ethanolic leaf and bark extracts of Piliostigma thonningii Schumach. Int. J. Biol. Chem. Sci, 2017. 11(2), pp. 768-776.

[27] Djeridane, Y., et al., Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem, 2006. 97, pp. 654-660.

[28] Heimler, D., et al., Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chemistry, 2006. 99, pp. 464-469.

[29] Larmond, E., Laboratory Methods for Sensory Evaluation of Foods. 1st Edn., Department of Agriculture Publication, Otawa, Canada, 1977. ISBN-13: 978-0662012719, 73p.

[30] Tchekessi, C.K.C., et al., Physico-chemical and sensory characterizations of three types of "dèguè", a local fermented drink made from milk in Benin. Int. J. Biosci., 2014. 5: 36-43.

[31] Tchekessi, C. K. C., et al., Microbiological, Nutritional and Sensory Characteristics of the Foodstuff “Foura” Produced From Millet in Benin. Pakistan Journal of Nutrition, 2021. 20 (4), pp.127-134.

[32] Singh, B. R., et al., Antimicrobial activity of lemongrass (Cymbopogon citratus) oil against microbes of environmental, clinical and food origin. International Research of Pharmacy and Pharmacology, 2011. 1(9), pp. 228-236.

[33] Sadiq, I. S., et al., Phytochemistry and Antimicrobial Activities of Cassia Occidentalis Used for Herbal Remedies. Journal of chemical engineering, 2012. 1(1), 38-41.

[34] Reygaert, W., The antimicrobial possibilities of green tea. Frontiers in Microbiology, 2014. 5(434), pp. 1-8.

[35] Canadian Food Inspection Agency, Bacterial Pathogens in Fermented Tea (Kombucha)-April 1, 2018 to March 31, 2019; Food microbiology -Targeted surveys – Final report. 2019. [https://inspection.canada.ca/food-safety-for-industry/food-chemistry-and-microbiology/food-safety-testing-bulletin-and-reports/bacterial-pathogens-in-fermented-tea/eng/1577143157877/1577143158283].

[36] Kayisoglu, S. and Coskun, F., Determination of physical and chemical properties of kombucha teas prepared with different herbal teas. Food Science and Technology, 2020. 41 (1), pp. 393-397.

[37] Tchekessi, C. K. C., et al., Health and Nutritional Qualities of Drinks Produced in the Beninese Brewery Company and Those Sold on the Benin Market. Asian Food Science Journal, 2021. 20(1), pp. 40-50.

[38] Czernicka, M., et al., Study of nutritional value of dried tea leaves and infusions of black, green and white teas from Chinese plantations. Roczniki Państwowego Zakładu Higieny, 2017. 68(3), pp. 237-245.

[39] Manikandaselvi, S., et al., Studies on physicochemical and nutritional properties of aerial parts of Cassia occidentalis L. Journal of food and drug analysis 2016. 24 (2016), pp. 508-515.

[40] Ranade, S.S. and Thiagarajan, P., Lemon grass. International Journal of Pharmaceutical Sciences Review and Research, 2015. 35(2), pp. 162-167.

[41] Soto, S.A.V., et al., Understanding Kombucha Tea Fermentation: A Review. Journal of Food Science, Wiley, 2018. 83 (3), pp.580-588.

[42] Loncar, E., et al., Influence of working conditions upon Kombucha conducted fermentation of black tea. Food and Bioproducts Processing, 2006. 84(C3), pp 186-192.