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Risks Related to the Consumption a Traditional Drink (*Koutoukou*) Obtained by Distillation of Palm Wine or Sucrose

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Abstract *Koutoukou* (KTK) is one of the most consumed alcoholic drinks in Côte d'Ivoire. This artisanal liquor is made in inadequate hygienic conditions with undefined alcohol levels. Thus the abuse of *Koutoukou* is associated with a range of social problems, physical and mental disorders, including depression and anxiety, obesity and the risk of accidents. this traditional alcoholic beverage represents a potential danger for the consumer. This study was to determine the characteristics of *Koutoukou* and the discomfort associated with its consumption. Thus a survey and analysis of the biochemical and microbiological characteristics of this traditional drink was carried out. Results showed that 92% of respondents were consumers of *Koutoukou*. However, 28.57% were hospitalized after consuming *Koutoukou*. The most common symptoms are headaches and vomiting. According to the biochemical analysis, *Koutoukou* produced with palm wine is the most acidic with a concentration of 0.16 ± 0.09 % for a pH of 3.90 ± 0.47. However, the soluble sugar (10.58 ± 0.60 ° Brix) and ethanol (51.36 ± 2.7 %) contents are higher in *Koutoukou* produced from sugar water and yeast. Hydroquinone was detected in *Koutoukou* produced with palm wine at a low concentration of 0.07 ± 0.01%. In addition, the presence of mesophilic aerobic, especially *staphylococci* and *Bacillus* was observed in all the beverages analyzed. In view of the problems associated with the consumption of this liquor, a national information campaign on the consumption of *Koutoukou* would be beneficial.

Keywords: *discomfort, Koutoukou, traditional liquor, biochemical, microbiological*

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1. Introduction

In West Africa, particularly in Côte d'Ivoire, the introduction of highly alcoholic beverages dates back to the slave trade and the beginning of the colonial period when alcohol was used to conclude alliances and territorial agreements with the customary chiefs [1]. Prior to this period, the populations had only low-alcohol fermented drinks, the most common of which were palm wines and beers of corn, millet and sorghum [2]. As for local brandies, called *Koutoukou* (KTK), it was only late that they were produced in Côte d'Ivoire, thanks to the introduction of the still from neighboring Ghana around 1940 [3]. It is obtained by distillation of musts from fermented products such as palm wine, sugar cane juice or sugar water with added yeast [4]. Thus, this distillation has long remained clandestine and considered a secondary activity to agriculture, especially for young farmers. However, the production of this drink has been sustained by the increase in oil palm plantations [5]. According to [6], the use of artisanal brandies obtained from palm wine, sugar cane juice and sugar water with yeast added, has also become permanent on the occasion of ancestor worship ceremonies, funerals and weddings. According to [3], *Koutoukou* is one of the most widely consumed alcoholic beverages in Côte d'Ivoire because of its low cost, especially after the devaluation of the CFA franc in January 1994. Since then, the impoverishment of the social strata has favored the proliferation of this artisanal liquor and its substitution for industrial alcoholic beverages whose selling price is constantly increasing. However, the production and marketing of the *Koutoukou* is beyond the control of the state. Indeed, several types and forms of *Koutoukou* are found on the market (*Koutoukou* + root, *Koutoukou* + bark) whose biochemical and microbiological characteristics of the additives are not

determined. Moreover, the production of Koutoukou is carried out under inadequate hygienic conditions with undefined alcohol levels. Thus made the biochemical and microbiological qualities of the finished product could be affected. Abuse of *Koutoukou* is associated with a range of social problems, physical and mental disorders, including depression and anxiety, obesity, and the risk of accidents [7]. Since the lifting of the ban on this drink by the Ivorian State in 1999, work has been carried out by several authors. [8] conducted a study on the impact of chronic short-term consumption of *Koutoukou* on certain hematological and biochemical parameters. The comparative effect of acute drinking of *Koutoukou* palm wine and pastis 45 on memorization in humans was carried out by [9]. Similarly, the work of [10] focused on the evolution of the consumption of alcoholic beverages sold in Côte d'Ivoire. Despite these different works, none of them has yet led to the stabilization and normalization of *Koutoukou*. Thus, the general objective of this work is to determine the characteristics and discomforts related to the consumption of *Koutoukou* in order to ensure consumer safety.

2. Material and Methods

2.1. Participants, Study Design, and Sampling

Two types of *Koutoukou* were used in this study: *Koutoukou* produced from sugar water and yeast, and *Koutoukou* produced with palm wine.These two types of drinks have been at *Koutoukou* saleswomen in the commune of yopougon (Abidjan, Côte d'Ivoire).

2.2. Survey Form

A survey form was developed and used to conduct surveys on the consumption of *Koutoukou* in the city of Abidjan, particularly in the commune of Yopougon (District where this drink is more widely consumed in Côte d'Ivoire).

2.3. Execution of the Survey (Consumer Survey Sheet)

The sampling method adopted for this work is three-stage cluster sampling with the survey site (the commune of Yopougon) at the primary level, the sales sites at the secondary level, and the respondents at the tertiary level. The survey was conducted from August to October 2019. The commune of Yopougon was divided into 20 sites, and with 5 people per site, a total of 100 peoples were interviewed. The questionnaire was explained chapter by chapter to respondents. In the sites where the respondents could not read and write, a person from the site who could read and write and who spoke the same ethnic group as the respondents was asked to be an interpreter. The questions were either multiple-choice questions with 2 to 6 suggested answers or questions with yes or no answers. The questionnaire is composed of 3 parts. The first part focused on the level of knowledge of the *Koutoukou*, the second part on the profile of consumers and the last part on the discomforts related to the consumption of the *Koutoukou*.

2.4. Sampling

The various samples from *Koutoukou* were collected in the commune of Yopougon. In this commune six (6) sellers were chosen because of their availability in the marketing of *Koutoukou*, including three in the marketing of *Koutoukou* produced from sweet water and yeast and three for *Koutoukou* produced with palm wine. For one passage, three 80 mL samples were taken per vendor (one sample from the control, one sample from *Koutoukou* mixed with roots and one sample from *Koutoukou* mixed with bark) and packaged in sterile 100 mL bottles, kept in a cooler. containing carboglaces at a temperature of 4°C and sent to the Laboratory of Microbiology and Food Biotechnology of the University of Nangui Abrogoua (UNA). A total of three (3) passages were performed at each vendor and fifty-four (54) samples were taken under the same conditions to be analyzed in the laboratory.

2.5. Biochemical Analysis

2.5.1. Determination of pH and Total Titratable Acidity

The pH of *Koutoukou* (KTK) samples was determined directly using a pH-meter (pH-meter P 107, CONSORT, Bioblock, France) after calibration with standard buffers. Total titratable acidity was determined by titrating samples against 0.1 M NaOH using 1% phenolphthalein as indicator as described by [11] and expressed as percentage of lactic acid**.**

2.5.2. Measurement of the Refractometric Dry Extract

The Refractometric Dry Extract was determined by [12]. A drop of *Koutoukou* was deposited on the glass of the Model ATAGO POCKET REFRACTOMETER to evaluate the amount of suspended solids. The value of the Dry Extract Refractometer was read with light, at the level of the eyepiece of the apparatus.

2.5.3. Organic Acids and Ethanol Determination

Samples were centrifuged at 4000 rpm for 20 min and supernatants were filtered through a 0.20 mm Millipore membrane filter (Sartorius AG, Goëttingen, Germany) and then stored at -20°C until analysis. Organic acid were determined by High-Performance Liquid Chromatography (HPLC) as previously described by [13]. Analyses were carried out with an ion-exclusion ORH-801 column (300 × 6.5 mm) (Interchrom, France) preceded by a Universal Guard Cartritge-Holder column. The High-Performance Liquid Chromatograph system (LC-6A, Shimadzu Corporation, Japan) was equipped of a Shimadzu LC-6A pump. Column effluents were monitored by a UV detector (SPD-6A, Shimadzu Corporation, Japan) set at 210 nm. The mobile phase (0.004 N H2SO4) used at a low rate of 0.8 ml/min was filtered through a 0.45 mm Millipore membrane filter (Sartorius AG, Goëttingen, Germany). A 20 µl injection volume was used for HPLC samples and the analyses were done in duplicate.The organic acids standards were dissolved in distilled water at concentrations ranging to 0.05 - 0.4 g/l, filtered and injected as the samples. Organic acids were identified and quantified by comparison of

their retention times and peak areas with those of standards. Ethanol content was determined by gas chromatographic analyses, carried out using a Shimadzu CG-14A gas chromatograph. Filtered samples (2 µl) were directly injected into the system. The temperature was set at 8°C/min. Injector and detector temperature were 200°C and 250°C, respectively. Helium at 2 kg/cm^2 was used as a carrier gas and the external standard method was used for the quantitative determination of ethanol.

2.6. Enumeration of Micro-organisms

The *Koutoukou* samples were shaken by hand in the stomacher bag and tenfold serial dilutions were prepared and spread-plated for determination of micro-organism counts. After dilutions, enumeration of total aerobic mesophile was carried out using plates of Plate Count Agar (PCA, Difco 0479-17-3; Difco Laboratories, Detroit, MI, USA) which were incubated at 30 °C for 2 days. The research and counting of *Staphylococcus aureus* was done on Baird Parker agar after one (1) day incubation at 30°C using [14] method. *Bacili* species were enumerated on plates Mossel agar (AES Laboratoire, COMBOURG France) after incubation at 30°C for 2 days. Sulfito-reductor bacteria were enumerated using tubes of Trypton Sulfite Neomycin agar (Biorad, Marnes-La-Coquette, France) at 37°C for 24-48 h.

2.7. Statistical Analysis

Software R. 3.01, ANOVA method with Duncan's post-hoc test, significance level 5% was used. This software was used to calculate the means and standard deviations of biochemical and microbiological parameters. It was also used to compare the means of the sample parameters and to determine whether the differences observed in the means of the biochemical and microbiological parameters were significant at the 5% significance level. The survey data were processed using IBM SPSS software (statistics 20).

3. Results and Discussion

The survey on *Koutoukou* (KTK) consumption has set itself the objective not only to know the preference of consumers for the different types and forms of *Koutoukou* (KTK) but also to evaluate the biochemical and microbiological qualities of these different types and forms of *Koutoukou* in the commune of Yopougon. In this regard, this study revealed that the proportion of subjects consuming *Koutoukou* was 92% (Figure 1).

These results are higher than those obtained by [9] which was 52.5%. This high consumption of *Koutoukou* is thought to be due to the low cost and accessibility of this artisanal liquor compared to industrial alcoholic beverages. The majority (73.9%) of these consumers drink at least one to five glasses a day, mostly in the evenings (22.8%) and mornings (19.6%). The proportion of women (10.9%) who usually drink alcohol is lower than that of men (89.1%) because of women's greater susceptibility to the harmful effects of alcohol [15]. However, it should be noted that regular consumption of *Koutoukou* is higher among single (77.2%) than married (22.8%) women (Figure 2).

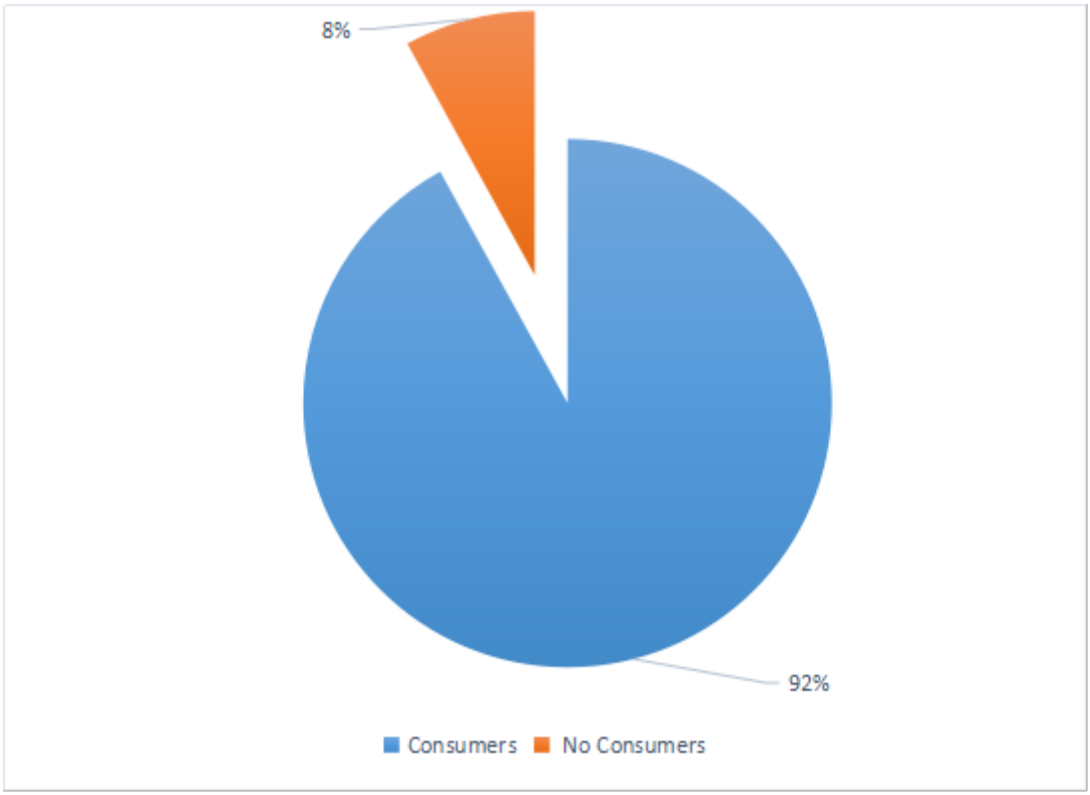

Figure 1. Consumption of Koutoukou

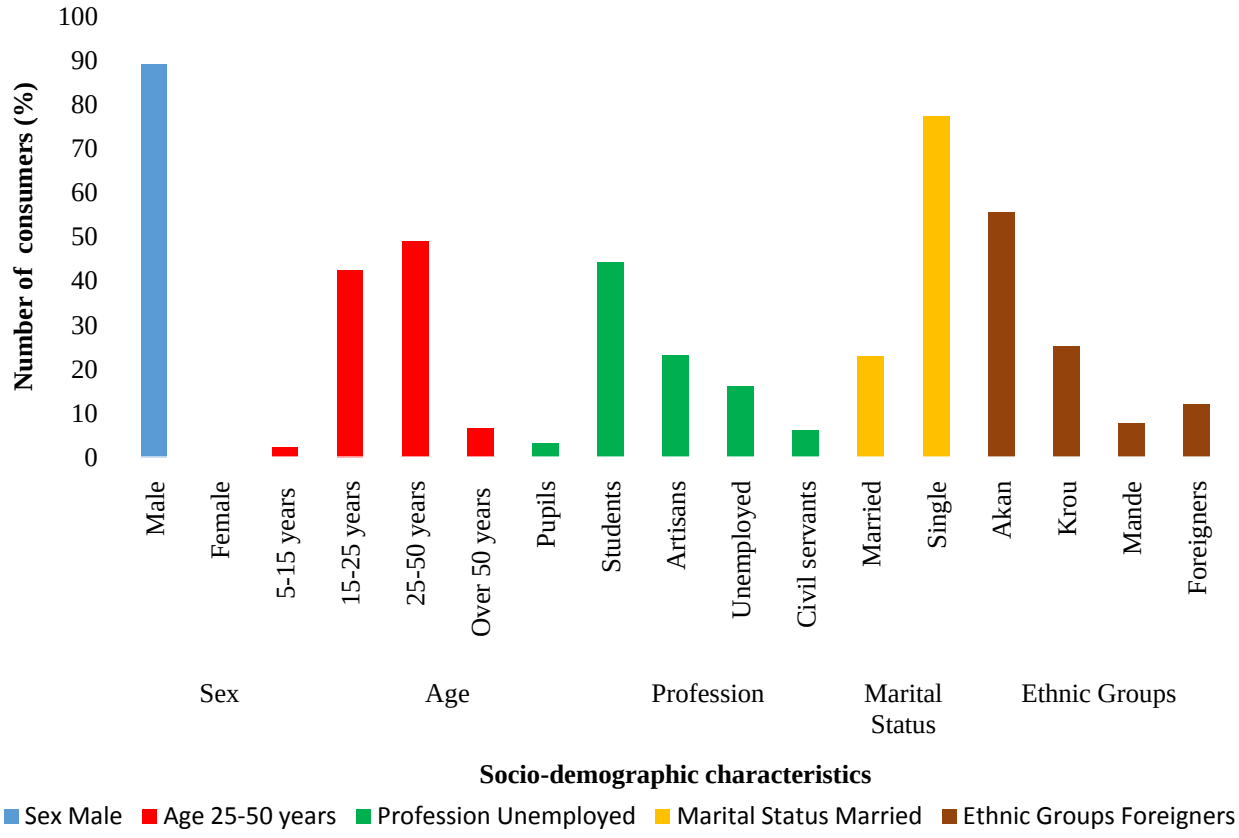

Figure 2. Socio-demographic distribution of consumers

In addition, the position of students in this consumption of *Koutoukou* is consistent with what is usually observed in Côte d'Ivoire [16]. Students (47.8%) consume more than artisans (25%), the unemployed (17.4%), civil servants (6.5%) and students (3.3%). Both students and single people with no family responsibilities do not hesitate to use the financial means available for alcohol consumption. Thus, the age group between 25 and 50 years, with 48.9% of declared drinkers, constitutes the most excessive drinkers in the *Koutoukou*. It is followed by the 15-25 age group, with 42.4% (Figure 2). These proportions are in line with the results obtained by [17] in the United States concerning the consumption of alcoholic beverages. Excessive consumption of *Koutoukou* by young people is thought to be due to the availability of *Koutoukou* at a more moderate price compared with industrial alcoholic beverages As well, adolescents aged 5 to 15 years made up the smallest proportion of users. However, this age group is of particular concern because of their greater vulnerability to the adverse effects of alcohol at the body level [18]. In addition, this may be at the root of problems in the country such as violence and exposure to new substances such as cigarettes and illicit drugs. However, 23.9 % of users experienced at least one discomfort following Koutoukou consumption. The most common symptoms encountered are headaches, vomiting, stomach aches, and fever. The duration of these symptoms was estimated to last from 1 to more than 3 days. Most of these symptoms lasted one day in 66.66 % of users, but (6) or 28.57 % of patients were hospitalized (Table 1).

These discomforts would be due to the fact that the production of *Koutoukou* is artisanal and is done in deplorable hygienic conditions. In addition, its production is still based on the use of artisanal materials and the lack of training of producers, which results in high toxicity of the product that can lead to the death of consumers [19]. However, consumers (10 peoples) of *Koutoukou* produced with palm wine are the most exposed to headaches, while consumers (7 peoples) of *Koutoukou* produced from sugar water and yeast are more exposed to vomiting and headaches (Table 2).

Table 1. Discomfort after the consumption of the *Koutoukou*

	Number of consumers	Frequency (%)
Discomfort (n= 92)		
Yes	22	23.9
No	70	76.1
Symptoms (n=92)		
Fever	2	2.2
Vomiting	7	7.6
Headache	8	8.7
Stomach pain	2	2.2
Others	2	2.2
None	71	77.2
Duration (n=21)		
1 day	14	66.66
2 days	5	23.80
3 days	1	4.76
More than 3 days	1	4.76
Hospitalization (n=21)		
Hospitalized	6	28.57
No hospitalized	15	71.42

Table 2. Symptoms of Koutoukou (KTK) post-consumption illness

Consumers of KTK	Symptoms						Total
	Fever	Vomitings	Headaches	Stomach aches	Others	Vomiting + Stomach aches	
KTK made with palm wine	1	1	5	0	2	1	**10**
KTK made with sugar + yeast	1	3	3	0	0	0	**7**
KTK manufactured with other substrates	0	2	0	0	1	0	**3**
KTK made with palm wine and KTK made with sugar + yeast	0	1	0	1	0	0	**2**

KTK: Koutoukou.

In fact, the contamination of *Koutoukou* produced with palm wine could be explained by human or animal fecal contamination at the farm site, as well as by the presence of insects attracted by the sugars contained in the palm wine, as observed by [20]. In addition, the presence of these symptoms could be evidence of poor hygienic conditions during the collection of palm wine. Microorganisms may be contaminants from the untreated container that is immediately used for the exploitation of palm wine by the harvesters [21]. These practices can affect the finished product and cause problems for consumers. On the other hand, discomfort related to the consumption of *Koutoukou* produced from sugar water and yeast is due to the quality of the water used for fermentation, which could be contaminated.. In addition to the survey, biochemical analyses were carried out on both types of *Koutoukou*. From this analysis it appears that the *Koutoukou* produced with palm wine is the most acidic (0.16±0.09%) with a pH of 3.90 ± 0.47 (Table 3). These results are consistent with those of [22], who obtained a titratable acidity content between 0.10 and 0.13% on palm wine distillate in Dogbo, a locality located in Benin. This acidity would be attributed to the lactic acid bacteria present in the palm wine which play the role of rapid acidification during fermentation [23]. The low level of titratable acidity (0.04 ± 0.01%) obtained in *Koutoukou* produced from sugar water and yeast is due to the fact that the raw material of this beverage was sugar and its fermentation was ensured solely by a yeast (*Saccharomyces*) that produces ethanol and CO2 during alcoholic fermentation [24]. As a result, the levels of ethanol (51.36 ± 2.7%) and soluble sugars (10.58 ± 0.6 °Brix) obtained in the *Koutoukou* produced from sugar water and yeast are much higher than those obtained in the *Koutoukou* produced from palm wine with (17.68 ± 3.1%) and (8.82 ± 1.05 °Brix) respectively (Table 3). In fact, alcoholic fermentation is carried out by yeasts during which sugars (mainly glucose) are transformed into alcohol (ethanol) [24]. Indeed, the variation in the alcohol content of these beverages is related to the raw material, the type of distillate (first, second or third distillate) or to the dilution in water. These artisanal "*Koutoukou*" products are produced without any measuring and control equipment for the final product [4]. Concerning organic acids, two were detected in the two types of beverages analyzed. These were coumarin and caffeic acid. In addition to these two organic acids, hydroquinone was also present in *Koutoukou* produced with palm wine at a low concentration of 0.07 ± 0.01% (Table 3).

Table 3. Biochemical parameters of *Koutoukou* samples

Biochemical parameters	Beverages	
	KTK made with sugar + yeast	KTK made with palm wine
pH	4.68±0.29^{a}	3.90±0.47^{b}
Titratable acidity (%)	0.04±0.01^{b}	0.16±0.11^{a}
Ethanol (%)	51.36 ± 2.7^{a}	17.68 ± 3.31^{b}
Refractometric dry extract (° Brix)	10.58±0.60^{a}	8.82±1.05^{b}
Gallic acid (%)	nd	nd
Catechin (%)	nd	nd
Quercetin (%)	nd	nd
Coumarin (%)	0.9 ± 0.06^{a}	0.2 ± 0.03^{b}
Flavanone (%)	nd	nd
Hydroquinone (%)	nd	0.07 ± 0.02
Na Cinnamate (%)	nd	nd
Caffeic acid (%)	0.7 ± 0.02^{a}	0.1 ± 0.02^{b}
Tannin-ol (%)	nd	nd
Tannin H2O (%)	nd	nd

In the same line, mean values followed by different alphabetical letters are statistically different (P<0.05) Duncan multiple t-test. **nd**: no detected, **KTK:** *Koutoukou.*

Table 4. Microbial load in beverages

Beverages		Microbial load (CFU / mL)			
		AM	*Staphylococcus*	*Bacillus*	*Clostridium*
KTK made with sugar + yeast	**Control**	(6.1 ± 0.6)×10^{1a}	(0.5 ± 0.06)×10^{1a}	< 1^{a}	nd
	Roots	(1.1± 0.8)×10^{2c}	(0.9 ± 0.1)×10^{1b}	(0.6 ± 0.2)×10^{1b}	nd
	Barke	(2.5 ± 0.5)×10^{2e}	(0.8 ± 0.2)×10^{1b}	(0.7 ± 0.06)×10^{1b}	< 1^{a}
KTK made with palm wine	**Control**	(9.8 ± 3.7)×10^{1b}	(0.6 ± 0.4)×10^{1a}	(0.1 ± 0.08)×10^{1c}	nd
	Roots	(1.3 ± 0.5)×10^{2c}	(1 ± 0.1)×10^{1b}	(0.8 ± 0.3)×10^{1b}	< 1^{a}
	Bark	(1.7 ± 0.8)×10^{2d}	(0.7 ± 0.2)×10^{1ab}	(0.2 ± 0.1)×10^{1c}	< 1^{a}

In the same column the mean values followed by the alphabetical letters are statistically different (P<0.05) Duncan multiple t-test.,
KTK : *Koutoukou* , **AM :** mesophilic aerobic; **nd :** no detected.

Indeed, during the preparation of *Koutoukou*, a condensation reaction occurs. In this reaction, activated phenols and ethyl acetoacetate yield polyphenols such as coumarins, hydroquinone, caffeic acid and by-products such as ethanol and water etc [25,26]. These polyphenols could have beneficial effects on the health of the consumer. Indeed, their role as natural antioxidants would arouse more and more interest in the prevention and treatment of cancer, inflammatory, cardiovascular and neurodegenerative diseases [25] especially Coumarin. *Koutoukou* produced from sugar water and yeast contains more coumarin (0.9 ± 0.04%) than *Koutoukou* produced with palm wine (0.2 ± 0.01%) (Table 3). It could be said that consumption of *Koutoukou* produced from sugar water and yeast would have a beneficial effect on the health of the consumer. However, at high doses, phenolic derivatives with anticoagulant properties would be toxic by ingestion [27] and could decree cases of acute cytolytic hepatitis [28]. Also these acids heated to decomposition can give off toxic fumes of carbon monoxide and dioxide, especially hydroquinone. Thus its consumption could lead to nervous disorders, eye damage, skin allergies, chest pain, cough, headaches, dizziness, salivation, convulsions, nausea and vomiting [29]. *Koutoukou* produced with palm wine contains a low concentration of 0.07±0.01% hydroquinone. In addition, mixing simple *Koutoukou* with either roots or bark could alter the microbiological quality of these *Koutoukou*. Thus, as these beverages were distilled, it was expected that these liquors would be free of microbial contamination as recommended by the WHO [30]. However, there are several types of *Koutoukou* (KTK) depending on the substances (roots, stems, bark, and other ingredients) added to the *Koutoukou*. These substances are immersed in the drink and are involved in the treatment of several diseases such as malaria, fever, headaches, stomach aches, hemorrhoids, etc. [6]. It should be noted that *Koutoukou* added to roots, stems, bark, and other ingredients could be a medium for the development of microorganisms. Thus, the presence of mesophilic aerobic (AM) including staphylococci and *Bacillus* was observed in all the beverages analyzed. *Koutoukou* mixed with bark is most contaminated with mesophilic aerobic loads of (2.5± 0.5). $\times 10^2$ CFU/mL in *Koutoukou* produced from sugar water and yeast and (1.7± 0.8) $\times 10^2$ CFU/mL in *Koutoukou* produced with palm wine (Table 4).

The contamination of the *Koutoukou* by these microorganisms after distillation would be due to the fact that these microorganisms would be found in the drums or bottles used to collect the drinks, either on the roots and bark used to mix the drinks, on the hands of the staff, in the water used to dilute the pre-sales drinks and especially in the production environment. The presence of *Bacillus* and staphylococci in these beverages poses a risk to the consumer. In addition, these microorganisms are producers of heat-resistant toxins and once ingested by humans they can cause gastroenterics, vomiting and often death [31].

4. Conclusion

This study on the consumption of *Koutoukou* in Yopougon (Abidjan) initially revealed that this alcoholic drink is widely consumed (92% of the respondents). It is mainly consumed by single men aged between 25 and over 50 years. In addition, some consumers claim to be suffering from discomfort such as headaches, vomiting, stomach aches and fever. Secondly, biochemical analysis of the drinks showed that *Koutoukou* produced with palm wine is more acidic and contains less ethanol than that produced from sugar water and yeast. At the microbiological level, both types of *Koutoukou* do not comply with WHO recommendations that alcoholic beverages should be free of microorganisms.

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Conflict of Interest

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Authors Contribution

All authors have contributed equally during the manuscript preparation.

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Consumer survey sheet

A survey form was developed and used to conduct surveys on the consumption of *Koutoukou* in the city of Abidjan, particularly in the commune of Yopougon (District where this drink is more widely consumed in Côte d'Ivoire). The questionnaire is composed of 3 parts. The first part focused on the level of knowledge of the *Koutoukou*, the second part on the profile of consumers and the last part on the discomforts related to the consumption of the Koutoukou

1-Sex : 1-M ☐ 2-F ☐

2- How old are you?
1. 5-15 years ☐ 2. 15-25 years ☐ 3. 25-50 years ☐ 4. 50 years and over ☐

3-Level of study: 1. None ☐ 2. Primary ☐ 3. Secondary ☐ 4. Higher ☐

4-Profession: 1. Pupil ☐ 2. Student ☐ 3. Civil servant ☐ 4. Artisans ☐
5. Unemployed ☐ 6. Other (to be specified) ☐

5-Maritial status : 1. Married ☐ 2. Single ☐

6-Ethnic group : 1. Akan ☐ 2. Krou ☐ 3. Mande ☐ 4. Foreigner. ☐

7-Do you know the *Koutoukou*? 1. Yes ☐ 2. No ☐

8-Do you consume *Koutoukou*? 1. Yes ☐ 2. No ☐

9-If yes, what type of Koutoukou do you like to consume ?
1. Palm wine ☐ 2. With sugar ☐ 3. Other… ☐

10-Have you ever had any discomfort (an infection) after consuming Koutoukou?
1. Yes ☐ 2. No ☐

11-If so, what were the symptoms?
1. Fever ☐ 2. Vomiting ☐ 3. Headache ☐
4. Stomach pain ☐ 5. Other (specify) ☐

12- How long did the discomfort last?
1. 1 Day ☐ 2. 2Days ☐ 3. 3 Days ☐ 4. Other ☐

13-Did the discomfort require hospitalization? 1. Yes ☐ 2. No ☐