Original Research Article

Assessment of health risks linked to consumption of Bissap, a nonalcoholic drink produced in an artisanal manner sold in the streets of the town Daloa (Côted'Ivoire)

ABSTRACT

Ready-to-eat Bissap juice is often classified in the street food group. The unhygienic conditions in which Bissap juice is prepared pose risks, sometimes leading to microbial contamination. The general objective of this work is to evaluate the diversity of pathogenic microorganisms in Bissap juices sold in the city of Daloa. To achieve this objective, a consumption survey was carried out. Counts and isolations were carried out to assess the microbiological quality of juices sold in the streets. Physicochemical parameters were determined. These are Brix and pH. *Salmonella* was screened for in the juices sold using the NF-V08-52 standard. The survey revealed that women consume more *Bissap* juice sold in the streets than men with a rate of 52% respectively compared to a rate of 48%. The Mandé represent half of the consumers with 50%, followed by the Akan with a rate of 30%, the Krou with a rate of 14.70%. Young people aged between 20 and 30 consume more Bissap juice with a rate of 47.30%. Consumers of Bissap juice come from various professions. Consumers have experienced discomfort characterized by diarrhea, vomiting, fever and stomach bloating. Microbiological analysis revealed the presence of microbial flora of alteration and contamination. These are total flora, fungal flora and enterobacteria. There was also the presence of flora of fecal origin such as fecal coliforms. The Bissap juice samples analyzed contain potentially pathogenic bacterial species in particular *Staphylococcus aureus* and*Bacillus cereus*.The samples analyzed overall are compliant for fecal streptococci,*Escherichia coli andClostridium perfringens*.As for Mesophilic Aerobic Germs, fungal flora, enterobacteria, fecal coliforms, *Bacillus cereus*the results are unsatisfactory. The pH of the different juices is very acidic, ranging from 1.59±0.46 to 2.45±0.65. Also some of the juice samples analyzed have very high sugar levels (34.67 ± 2.52). Bissap juices sold in the streets represent a danger for the population of Daloa.

Keywords: Bissap, contamination, microbial, fungal, fecal streptococci

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Same is true for Bacillus

1. INTRODUCTION

Rapid urbanization and multiple constraints including the distance between workplaces and homes, poverty, the development of women's activity, the breakdown of family solidarity and the appearance of new food styles have made street food essential in major African cities. These foods are defined as ready-to-eat foods and beverages prepared and/or sold by street or stationary vendors [1]. The consumption of these street drinks has grown considerably in all the major cities and towns of the West African sub-region [2] [3]. On the streets of the town of Daloa, refreshing drinks of local production are commonly encountered. It's about "Bissap".The beverage "Bissap» is made from the flowers of*Hibiscus sabdariffa*. This refreshing drink can be consumed alone or with family and even during ceremonies such as weddings or baptisms [2].

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However, during processing and artisanal manufacturing processes, this drink can be contaminated by various microorganisms including pathogens. Indeed, the preparation equipment and the lack of application of the rules of good production hygiene practices can negatively influence the microbiological quality of this drink. The consequence of these uncontrolled productions often generates health problems, sometimes with economic effects that are difficult to manage [4] [5]. In addition, street foods are frequently associated with infectious diseases and several microbiological ~~analyzes~~ have revealed the presence of numerous pathogenic microorganisms with loads exceeding standards [6][7] [8]. Moreover, several disease outbreaks have been attributed to this drink in various places around the world and certain infections have been reported in populations consuming these street foods [9] [10]**.**However, it is necessary to guarantee the health of populations and promote the quality of such products given the multiple issues linked to local production and consumption.

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Although known to the populations of Daloa, there is little information on the artisanal soft drinks sold in the streets and the health risks linked to their consumption, more specifically on theBissap. The theme of this study is therefore: “Study of microbiological contamination and assessment of health risks linked to the consumption of a nonalcoholic drink: theBissapcraft sold in the streets of Daloa. The general objective of this study is to study the microbial contamination ofBissapof the city of Daloa and to assess the health risks linked to its consumption.

2. MATERIAL AND METHODS

2.1. Collection of data

To carry out the survey, a consumption survey sheet dedicated to juice consumersBissapwas conceived. The population surveyed is made up of passers-by encountered in the main streets and secondary streets of the abattoir 2 (Adja mosque) and University of Daloa and bus station districts, and volunteering to answer the questionnaire. In total, 150 people were interviewed. The survey consisted of a direct interview with the volunteers. This interview focused, among other things, on the socio-demographic profile (age, level of education, etc.) of the consumer, their knowledge of juiceBissap, its mode and frequency of consumption of this non-alcoholic drink and the appearance of possible symptoms of food poisoning (vomiting, diarrhea, etc.) linked to its consumption.

2.2 .Sampling

Non-alcoholic drinks were sampled at the different sites selected for the study. A total of 30 juice samples from *Bissap*of 10 ml each was taken. Juice samples from *Bissap*were collected at six sites. These are three sites in the Abattoir district, two sites in the shopping district and one site in the Tazibouo district. Approximately five samples were collected per purchase per site. Once collected, the samples were carefully labeled with an individual identification number and transported in coolers containing ice packs to the laboratory for analysis.

2.3. Microbiological analyzes of collected samples

The NF EN ISO 6887-1, 2017 standard was used as a reference for producing the stock suspension and decimal dilutions. To do this, a quantity of 25 grams of each sample ~~is~~ waseighed using a balance then added to a sterile stomacher paper containing 225 ml of Buffered Peptone Water. The mixture obtained was homogenized for 1 minute to obtain the mother suspension which was left to stand for 30 min at laboratory temperature for revivification of the microorganisms.

2.4. Culturing of microorganisms

- During work, seeding took into account the different flora microbial alterations such as fungal flora, mesophilic flora and coliforms. To do this, one milliliter of each dilution obtained

is introduced into the Petri dishes. A quantity of 20 ml of previously prepared medium is poured into the Petri dish. The whole thing ~~is~~ waswell homogenized. The seeded plates ~~are~~ wereleft on the bench for solidification agar. The plates thus solidified ~~are~~ wereincubated at 25°C for 7 days for the counts of yeasts and molds, at 30°C for 24 hours for thermotolerant coliforms and at 30°C for 72 hours for mesophilic aerobic germs. Another quantity of 0.1 ml of each decimal dilution concerned ~~is~~ was placed in a Petri dish containing 20 ml of agar previously prepared and poured. Then the 0.1 mL ~~are~~ wasspread on the surface of the agar using a sterile spreader. The inoculated plates ~~are~~ were incubated at 45°C for 24 h for the research and enumeration ofE.coli, at 37°C for 48 hours for streptococci, at 30°C for 24 to 48 hours for the enumeration of vegetative forms ofBacillusand at 37°C for 24 to 48 hours for the research and enumeration of*Staphylococcus aureus*.

- For the search and enumeration of spores ofClostridium perfringensand of Bacillus cereus, 10 mL of the inoculum is transferred to a screw test tube. The tube is then treated at 80°C for 10 minutes in a water bath and cooled in water containing ice. This treatment creates a thermal shock which causes the spores to explode. A quantity of 1 mL of the dilutions retained after treatment ~~of the treated inoculum~~ is inoculated ~~respectively~~ in 15 mL of agar previously prepared and poured into a tube for *Clostridium perfringens* and 0.1 mL is spread on the Mossel medium previously prepared and poured into boxes of Petri. The tubes are then returned to water containing ice for rapid solidification. The tubes thus inoculated are incubated at 45°C for 48 hours for the search and enumeration of *Clostridium perfringens* and at 37°C for 24 hours for*Bacillus cereus*.

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Same for bacillus, this one is more fast growing

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The media used and the identifcation methods are not mentioned – add it

2.5. Research of*Salmonella*

The research ofsalmonellawas carried out in four stages according to the reference standards NF/ISO 6579 (2002) and NF-V08-52. These are pre-enrichment, enrichment, isolation and identification.

2.6. Expression of enumeration results

The number N which represents the estimate of the microbial population was calculated according to the following equation

$$N\ (UFC / g) = \frac{\sum Ci}{(N_1 + 0{,}1N_2)\ d.V}$$

N (CFU/g): Number of germs per gram of product;

∑Ci: Sum of colonies counted on all plates retained from successive dilutions;

V: Volume of inoculum applied to each plate (in ml);

n1: Number of boxes retained at the first dilution considered;

n2: Number of boxes retained at the second dilution considered;

d: Dilution factor corresponding to the first dilution retained.

2.7. Statistical analysis

The results were analyzed by Excel 2013 software for descriptive analyzes and STATISTICA 7.1 software for one-way analysis of variance (ANOVA) at the threshold of $\alpha = 0.05$ and Tukey's HSD test were used respectively. For the calculation and classification of averages. The data collected during the survey were entered with the SPHINX-LEXICA software and then processed and analyzed with the SPHINX-LEXICA software

3. RESULTS

3.1. Profile of juice consumers Bissap

The survey revealed that both sexes consume the juice of Bissap. However, females consume more juice fromBissapthan those of the male sex with a rate of 52% versus 48% respectively (Figure 1A). As for the different ethnic groups who consume the juiceBissap, the Mandé represent half of the consumers with 50%, followed by the Akan with a rate of 30%, the Krou with a rate of 14.70%, the foreigners with a rate of 3.30% and the Gours with a relatively low rate 2% (Figure 1B). Taking into account the level of ~~study~~, consumers at the secondary level are the most numerous with a rate of 37.30%, followed by those at the higher level 30.70%. Illiterate people have a consumption rate of 19.70% and those at the primary level 12.70% (Figure 2A). Young people aged between 20 and 30 years, consume more juiceBissapwith a rate of 47.30%, followed by those under 20 years with a rate of 42.70%. Other age groups consume less juiceBissapwith respective rates of 7.30% for those aged between 30 and 40 years and 2.70% for those aged over 40 (figure 2B). Consumers of juiceBissapare from various professions. There are traders who consume much more with a rate of 37.30%, followed by students with a rate of 34.70%. Students with a rate of 23.3% consume juice on average. Bissap. As for civil servants, consumption is relatively low with a rate of 0.70% and the unemployed with a rate of 4.00% (Figure 3).

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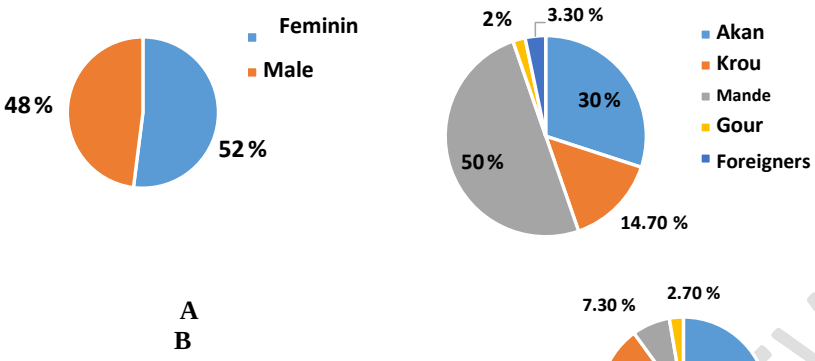


Figure 1: Distribution of Bissap juice consumers according to gender (A) and ethnic group (B)

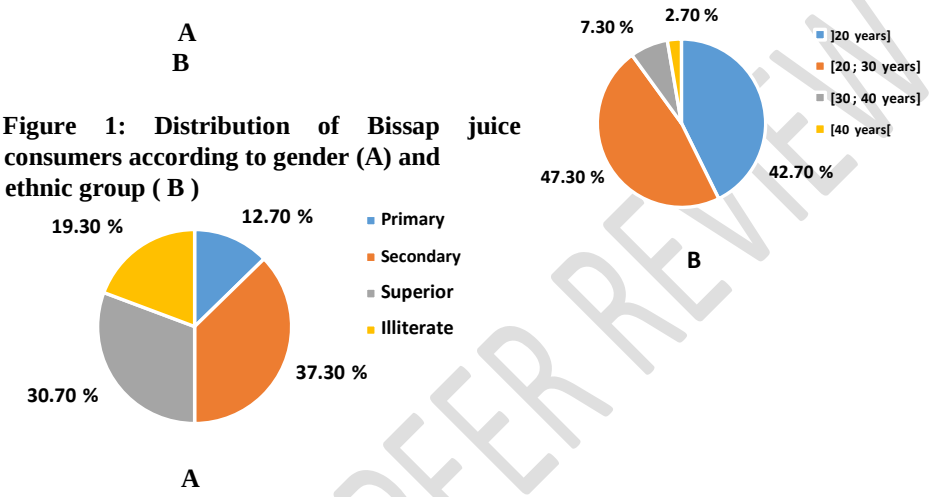


Figure 2: Distribution of juice consumersBissapaccording to the level of studies (A) and the age group (B)

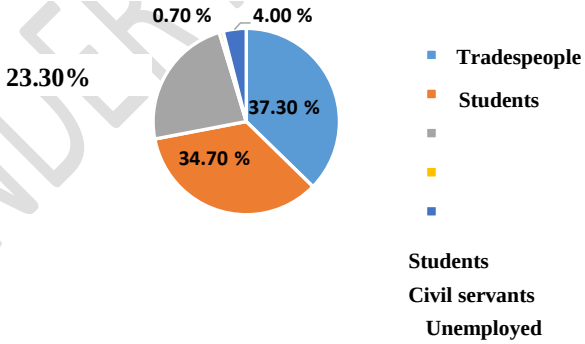


Figure 3: Distribution of juice consumers*Bissap*depending on the profession

3.2. Risks associated with consumption of juice*Bissap*

Analysis of the data collected after the survey revealed that 27.30% of the population experienced discomfort following the consumption of juice.Bissapagainst 72.70% (figure 4).

~~By takinggender,~~ more women had discomfort after consuming Bissap juice with a rate of 36.10% compared to fewer men with a rate of 19.20% (Figure 5). The survey found that people who are younger than 40 years old showed less affection compared to people older than 40 years old. The rates were respectively 23.40% for the age group of less than 20 years, 29.90% for the interval between 20 and 30 years, 18.20% for the age group between 30 and 40 years and 75% for people aged over 40 (Figure 6). The appearance of discomfort does not depend on the level of education of consumers. In fact, consumers with higher level of education~~consumers~~ have higher levels of discomfort with a rate of 32.6% than those in primary school and illiterates with similar rates of 31%. Also those in secondary education have a rate estimated at 19.6% (Figure 7). These discomforts are characterized by conditions such as fever with a rate of 3.30%, diarrhea with a rate of 20.70%, stomach bloating with a rate of 2.70%. Around 0.70% of consumers observed other undetermined symptoms (Figure 8).

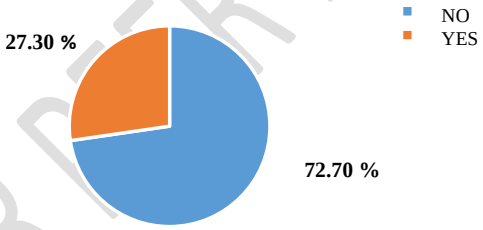


Figure 4: Frequency of consumers having experienced discomfort after consumption

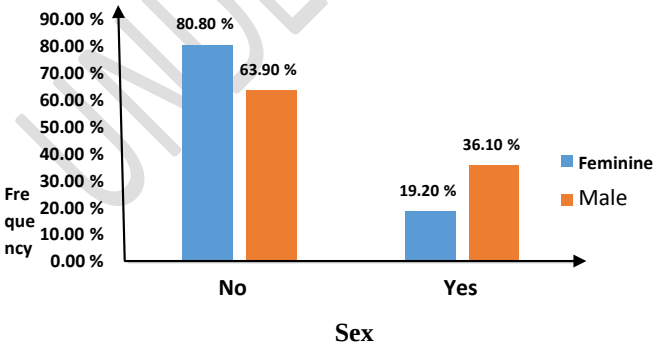
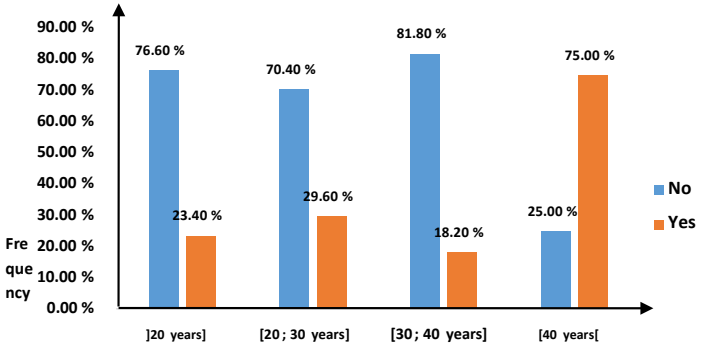


Figure 5: Appearance or not of discomfort according to sex



Age range

Figure 6: Appearance or not of discomfort according to age group

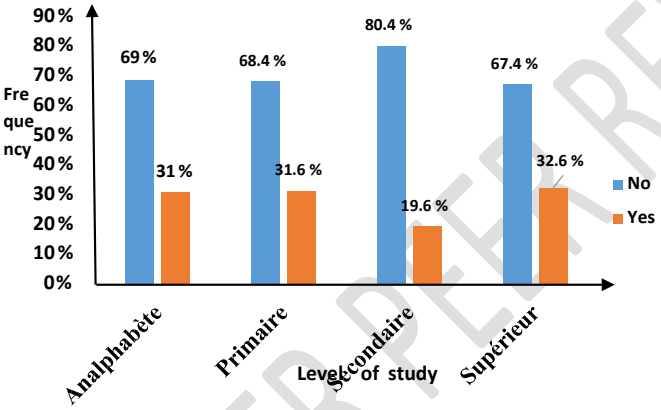
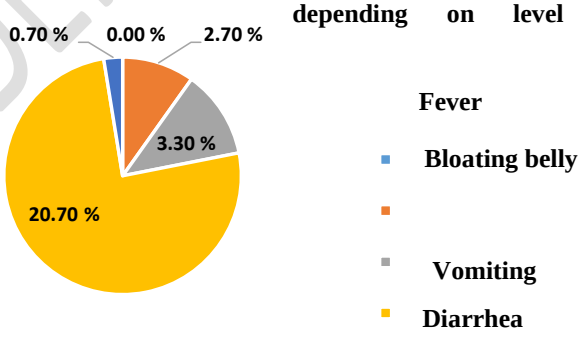


Figure 7: Appearance or not of discomfort depending on level ofstudy



Diarrhea

Figure 8: Symptoms observed after consumption of Bissap juice

3.3.Evaluation of the microbiological quality of Bissap juice

3.1.1. Germs of contamination and alteration

The microbiological analysis carried out on the different samples of the juiceBissap revealed the presence of microbial flora of alteration and contamination. This is the total flora represented by aerobic mesophilic germs with average loads which vary from 0±0 CFU/g to 9.7.10 ^{4}±4821 CFU/g; fungal flora designated by yeasts and molds with average loads oscillating between 0±0 and 10.5.10 ^{5}±19284 and enterobacteria with loads varying between 0±0 and 4.1.10 ^{5}±6.4.10 ^{3} CFU/g (Table **I**).

Table I: Average loads of juice contamination germsBissap

Samples	Yeasts and Mold	Enterobacteria	Aerobic Germs Mesophiles
SITE I	1,5.10^{3}±240,7 ^{a}	7,6.10^{3}±482,1 ^{a}	4,6.10^{3}±642,8 ^{d}
SITE II	6,5.10^{3}±805, 3^{b}	3.10^{5}±2,7.10^{3},0 ^{b}	5,9.10^{3}±482,1^{e}
SITE III	2,1.10^{3}±160,7 ^{a}	1,3.10^{3}±482 ,1 ^{b}	1,9.10^{3}±642,8 ^{b}
SITE IV	1,2.10^{3}±160,7 ^{a}	2,1.10^{3}±160,7 ^{a}	2,3. 10^{3}±1,4. 10^{2}
SITE V	1,5.10^{3}±160,7 ^{a}	3.10^{3}±2,7.10^{3},0 ^{b}	1,3.10^{3}±482 ,1 ^{b}
SITE VI	1,2.10^{3}±160,7 ^{a}	1,4.10^{3}±140,2 ^{a}	4,6.10^{3}±642,8 ^{d}
Microbiological criteria	**10^{4} UFC/g**	**10^{5} UFC/g**	**10^{2} FC/g**

3.1.2. Contamination germs of fecal origin

The average loads of juicesBissapat the level of the fecal flora analyzed vary from one sample to another taking into account the parameters studied. All loads of different juice samplesBissapanalyzed are below the criteria set by the microbiological quality standard in force for fecal streptococci. Concerning fecal coliforms except sample E3, E6 and E7 which have high loads of 1.5.10 respectively$_{3}$ ±321.4 CFU/g, 6.10$_{4}$±2.2.10 CFU/g and 9.7.10^{4}±1.7.10^{3}CFU/g all other samples are free of fillers (Table **II).**

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Table II: Average loads of germs of fecal origin in Bissap juice

Sampling Sites	Fecal coliforms	Fecal streptococci
SITE I	0±0 [a]	0±0 [a]
SITE II	0±0 [a]	0±0 [a]
SITE III	0±0 [a]	0±0 [a]
SITE IV	0±0 [a]	0±0 [a]
SITE V	0±0 [a]	0±0 [a]
SITE VI	0±0 [a]	0±0 [a]
Criteria microbiological	**10^2 UFC/g**	**Absence**

3.1.3. Potentially pathogenic germs

Juice samplesBissapanalyzed contain potentially pathogenic bacterial species, in particular*Staphylococcus aureus*~~A.~~and*Bacillus cereus*. On the other hand, no charge Clostridium perfringensnor of*Escherichia coli*was not found in the juice samples Bissap analyzes. Furthermore, the average costs ofStaphylococcus aureusvary from 0±0 cfu/g to 6.10^6±$2.5.10^4$CFU/g. At the level of*Bacillus cereus*, the highest load is $1.1.10^6$±$7.9.10^4$cfu/g and the lowest is 00±00 cfu/g (Table **III).**

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Table III: Average loads of potentially pathogenic species in Bissap juice

Samples	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Clostridium perfringens*
SITE I	0±0	$2,8.10^3$±160	$1,1.10^5$±$1,2.10^{3\ b}$	0±0
SITE II	0±0	$1,2.10^2$±$8.10^{3\ a}$	$1,9.10^3$±$4,8.10^{2\ a}$	0±0
SITE III	0±0	$1,4.10^2$±24034 [a]	$3,1.10^3$±96 [a]	0±0
SITE IV	0±0	$2,5.10^2$±1607 [a]	2.10^4±$3,2.10^{2\ a}$	0±0
SITE V	0±0	$1,1.10^2$±$8.10^{3\ a}$	$3,4.10^2$±48 [a]	0±0
SITE VI	0±0	$3,7.10^2$±24034 [a]	10^5±$1,2.10^{3\ b}$	0±0
Criteria microbiological	**10^2 UFC/g**	**10^2 UFC/g**	**10^3 UFC/g**	**10^2 UFC/g**

4. DISCUSSION

The juice of*Bissap*is a street food consumed daily by the population of Daloa in the majority of ceremonies. This is why 100% of the people surveyed said they regularly consumed B juice.issap. They generally consume it at least once a day.The survey revealed that both sexes consume the juice ofBissap. However, 52% of women consume more juiceBissapsold in the streets than men. According to studies carried out in Burkina,women are mostly involved in the sale and consumption of street foods that come from the informal sector [11]. In addition, traders consume more food than other social strata according to Barro's work [12] in Burkina and Benin [13]. Furthermore, 37.30% of those under 20 years of age consume more juice Bissapaccording to the study carried out in Daloa. This could be explained by the sweet taste of the juiceBissapbecause during manufacturing, sugar is added to the juice obtained after maceration of the petals of guinea sorrel. According to studies, young subjects tolerate sugar more if they do not have a medical history than subjects over 40 years ~~old~~[14] [15].

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It should be noted that approximately 27.30% of the people surveyed had discomfort following the consumption of juice Bissap. These discomforts are most often characterized by abdominal pain, fever, vomiting and end with diarrhea. According to previous work of a researcher, these symptoms are generally attributed to the ingestion of poorly cooked food and/or having been subjected to intense handling without any precautions [16]. These observed conditions are due to food poisoning caused by certain bacteria such as*Clostridium perfringens*, *Staphylococcus aureus*, *Escherichia coli*and others [17].

The present study makes it possible to assess the microbiological quality of juices *Bissap*sold in the streets of Daloa, but also to assess the health risks linked to the consumption of *Bissap*. Microbiological analyzes of samplesBissaptaken from the different sites revealed the presence of various microorganisms. These are spoilage and contamination flora, contamination flora of fecal origin, potentially pathogenic bacterial species and strict pathogenic species.

As for spoilage and contamination flora, GAMs are counted in the juice ofBissap with loads that exceed microbiological standards. These charges are estimated respectively between $1.3.10^3$UFC/g and $9.7.10^4$CFU/g. Thus in Daloa, the juices of Bissap contain Mesophilic Aerobic Germs. This would be linked to the conditions inwhich juices are made and sold.

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Indeed, in these streets, the juices*Bissap*are sold and manufactured in the open air and sometimes near garbage piles and even public toilets with the presence of large flies around the place where these juices are put in the bags and containers for sale. These results are similar to those of a previous study carried out by Gouen in Côte d'Ivoire and by Abeid [18] [19] on some street foods sold in Abomey Calavi in Benin.

As for yeasts and molds, they are present in the juices ofBissapwith moderately high loads that vary between $1.2.10^{3}$U FC/g and $10.5.10^{2}$CFU/g which is higher than microbiological standards. This presence is explained by the fact that the juices of Bissapare not kept in constant temperature boxes containing ice. Furthermore, prolonged stay at ambient temperature as well as exposure to open air constitute two major factors of contamination and multiplication of the total flora and fungal flora in juices Bissap.According to previous studies carried out by Barro [12], these germs indicate the state of freshness and general hygiene of the food and the presence of sugar in high quantities should constitute a good protection against the attack of contaminants in the food exception of mold.

Regarding flora of fecal origin, all loads are higher than the criteria set by the microbiological quality standard. Fecal coliform loads from juiceBissapare included from $1.5.10^{3}$±321.4 to $9.7.10^{4}$±1.7.10 CFU/g while those of fecal streptococci is 0±0 which agrees with the standard which predicted a total absence of germs in fecal streptococci. Microbiological quality standard of fecal coliform 10^{2}CFU/g. More fecal coliforms are observed in the juice of*Bissap*. The strong presence of these flora could be explained by fecal contamination of humans and animals. These data agree with those of Agbabiaka's work [20] who counted germs of fecal origin, notably fecal coliforms in street foods. Also the irregular presence of hygiene germs such as fecal coliforms is almost automatically attributed to poor personal and clothing hygiene of sellers as well as the unsanitary nature of work equipment such as knives, utensils, papers packaging [12].

In fact, the juice ofBissapbeing a perishable food, must be preserved and processed in such a way as to guarantee the health of the consumer. This objective can be achieved when strict hygiene rules are applied. This will allow sellers to market a quality product that is safe for consumers. In addition, the techniquesused in processing methods ~~currently used~~ are traditional and do not make it possible to present a quality product on the market.

The different analyzes carried out on the samplesBissapdid not reveal the absence of salmonellain the samples. This is explained by the fact that the water used for manufacturing is

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Can be replaced by microorganisms

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unpolluted water. These results are consistent with some research that has shown the absence of these germs in certain foods sold in the streets [21].

For potentially pathogenic species, juice samplesBissapanalyzed are contaminated byStaphylococcus aureusand an absence of*E.coli*. Indeed,the juice of Bissapcounts more germsStaphylococcus aureuswith a load between $1.4.10^3 \pm 1607$ and $6.10^6 \pm 2.5.10^4$CFU/g. These results are not in compliance with the standard (10^2CFU/g). Our results are similar with research who showed through recent studies that the presence of*Staphylococcus aureus*could also be due to human contamination of food since this bacteria is commensal to human skin and mucous membranes [22]. Which would show the non-compliance with good hygiene practices and the ineffectiveness of the product in these different markets.

The results of microbiological analyzes of*Escherichia coli*revealed an absence of these germs in samples of juiceBissap,these results meet the microbiological criteria (10CFU/g). The absence of Escherichia coliin all samples attests to noncontamination of fecal origin.The results of this studyagree with those made by Degnon [23]

5. CONCLUSION

This study was carried out to assess the health risk linked to the consumption of juices. Bissapsold in the streets of Daloa. The juice trade*Bissap*is a reality in the city of Daloa. Thus, an investigation was carried out to determine the different discomforts observed after consumption of juice.Bissap, followed by microbiological analyses. The lack of good hygiene practices in places of sale and manufacture of juiceBissap predispose to all forms of contamination. This survey revealed that the majority of consumers of juice*Bissap*have an age between 20 and 30 years old, dominated by women. The technical manufacturing itineraries described by these juice sellers*Bissap* remained very empirical. Poor preparation, the use of water for juice, sieves, the use of hands for mixing are subject to all microbial contamination. Microbiological analyzes showed that the juices of*Bissap*sold in theDaloa streets were heavily contaminated by spoilage flora and other flora of fecal origin. It should be noted the presence of pathogenic species such as*Staphylococcus aureus* coagulase positive and*Bacillus cereus*in the juices ofBissapsold in Daloa. The strong presence of germs of fecal origin would reflect a lack of good juice manufacturing practices and good juice hygiene practices.Bissap, which would represent a danger for consumers. The juices of*Bissap*sold in the streets would represent a danger for the population of Daloa.

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6. REFERENCES

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