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Microbial Contamination and Hygiene Risks in Street-Sold *Bissap* Juice: A Case Study in Daloa, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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.

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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 Thermotolerants coliforms. The *Bissap* juice samples analyzed does not contain potentially pathogenic bacterial species. The results regarding Mesophilic Aerobic Germs, fungal flora, enterobacteria, fecal coliforms are not satisfactory. The pH of the different juices is very acidic, ranging from 4.41 ± 0.18 to 2.18 ± 0.03. Also some of the juice samples analyzed have very high sugar levels (34.83 ± 0,28) *Bissap* juices sold in the streets represent a danger for the population of Daloa.

Keywords: Bissap; contamination; microbial; fungal; fecal streptococci.

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].

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 analysis 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.

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 the*Bissap*. The theme of this study is therefore: "Microbial cintamination and hygiene risks in street-sold *Bissap* juice : A case study in Daloa, Côte d' Ivoire. The general objective of this study is to study the microbial contamination of *Bissap* of the city of Daloa and to assess the health risks linked to its consumption.

2. MATERIALS AND METHODS

2.1 Materials

a. Biological material

b. Technical equipment

For physicochemical analyses, a beaker, a pH meter (PHS-38W) and a portable refractometer (ATAGO N-1α) were used. During the microbiological analyses, a balance (KERN having a sensitivity of 0.0001 g) was used to weigh the masses of culture media to be prepared and the test portions. An autoclave (NÜVE model OT 012) was used to carry out the sterilizations. The water bath that was used is of the Fisher Scientific type (polytest 12) for the different preparations of the media. The different incubators used for the analyzes are: MEMMERT model UF750, MEMMERT Beschickung/loadingmodell 100-800 and Neo-Tech SA for the different incubations of the seeded culture media.

Fig. 1. Sample of *Bissap*

Vials with autoclavable caps for media preparation, test tubes for decimal dilutions, and Erlenmeyer flasks for stock solutions were used. In addition to the classic equipment of a biology laboratory, other specific equipment such as platinum handles, spreaders, and a gas cylinder with a Bunsen burner were used.

c. Culture media

Different culture media have been used for research, isolation and identification of potentially pathogenic species and microbial spoilage flora.

- **Culture media for research, isolation and identification of potentially pathogenic species Several culture media have been used to detect potentially pathogenic germs**

-**Buffered Peptone Water (EPT) broth**

It is a diluent commonly used for food sample preparation. It is used in the preenrichment phases and regeneration of microorganisms for microbiological analyzes (ISO, 2011).

-**Rappaport broth from Vassiliadis Soya Broth**

It is a selective broth. It has been used for enrichment of samples for research of *Salmonella*.

-**Hektoen Enteric Agar**

It is a selective isolation medium for non-demanding Gram-negative bacilli and used for the research of*Salmonella*. Sodium deoxycholate in the medium inhibits the growth of Gram-positive bacteria. This medium makes it possible to simultaneously detect the use of lactose, sucrose and salicin. This use results in acidification and therefore a change from bromothymol blue to yellow (colonies) or orange: there is acidification of the medium by fermentation when at least one of the carbohydrates is present in the medium (green or blue colonies) . There is no acidification of the environment when none of the carbohydrates have been used (ISO, 2007).

-**Bile Esculin Azide Agar (BEA)**

BEA agar is a selective isolation medium used for the detection of Enterococcus and Group D Streptococcus. Sodium azide inhibits Gram-negative bacteria and ox bile inhibits most Gram-positive bacteria. Enterococcus and group D Streptococcus secrete beta-glucosidase. This enzyme hydrolyzes esculin and the product of this hydrolysis, called esculetin, reacts with ferric ions to form a black precipitate which diffuses into the medium (ISO, 2000).

-**Rapid E. coli 2 agar**

Rapid E. Coli 2 is a selective chromogenic culture medium for directly enumerating Escherichia coli and other coliforms in a product intended for human and animal food. The principle of the medium is based on the simultaneous demonstration of two enzymatic activities, namely β-D-Glucuronidase (GLUC) and β-D-Galactosidase (GAL). The medium also contains two chromogenic substrates, a specific GAL substrate which causes the blue coloring of colonies positive for this enzyme and a specific GLUC substrate which causes the pink coloring of positive colonies for this enzyme. Coliforms (GAL+/GLUC-) form blue to green colonies. E. coli (GAL+/GLUC+) form purple to pink colonies (ISO, 2014).

-**Tryptone Sulfite Neomycin (TSN) agar**

TSN medium is a selective agar. It exploits the fact that Clostridium perfringens is resistant to

polymyxin, neomycin (antibiotics) and has the power to reduce sulphites. The growth of other sulfito-reducing Clostridia is almost completely inhibited because the accompanying flora is largely suppressed. This medium contains a criterion of differentiation: sodium sulphite, the reduction of which is revealed by iron (precipitation of iron sulphide), forms a black colony. The incubation temperature makes it possible to select: at 37°C, enumeration of vegetative forms of Clostridium perfringens and at 46°C, enumeration of Clostridium perfringens. Incubation takes place anaerobically (Claeys, 2002).

-**Mossel agar**

Mossel Selective Agar is used for the detection and enumeration of spores and vegetative forms of Bacillus cereus in food products. It is used for the enumeration of presumptive Bacillus cereus at 30°C. The use of this medium must be oriented beforehand. The bacteria must use mannitol. If the medium contains egg yolk, the presence of lecithinase can be detected. This medium must only select Bacillus cereus, other species could have the same orientation profiles. It is therefore added to the middle of polymyxin (ISO, 2012).

-**Baird Parker Agar**

It is a selective medium to which potassium tellurite egg yolk and an antibiotic (0.2% sulphamethazine) have been added. Baird-Parker agar is a selective and differential culture medium used in microbiology for the isolation and identification of the pathogen Staphylococcus aureus. The principle of the medium is based on the ability of Staphylococcus aureus to reduce tellurite (black colonies), to cause proteolysis of egg yolk (clear halo around the colonies), and to opacify the proteolysis zone (lipase activity). The medium is made inhibitory to other bacteria by lithium chloride and potassium tellurite.

- **Culture media for the demonstration of spoilage germs Several media have been used to demonstrate the flora of interest**

-**CONDA Plate Count Agar (PCA)**

It's a basic environment. PCA agar is an agar medium used in food bacteriology for the enumeration of aerobic psychrotrophic and mesophilic bacteria in milk, meats, meat products, other food products, as well as for the analysis of pharmaceutical products.

-**Sabouraud + Chloramphenicol agar (Alpha Biosciences)**

It is a general-use medium, allowing the growth and isolation of a wide variety of yeasts and molds. Chloramphenicol, a thermostable antibiotic with a broad antibacterial spectrum, inhibits the development of contaminating microflora. The addition of chloramphenicol inhibits the growth of Gram-positive and Gram-negative bacteria (Sabouraud, 1910).

-**Violet Red Neutral Bile Lactose Agar (VRBL) (Diagnostici-Liofilchem)**

It is a selective medium which has been used for the research and enumeration of total coliforms. The principle of the medium is based on the ability of coliforms to ferment lactose. The medium is made inhibitory to Gram-positive bacteria and certain Gramnegative bacteria by the simultaneous presence of crystal violet and bile salts. Neutral red is a pH indicator.

-**Neutral Purple Red Bile Glucose Agar (VRBG)**

The VRBG culture medium is a selective medium intended to determine the presence and estimate the quantity of enterobacteria present in various products, particularly in food products. The principle of the medium is based on the ability of enterobacteria to ferment glucose. The medium is made inhibitory to Gram-positive bacteria and certain Gram-negative bacteria by the simultaneous presence of crystal violet and bile salts. Neutral red is a pH indicator. Enterobacteriaceae present purple colonies, surrounded or not by a purple halo of precipitated bile salts (ISO, 2017).

2.2 Methods

2.2.1 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.

2.2.2 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. The samples of juiceBissap »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.

2.2.3 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 weighed 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.

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2.6 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 was well homogenized. The seeded plates were left on the bench for solidification agar. The plates thus solidified were incubated at 25°C for 7 days for the counts of yeasts and molds, at 44°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 placed in a Petri dish containing 20 ml of agar previously prepared and poured. Of what was spread already 0.1 mL were added. The inoculated plates are incubated at 44°C for 24 h for the research and enumeration of *E.coli*, at 37°C for 48 hours for streptococci, at 30°C for 24 hours for the enumeration of

vegetative forms of Bacillus and at 37°C for 24 to 48 hours for the research and enumeration of *Staphylococcus aureus*.

-For the search and enumeration of spores of Clostridium perfringens and of Bacillus cereus, 10 mL of the inoculum was transferred to a screw test tube. The tube was then treated at 80°C for 10 minutes in a water bath and cooled in water containing ice. This treatment created a thermal shock which caused the spores to explode. A quantity of 1 mL of the dilutions retained after treatment in were oculated in 15 mL TSN agar previously prepared and poured into a tube for *Clostridium perfringens* and 0.1 mL was spread on the Mossel medium previously prepared and poured into boxes of Petri for *Bacillus cereus*. The tubes were then returned to water containing ice for rapid solidification. The TSN tubes and Mossel boxes thus inoculated were respectively 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*. Clostridium perfringens being an anaerobic bacterium, the incubation was done in anaerobic plastic jars hermetically sealed to create anaerobic.

2.7 Research of *Salmonella*

The research of*salmonella*was 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.

2.8 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.

2.9 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 α = 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 from Bissap than those of the male sex with a rate of 52% versus 48% respectively (Fig. 1A). As for the different ethnic groups who consume the juice Bissap, the Mandé represent half of the consumers with 50%, followed by the Aken with a rate of30%, 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% (Fig. 1B). Taking into account the level of education, 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% (Fig. 2A). Young people aged between 20 and 30 years consume more juice Bissap with a rate of 47.30%, followed by those under 20 years with a rate of 42.70%. Other age groups consume less juice Bissap with respective rates of 7.30% for those aged between 30 and 40 years and 2.70% for those aged over 40 (fig. 2B). Bissap consumers can be found in various professions, there are traders, civil servants, students. The traders consume much more with a rate of 37.30%, followed by hygh school students and university students with rates of 34.70% and 23.3%. As for civil servants, consumption is relatively low with a rate of 0.70% and the unemployed with a rate of 4.00% (Fig. 3).

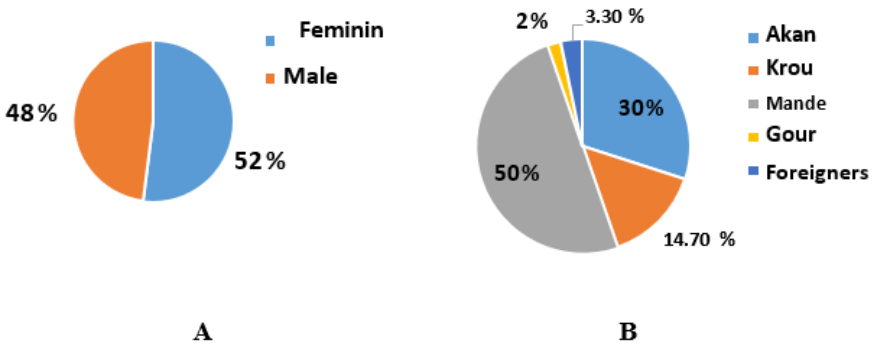

Fig. 2. Distribution of *Bissap* juice consumers according to gender (A) and ethnic group (B)

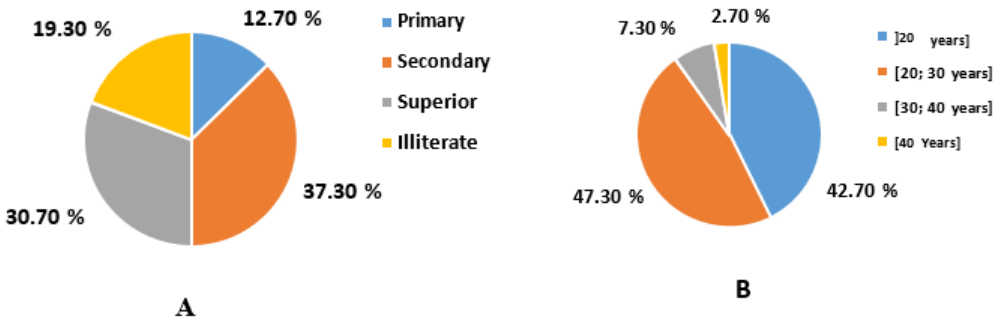

Fig. 3. Distribution of juice consumers *Bissap* according to the level of studies (A) and the age

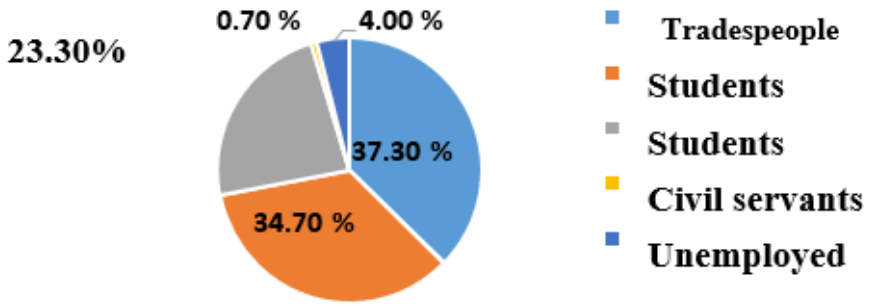

Fig. 4. Distribution of juice consumers *Bissap* depending on the profession

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. *Bissap* against 72.70% (Fig. 5). More women had discomfort after consuming *Bissap* juice with a rate of 36.10% compared to fewer men with a rate of 19.20% (Fig. 6). 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 (Fig. 7). The appearance of discomfort does not depend on the level of education of consumers. In fact, consumers

with higher level of education 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% (Fig. 8). 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 rateof 2.70% Around 0.70% of consumers observed other undetermined symptoms (Fig. 9).

3.3 Physicochemical Characteristics

The different samples analyzed havevarying pHs. Thus, theBissapis acidic with a pH varying from 4.41 ± 0.18 to 2.18 ± 0.03. The Brix degrees obtained after analysis of the different juices Bissap were very varied. Thus, the Brix degree varied from 34.83 ± 0.28 to 24.86 ± 0.70 (Table 1).

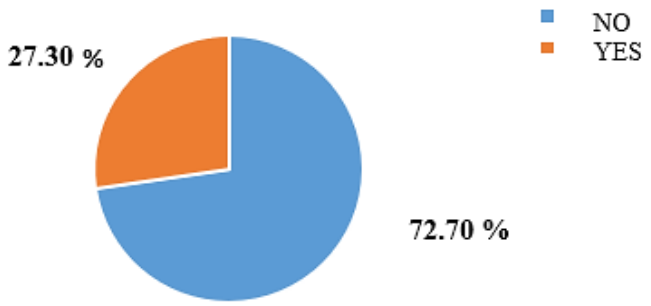

Fig. 5. Frequency of consumers having experienced discomfort after consumption

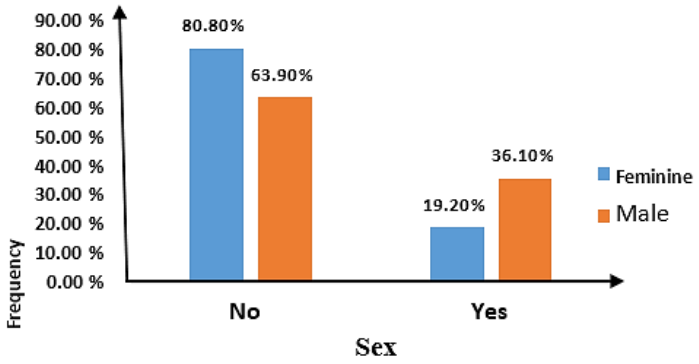

Fig. 6. Appearance or not of discomfort according to sex

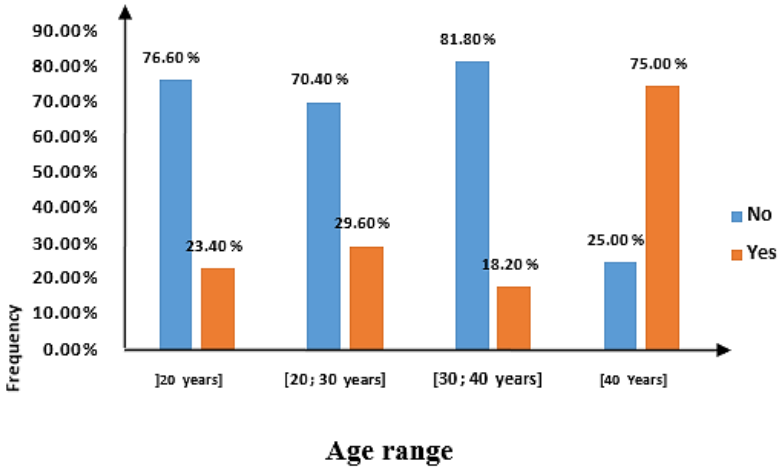

Fig. 7. Appearance or not of discomfort according to age group

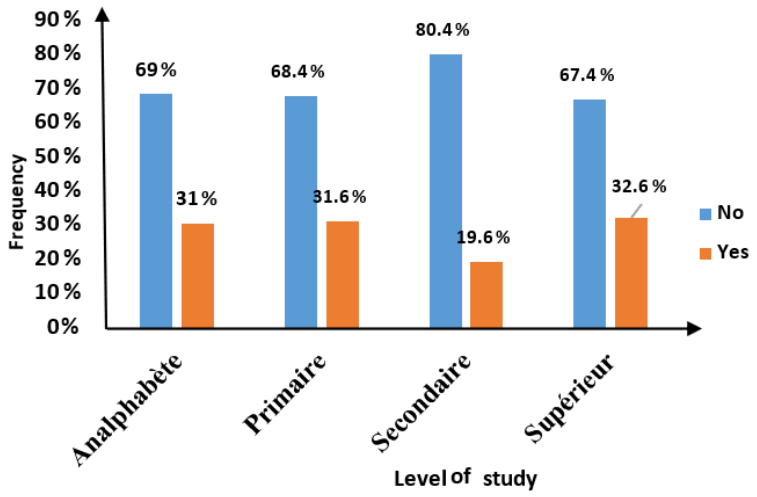

Fig. 8. Appearance or not of discomfort depending on level ofstudy

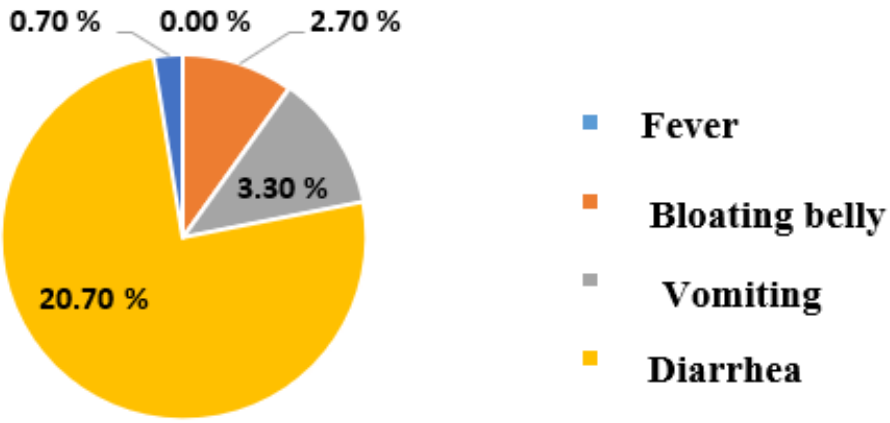

Fig. 9. Symptoms observed after consumption of *Bissap* juice

Table 1. Some physicochemical parameters of the samples analyzed Samples

Samples	Physico-chemical parameters	
	pH	Degree Brix
SITE 1	3.50±0.01	34.83±0.28
SITE 2	3.04±0.02	34.66±0.57
SITE 3	2.83±0.11	26.16±0.76
SITE 4	2.89±0.01	24.86±0.70
SITE 5	2.18±0.03	27.33±1.52
SITE 6	4.41±0.18	34.50±0.86
Average	3.65±0.15	31.05±0.78

3.4 Evaluation of the Microbiological Quality of *Bissap* Juice

3.4.1 Germs of Contamination and Alteration

The microbiological analysis carried out on the different samples of the juice *Bissap* 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 2).

3.4.2 Contamination germs of fecal origin

The average loads of juices *Bissap* at the level of the fecal flora analyzed vary from one sample to another taking into account the parameters studied. All loads of different juice samples *Bissap* analyzed 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^{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 (0±0 CFU/g) (Table 3**).**

3.4.3 Potentially pathogenic germs

Juice samples *Bissap* analyzed do not contain potentially pathogenic bacterial species. No charge *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus* nor of *Escherichia coli* was not found in the juice samples *Bissap* analyzes. (Table 4).

Table 2. Average loads of juice contamination germs *Bissap*

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} ^{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} ^{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**

Table 3. Average loads of germs of fecal origin in *Bissap* juice

Sites	Samples	Fecal coliforms	Fecal streptococci
SITE I	**E1**	0±0 ^{a}	0±0 ^{a}
	E2	0±0 ^{a}	
	E3	1.5.10^{3}±321.4	
	E4	0±0 ^{a}	
	E5	0±0 ^{a}	
SITE II	**E6**	6.10^{4}±2.2.10	0±0 ^{a}
	E7	9.7.10^{4}±1.7.10^{3}	
	E8	0±0 ^{a}	
	E9	0±0 ^{a}	
	E10	0±0 ^{a}	
SITE III	**E11**	0±0 ^{a}	0±0 ^{a}
	E12	0±0 ^{a}	
	E13	0±0 ^{a}	
	E14	0±0 ^{a}	
	E15	0±0 ^{a}	
SITE IV	**E16**	0±0 ^{a}	0±0 ^{a}
	E17	0±0 ^{a}	
	E18	0±0 ^{a}	
	E18	0±0 ^{a}	
	E20	0±0 ^{a}	
SITE V	**E21**	0±0 ^{a}	0±0 ^{a}
	E22	0±0 ^{a}	
	E23	0±0 ^{a}	
	E24	0±0 ^{a}	
	E25	0±0 ^{a}	
SITE VI	**E26**	0±0 ^{a}	0±0 ^{a}
	E27	0±0 ^{a}	
	E28	0±0 ^{a}	
	E29	0±0 ^{a}	
	E30	0±0 ^{a}	
Criteria microbiological		**10^{2} UFC/g**	**Absence**

Table 4. Average loads of potentially pathogenic species in Bissap juice

Samples	Escherichia coli	Staphylococcus aureus	Bacillus cereus	Clostridium perfringens
SITE I	0±0	0±0	0±0	0±0
SITE II	0±0	0±0	0±0	0±0
SITE III	0±0	0±0	0±0	0±0
SITE IV	0±0	0±0	0±0	0±0
SITE V	0±0	0±0	0±0	0±0
SITE VI	0±0	0±0	0±0	0±0
Criteria microbiological	**10^{2}CFU/g**	**10^{2}CFU/g**	**10^{3}CFU/g**	**10^{2}CFU/g**

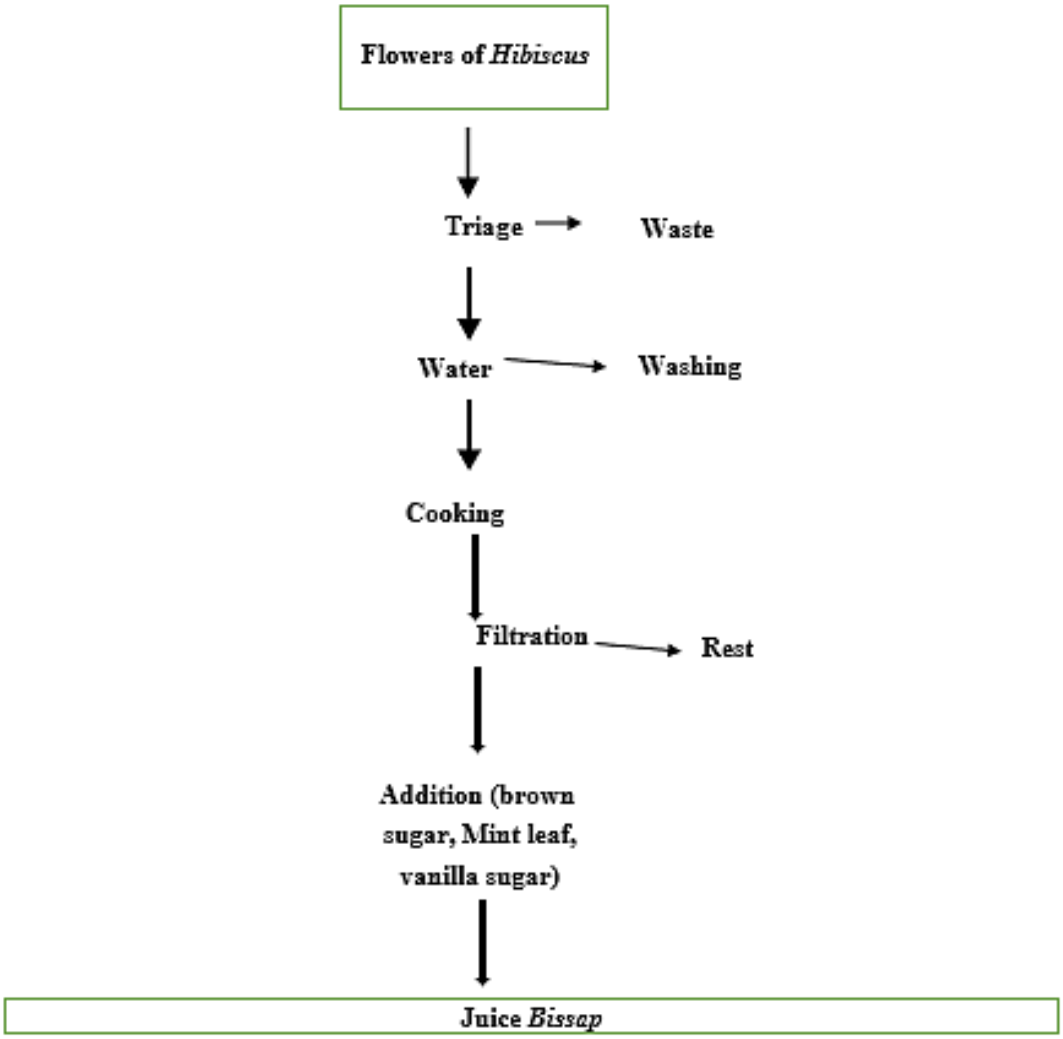

Fig. 10. Production diagram *BISSAP*

4. DISCUSSION

The juice of *Bissap* is a street food consumed daily by the population of Daloa in the majority of ceremonies. *Bissap* have antispasmodic, hypotensive, antiinflammatory and venoprotective activities. They are also antiasthenic and anthelmintic. They are also attributed with laxative, diuretic and depurative properties. On the other hand, hibiscus has antibacterial properties This is why 100% of the people surveyed said they regularly consumed *Bissap* juice. They generally consume it at least once a day.The survey revealed that both sexes

consume the juice of *Bissap*. However, 52% of women consume more juice *Bissap* sold 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 strat a according to Barro's work [12] in Burkina and Benin [13]. Furthermore, 37.30% of those under 20 years of age consume more juice *Bissap* according to the study carried out in Daloa. This could be explained by the sweet taste of the juice *Bissap* because 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 [14,15].

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 subject 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 samples *Bissap* taken 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 of *Bissap* with loads that exceed microbiological standards. These charges are estimated respectively between $1.3.10^3$ CFU/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 in which juices are made and sold. 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 of *Bissap* with 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 *Bissap* are 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 microorganisms 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 juice *Bissap* are 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. This contamination would be due the processing process of *Bissap*. Like simple washing, no disinfection operation has been undertaken to neutralize the microorganisms. In addition, the hygienic state of the containers is never controlled. Also, the juice extraction operation would be carried out with bare hands. Hands most of the time carry contamination germs, even fecal ones, when hygiene is not practiced. 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 of *Bissap* being 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 techniques used in processing methods are traditional and do not make it possible to present a quality product on the market.

The different analyzes carried out on the samples *Bissap* revealed the absence of *salmonella* in the samples. This is explained by the fact that the water used for manufacturing is 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 samples *Bissap* analyzed are not contaminated. These results are in accordance with the standard (10 ^{2}CFU/g). Our results are different from those of Loir and Gautier who showed through recent studies the presence of *Staphylococcus aureus* in street foods [22].

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

5. CONCLUSION

This study was carried out to analysis the health risk linked to the consumption of juices. *Bissap* sold 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 juice *Bissap* 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 poor quality 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 the Daloa streets were heavily contaminated by spoilage flora and other flora of fecal origin. It should be noted the absence of pathogenic species in the juices of *Bissap* sold in Daloa. The strong presence of germs of fecal origin would reflect a lack of good hygiene practices and good manufacturing practices of *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.

6. RECOMMENDATIONS

- The competent authorities must inform and raise awareness to raise awareness of the health risks incurred by consumers in connection with street foods.
- Implementing street food regulations could limit the risks of contamination.
- Adequate measures must be taken during manufacturing to produce juice Bissappresenting less risk. These measures may include disinfection of the raw material with all the equipment used, hot packaging, wearing gloves, pasteurization at the end of preparation of the drink which would limit any transmission of germs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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