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African Journal of Microbiology Research

21 September 2018
ISSN 1996-0808
DOI: 10.5897/AJMR
www.academicjournals.org

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Vol. 12(35), pp. 849-856, 21 September, 2018
DOI: 10.5897/AJMR2018.8858
Article Number: C6B1D9558749
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation and characterization of actinomycetes with antimicrobial activity from the soil and the effect of the environmental factors on their antimicrobial activity

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Received 1 April, 2018; Accepted 12 September, 2018

This study aimed at isolating and characterizing antibiotic producing actinomycetes from a specific geographic location Alba'qa, Jordan, with a known soil character, and study the effect of the environmental factors on their antimicrobial activity, in order to relate the actinomycetes flora to the environmental characteristics. Soils of the Alba'qa region are of uniform composition and contain antimicrobial producing microorganisms. Although the initial aim was to isolate organisms of the genera *Actinomyces*, other organisms such as *Aspergillus* and other antibiotic producing microorganisms "mostly bacteria" were also isolated. Actinomycetes showed antimicrobial effect on all tested bacteria "*Escherichia coli* SQ21, *Staphylococcus aureus* SQ31, *Streptococcus pyogenes* SQ33, *Serratia marcescens* SQ22, *Staphyloccocus epidermidis* SQ32 and *Bacillus* spp. SQ41" depending on the media they were cultured on and the temperature they were incubated at. It was established that the optimum antibacterial activity is shown when cultivated on GYE medium at 30°C. This made a beneficial first step to find treatments for the diseases caused by the pathogenic bacteria.

Key words: Actinomycetes, antibiotics, environmental factors, soil.

INTRODUCTION

Actinomycetes are a broad group of gram positive bacteria that form thread-like filaments in the soil. A few are normal inhabitants of the mouth and several species are pathogenic (Actor, 2012). They are responsible for the distinctive scent of freshly exposed moist soil. Antibiotics are the best known products of actinomycetes. Over 5,000 antibiotics have been identified from the cultures of Gram positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases. The genus, *Streptomyces*, is responsible for the formation of more than 60% known antibiotics while a further 15% are made by a number of related *Actinomycetes*, *Micromonospora*, *Actinomadura*, *Streptoverticillium* and *Thermoactinomycetes* (Waksman, 1954). Actinomycetes centered mainly on their ability to

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Table 1. Properties of the soil samples

Sample	1	2	3
Top Depth (cm)	0	0	0
Bottom Depth (cm)	10	10	10
Moisture status	wet	wet	wet
Moisture content	2.39g	1.15g	1.26g
Structure	Single grained	Granular	Massive
Main color	7.5 YR 3/4	7.5 YR 4/6	7.5 YR 4/4
Second color	-	-	7.5 YR 5/4
Consistency	Friable	Friable	Loose
Rocks	Few	Few	Many
Roots	Few	Few	Many
Carbonates	Slight	Slight	Strong
pH	7.79	7.76	7.44
Temperature (°C)	23	21	22
Particle density (mg m^{-3})	2.222	2	2
Bulk density (ml)	8.6	1	1
Pore space	-2.69	0.5	0.5
Soil particle size distribution	Clay loam	Clay loam	Slit clay loam

form antibiotics. Beginning with the discovery of actinomycin in 1940, the interest in the antibiotics produced by actinomycetes has been wondrous. Srtreptothricin, streptomycin, nystatin, chloramphenicol, tetracycline, erythromycin, the chain of the discoveries are endless (Mahajan and Balachandran, 2012).

Actinomycetes can be grown on the common bacteriological media used in the laboratory, such us nutrient agar, trypticase soy agar and blood agar (Nanjwade et al., 2010b). The actinomycetes have universal occurrence and play an active part in the cycle of nature. The morphology of actinomycetes growing on agar (the presence or absence of spores on the substrate mycelium, the formation of zoospores in specialized spore vesicles or sporangia), their spores arrangement and the features of their colonies can provide useful and rapid clues to their identity (Oskay et al., 2004).

Actinomycetes presence in soil is affected by the geographical location and the prevailing abiotic factors (soil temperature, soil type, depth, soil pH, organic matter content, cultivation, aeration and moisture content). Therefore, we believe that when attempting to isolate actinomycetes it is important to specify the soil type and related atmospheric conditions at the time of sampling. In the past years, focus on natural product discovery from actinomycetes shifted from the extensively investigated soil-dwelling isolates towards underexplored habitats of rare actinomycetes from unusual ecosystem. This strategy has an impact on the discovery platform for novel compounds with promising bioactivities despite the fact that a large amount of the reservoir of habitats still awaits exploration (Hug et al., 2018). Our interest was focused on screening the soil samples for actinomycetes with antibiotic production potential from Alba'qa area, Jordan, in addition to study the effect of the environmental factors on their antimicrobial activity.

MATERIALS AND METHODS

Soil characterization

Alba'qa Region in Jordan was the geographic location of the soil samples. They were obtained from previous site studies applying the site characterization GLOBE protocol; the coordinates were determined to be 32.03234° north (Tables 1 and 2) and 35.90517° East at an elevation of 920 m. All readings were taken with a Garmin GPS12.

Isolation of antimicrobial substances producing microorganisms from soil

Actinomyces and other microorganisms such as Aspergillus and Gram positive bacteria" were isolated from soil samples collected from Alba'qa, Jordan (depending on their morphologically and cultural properties of the isolates shown in Table 3). For the isolation, 1 g of rhizosphere soil sample was dispensed into 10 ml of sterile deionized water. The soil suspension was then shaken on a rotary shaker (Sanyo Gallenhamp PLC, Leicester, LE 3 2uz, UK) at 180 rpm and 27°C for 30 min. Ten-fold dilutions were made in sterile saline solution and 100 µl aliquots were spread with a sterile glass rod over the surface of nutrient agar plates (per liter of distilled water) (peptone 5 g, sodium chloride 5 g, yeast extract 1.5 g, beef extract 1.5 g and agar 15 g) (HiMedia Laboratories Pvt. Limited, Bombay, India). Six plates were used per dilution and dried in a laminar flow-cabinet for 60 min before incubation at 27°C in the dark for 48-72 h. Bacterial colonies were sub-cultured and transferred onto nutrient agar plates. Single colonies were isolated and screened for antimicrobial activity using the agar streak method (El-Banna and Winkelman, 1998).

Table 2. Calculations done for the soil particle size distribution examination.

Calculation	Sample 1	Sample 2	Sample 3
2 minutes hydrometer reading	1.010	1.011	1.012
2 minutes temperature reading ° C	19	19	19
Grams/L of soil (slit + clay) from table (8)G/L	14	14	15.5
Temperature correction [0.36 * (B-20)] G	-0.36	-0.36	-0.36
Corrected slit and clay in suspension (C+D) G	13.64	13.64	15.14
Grams of soil (slit + clay) in 500 mL: E * 0.5 G	6.82	6.82	7.57
Grams of sand in sample: 12 gram -f G	5.18	5.18	4.43
Percent sand: [(g / 25) * 100] %	20.72%	20.72%	17.72%
24 hours hydrometer reading	1.010	1.011	1.012
24 hours temperature reading °C	23	22.5	23
Grams/L of soil (clay) from table (8) G	14	14	15.5
Temperature correction [0.36 * (j-20 C)] G	1.08	0.9	1.8
Corrected clay in suspension (k + l) G	15.08	14.9	16.74
Grams of soil (clay) in 500 mL: m * 0.5G	7.54	7.45	8.37
Percent clay: [(n/25) * 100] %	30.16%	29.8%	33.48%
Grams of slit: [25-(g + n)] G	12.28	12.37	12.2
Percent slit: [(p/25) * 100] %	49.12%	49.48%	48.8 %

Table 3. The morphological properties of the isolates and their antimicrobial effect.

Isolate	Appearance of colonies	Gram stain	Spore arrangement	Antimicrobial activity
1	Creamy/orange, slightly elevated, opaque	G+ve	Flexous spores	G+ve and G-ve bacteria
2	Pink, slightly elevated, opaque	G+ve	Facsicled spores	G+ve bacteria
3	White, powdery, elevated, concave	G+ve	Conodia in conodiophores	G+ve and G-ve bacteria
4	Creamy slightly elevated, opaque	G+ve	Long rods seen /no spores	G+ve bacteria

Bacterial strains

Escherichia coli SQ21*, Staphylococcus aureus* SQ31*, Streptococcus pyogenes* SQ33*, Serratia marcescens* SQ22*, Staphylococcus epidermidis* SQ32 and *Bacillus* spp. SQ41 were used as test microorganisms.

Culture media

Nutrient agar (NA), trypticase soya agar (TSA.), glycerol yeast extract (GYE), nutrient broth (NB) and GYE (5 ml of glycerol, 2 g of yeast extract, 1 g of dipotassium phosphate, 15 g agar, 1000 ml water).

Agar streak method

The microbial sensitivity of the soil isolates was analyzed by agar streak method. Each of the isolate was streaked as a straight line on medium and incubated at 27°C for 6 days (144 h). After the 6th day, different strains of microorganisms were streaked at right angle, but not touching each other, and then incubated at 37°C for 24 h in the case of bacteria. If the organism is susceptible to the antibiotic produced by actinomycetes, then it will not grow near the actinomycetes (Nanjwade et al., 2010a). The zone of inhibition against each test organism was noted. The isolated actinomycetes were screened against test microorganisms (Haque et al., 1996). Based on their antimicrobial properties, isolates were chosen for the further biochemical characterization.

Morphological and cultural characterization

The effect of the type of media on the antimicrobial activity of the isolated strains

Streaks of six bacterial species were inoculated on the plates of 6 days old cultures of isolates 1, 2, 3 and 4 incubated at 30°C for 24 h.

The effect of the temperature

The effect of the incubated temperature on the antimicrobial activity of the strain 1, 2, 3 and 4 incubated at different temperatures for 24 h.

Effect of isolate culture age

Isolates of different age were used to determine the effect of the culture on the potency of their antibiotic productivity.

Table 4. The effect of the type of media on the antimicrobial activity of the isolate 1.

Isolate (1) grown on different types of media	Test microorganisms					
	(1)	(2)	(3)	(4)	(5)	(6)
N.A			+		+	
T.S.A			+			+
G.Y.E			+		+	+

Test microorganisms (1*): Bacillus spp* SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 *(5): Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32 +: showed antibiotic sensitivity.

Table 5. The effect of the type of media on the antimicrobial activity of the isolate 2.

Isolate (2) grown on different types of media	Test microorganisms					
	(1)	(2)	(3)	(4)	(5)	(6)
N.A			+			
T.S.A		+	+			
G.Y.E			+			+

Test microorganisms (1*): Bacillus spp* SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 *(5): Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32, +: showed antibiotic sensitivity.

Figure 1. Antimicrobial activity of the isolate 4 against (1*): Bacillus* spp SQ41 (2)*: S. marcescens* SQ22 (3)*: S. pyogenes* SQ33 (4)*: S. aureus* SQ31 *(5): E.coli* SQ21 (6): *S. epidermidis* SQ32.

Figure 2. The antimicrobial activity of the isolate strain 3 on GYE.

RESULTS

Soil characterization

The results of the soil samples which were collected from Alba'qa, Jordan are shown in Tables 1 and 2.

Isolation of antibiotic producing organisms

During the initial isolation attempts, four morphologically different isolates were obtained (Table 3), showing significant antimicrobial activity (Figure 1). Variables that may affect the efficiency of antibiotic production were narrowed down to three factors that were examined separately. These were the type of medium, the incubation temperature and the age of isolate.

The effect of the type of media on the antimicrobial activity of the isolated strains

Streaks of six bacterial species were inoculated on the plates of 6 days old cultures of isolates 1, 2, 3 and 4 as shown in Tables 4, 5, 6 and 7 incubated at 30°C for 24 h. Figures 2, 3 and 4 showed the agar streaks test of strain

Table 6. The effect of the type of media on the antimicrobial activity of the isolate 3

Isolate (3) grown on different types of media	Test microorganisms					
	(1)	(2)	(3)	(4)	(5)	(6)
N.A	+		+		+	
T.S.A	+		+			
G.Y.E	+		+	+	+	+

Test microorganisms (1*): Bacillus spp* SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 *(5): Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32 +: showed antibiotic sensitivity.

Table 7. The effect of the type of media on the antimicrobial activity of the isolate 4.

Isolate (4) grown on different types of media	Test Microorganisms					
	(1)	(2)	(3)	(4)	(5)	(6)
N.A			+			
T.S.A	+		+			+
G.Y.E			+			+

Test microorganisms (1*): Bacillus spp* SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 *(5): Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32. +: showed antibiotic sensitivity.

Figure 3. The antimicrobial activity of the isolate strain 3 on TSA.

Figure 4. The antimicrobial activity of the isolate 3 on NA.

3 on different types of media.

The effect of the temperature

The effect of the incubated temperature on the antimicrobial activity of strains 1, 2, 3 and 4 incubated at different temperatures for 24 h are shown in Table 8 and Figures 5, 6, 7 and 8. The effect of the incubated temperature on the antimicrobial activity of the isolated strains 1, 2, 3 and 4 incubated at 37°C, for 24 h are shown in Tables 8 and 9, and Figures 9, 10, 11 and 12.

Effect of isolate culture age

The age of the culture seemed to have no effect on the potency of its antibiotic product.

DISCUSSION

The soil samples characterization in Table 1 showed no obvious difference between the 3 types of soil which were taken from the 3 different areas of the Alba'qa, Jordan. So even from different locations that are distant from each other in the region, they were almost the same type

Table 8. The effect of the incubated temperature on the antimicrobial activity of isolated strain 1, 2, 3 and 4 incubated at 30° C, for 24.

Isolate	Type of media	Test cultures					
		(1)	(2)	(3)	(4)	(5)	(6)
1	T.S.A			+			+
2	T.S.A		+	+			
3	T.S.A	+		+			
4	T.S.A	+		+			+

Test cultures (1*): Bacillus spp* SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 (5): *Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32. +: showed antibiotic sensitivity.

Table 9. The effect of the incubated temperature on the antimicrobial activity of the isolated strain 1, 2, 3 and 4 incubated at 37° C, for 24.

Isolate	Test cultures					
	(1)	(2)	(3)	(4)	(5)	(6)
Isolate 1			+			+
Isolate 2						
Isolate 3	+					
Isolate 4						

Test cultures (1): *Bacillus* spp. SQ41 (2)*: Serratia marcescens* SQ22 (3)*: Streptococcus pyogenes* SQ33 (4)*: Staphylococcus aureus* SQ31 *(5): Escherichia coli* SQ21 (6): *Staphyloccocus epidermidis* SQ32 +: showed antibiotic sensitivity.

Figure 5. The effect of the incubated temperature on the antimicrobial activity of the strain 1 incubated at 30° C, for 24.

Figure 6. The effect of the incubated temperature on the antimicrobial activity of the strain 2 incubated at 30°C, for 24.

which had no significance in the isolation process. The presence of relatively large populations of actinomycetes in the soil samples of Alba'qa region indicates that it is a suitable ecosystem that promotes the isolation of actinomycetes during screening programmes.

Actinomycetes have been, for decades, one of the most important sources for the discovery of new antibiotics; an important number of drugs and analogs have been successfully introduced into the market and are still used today in clinical practice (Genilloud, 2017). In the course of screening for antimicrobial substances producing actinomycetes, four antibiotic-producing isolates (isolate 1, 2, 3 and 4) were recorded from soil samples taken in Alba'qa, Jordan. The isolate 1 had an antimicrobial effect on *Streptococcus pyogenes* SQ33*, Escherichia coli* SQ21 and *Staphyloccocus epidermidis*

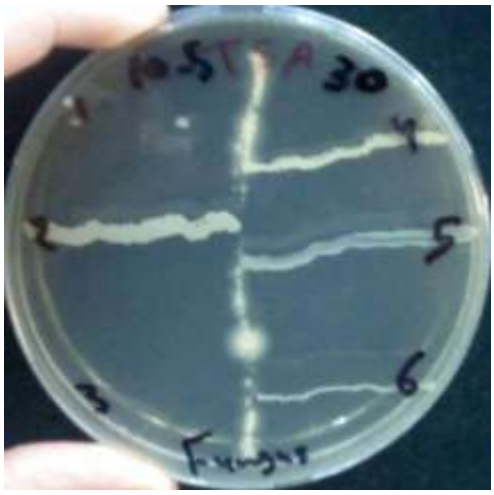

Figure 7. The effect of the incubated temperature on the antimicrobial activity of the strain 3 incubated at 30°C, for 24.

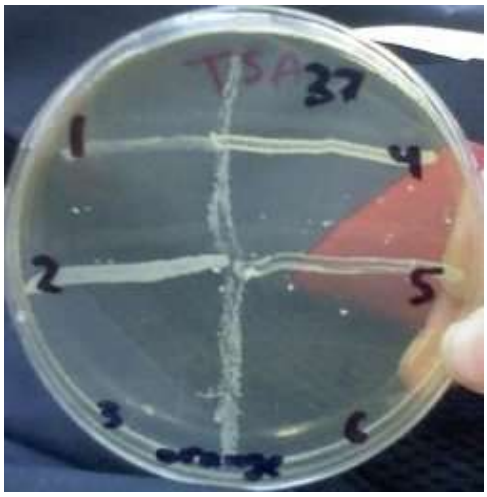

Figure 9. The effect of the incubated temperature on the antimicrobial activity of the strain 1 incubated at 37°C, for 24.

Figure 8. The effect of the incubated temperature on the antimicrobial activity of the strain 4 incubated at 30°C, for 24.

Figure 10. The effect of the incubated temperature on the antimicrobial activity of the strain 2 incubated at 37°C, for 24.

SQ32. Isolate 2 had an antimicrobial effect on *Serratia marcescens* SQ22 and *S. epidermidis* SQ32. Isolate 3 had an antimicrobial effect on *Bacillus* spp SQ41, *Staphylococcus aureus* SQ31, *E. coli* SQ21, *Streptococcus pyogenes* SQ33 and *S. epidermidis* SQ32. While isolate 4 had an antimicrobial effect *on Bacillus spp* SQ41, *Streptococcus pyogenes* SQ33 and *S. epidermidis* SQ32. A-4 isolated by Nanjwade et al. (2010a) showed broad spectrum of activity against both Gram-positive and Gram-negative organisms as well as antifungal activity. Three isolates (Ab18, Ab28 and Ab43) have shown high antagonistic activity against resistant pathogens, during the primary screening (Bizuye et al., 2013). As stated earlier, actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances (Waksman, 1954). The results demonstrate that the type of culture medium, and incubation temperature have a significant effect on the antimicrobial agent production capability of antibiotic producing organisms. The preferred medium in this experiment was GYE, may be due to its enriched nature, and the preferred temperature being 30°C. This is in correlation with prevailing climate at the site of isolation, since the average maximum temperature at the site is approximately 30°C. In order to achieve maximum antibiotic production, experiments were conducted by James et al. (1991) and Nanjwade et al. (2010b to optimize the various parameters such as carbon source, nitrogen source, temperature, pH, DO2, and micronutrients

Figure 11. The effect of the incubated temperature on the antimicrobial activity of the strain 3 incubated at 37°C, for 24.

Figure 12. The effect of the incubated temperature on the antimicrobial activity of the strain 4 incubated at 37°C, for 24.

etc. Isolating and screening action myceters from such areas in optimum conditions may contribute to the discovery of new antibiotics (Bizuye et al., 2013).

Conclusion

The findings from this investigation reveal that strains *Actinomyces* 1 and 2, *Aspergillus* and Gram positive bacterial rods were isolated from soils of Alba'qa region, Jordan. Actinomycetes showed antimicrobial effect on all the 6 bacteria used "*E. coli* SQ21, *S. aureus* SQ31, *S. pyogenes* SQ33, *S. marcescens* SQ22, *S. epidermidis* SQ32 *and Bacillus* species SQ41" depending on the media they were incubated on and the temperature they were incubated at. And it was established that the optimum antibacterial activity is shown when cultivated on GYE medium at 30°C.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(35), pp. 857-865, 21 September, 2018
DOI: 10.5897/AJMR2018.8951
Article Number: A29109358751
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Microbial contamination of the non-alcoholic beverage *Gnamakoudji* made from *Zingiber officinale* in Daloa, Côte d'Ivoire

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Received 30 July, 2018; Accepted 29 August, 2018

Street food has become unavoidable in the current scheme of restoration in urban Africa. This study aimed to study the microbiological contamination of a refreshing artisanal drink, *Gnamakoudji*, made from *Zingiber officinale* sold in the city of Daloa (Côte d'Ivoire). Physicochemical tests showed that *Gnamakoudji* is an acidic drink (pH ≈ 3.83) and very sweet (0.2 gmL^{-1}). Microbiological analyses revealed a high level of contamination. For mesophilic aerobic germs, the CFU/mL load ranged from 6.5×10^9 to 3.9×10^{10}. The CFU/mL load for yeasts and molds ranged from 1.5×10^6 to 1.4×10^7. Total coliforms and enterobacteria ranged between 2.2× 10^4 to 1.7×10^5 CFU/mL for the first and 1.7× 10^4 to 9.5x 10^4 CFU/mL for the second. Worse, pathogenic bacterial species have been found in the *Gnamakoudji* of all neighborhoods. Gbokora and Grand-marche *Gnamakoudji* were contaminated with *E. coli* alone with 5x10^5 and 4x10^5 CFU/mL, respectively. That of the other neighborhoods contained both *E. coli*, *S. aureus* and *Salmonella* sp. The charges in *E. coli* and *S. aureus* oscillated between 4×10^4 and 1.4×10^5 for the first and 2.5×10^4 to 5×10^4 CFU/mL for the second. *Salmonella* sp was found in samples from the other four quarters (Commerce, Tazibouo, Labia and Soleil) with loads that ranged from 10^5 to 1.4×10^5 CFU/mL. These colony counts were unequally distributed and far exceeded the microbiological standards for juices and fruit drinks. The empirical manufacture of *Gnamakoudji* and the difficult sales conditions would increase the risk of contamination. The street *Gnamakoudji* is unfit for human consumption. Strategies to ensure the availability of a healthy *Gnamakoudji* must be put in place with government authorities, the private sector and consumers. This scientific data could help to develop codes of practice for the safety of *Gnamakoudji* in order to avoid diseases transmitted by street foods.

Key words: Microbial *contamination*, *gnamakoudji*, *street food, Daloa*, *Côte d'Ivoire.*

INTRODUCTION

In recent decades, street food has become an essential source of food in the major cities of developing countries. These foods are defined as ready-to-eat foods and beverages prepared and/or sold by street vendors or fixed vendors (FAO, 2014a). Rapid urbanization and the multiple constraints associated with it such as the distance between workplaces and home, poverty, the development of women's activity, the breakdown of family

solidarity, and the emergence of new food styles would explain the expansion of this activity in Africa (Compaoré et al., 2008). The city of Daloa with 5,305 km^2 of area is the third most populous city of Côte d'Ivoire after Abidjan and Bouake. Its population increased from 163,537 in 1998 to a population of 245,350 at the last general census in 2014 (RGPH, 2014; Zah, 2015). Today, the population is estimated at more than 288,000 inhabitants.

With rampant urbanization, Daloa is also confronted with profound changes in lifestyles, work activities, family and social relations, which crystallize the problem of food security. Thus, this constantly growing population with the many administrative changes (commune, sub-prefecture, department and region, university town), has a clearly growing need for food, and street food has become a major source of food. Local craft drinks made from local fruits or vegetables are part of street foods. Of these, the drink *Gnamakoudji* (based on ginger (*Zingiber officinale*)) is very well known. This non-alcoholic, hot-tasting traditional drink is appreciated and abundantly well-known in many countries of the sub-region, as is the case in Côte d'Ivoire (Nandkangre et al., 2015; Mpondo et al., 2017). This drink, extracted from the rhizome, is a refreshing drink during weddings or baptisms. It is consumed alone as in family (Nandkangre et al., 2015). In Daloa, it is sold along roadsides, in markets and areas with high population densities such as schools and bus stations. Because of its cost accessible on all budgets, this refreshing drink is an important part of people's eating habits. However, during processing, this drink can be contaminated by various microorganisms including pathogens.

In addition, street foods are known to be frequently associated with diseases and several microbiological analyses have revealed the presence of many pathogenic microorganisms with loads exceeding the standards (Barro et al., 2007; Chenouf et al., 2014; Mbadu et al., 2016). In addition, several outbreaks of disease have been attributed to beverages in various parts of the world and some infections have been reported in populations consuming these street foods (WHO, 2010; Mihajlovic et al., 2013; Kouassi et al., 2018). If the diet of this drink has proven positive aspects, the microbiological quality remains doubtful (Kouassi et al., 2012; Bayoï et al., 2014; Mbadu et al., 2016). Except, to our knowledge, no study on the quality of this soft drink sold in the city of Daloa has yet been the subject of scientific study. It is in this context that this study falls under the theme: Microbial contamination of a non-alcoholic soft drink sold in the streets of Daloa: the case of *Gnamakoudji* (*Z. officinale*). The overall objective of this study is to evaluate the microbial contamination of this non-alcoholic refreshing drink sold in the streets of Daloa. It also aims to diagnose the manufacturing process, looking for physicochemical parameters of this refreshing drink sold in the streets of Daloa. The information obtained can be used to educate producers and consumers about the quality and safety of this drink produced in the city of Daloa, or even throughout the country.

MATERIALS AND METHODS

The study material consists of the non-alcoholic drink called *Gnamakoudji* sold in the streets of Daloa. This drink is made from ginger (*Z. officinale*).

Investigation for the diagnosis of the manufacturing process of *Gnamakoudji*

Information on *Gnamakoudji* drink manufacturing processes was collected from 10 producers in the city of Daloa. The information concerned the different stages of the manufacture of the *Gnamakoudji* drink.

Sampling

Samples of *Gnamakoudji* were harvested in six (6) main districts of Daloa City (Commerce, Tazibouo, Gbokora, Labia, Grand marche and Soleil). In each neighborhood, three samples each consisting of three bottles (250 to 330 mL) were collected at the same point of sale. The various points of sale were either on the roadsides, in the markets or in areas with high population densities such as schools and bus stations. In these different outlets, drinks are exposed to room temperature. Samples once taken are stored in a cooler with dry ice and transported to the laboratory for analysis. These analyses were done at the Laboratory of Host-Microorganisms and Evolutions Interactions (LIHME) of University.Jean Lorougnon Guede.

Physicochemical characterization of *Gnamakoudji*

Determination of the pH

The determination of the pH is carried out using a pH-meter (PHS-38W). A quantity of 150 mL of the sample was used for this purpose. Three (3) measurements from one sample of 150 mL were performed.

Determination of Brix degree and amount of sugar in Gnamakoudji

The degree of Brix was measured using a portable refractometer (ATAGO N-1α) measuring up to 30 Brix. Three measurements were performed per sample. For the determination of the amount of sugar in the beverages, the volume of beverage contained in the

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bottle was spilled in a beaker and weighed. From Brix degrees and determined beverage masses, the amount of total sugars contained in a bottle of *Gnamakoudji* was calculated according to the formula described by Monrose (2009):

Quantity of sugar in the product (g) = (Brix degree of the product / 100) × Amount of product (g)

Microbiological characterization

Methods of analysis

Buffered Peptone Water (BPW) broth was used in the preparation of stock solutions as described in ISO 6887-4: 2011. Decimal dilutions were performed with Tryptone Sel broth as recommended in ISO 6887-1: 1999. Plate Count Agar (PCA) was used to count mesophilic aerobic flora at 30°C for 72 h as recommended in NF/ISO 4833: 2003. Enterobacteria count was performed at 37°C for 24 h on Violet Red Neutral Bile Glucose (VRBG) agar according to ISO 21528-2: 2004. Violet Neutral Bile Lactose (VRBL) agar was used for total coliform count at 30°C for 24 h as described in ISO 4832: 2006. For the search, isolation and enumeration of *Salmonella*, media Buffered Peptone Water (BPW), broth Rappaport of Vassiliadis Soya Broth and Hecktoen Enteric Agar were used as described in the reference standard NF/ISO 6579: 2002 Amd 1: 2007. Baird-Parker Agar with Telluride Egg Yolk and 0.2% Sulphamethazine served the identification of *Staphylococcus aureus* at 37°C for 48 h according to the French standard NF/ISO 6888: 2004. Rapid'E coli 2 agar served the isolation and enumeration of *Escherichia coli* at 44°C for 24 to 48 h as recommended in standard NF/ISO 16140: 2013. Yeasts and molds were counted with Sabouraud agar chloramphenicol 25°C for 5 days according to the NF/ISO 16212: 2011 standard. The different culture media used were prepared according to the manufacturers' instructions.

Preparation of the stock solution and decimal dilutions

Twenty-five milliliters (25 mL) of *Gnamakoudji*'s sample was aseptically transferred to an Erlenmeyer flask containing 225 mL of sterile (BPW) medium to prepare the stock solution. After a 1 hour rest on the bench at room temperature, the stock solution was decimally diluted in sterile Tryptone Salt medium up to 10^{-6}.

Inoculations and incubations

Research of mesophilic aerobic germs, yeasts and molds, total coliforms, enterobacteria and Escherichia coli

According to the prescriptions of the standards adopted, the pour plate method was applied. Thus, one milliliter (1 mL) of the diluted *Gnamakoudji* sample to be analyzed is aseptically transferred to a sterile Petri dish and mixed with 20 mL of the respective agar. After solidification, the dishes are inverted and incubated at temperatures as given in the respective standard. Three Petri dishes were inoculated per dilution. The characteristic colonies according to the different media are then counted taking into account the calculation standard (NF/ISO 7218: 2007).

Research of Staphylococcus aureus

The surface spreading method was used for the detection of *S. aureus*. It consisted of taking 0.1 ml of the stock solution or a dilution of the *Gnamakoudji* sample to be analyzed, using a sterile pipette, and transferring to a Petri dish containing the Baird Parker agar medium already poured and solidified. The dilution is spread on the agar using a spreader rake. These manipulations are all performed under aseptic conditions near the Bunsen burner flame. Petri dishes are then inverted and incubated at 37°C for 48 h. Two plates of petri were seeded by dilution. Black colonies with a clear halo (action of lecithin) and an opaque zone (action of lipase) are counted (15-150 characteristic colonies) taking into account the dilution.

Highlighting Salmonella sp.

It is done in three stages. Pre-enrichment is performed by incubating the stock solution at 37°C for 24 h. The enrichment consisted of taking 0.1 mL of the stock solution (pre-enriched) and transferred to a tube containing 10 mL of Vassiliadis Rappaport previously prepared and sterilized. After homogenization, the tube is incubated at 42°C for 24 h. Finally the isolation was carried out from the enrichment medium incubated on a solid selective medium: Hecktoen agar. A drop is taken using a Pasteur pipette and then seeded by streaks on the surface of the Hecktoen agar. The dish is incubated at 37°C for 24 h, and sometimes even for 48 h, in the absence of characteristic colonies after the first incubation. On Hecktoen agar, the typical *Salmonella* colonies observed are green or blue with a black center.

Enumeration

The number of Colony Forming Units per milliliter of sample (CFU/mL) from the number of colonies obtained in the Petri dishes is carried out according to standard NF/ISO 7218: 2007

$$N = \frac{\Sigma C_i}{(N_1 + 0.1N_2)\, d \cdot V}$$

ΣCi: Sum of characteristic colonies counted on all retained Petri dishes;
N_1: Number of Petri dishes retained at the first dilution;
N_2: Number of Petri dishes retained at the second dilution;
d: Dilution rate corresponding to the first dilution;
V: Inoculated volume (mL);
N: Number of microorganisms (CFU/mL).

Standards for assessing the microbiological quality of Gnamakoudji

The microbiological quality assessment standards for *Gnamakoudji* are taken from the “Microbiological Criteria for Foodstuffs - Guidelines for Interpretation of 2015 of Luxembourg”.

Statistical analyses

The different parameters analyzed were then subjected to an analysis of variance (ANOVA) with the software Statistica, 99 Edition. For this purpose, a single-factor ANOVA and Duncan's multi-extended tests were used. ANOVA was used to test, on the one hand, the variability between the different samples. As for Duncan's test, he later made it possible to first locate the differences between the samples and then the differences between them. Statistical differences with P-values under 0.05 were considered significant.

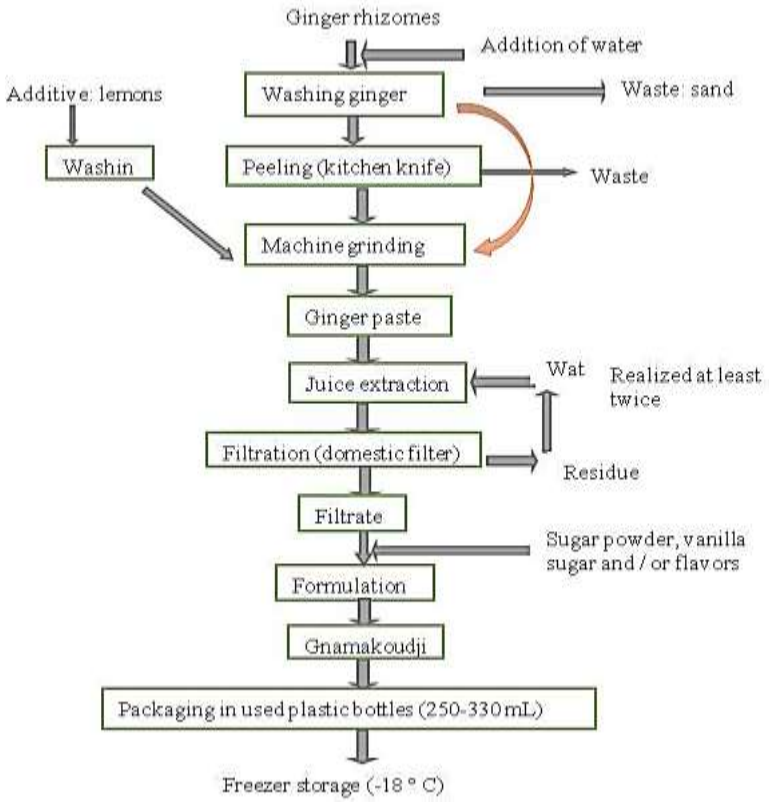

Figure 1. Flow-Diagram of homemade *Gnamakoudji*.

Table 1. Physicochemical characteristics of *Gnamakoudji* sold in the main districts of Daloa.

Variable	pH	Brix degree	Sugar level (g / mL)
Commerce	3.90 ± 0.18[b]	19.53 ± 7.51[d]	0.22 ± 0.03[b]
Tazibouo	3.84 ± 0.49[b]	19.73 ± 4.40$_d$	0.22 ± 0.04[b]
Gbokora	3.61 ± 0.09[a]	18.07 ± 1.11[b]	0.19 ±0.01[ab]
Labia	3.68 ± 0.10[a]	16.93 ± 1.38[a]	0.17 ± 0.01[a]
Grand-marche	3.78 ± 0.20[a]	18.60 ± 0.67[c]	0.20 ± 0.03[ab]
Soleil	4.04 ± 0.11[b]	18.87 ± 0.29[c]	0.21 ±0.01[b]
Mean ± standard deviation	3.81 ± 0.12	18.62 ± 1.10	0.20±0.01[ab]

In columns, the values assigned to the same letter are not significantly different from (P> 0. 05).

RESULTS

Evaluation of the manufacturing process of *Gnamakoudji*

All the information collected from the manufacturers, unit processing operations are essentially the same, with the exception of the rhizome peeling stage which is not carried out by some manufacturers. These operations are carried out on the same production site except the grinding phase which is carried out in another site (market, or grinding machine site). The essential operations are illustrated by the *Gnamakoudji* manufacturing diagram (Figure 1). However, no heat treatment is associated with the manufacture of this drink.

Physicochemical characteristics of *Gnamakoudji*

The physicochemical studies carried out on the various drinks made it possible to determine the Brix degree and the pH summarized in Table 1. *Gnamakoudji* is an acidic drink with an average pH of 3.81 ± 0.12. The Brix degree ranged between 16.93 and 19.73. The estimated

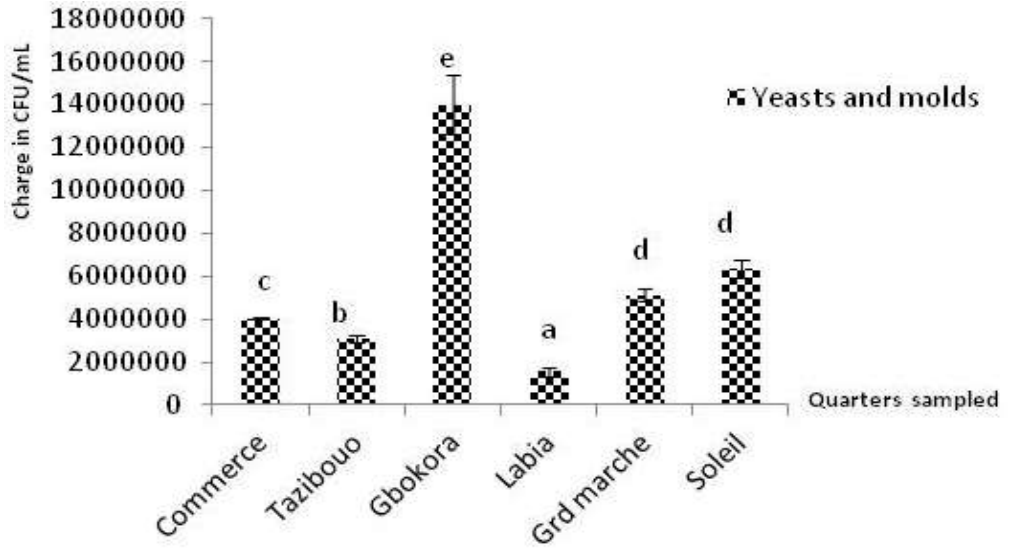

Figure 2. Numbers in CFU/mL of yeasts and molds in *Gnamakoudji* according to neighborhoods. Values with the same letters are not significantly different (P>0.05).

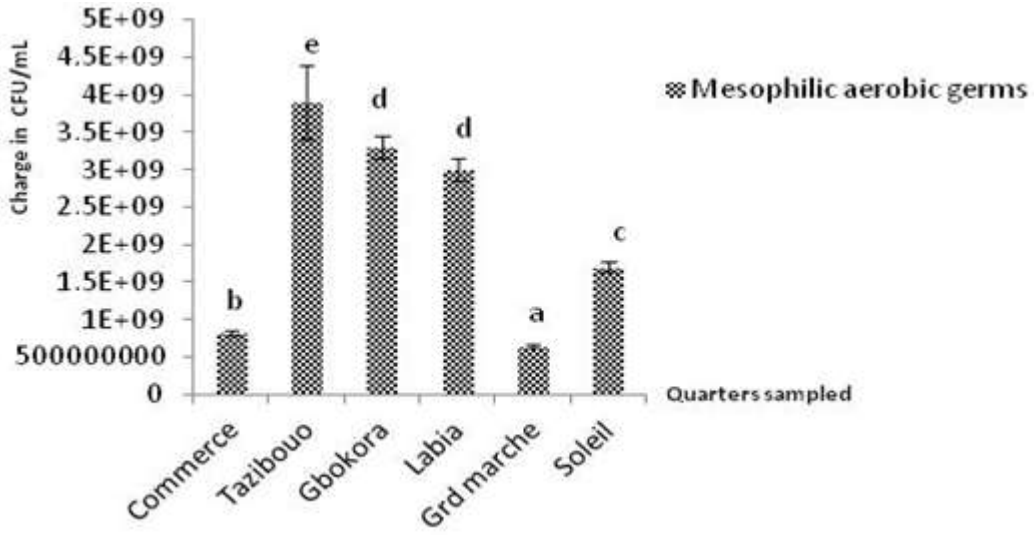

Figure 3. Numbers in CFU/mL of mesophilic aerobic germs in *Gnamakoudji* according to neighborhoods. Values with the same letters are not significantly different (P>0.05).

Gnamakoudji sugar level from Brix degrees and drink masses is about 0.20 g/mL

Microbiological characteristics of *Gnamakoudji*

The microbiological studies carried out allowed the counting of microorganisms, the main weathering flora or flora evoking a lack of hygiene (yeasts, molds, mesophilic aerobic germs, total coliforms and enterobacteria) and pathogenic species in *Gnamakoudji*. Drinks from all sampled neighborhoods were heavily contaminated by these different floras. In addition, all flora charges (CFU/mL) were all well above the expected microbiological quality standards (norm NF in 2073/2005/CE). The CFU/mL load for yeasts and molds ranged from 1.5×10^{6} to 1.4×10^{7}, whereas the standard predicts 10^{5} (Figure 2). The counts found in the *Gnamakoudji* neighborhood Soleil and Grand-marche were not statistically different (p> 0.05). For mesophilic aerobic germs, the CFU/mL load ranged from 6.5×10^{9} to 3.9×10^{10} while the standard indicates 10^{6} (Figure 3),

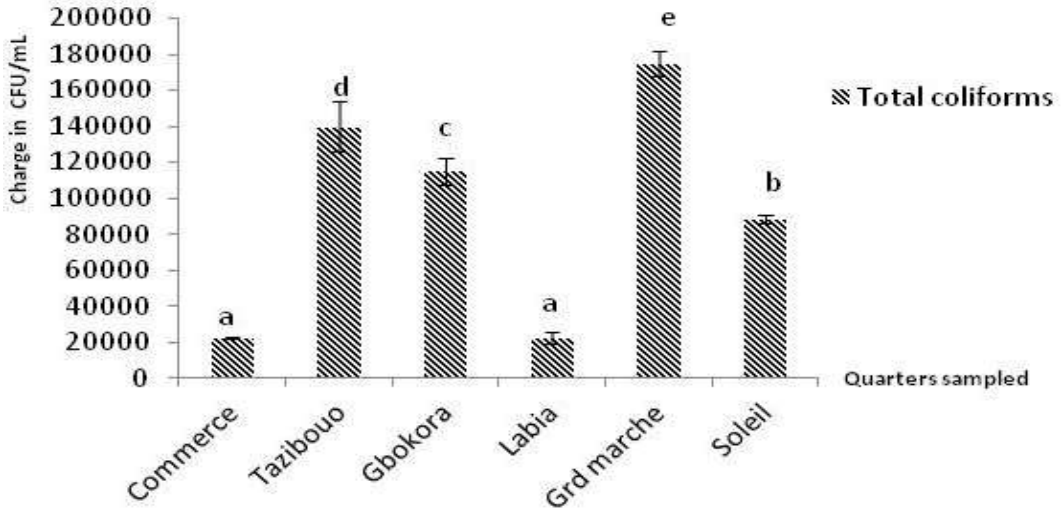

Figure 4. Numbers in CFU/mL of total coliforms in *Gnamakoudji* according to neighborhoods. Values with the same letters are not significantly different (P>0.05).

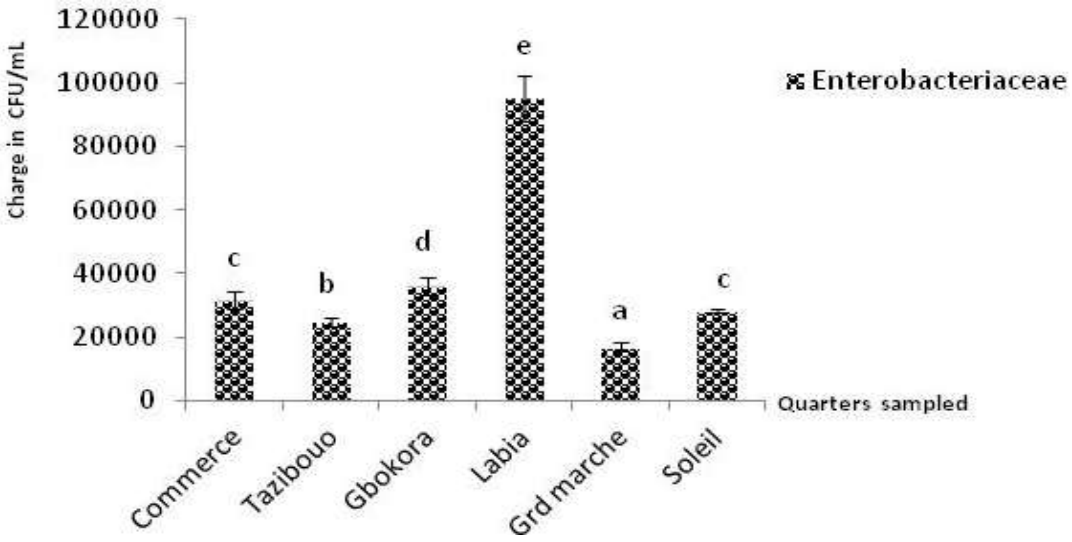

Figure 5. Numbers in CFU/mL of enterobacteria in *Gnamakoudj*i according to neighborhoods. Values with the same letters are not significantly different (P>0.05).

however, *Gnamakoudji* loads from Gbokora and Labia neighborhoods were not statistically different (p> 0.05).

Total coliforms and enterobacteria ranged between 2.2×10^4 to 1.7×10^5 CFU/mL for the first and 1.7×10^4 to 9.5×10^4 CFU/mL for the second while the expected standard is 10^3 CFU/mL (Figures 4 and 5). For total coliform loads in the Commerce and Labia neighborhoods, there was no statistically significant difference (p> 0.05); those in enterobacteria of the Soleil and Commerce districts also did not statistically show any difference (p> 0.05). In addition, the colony counts of the flora were also unevenly distributed. If *Gnamakoudji* from the Tazibouo district was more contaminated by mesophilic aerobic germs (3.9×10^{10} CFU/mL), the Gbokora District contained the highest yeast and mold load (1.4×10^7 CFU/mL); *Gnamakoudji* in the Grand Marche District had the highest total coliform load (1.7×10^5 CFU/mL) and the Soleil district, the highest level of enterobacteria (9.5×10^4 CFU/mL).

Pathogenic bacteria species were detected all the drinks of different neighborhoods (Figure 6). Gbokora and Grand-marche *Gnamakoudji* were contaminated only with *E. coli* with 5×10^5 and 4×10^5 CFU/mL, respectively; loads higher than the standard of 10^2. Other beverages from

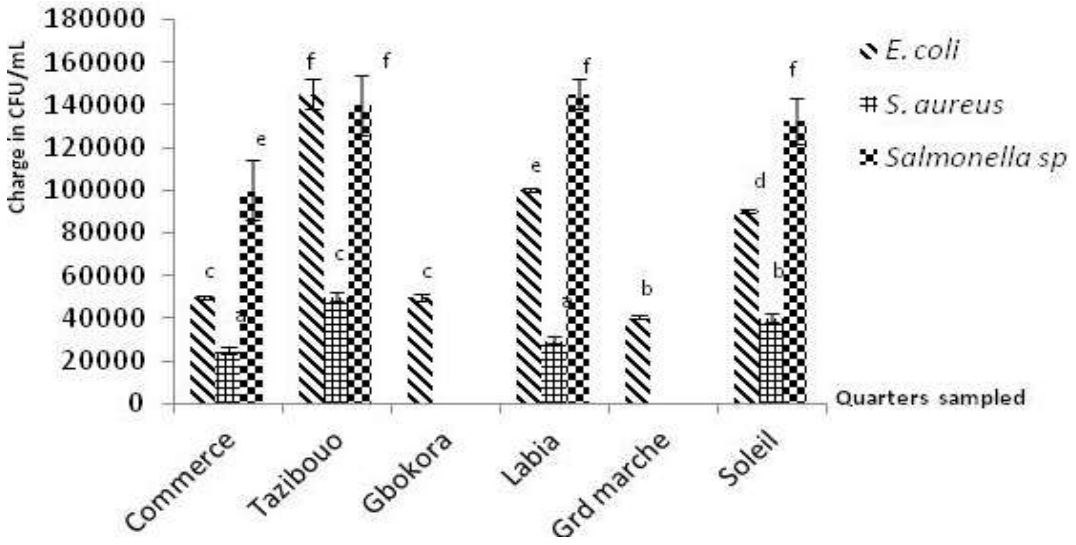

Figure 6. Numbers in CFU/mL of pathogenic bacterial species in *Gnamakoudji* by neighborhood. Values with the same letters are not significantly different (P>0.05).

other neighborhoods contained both *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp with heavy loads exceeding the microbiological standards for juices and fruit drinks. The *E. coli* and *S. aureus* loads of all beverages ranged from 4×10^4 to 1.4×10^5 for the first and 2.5×10^4 to 5×10^4 CFU/mL for the second while the standard tolerated only 10^2 for both species. Where the microbiological standard for *Salmonella* sp is a total absence in 25 mL of beverage, loads of 10^5 to 1.4×10^5 CFU/mL were noted for Commerce, Tazibouo, Labia and Soleil samples. The burden of pathogenic species varied from one district to another. Tazibouo's *Gnamakoudji* was more contaminated than those in other neighborhoods; it contained both the largest *E. coli* and *S. aureus* loads of 1.4×10^5 and 5×10^4 CFU/mL, respectively. Labia's had the largest load of *Salmonella* sp. (1.4×10^5 CFU/mL). The *E. coli* loads of *Gnamakoudji* from Commerce and Gbokora were not statistically different (p> 0.05). Those in *S. aureus* from the Commerce and Labia neighborhoods were not as statistically different (p> 0.05). *Salmonella* sp (CFU/mL) contamination of *Gnamakoudji* from Tazibouo, Labia and Soleil neighborhoods was not statistically different (p> 0.05). The *Gnamakoudji* sold in the streets of Daloa is identified as a source of microbiological hazards, which would cause multiple diseases such as diarrhea, gastroenteritis, typhoid and paratyphoid fevers. Its consumption constitutes a real risk of infection or a source of food poisoning which can lead to a public health problem.

DISCUSSION

The flow diagram of artisanal production of *Gnamakoudji* compared with those presented in the work of Agassounon et al. (2011) and those of Ndiaye et al. (2015) on other close-up craft drinks were almost the same. Moreover, according to the work of Ndiaye et al. (2015), throughout West Africa, fruit juice and beverage product flow diagrams show the same unit operations with some differences in formulations. But, these empirical manufacturing methods do not guarantee the stability of the productions. However, the physicochemical stability and the microbiological quality of *Gnamakoudji* depend in particular on the raw material, the formulations and the various technological treatments including pasteurization, chemical preservatives and stabilized final pH. Physicochemistry showed that *Gnamakoudji* was an acidic drink. This acidity is due to the liberation of many organic acids (contained in the rhizomes of ginger in the juice (Ali et al., 2008; Park et al., 2008; Shimoda et al., 2010). The pH of this non-alcoholic beverage is satisfactory because according to CODEX-STAN 243-2003, the pH of fruit juices and beverages must be less than 4.5. With regard to the sugar content of *Gnamakoudji*, consumption approximately 330 mL (more than 50 g of sugar) would put the consumer at the limit of the tolerable amount recommended by the WHO (less than 50 g of sugar per day).

Over-consumption of non-alcoholic but very sweet *Gnamakoudji* could have adverse effects on health and lead to cardiovascular disease (Johnson et al., 2009, Yang et al., 2014), obesity (Sievenpiper et al., 2012; Wang et al., 2013), diabetes (Basu et al., 2013) and dental caries (Moynihan and Kelly, 2014). This 18% sugar content is lower than the levels that would limit microbial growth (between 65 and 67%) based on Boumendjel's work (2005). Microorganisms could therefore develop easily in said drinks despite relatively low pH. Indeed, at pH close to 4, germs are not

eliminated but have a slow growth. The microbiological study showed the contamination of *Gnamakoudji* by flora of deterioration and insalubrity resulting in a deficit of good production practices. Worse, pathogenic species are found there. This contamination is due, on the one hand to the process of transformation of the drink and on the other hand to the intrinsic characteristics of this drink.

According to the manufacturing process, ginger is often ground with the skin during the manufacture of *Gnamakoudji*. Like simple washing, no disinfection operation has been undertaken to neutralize the microorganisms of the soil on this skin. In addition, the hygienic state of mechanical grinders in community markets is never mastered. The multiplicity of products crushed by these machines could be considered as a cross-contamination site par excellence. Also, the operation of extraction of the juice would be carried out with bare hand. The hands would carry most of the germs of contamination, even faecal, when hygiene is not practiced. It should be noted, however, that the drink obtained was always packaged in jars already used or recycled; anything that does not guarantee their safety.

Moreover, according to the work of Bayoï et al. (2014), manufacturing processes greatly influence the microbiological quality of artisanal beverages. In a study on food packaging, recycled packaging is strongly discouraged as it may contaminate food (FAO, 2014b). Hygiene indicators such as *E. coli*, coliforms and enterobacteria have been detected at loads well in excess of standards. These germs could be responsible for serious food infections. Moreover, in the recent work of Kouassi et al. (2018), street food consumers in the same city reported gastroenteritis, diarrhea, vomiting and fevers from consuming foods such as fruit juices and drinks, or dairy products. These microorganisms indicative of a lack of hygiene have also been highlighted in other artisanal drinks by other authors such as Aawi (2000), Kruy et al. (2001) and Baba-Moussa et al. (2006).

In addition, the total absence of heat treatment throughout the manufacturing process would explain the very high load of indicator bacteria mesophilic aerobic germs in *Gnamakoudji*. The presence of these germs in artisanal beverages has also been reported by Mbadu et al. (2016). Poor conservation would favor the process of alteration of *Gnamakoudji*. Indeed, this drink is sold in coolers and jars without ice to maintain the temperature at 4°C, without omitting the phases of freezing and thawing. The breaking of the cold chain could cause the accelerated growth of certain microorganisms.

According to the work of Agassounon et al. (2006) and those of Folefack et al. (2008), in addition to manufacturing processes, the contamination of artisanal juices and beverages is also related to the socio-geographical environment of manufacture and / or sale of these juices or drinks. The fabrications were made in homes with uncontrolled environment. Under these conditions, these drinks would be subject to all kinds of contamination according to Koné (2014) in a study of the control of contamination of fresh drinks. The less acidic pH could help the growth of these germs. The presence of pathogenic bacterial species such as *S. aureus*, *E. coli* and *Salmonella* sp is a real danger for consumers of this drink. Apart from samples from Gbokora and Grand-marche districts, where the drinks were contaminated with only *E. coli,* the *Gnamakoudji* from the other quarters was contaminated by the three pathogenic species in combination: *S. aureus*, *E. coli* and *Salmonella*; anything that would not guarantee food security. Similar studies have reported not only common but also pathogenic germs in beverages (Agassounon et al., 2009; Amusa et al., 2009). The consumption of street *Gnamakoudji* poses a real risk of dietary infection for Daloa populations.

Conclusion

Street food has become unavoidable in the current development of urban catering, as is the case in Daloa. The physicochemical and microbiological analyses revealed that the consumption of the *Gnamakoudji* drink sold in the city of Daloa would pose a risk to the health of the consumer. High loads of coliforms, enterobacteria and mesophilic aerobic germs have been discovered. Pathogenic bacterial species such as *E. coli*, *S. aureus* and *Salmonella* sp even in high numbers have been detected in street *Gnamakoudji*. As a result, consumption poses a real danger to the health of consumers. The upstream empirical manufacturing process and the difficult sales conditions would increase the risk of contamination and growth of microorganisms. Thus, the competent authorities must inform and raise awareness of the health risks to consumers. A regulation of street food could limit the risk of contamination. Awareness of hygienic measures such as routine hand washing before and during production would reduce the risk of contamination. Adequate measures must be taken during manufacture to produce a less risky *Gnamakoudji*. These measures may include decontamination of the raw material with all equipment used, hot packaging, wearing gloves, heat treatment at the end of the preparation of the drink; which would limit transmissions of germs. Good communication about the dangers of eating street foods would help to improve the well-being of consumers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 12(35), pp. 866-872, 21 September, 2018
DOI: 10.5897/AJMR2016.7934
Article Number: 974036958753
ISSN: 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Handling conditions and microbial contamination of fish from Ouagadougou markets in Burkina Faso

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Received 26 January, 2016; Accepted 17 June, 2016

The purpose of this study was to analyze the handling conditions and microbial contamination of fish from Ouagadougou markets. In spite of the fact that fish consumption is linked to several events of alimentary infection in Burkina Faso, fewer studies were dedicated to this issue. The study was conducted using two approaches. First, an investigation conducted with fish sellers to evaluate handling hygienic conditions of fish. Secondly, microbiological analysis conducted to assess the microbiological quality of fish. Total and faecal coliforms, *Staphylococcus aureus*, fungic flora, *Salmonella* sp., *Shigella* sp. and *Escherichia coli* were the main groups found on fish specimens from Ouagadougou markets. The results showed also a predominance of women in the fish handling domain, sellers mainly illiterate, without any training in good hygienic practices (GHP). The consequences on microbiological level, was an abundance of *S. aureus* mainly on smoked fish. 57.5, 55 and 7.5% of samples have been found unsatisfactory (not good for consumption) respectively according to their contamination by *S. aureus*, faecal coliforms and fungus. Besides, *Shigella* sp. was found on 1% of sample, *E. coli* 15% and *Salmonella* sp. 25%.

Key words: Fish, coliforms, staphylococcus, market, Ouagadougou.

INTRODUCTION

Fish is an important dietary component of people all around the world and represents a relatively cheap and accessible source of high quality protein for poorer households (Ikutegbe and Sikoki, 2014). In the world, fish consumption per habitant raised up from 10 kg in the 60s to 19 kg in 2012 (FAO, 2014). In Africa, more than 200

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million of persons consumed regularly fresh, smoked and dried fish (World Fish Center, 2005). In Burkina Faso, due to financial and economic crises (like devaluation which happened in January 1994), livestock exportation has considerably increased. This situation induced an expensiveness of meat and a shift from meat to fish consumption (Coulibaly et al., 1995). Fish is consumed fresh or transformed (smoked, fried or roasted). In urban area, fish is mainly sold fresh on markets. In rural areas, transformed fish is the most sold. In Burkina Faso, fish consumption was 1.4 kg/habitant in 2003 (FAO, 2009). However, due to the microbial contamination already found by some authors, the consumers are in risk. Barro et al. (2006) and Abdelrahim et al. (2012) reported some contamination of transformed fish by coliforms, *Escherichia coli* and some enteropathogenic bacteria, in street in Burkina Faso. In spite of this, microbiological quality of fish is not sufficiently analyzed in Burkina Faso and there is no data about handling condition and microbiological contamination of fresh fish produced and imported in Burkina Faso, this is why we sought into it. People selling fish on the street in a lack of hygiene practices could be the related factor of fish contamination. Using survey and microbiological analysis, this study aimed to highlights the bad handling conditions of fish in Ouagadougou markets and it consequence on fish contamination. Specifically, it evaluated the handling hygienic conditions of fresh, roasted, fried and smoked fish, and the microbiological quality of those fish.

MATERIALS AND METHODS

Study area and handling condition survey

The study was carried out in Ouagadougou, the capital city of Burkina Faso. Fresh, smoked, fried and roasted fish samples were collected from May to November 2013, on eleven locations throughout the city (Figure 1). Fifty one (51) fish sellers were interviewed using a set of questions, aiming to determine sellers' population characteristics (age, sex, level of instruction, level in training in Good Hygiene Practices (GHPs). Handling condition were analyzed with regard to the Sanitation Standard Operating Procedures (SSOPs): Hair protection, hands washed before selling, use of gloves and kitchen smock; the hygienic conditions of trading place (surrounding wastes, tap water availability, implement and utensil conditions).

Sampling

Sampling was done by making a purchase of fish from seller at the sales peak hours (7 am to 6 pm for fresh and smoked fish; 6 pm to 11 pm for fried and roasted fish). A total of 40 fish samples were collected under aseptic conditions. They were put in sterile freezer bags, kept cold to under 4°C with an icebox and directly transported to laboratory for microbiological analysis. Once in the laboratory, each specimen was identified according to Paugy et al. (2004), and Diouf (1991) keys. The different types of fish sampled were fresh water fish (fresh and roasted *Oreochromis niloticus*, fresh *Saroterodon galilaeus*, smocked *Clarias anguilaris*) and marine fish (fried *Mugil cephalus* and *Liza* sp.).

Microbiological analysis

Bacteria were isolated and counted using several culture media, with petri dishes. Depending on the type of germ, different culture media have been used. The Violet Red Bile Lactose (VRBL) was used for total and thermo tolerant coliforms and *Escherichia coli* isolation. Mannitol Salt Agar was used for *Staphylococcus aureus,* Sabouraud enriched with Chloramphenicol for toadstool (yeasts and moulds) and Salmonella/Shigella (SS) for *Salmonella* sp. and *Shigella* sp. isolation. Culture media have been sterilized at autoclave 121°C for 15 min, except VRBL and SS which have been warmed to boiling point. They were cooled to about 50°C and poured into petri boxes (15 to 20 ml/box) previously sterilized at 180°C.

Total viable count (TVC)

For total and thermo tolerant coliforms NF.V08-50 and NF.V08-60 was used respectively. Ten (10) gram of different part of each fish sampled was mixed in 90 ml of NaCl 9‰ water with a stomacher. Serial dilutions from 10^{-1} to 10^{-4} were prepared by diluting this mixture. 0.2 ml of each dilution (10^{-2}, 10^{-3} and 10^{-4}) was poured and spread with a sterile small rake on the agar (VRBL) in aseptic conditions. Boxes were incubated in aerobic at 37°C for 24 to 48 h for total coliforms, 44 ± 0,5°C for thermo tolerant coliforms. Fungi (yeasts and moulds) were isolated and grown on Sabouraud Chloramphenicol Agar, incubated aerobically for 48 h at 30° C. For *S. aureus,* NF.V08-57-2 was used. The agar (Mannitol Salt Agar) was incubated in aerobic conditions at 37°C for 24 to 48 h. Presumed *S. aureus* showed shining and pigmented colonies surrounded with a yellow halo due to mannitol fermentation. Nonpathogenic *Staphylococcus* constituted generally red colonies which do not change agar colour. Coagulase test have been done to confirm the presence of *Staphylococcus aureus.*

Salmonella sp. and *Shigella* sp. have been isolated following normalized method (ISO 6579: 2002). 25 g of fish tissues have been homogenized in 225 ml of buffered peptone water, incubated at 37°C for 24 h. One milliliter of this solution was introduced in 10 ml of Rappaport Vasiliadis, incubated at 42°C for 24 h. With a pastor pipette sterilized in alcohol then backling, few microliters were seeded in scores on SS agar, incubated at 37°C for 24 h. Presumed *Salmonella, Shigella* and *E. coli* have been characterized with minimal gallery: Kligler Iron Agar, Simmons Citrate Agar, and Mannitol-Motility Test Medium. Pure bacteria colonies obtained from Müller Hinton agar have been seeded on those culture media, incubated at 37°C for 18 to 24 h. The identification has been confirmed with API 20E gallery, which is a standard method with 20 micro tubes, aiming to realize 20 biochemical tests.

Bacteria count

Counting was done with a colony counter. The technic is based on the principle that each viable bacteria produced one colony, thus the number of colonies on the petri box corresponded to the number of living bacteria contained in the seeded solution. The number of bacteria was obtained using the formula below, where *N* designate the numbers of bacteria, *Nc* the numbers of colonies in each petri dishes, V_{ml} the volume of the inoculum, d_1 the dilution factor and n1 and n2 the numbers of dishes.

$$N = \frac{\sum Nc}{V_{mL\times}(n_1 + 0.1\, n_2) \times d_1}$$

Data analysis

The average numbers of bacteria counted on fish according to their

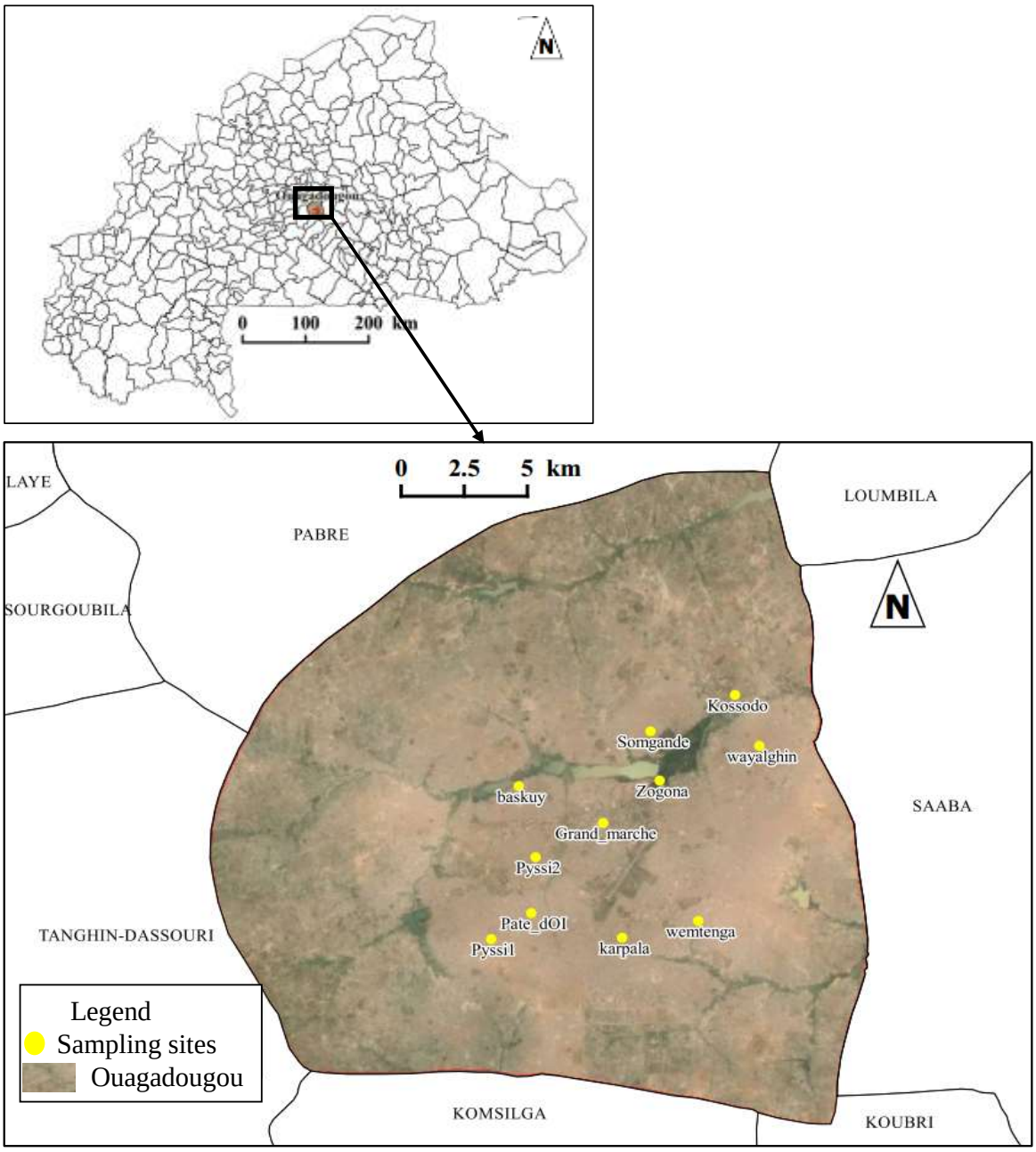

Figure 1. Sampling site in Ouagadougou town.

Table 1. Standards of the European Commission of the appreciation of raw fish and shellfish, fresh or frozen, of smocked fish ready for consumption (Regulation 2073/2005/).

Bacteria	Total coliform (1 g)	Faecal coliform (1 g)	*S. aureus* (1g)	*Salmonella/Shigella* 25 g
Level tolerated	10^3	10	10^2	Absence

type of cooking and the sampling sites were compared using Kruskal-Wallis and Mann-Whitney, respectively. The occurrences of phatogenic germs were assess for cooking type, sampling sites using χ-square test. Assessment of samples microbiological quality was done using European Communities' Commission standards No 2073/2005 of 15 November 2005 (Table 1) because a local regulation aiming to appreciate the food microbiologic quality was not in existence yet in Burkina Faso. Statistical analyses were

Figure 2. Photoes of four fish selling sites in Ouagadougou.

performed with R software version 2.15.1. The significance level was set at p=0.05.

RESULTS

Fish handling condition

For Sanitation Standard Operating Procedures (SSOPs) for fish handling, the study reported that more than fifty percent (50%) of the sellers had no hair protection; over a quarter of this population do not care about hands washing. Moreover, less than twenty five percent (25%) used protection like apron, gloves or bibs during the selling (Figure 3a). Nearly seventy five percent (75%) of sellers handled money and fish at the same time. In addition, a total of 39 selling sites prospected, more than sixty percent (60%) did not have potable water supplied, most of them were near uncovered gutters. Insect were often present in the disposed fish that were sometime uncovered (~40%). The most used packing method was the plastic. In most case there was no waste disposal near the selling site.

For fresh fish conservation and possessing conditions selling (Figures 2b and c), twelve (12) fish shop were prospected. About twenty five percent (25%) of them were broken-down and above 20% did not have a fridge. Most of sellers stored the wastes (liquid and solid) and threw them later in the gutter; some of sellers threw the wastes directly on the selling site. There were animals (sheep, goats, dogs, cats) on all prospected sites. The mean storage time was 24 h and the mean temperature for conservation (for sellers who have fridge) was 4.2°C.

Microbiological contamination

Bacteriological analysis underlined contamination by total coliforms on 67.5% of samples, fecal coliforms on 55%, *S. aureus* on 97.5% and fungi on 87.5%. We found *Salmonella* sp., *Escherichia coli* and *Shigella* sp. respectively on 25, 15 and 1% of fish sample. Smoked fish were the most contaminated. Fresh fish were slightly less contaminated than smoked one. Fried and roasted fish had the lower microbial load. Total and faecal coliforms, and *S. aureus* densities were different according to cooking process with the highest values being observed in smoked fish followed by fresh ones (Figure 3C). However, yeast and mold density did not show significant variation ($p= 0.14$) even if smoked fish present high density of fungi. For pathogenic germs occurrence, fresh fish had the high prevalence in *Salmonella* sp. (p= 0.02). However, the other pathogens prevalence did not show significant difference according

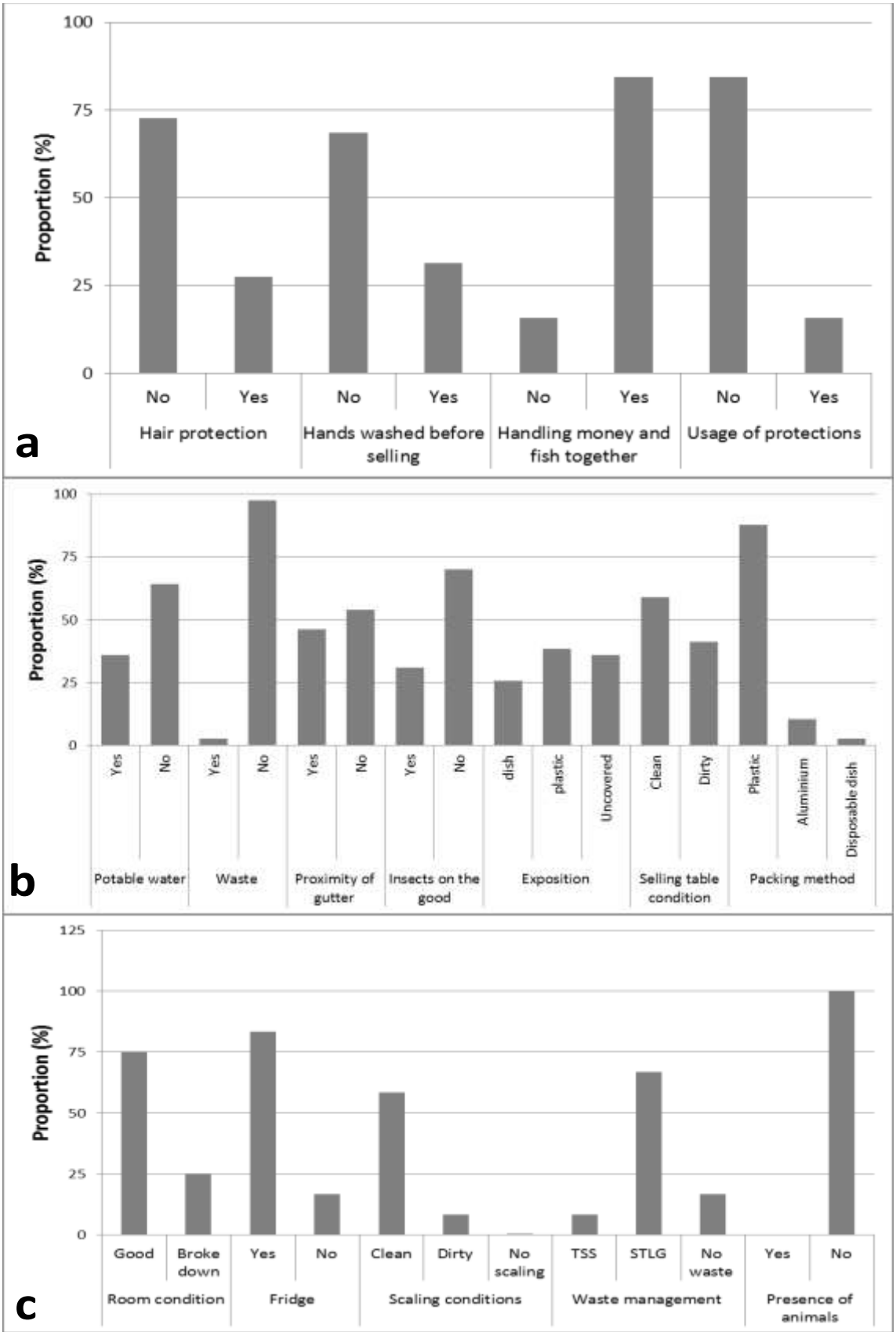

Figure 3. Characteristics of fish sellers' population (a), sellers' attitudes during the selling (b) and selling site conditions (c).

to the cooking type.

Influence of selling conditions

According to the type of site (fixed and semi-fixed site), total coliform and *S. aureus* densities significantly vary (p=0.005 and p=0.013, respectively). However, fecal coliforms (p=0.35) and fungi (p=0.38) mean densities were quite the same between fixed and semi-fixed site. In regard to pathogenic germs, whereas a large predominance of *salmonella* sp. on fixed site (p=0,049) was observed; *Shigella* sp. and *E. coli* occurrence did not depend on site (Table 1).

Food safety assessment

In line with the unhealthy handling conditions of fish underlined by this survey, the microbiological analyses showed according to the European Union Standards, that 55.0 and 57.5% of samples were unsatisfactory respectively regarding fecal coliforms and *S. aureus.* In equal, samples contaminated by *Salmonella* sp, *Shigella* sp. and *E. coli* (respectively 25.0, 1.0 and 15.0%) were also unsatisfactory (Table 1). Unsatisfactory sample were those unappropriated to consumption.

DISCUSSION

Sellers do not respect strictly the good hygienic practices rules because of low instruction level and the lack of training. This have been considered like the main factor of fish contamination in this study, but also in Barro et al. (2006), Tidjani et al. (2011), and El Marnissi et al. (2012). The cooking and the selling of fish occured generally in unhealthy places. This is the same remark with Umoh and Odoba (1999), who have underlined presence of animals, insects and wastes on roasted fish selling site in Nigeria. Particularly fresh fish were processed and sold in unhealthy conditions. Consequently, the microbial loads underlined were above the acceptable levels regarding hygienic indicator germs, and varied considerably according to the cooking type. This result was confirmed not only by Ahmed et al. (2011) during their researches on the impact of smoking on fish in Cameroun, but also by Rebecca (2013) when they studied smoked fish bacteriology in Nigeria. Indeed, Abolagba and Uwagbai (2011), and Adeyeye et al. (2014) underlined a significant difference between fresh and smoked fish microbial load in Nigeria. On all the selling sites prospected, smoked fish were exposed and uncovered, in consequence, they made contact with the wind and dust germs. The high occurrence of *S. aureus* could be explained by the continuous handling of fish not only by the customers, but also by the sellers. This has been demonstrated by Sylla and Seydi (2003) when they studied the fish used in collective catering at Dakar University. The microbial load of smoked *Clarias anguilaris*, is less than that of Rebecca (2013). They counted 4×10^5 UFC/g and showed that this number varied according to the type of site and season. Fresh fish presented a contamination level lower than smoked one. Abolagba and Uwagbai (2011), underlined that microorganisms isolated from fresh fish could be considered like normal flora. These germs are generally nonpathogenic and can produce bacteriocins against other invader bacteria. One hundred percent (100%) of fresh *Sarotherondon galilaeus* were contaminated by *Salmonella* sp. because of a primordial contamination in their living environment (river's water). Indeed, bad handling conditions during the selling (dirty and uncovered table, non-hygienic defrosting and evisceration process) could also explain this high occurrence. This is the same remarks by Akaki et al. (2012) when they studied roasted fish bacteriology in Yamoussocro (Côte d'Ivoire). Frying and roasting destroy efficiently microorganisms of fish (Oladipo and Bankole, 2013). Theoretically, fried and roasted fish do not have much microbial load. Actually, this is impossible because they are submitted to many handlings, multiplying risks of contamination from sellers, like Barro et al. (2006) showed in their study on the hygienic conditions of processing and selling sites of street food in Ouagadougou.

Conclusion

In Burkina Faso, particularly in Ouagadougou, due to meat expensiveness, fish have been progressively the main important source of animal proteins. Then, since 1994, his consumption is becoming more and more important. Fish is consumed generally after several cooking methods: Smoking, roasting, frying. Unfortunately, majority of consumers have no information about microbiological quality of fish consumed. In consideration of results obtained in this study, we aim to evaluate the nutritional value of fish just after the capture and just before the consumption in order to underline the effect of conservation and transformation proceedings. This different analysis will produce results usable in population sensitization about dangers linked to fish consumption.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank SUSFISH project (Sustainable

Management of Water and Fish Resources in Burkina Faso) for financial support during this study. They thanks also Dr Magloire Boungou, Dr Malika Kangoyé, Laboratoire de Biologie et Ecologie Animales, Université de Ouagadougou, Burkina Faso, Serge Bagré, Salomon Bouda, Laboratoire de Technologie Alimentaire, Université de Ouagadougou, Burkina Faso, for support and frank collaboration during this work. They also thank Rosalie Ilboudo for correcting this paper and fish sellers for responding to our questionnaire.

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