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Microbial quality of artisanal yoghurt and Dèguè products collected in schools of Cotonou and Abomey-Calavi (Benin)

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The main aim of this study is to evaluate the microbiological quality of yoghurt and two types of dèguè (couscous and millet) sold in Cotonou and Abomey-Calavi. Samples of dairy products were collected in 15 schools of Abomey-Calavi and Cotonou from vendors located inside the schools. In this study, 180 samples (60 yoghurt, 60-dèguè millet and 60-dèguè couscous) were analysed. The microbial quality of collected samples was investigated using conventional methods for lactic acid bacteria, staphylococci, faecal and total coliforms and thermo-tolerant Gram-negative bacilli bacteria. The results of the microbiological analyses of the collected samples revealed a contamination of all the samples of yoghurt, dèguè millet and dèguè couscous. The microorganisms load varies according to not only the sampling sites, the sampling period but with the type of microorganisms. The presence of potentially pathogenic bacteria particularly the total coliforms (38%), faecal coliforms (21%), and thermo-tolerant Gram-negative bacilli bacteria (20%) and staphylococci (21%) were observed. In addition, it should be noted that the different samples of yoghurt, dèguè millet and dèguè couscous taken in the morning have a high microbial load compared to that of the evening with regard to faecal coliforms for the two sites. Dairy products sold to school children in Cotonou and Abomey-Calavi are potentially sources of food poisoning.

Key words: Yoghurt, dèguè, microbiological quality, schools, South Benin.

INTRODUCTION

Dairy products have exceptional qualities, which are very important in the diet for both humans and animals. They are rich in a wide variety of essential nutrients, easily digestible minerals, vitamins and proteins (Haug et al., 2007). The production of milk is not high in Benin (about 112.302 tons) compare to other countries (Kassa et al.,

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2016). However, a significant part of production sold to people is found in the informal sector without any control. Dairy products occupy an important place in the diet of the Beninese population because of its richness in proteins of animal origin and its high concentration of nutrients (Anihouvi et al., 2019). Among those products, yoghurt is consumed by both children and adults because it provides energy and essential elements for growth and maintenance (Dror and Allen, 2014). It also contains probiotics and elements with immune-stimulant properties that help them adapt to environmental variations (Aspri et al., 2017). According to Standard N°. A- 11 of the Codex Alimentarius Commission (1975), yoghurt is a coagulated milk product obtained by lactic fermentation through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* from fresh milk and pasteurized milk with or without addition. Milk and its derivatives are mostly sold as ready to eat food dubiously in the local markets and particularly on roadsides and in schools (Tchekessi et al., 2014). In such cases, raw milk is processed and spontaneously fermented at home, before it is transported to the market in calabashes (Tankoano et al., 2016). There are various kinds of dairy products but the most consumed in West African countries are dèguè (millet and couscous), yoghurt, gappal, tchobal (Tankoano et al., 2016) which are foods rich in vitamins and minerals, especially calcium.

Despite their nutritional interest, milk products are excellent environments for the multiplication of all kinds of microorganisms (Bonfoh et al., 2003). They can present a health hazard due to the possible contamination with pathogenic bacteria when consumed unpasteurized or exposed to contaminated environment (Angulo et al., 2009). The microorganisms in the final product must be viable and abundant (FAO, 2008). Thus, more than 200 diseases can be transmitted through food contaminated by pathogenic bacteria, fungi, viruses and parasites (Zagare et al., 2012). The dairy products are mostly packaged in recycled bottles after fermentation of unpasteurized raw milk (Bagré et al., 2014). Several studies reported the presence of *Enterobacteria*, *Salmonella*, yeast and *Staphylococcus aureus* in the raw milks and other milk products sold in Burkina Faso (Bagré et al., 2014; Millogo et al., 2018). The prevalence of food-borne pathogens in milk is influenced by numerous factors such as farm size, hygiene, sampling, and samples analyse methodology, (Zagare et al., 2012). Indeed, to guarantee the safety of food offered for sale, good maintenance and adequate storage of preparation and sales materials and equipment are essential (Becila, 2009). Traditional techniques for improving the production and preservation of yoghurt and dèguè are expensive and less accessible to processors in Africa, including Benin (Akouedegni et al., 2013; Sessou et al., 2013). The quality of the ingredient used in the manufacture and non-compliance with hygiene rules are among factors that can affect the microbiological quality of the final product that can be source of public health problems (Bagré et al., 2014).

Preventing school health is important, as nutritional deficiencies and infections have serious consequences for school participation and learning (UNESCO, 2000; UNICEF, 2013). Nevertheless, food poisoning has been reported among young schoolchildren in Benin, Ivory Coast and other West African countries (Pinstrup-Andersen and Watson, 2011; Bsadjo-Tchamba et al., 2015). Foodborne disease is a major public health problem worldwide causing several deaths in developing countries (Baba-Moussa et al., 2006; Havelaar et al., 2015; Van de Venter, 2000). Thus, this study is a contribution to improving of young people's health. It is aimed at evaluating the microbiological quality of yoghurt and its two derivate product (dèguè couscous and millet) sold in secondary schools of Abomey-Calavi and Cotonou in Benin.

MATERIALS AND METHODS

Sampling and samples collection

Samples of dairy products (Yoghurt, Dèguè millet and Dèguè couscous) were randomly collected in 15 schools of Cotonou and Abomey-Calavi from vendors located inside the schools or outside within a radius of 20 m. For each type of dairy products, two samples were taken twice a day (morning and evening) and twice during the week with one day difference. A total of 180 samples were collected from the two sites: 60 yoghurt samples, 60 dèguè millet samples and 60 dèguè couscous samples. Once collected, the samples were transported in ice (4°C) to the laboratory for microbial analysis.

Microbiological analyses

Once in the laboratory, 10 g of each collected samples were homogenized into sterile stomacher bag with 90 ml of sterile tryptone salt water to obtain the stock solution. From this solution, a serial decimal dilution was made with tryptone salt water. The dilutions 10^{-1} to 10^{-3} were used for the detection and enumeration of total and faecal coliforms, *Staphylococcus* sp., and thermo-tolerant Gram-negative Bacilli bacteria whereas dilutions 10^{-1} to 10^{-7} were used for the detection and enumeration of lactic acid bacteria.

Lactic acid bacteria

The Man Rogosa and Sharpe Agar (MRSA) medium was used for the enumeration of *Lactobacillus* in dairy products (de Mesquita et al., 2017). One millilitre of each dilution was taken and spread on sterile Petri dishes and 15 ml of the medium was poured into the samples and homogenized. After solidification, 5 ml of the medium was added to the surface of the previous stratum. The dishes were then incubated in anaerobiosis at 30°C for 48 to 72 h (de Mesquita et al., 2017).

Total and faecal coliforms

The enumeration of faecal coliforms was performed on Violet Red Bile Glucose Agar (Oxoid CM0485, England) medium (Milani et al., 2011). One millilitre of 10^{-1} to 10^{-3} dilutions was first spread on

Table 1. Average number of microbial load per germ on dairy products in colony forming units per gram (CFU / g).

	Yogurt (CFU/g)	Dèguè millet (CFU/g)	Dèguè couscous (CFU/g)
Lactic acid bacteria	3.710^{10}	$1.15*10^{4}$	$2.2*10^{10}$
Total Coliforms	$1.2*10^{5}$	$1.2*10^{6}$	$7.7*10^{6}$
Faecal Coliforms	$3.9*10^{4}$	$1.1*10^{6}$	$1.5*10^{6}$
Staphylococcus spp.	$7.5*10^{5}$	$1.2*10^{6}$	$7.7*10^{5}$
Thermo-tolerant Gram-negative bacilli	$7.2*10^{5}$	$3.1*10^{5}$	$1.1*10^{6}$

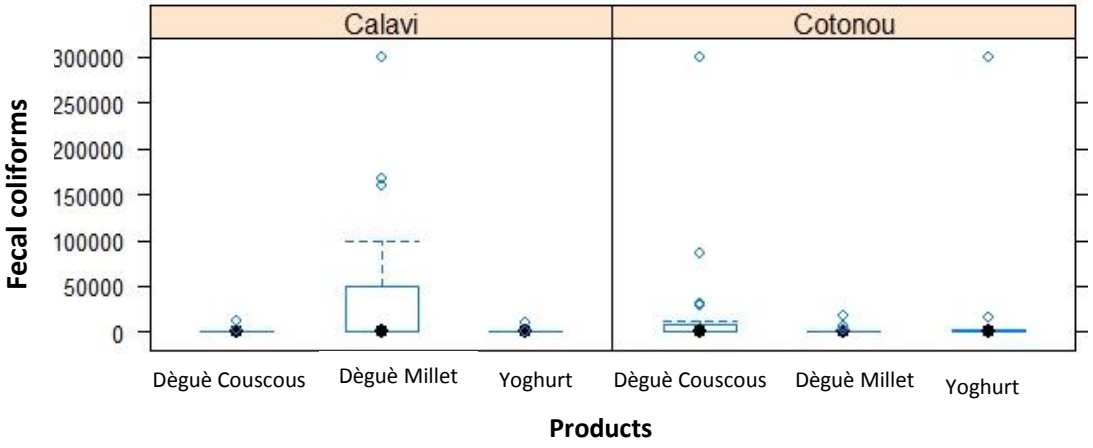

Figure 1. Distribution of the average faecal coliforms.

sterile Petri dishes and 15 ml of the VRBA medium was added and homogenized. A second stratum of about 5 ml was poured on the first before incubation at 30°C for 24 h for total coliforms and at 44°C for 24 h for faecal coliforms.

Thermo-tolerant Gram-negative bacilli

Thermo-tolerant Gram-negative bacilli were enumerated on Tryptone Bile X Glucuronide agar (Oxoid CM0595, England). Thus, 1 ml of the 10^{-1} to 10^{-3} dilutions was poured into petri dishes and 15 ml TBX was added, mixed and homogenized before incubation at 44°C for 24 h. The search for gram-negative bacteria was completed, after isolation on Eosin Methyl Blue Agar (Oxoid CM0069, England), by the indole test and the Api20E (bioMérieux, France) gallery (Riegel et al., 2006).

Staphylococci

Baird-Parker Agar enriched with egg yolk and potassium tellurite was used for the detection and enumeration of *Staphylococcus* sp strains (Baird-Parker, 1990). Briefly, 0.1 ml of the 10^{-1} at 10^{-3} dilutions was spread into petri dishes containing B Baird-Parker Agar (Oxoid CM0275, England) with with egg yolk. After 48 h incubation at 37°C, black, smooth or domed colonies with regular outlines with or without a halo were counted (Yehia et al., 2019).

Data analysis

To assess whether there is a significant difference in the measured parameters between the products, the period and place of collection, generalized linear models were used. The R 3.6.0 software was used for the realization of graphs (Pinheiro et al., 2019). The threshold of statistical significance was set at $p<0.05$.

RESULTS

Microbial load of dairy product

The results of the microbiological analysis show that 100% of dairy product (yogurt, Dèguè millet and Dèguè couscous) collected in the schools of Cotonou and Abomey-Calavi has unsatisfactory hygienic quality considering acetic acid bacteria, total coliforms, faecal coliforms, *Staphylococcus*, thermo-tolerant Gram-negative bacilli (Table 1).

Microbial loads in total coliforms and faecal coliforms

The average faecal coliform loadings of the products sampled are represented in Figure 1. It is noted that the average faecal coliform loads are similar for products such as Yoghurt and Dèguè couscous in the two collection cities. However, Dèguè millet samples from Abomey-Calavi mean loads are higher than those collected

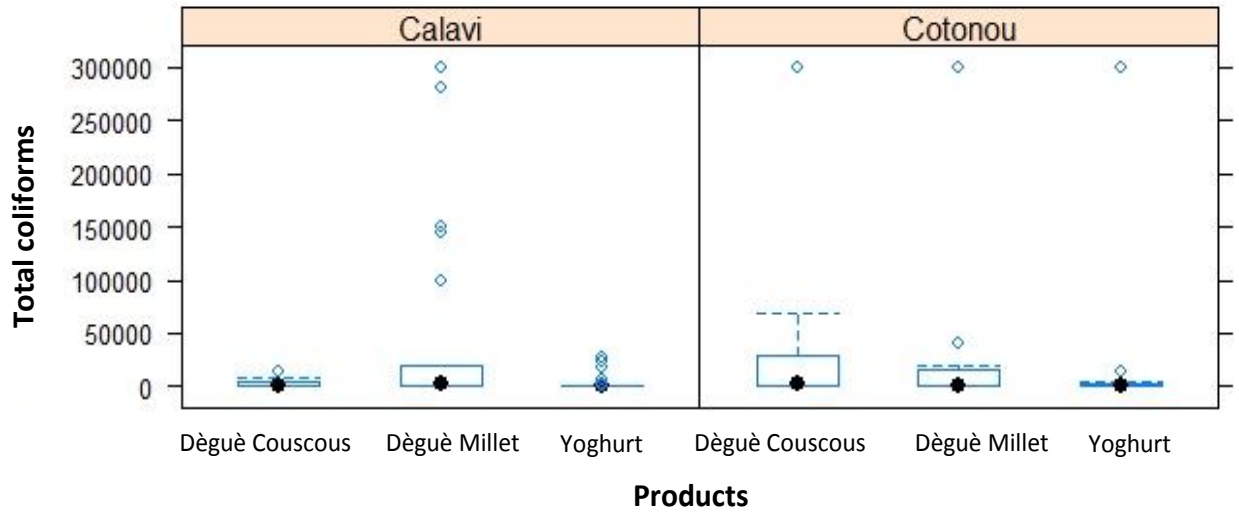

Figure 2. Distribution of the average total coliforms.

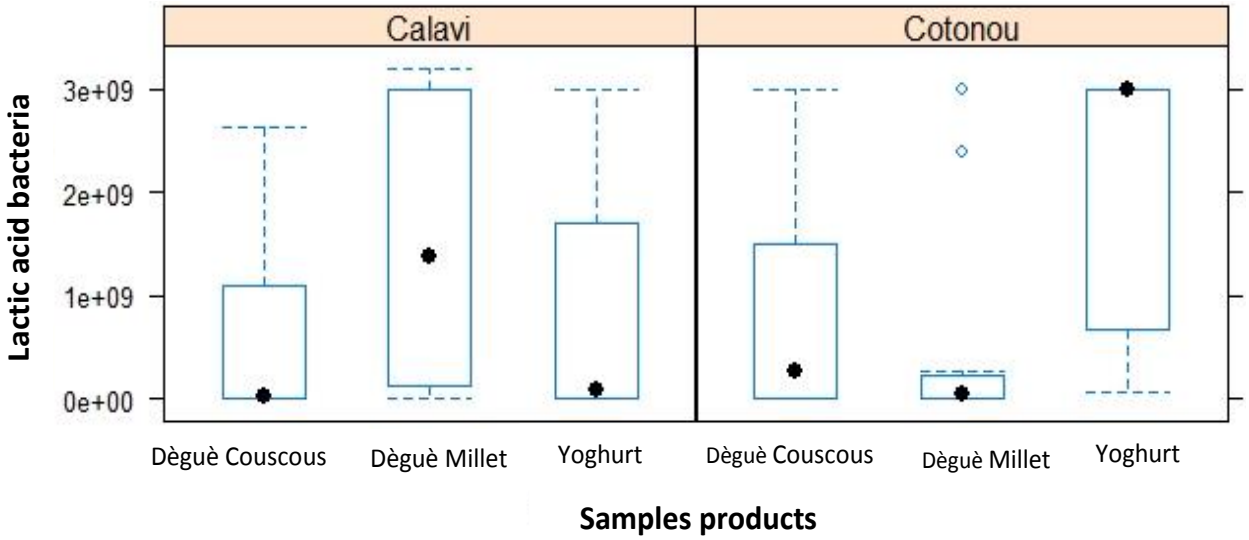

Figure 3. Distribution of the average lactic acid bacteria per product.

in Cotonou.

Concerning total coliforms, these averages are all similar between the two cities for yoghurt and Dèguè Millet and a variation is noted for Dèguè couscous (Figure 2).

Microbial loads of lactic acid bacteria

The Figure 3 shows the variation in lactic acid bacteria loads. It is founded that the average loads are higher in products such as yoghurt and Dèguè couscous in the city of Cotonou while these loads are higher in dèguè millet in the city of Abomey-Calavi.

Staphylococcal microbial loads

The changes in staphylococcal loads are represented in Figure 4. It is founded that the average *Staphylococcus* sp. loads are higher in products such as yoghurt and Dèguè couscous in the city of Cotonou while these loads are higher in dèguè millet in the city of Abomey-Calavi.

Thermo-tolerant Gram-negative bacilli microbial loads

It was found that the average load of thermo-tolerant Gram-negative bacilli (Figure 5) is similar between products

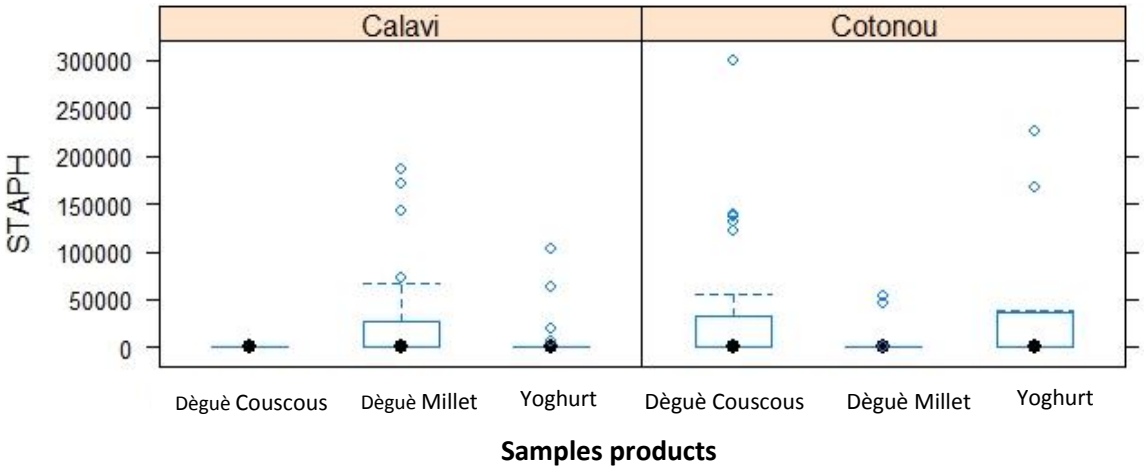

Figure 4. Average of *Staphylococcus* by product.

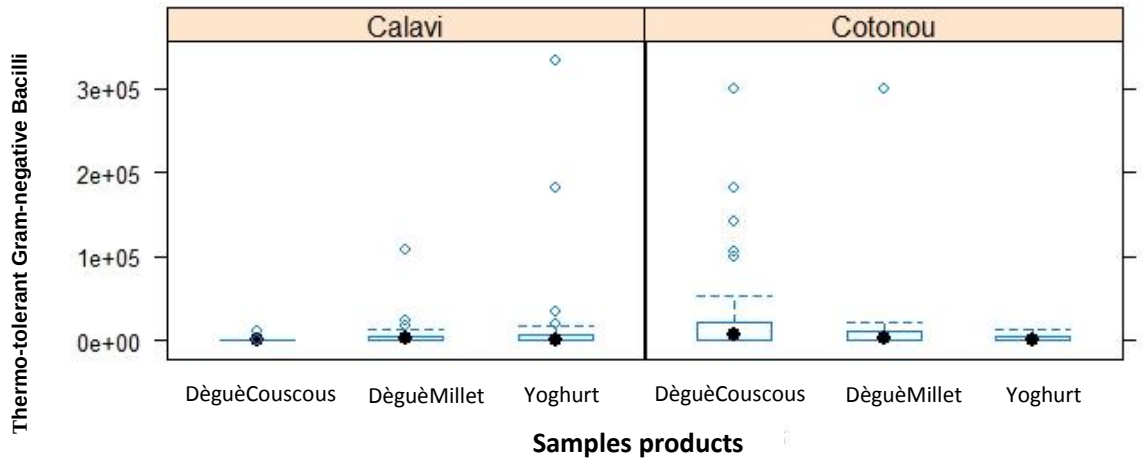

Figure 5. Average thermo-tolerant Gram-negative bacilli per product.

such as yoghurt and Dèguè millet in the two collection cities. However, these average loads are higher in the Dèguè couscous samples from Cotonou than those from Abomey-Calavi are.

DISCUSSION

A good fermented milk product must meet a number of criteria, particularly microbiological standards. These can only be achieved through the application of good hygienic practices and good manufacturing practices at all stages of product life. Thus, microbiological analyses revealed the presence of potentially pathogenic bacteria such as thermo-tolerant Gram-negative bacilli, *Staphylococcus* as well as faecal contamination index germs (total and faecal coliforms). Thus, the number of lactic acid bacteria varies according to the fermented milk product with an average of 8.33×10^{8} CFU/ml in the dèguè couscous samples, 1.14×10^{9} CFU/ml for the dèguè millet samples and 1.21×10^{9} CFU/ml for the yoghurt samples. These results are lower than the values (2.5×10^{8} to 25×10^{8} germs/g) reported by Ndiaye (2002) yoghurts in Dakar. This difference could be explained by the choice of quality and quantity of the lactic ferments used by the producers. Indeed, during investigation, it was noted that the salespersons did not always use suitable ferments when making yoghurt.

The environment of the sales sites prospected during this study allowed the observation of the presence of rubbish and open gutters draining wastewater at certain sites. It was found that fermented milk products were laid out on makeshift tables. The salespersons do not have adequate bins for garbage collection. This results in the attraction of flies, which are not only indicators of poor hygiene, but also vectors of faecal contamination germs

as mentioned by Samapundo et al. (2016). In addition, salespersons seldom change the water used to rinse service utensils. It was also noticed that the hands of the salespersons were in contact with the money and the food sold. These practices can lead to cross-contamination of the products served. All of this is detrimental to good practices in the preparation and sale of these desserts. Moussé et al. (2016) made almost the same observations in their study of street foods. The evaluation of the microbiological quality of fermented milk products reveals the presence of total coliforms, faecal coliforms, *Staphylococcus* sp. and thermo-tolerant Gram-negative Bacilli germs in fermented milk products; this illustrates a failure in hygiene and the implementation of good manufacturing practices collected in the survey results. This is in line with the work of Morou (2010) who notes that the search for these germs at the industrial level is a test of overall hygienic quality. The presence of coliforms in samples of fermented dairy products could also be explained by insufficient heat treatment or contamination during brewing, yoghurt packaging and even sale. Indeed, failure to respect the time/temperature couple during pasteurization can lead to the survival of coliforms in the yoghurt. In addition, the intake of sugar and flavourings at the end of the yoghurt manufacturing process by sealers could be a real source of contamination (Ndiaye, 2002).

Humans are the main vector of contamination during handling throughout the food chain. *Staphylococci* present in the fermented milk products studied can lead to intoxication due to the ingestion of staphylococcal enterotoxins, heat-resistant proteins performed in the food, in which *S. aureus* (or any other SE-producing *staphylococcus*) may have developed and produced its toxins. These toxins, if present in sufficient quantities in the food, can trigger nausea followed by characteristic uncontrollable vomiting (rocket vomiting), abdominal pain, diarrhoea, dizziness, chills, general weakness sometimes accompanied by moderate fever in the most severe cases, headaches, prostration and hypotension (ANSES, 2011).

Conclusion

The main objective of this study is to evaluate the microbiological quality of fermented dairy products, artisanal manufactured and sold in primary schools in Cotonou and Abomey-Calavi. This study had shown that fermented dairy products manufactured and sold by the sellers are contaminated with total coliforms, faecal coliforms, thermo-tolerant Gram-negative bacilli and *Staphylococcus* sp. Sellers do not respect the hygienic rules required during the manufacturing process of the products. Also noted are the inexistence of a quality approach, the sources of human contamination, and the inadequate framework for the sale of its products, the contamination of the raw material used in manufacturing and poor preservation of the products, all of which are factors that influence the microbiological quality of fermented dairy products. Special attention must therefore be paid to the sanitary quality of dairy products sold in streets and schools in Benin, as microbial contamination can make the product highly perishable, unfit for human consumption following organoleptic alteration, or present a danger to young children. Thus, the monitoring of the hygienic quality of artisanal produced dairy products is essential to safeguard the health of consumers in general and children in particular.

CONFLICTS OF INTERESTS

The authors have not declared any conflicts of interests.

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