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Diversity and microbiological quality of fruit juices produced in southern Benin

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The fruit juice production sector is growing rapidly in Benin's major urban centers and appears to have enormous hygienic insufficiencies. This study aimed to identify and evaluate the microbiological quality of the different fruit juices produced in southern Benin. The methodological approach to the first objective consisted of a semi-structured survey coupled with direct observations of fifty enterprises produced fruit juices in the Municipalities of Cotonou and Abomey-Calavi. The microbiological quality evaluation of pineapple juice (*Ananas cosmosus*) consisted in the enumeration of microorganisms that alter both hygienic and market ability qualities of foodstuffs, using standard methods. This study revealed a strong diversity of fruit juices. There are thirteen (13) different mono-fruity juices, fifteen (15) poly-fruity juices with pineapple and nine (09) poly-fruity juices without pineapple. The pineapple mono-fruity juice is produced by almost all the enterprises (98%). Poly-fruity juices with pineapple are the most popular among consumers. From the microbiological point of view, a strong variation of the microbial load was noticed from one enterprise to another. With the exception of a few enterprises, about 18% of pineapple juices are microbiologically non-compliant. These pineapple juices contain mycotoxigenic yeasts and moulds, lactic acid bacteria, thermotolerant coliforms and *Staphylococcus aureus*. The consumption of these poor sanitary quality juices poses a real public health problem, as it exposes consumers to the severe food poisoning risks.

Key words: Fruit juice, diversity, pineapple, *Ananas cosmosus*, microbiological quality, non-compliance.

INTRODUCTION

Fruit is often recommended to prevent and reduce the risk of chronic degenerative diseases, cardiovascular diseases and diabetes (Benton and Young, 2019). They remain an important source of minerals, vitamins, phytoconstituents and phenolic compounds (Asandé et al., 2020). Thus, many fruits contain phenolic amino acids, organic acids such as tartronic acid (Sun et al., 2018), minerals such as Ca, K, Mg, Fe and Se (Doharey et al., 2021) and vitamins such as vitamin C, riboflavin and niacin (Zaini et al., 2011). Several medicinal properties such as anxiolytic, anticonvulsant, antidepressant, anti-inflammatory, analgesic and anti-asthmatic effects have been associated with fruits (Tiwari et al., 2013). For a healthy diet and better overall health, the Food and Agriculture Organization of the United Nations recommends a daily fruits consumption of at least 400 g (FAO, 2020). However, the adoption of this recommendation, especially in most developing countries, is very difficult. Indeed, in these low-income countries the diet is essentially based on cereals and tubers (FAO, 2020).

In Benin, the abundant consumption of fresh fruit by the population faces several difficulties, namely: high prices, inadequate preservation technologies, high post-harvest losses due to poor sales, and the fragility and seasonality of fruit. Faced with this situation, fruit transformation into other by-products is essential. The processing of fruit into fruit juice is widespread in Benin. Indeed, fruit juice is a nutritious drink that not only contains vitamins, minerals, proteins and protective antioxidants, but also provides an additional source of water (Heyman and Abrams, 2017).

Noumavo et al. (2022) showed that the fruit juice production sector is growing rapidly in southern Benin. It creates huge employment and generates significant income for the actors in the sector. However, the production and conservation conditions of these juices do not guarantee the required food safety. Indeed, the production methods of these juices are often artisanal, under unsatisfactory hygienic conditions. The ingestion of food or drink contaminated by certain infectious or toxigenic agents is a public health problem throughout the world. Foodborne diseases affect one in ten people every year and 420,000 people die from them (Petruzzi et al., 2017). These foodborne infectious diseases can also have a significant economic impact because of treatment cost.

This study aims to identify and evaluate the microbiological quality of the different fruit juices produced in southern Benin.

MATERIALS AND METHODS

Geographical area

The present study was carried out in southern Benin, particularly in the municipalities of Abomey-Calavi (6˚26'9112 "N; 2˚21'3396 "E) and Cotonou (6˚21'9216 "N; 2˚25'0998 "E). These two municipalities contain the majority of fruit juice producing enterprises in southern Benin. Indeed, they are very close to Allada department, an area of large fruit production, particularly pineapple (*Ananas cosmosus*) in Benin.

Diversity evaluation of fruit juices

This study was conducted from November 2020 to February 2021. Fifty (50) fruit juice enterprises previously identified by Noumavo et al. (2022) were involved in the present study. These included semi-industrial (05) and artisanal (45) enterprises. Within the artisanal enterprises (A), several sub-groups can be distinguished: A1 enterprise have no mechanized equipment except for a manual capper; A2 enterprise have an extractor and/or grinder, all stainless steel or not, and then a manual capper; A3 enterprise have an extractor and/or grinder and a press, a cooker, all stainless steel, and then a manual capper; and finally A4 companies have a cooker, an extractor, a press, a pasteurizer all stainless steel, and then a manual capper. Different fruit juices census was carried out by a semi-structured survey coupled with direct observations.

Microbiological analysis of fruit juices

The microbiological investigation was carried out only on mono-fruity pineapple juice at the Laboratory of Microbiology and Food Technology (University of Abomey-Calavi, Benin). A total of 100 juice samples were collected (two samples per enterprise). The sampling, transport and storage of the samples were carried out under technical conditions that prevented any modification of their microbial flora. The preparation of decimal dilutions was done under aseptic conditions according to Speck (1976) method. Microorganism's enumeration was performed according to the method used by Ohin et al. (2018). Thus, Total Mesophilic Aerobic Microorganisms were counted on Plate Count Agar (Oxoid, England) after incubation at 30°C for 72 h. Total Coliforms and Faecal Coliforms were isolated on Violet Red Bile Lactose agar (Liofilchem Diagnostici, Italy) after incubation at 30 and 44°C, respectively for 24 h. Lactic acid bacteria were counted on MRS (de Man, Rogosa and Sharpe) agar after incubation at 37°C for 72 h. Tryptone Sulfite Neomycin agar (Biokar Diagnostics, France) was used to enumerate anaerobic sulfite-reducing bacteria (44°C for 24 h). Yeasts and molds were enumerated on Sabouraud agar (Biokar Diagnostics, France) supplemented with Chloramphenicol (25°C for 5 days). Finally, *Staphylococcus aureus* were isolated on Baird Parker agar (Biokar Diagnostics, France) supplemented with egg yolk and potassium tellurite after incubation at 37°C for 48 h.

Data management and analysis

Data from the survey forms and microbiological analysis were encoded using the Microsoft Excel 2013 spreadsheet for descriptive statistics (mean, proportion and standard deviation). The R.4.0.0 software was used to carry out the Analysis of Variance (probability level of 5%) and the Student-Newman-Keuls test.

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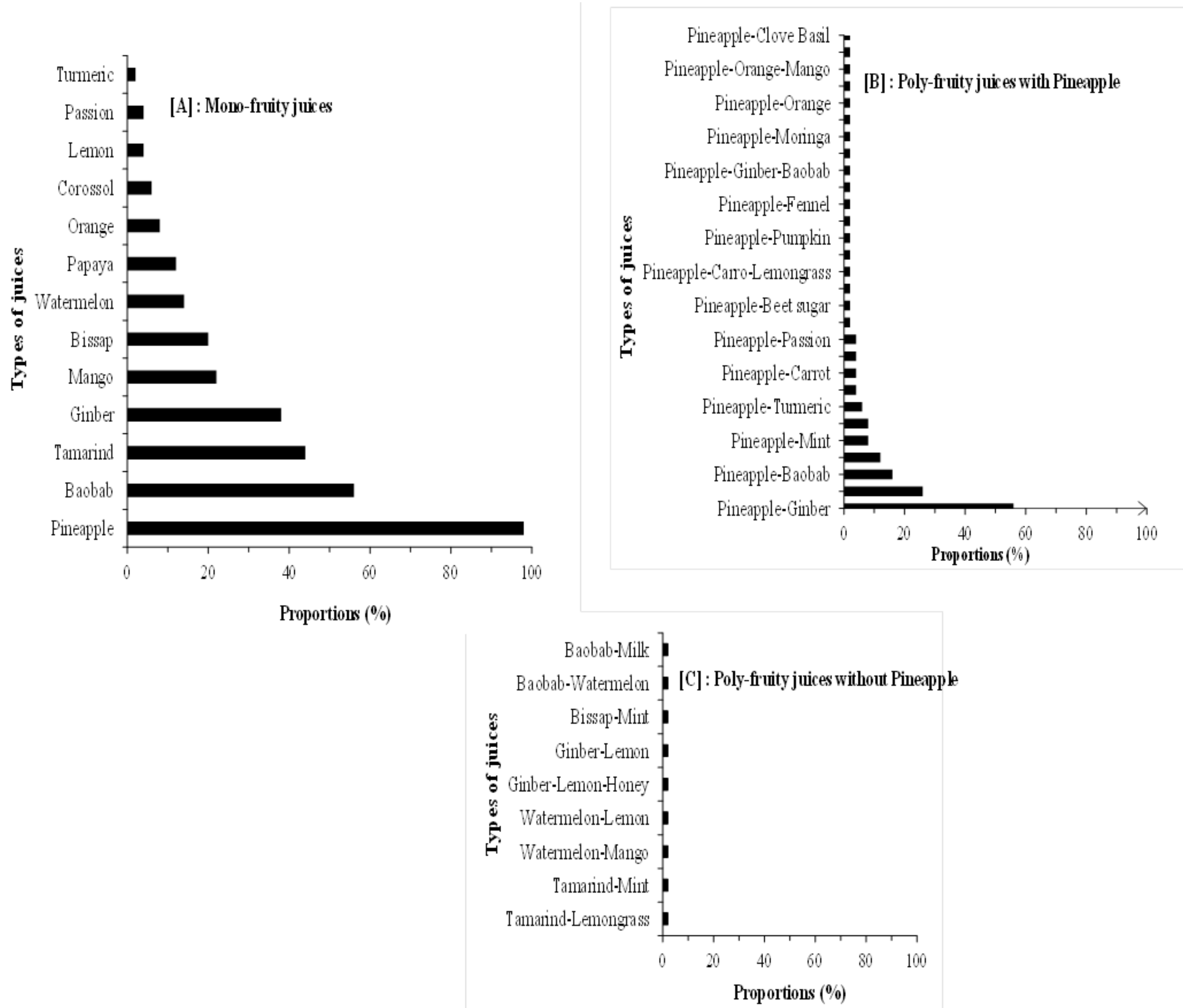

Figure 1. Diversity of fruit juices,
Source: Authors

RESULTS AND DISCUSSION

Diversity of fruit juices produced

The types of juice encountered during our surveys are verydiverse. There are thirteen (13) different mono-fruity juices, fifteen (15) poly-fruity juices with pineapple and nine (09) poly-fruity juices without pineapple. Indeed, among the most common mono-fruit juices (Figure 1A), we note pineapple (*A. cosmosus*) juice produced by almost all the enterprises surveyed (98%), baobab (*Adansonia digitata*) juice (56%), tamarind (*Tamarindus indica*) juice (44%) and ginger (*Zingiber officinale*) juice (38%).

Apart from mono-fruit juices, several types of cocktails (poly-fruit juices) have been recorded (Figure 1B). Among the cocktails with pineapple, Pineapple-Ginger is produced by just over half of the enterprises (56%). It is followed in order of proportion by Pineapple-Watermelon (26%), Pineapple-Baobab (16%) and Pineapple-Tamarind (12%) cocktails. Note that a few rare enterprises sporadically produce cocktails without pineapple (Figure 1C). Poly-fruity juices with pineapple are the most popular among consumers.

It should be noted that a diversity of juices is produced. Thus, apart from the pineapple used to sweeten the various cocktails, sour, honey or milk is also used for those who develop an allergy to pineapple. These particularities are therefore on special order of the customer. Apart from pineapple, other raw materials are obtained in local markets such as the Dantokpa and Abomey-Calavi markets.

Microbiological quality of pineapple juice

In this study, the evaluation of the microbiological quality of the pineapple juice collected consisted in the search for certain microorganisms responsible on the one hand, for the deterioration of the hygienic quality of food (Total Coliforms, Fecal Coliforms and *S. aureus*) and on the other hand, the deterioration of the marketable quality of food (yeasts, molds and lactic acid bacteria). Figure 2 presents the different microbial groups sought and their abundance according to the industrial size of the companies producing these juices.

More specifically, Figure 2A illustrates the variation in the load of Total Mesophilic Aerobic Flora (TMAF) of pineapple juice according to the type of business. Indeed, the TMAF load is a good indicator of hygiene, which makes it possible to assess microbial pollution and the general quality of a foodstuff (Ayadi and Touahmia, 2021). With regard to the statistical analyses carried out, the load in TMAF varies very significantly from one type of enterprises to another ($p < 0.01$). The juices from the A1 and A2 artisanal type enterprises harbor a very abundant TMAF in comparison to the juices from the other types of enterprises. This result corroborates the many hygienic shortcomings noted in craft businesses, particularly those of types A1 and A2. Within type A1 and A2 companies, all stages of the production process are carried out manually except for capping, which is done using a capping machine, and grinding, which is done using an extractor and/or a crusher (A2). On the other hand, production lines are a little more mechanized in other types of craft businesses and even better in Semi-Industrial (SI) businesses. It should be noted, however, that the TMAF load of the juices from SI companies is much higher than those of the A4 type artisanal companies which contain the lowest TMAF loads. This atypical result illustrates the difficulty that some SI companies have in respecting the rules of good hygiene practice. Indeed, they are often satisfied with a simple washing with soapy water before and after production without dismantling the devices for proper cleaning. This could favor the accumulation of water and fruit residues in non-accessible areas and consequently the proliferation of spoilage and pathogenic microorganisms.

Similar results were obtained by Ogodo et al. (2016) following the analysis of the microbiological quality of fruit juices packaged and marketed in South-East Nigeria. In this study, the bacterial load of pineapple juice reached 4.4×10^5 CFU/ml. In another study by Amin et al. (2018) on mango juice the bacterial load varied from 1.10^3 to 3.10^3 CFU/ml.

The microbiological profile of pineapple juice relative to the two groups of microorganisms affecting the marketability of food (yeast-molds and lactic acid bacteria) investigated in this study is presented in Figure 2B and C. A trend of the variation of the microbial load from one type of enterprise to another is practically similar to that of the TMAF. However, the juices from A4-type artisanal businesses and SI businesses contain a very negligible load of yeasts and molds and lactic acid bacteria. According to the guidelines and standards for the interpretation of analytical results in food microbiology of the State of Quebec (Dumont, 2019), the microbial load in yeasts and molds of pasteurized fruit juices must not exceed 10^3 CFU/ml. With regard to this threshold relating to yeasts and molds, we can say that approximately 20% of the samples of pineapple juice coming from artisanal enterprises type A1 and A2 and from SI businesses as well as 12.5% of those from artisanal businesses type A3 are of unsatisfactory microbiological quality, and therefore unfit for human consumption (Table 1). All the pineapple juice samples from A4-type artisanal businesses are of satisfactory quality with regard to their yeasts and molds content. These samples will lose their organoleptic characteristics more quickly than those from A4-type artisanal businesses. In fact, molds can produce pectinases which are degrading enzymes with a high potential for modifying the taste, smell and appearance of fruit juices. The presence of these molds may be due to insufficient hygiene within the production units with a high preponderance of heat-resistant molds (Konan, 2016).

Also, the consumption of these juices exposes consumers to real risks of food poisoning. Indeed, fungal contamination can lead to the formation of allergens and the production of toxigenic compounds such as mycotoxins (aflatoxins, ochratoxin A, patulin, etc). These toxigenic compounds pose a real public health problem because of their responsibility in the occurrence of acute and chronic toxicity phenomena. Their prolonged exposure through food has been associated with cancers and diseases of the kidneys, liver and immune system (Tovide et al., 2017). Unfortunately, there is no single solution in the fight against mycotoxins. It is therefore important to take all the necessary measures to reduce and control the level of mycotoxins in agri-food products (Gauthier, 2016).

The microbial loads of germs indicating fecal contamination (coliforms) and mucocutaneous contamination (*S. aureus*) are presented in Figure 2D, E and F. The analysis of variance revealed that there is a very highly significant difference in these microbial groups from one type of business to another. Samples from type A1 and A2 artisanal businesses contain more coliforms (Total and Fecal) than samples from type A3 and SI artisanal establishments. On the other hand, the samples from artisanal type A4 establishments do not contain coliforms at all. Thus, all samples (100%) of type

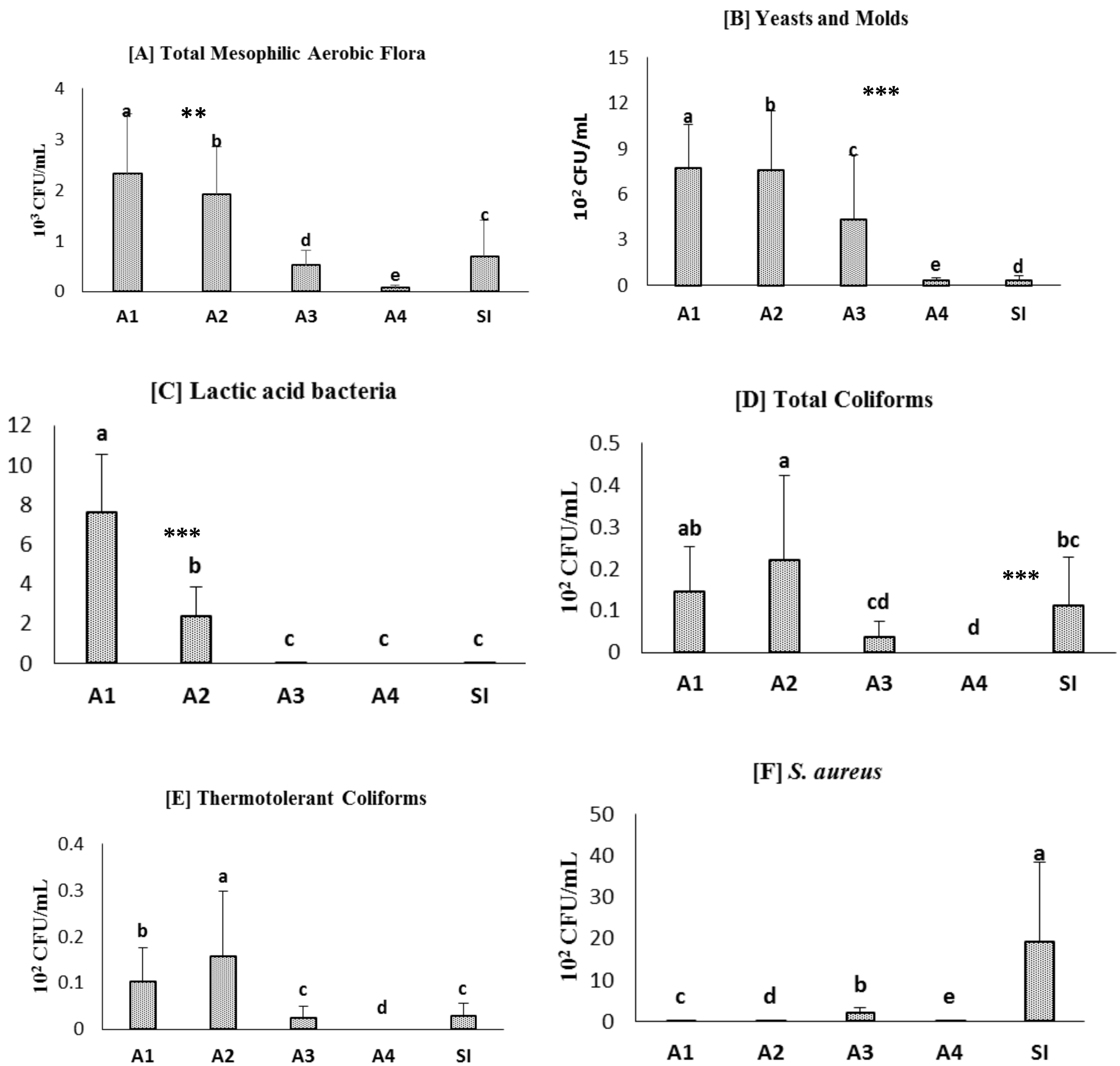

Figure 2. Diversity and microbial load variation of pineapple juices according to the industrial size of enterprises. A1: Artisanal enterprise type 1; A2: Artisanal enterprise type 2; A3: Artisanal enterprise type 3; A4: Artisanal enterprise type 4; SI: Semi-Industrial enterprise. ° = $p > 0.05$ (not significant), * = $p < 0.05$ (significant), ** = $p < 0.01$ (very significant), *** = $p < 0.001$ (very highly significant), CFU/g = Colony Forming Unit per milliliter of sample; on the same graph, the means with different letters are significantly different with probability level of 5% according to ANOVA.
Source: Authors

A4 artisanal establishments are suitable for human consumption if we stick to the guidelines mentioned earlier. About 20% of the samples from artisanal businesses A1 and SI and 10% of those from artisanal establishments A2 and A3 have a fecal coliform microbial load above the normative threshold (Table 1). Once again and in view of our results, the mechanization of pineapple juice production companies does not always guarantee good quality of the juices produced. Regardless of the industrial size of an agri-food processing establishment, the adoption of rules of good hygiene practice remains essential. Although we cannot be satisfied with our results, they are still lower than the 40% non-compliance obtained by Rahman et al. (2011) in a similar study. Asghar et al. (2018) also proved that apple, carrot, orange and sugar cane juices had a high

Table 1. Non-conformity rate of analysed pineapple juice samples.

Industrial size	Non-conformity (%)		
	Yeasts and molds	Fecal coliforms	General
A1	20	20	20
A2	22.72	9.52	22.72
A3	12.5	12.5	12,5
A4	0	0	0
SI	20	20	20

A1: Artisanal enterprise type 1; A2: Artisanal enterprise type 2; A3: Artisanal enterprise type 3; A4: Artisanal enterprise type 4; SI: Semi-Industrial enterprise.
Source: Authors

load of total and fecal coliforms. Similar results were obtained by Onuoha et al. (2018) on simple fruit juices and cocktails packaged and sold in the metropolis of Owerri (Nigeria). It should be noted that these last studies were carried out for the most part on non-pasteurized fruit juices. The presence of these germs in the pineapple juices analyzed in our study may also be due to a pasteurization defect. Indeed, pasteurization is a technique of preservation by heat treatment which makes it possible to considerably reduce the microbial load in a food. It is a simple and inexpensive technique for small agro-industries (Hounhouigan et al., 2020). However, at the level of some fruit juice production companies surveyed, there is a problem of non-compliance with pasteurization scales and lack of appropriate equipment to carry out this preservation treatment.

The presence of coliforms is on one hand an indicator of fecal contamination, by germs that are not directly pathogenic, but whose presence suggests the existence of pathogenic germs for human beings. With the exception of the genus *Klebsiella* and a few others, fecal coliforms are made up of nearly 80% *Escherichia coli*. Three major groups of *E. coli* are involved in diarrheal syndromes in humans and young children, especially in developing countries. These are enterotoxigenic *E. coli*, the cause of traveler's diarrhea and childhood diarrhea; enteropathogenic *E. coli*, agent of infantile gastroenteritis; and finally enterohaemorrhagic *E. coli*, agent of hemorrhagic colitis. *E. coli* serotypes *O157* and *O157: H7* are enterohemorrhagic *E. coli*, agents of serious food poisoning linked to the consumption of meat, unpasteurized apple juice, etc. The second serotype is also called verotoxigenic *E. coli* (VTEC) (Delarras, 2008).

A single germ indicator of mucocutaneous contamination was sought in pineapple juice. This is *S. aureus* known to be common to the bacterial flora of human skin (Alabi et al., 2021), whose microbial load in this study varied markedly ($p < 0.001$) between the juices of the different types of business. Contrary to the general trend observed with the other germs sought, juices from semi-industrial SI companies are highly contaminated with *S. aureus*. The *S. aureus* microbial load of these juices is at least 8 times higher than that of juices from other types of companies. This result remains a priori paradoxical because within semi-industrial companies, manual activities are quite limited compared to craft companies. However, it should be noted that one of the 5 SI companies investigated does not peel the pineapple fruits before grinding. Indeed, often not well-washed pineapple fruits are directly crushed with the skin using a crusher. When we refer to the method of transporting pineapple fruits from the fields or supply markets to the processing units and to the method of receiving and storing pineapple fruits, it is easy to see that the bare hand is heavily used. The fruits are even in many cases received on the ground. It should also be noted that within these companies, the washing of the packaging bottles, the filling and the capping are done by hand in conditions that are not often hygienic. To all this is added the defects of pasteurization often noted within companies in general. All these observations, although not exhaustive, could explain this high load of *S. aureus* in juices from SI enterprises.

This presence of *S. aureus* in the pineapple juice analyzed is very worrying. Indeed, food poisoning due to *S. aureus* does not result primarily from the ingestion of the bacterium itself, but rather from the toxins preformed in the contaminated food (Moloi et al., 2021). Staphylococcal food poisoning is mainly caused by a particular group of toxins called enterotoxins (Chebana et al., 2021). These enterotoxins are heat stable, highly toxic and persist in food even after adequate cooking (Ghalehnoo, 2018). Twenty-two (22) Staphylococcal Enterotoxins (ES) have been described and designated by the letters ESA to ESV in the chronological order of their discovery (Hennekinne et al., 2010). Staphylococcal Food Poisoning (SFP) is characterized by a sudden onset of symptoms (1 to 6 h after ingestion), with vomiting, abdominal pain and stomach cramps (Fetsch et al., 2014). SFP can lead to hospitalizations, especially among young, old, pregnant and immunocompromised (Murray, 2005).

Conclusion

This study of fruit juices diversity and quality in southern Benin provides important data for restructuring the sector. This study revealed a strong diversity of mono-fruity and poly-fruity juices. Mono-fruity juice of pineapple is produced by almost all the enterprises. Pineapple is incorporated in majority of poly-fruity juices. From the microbiological point of view, a strong variation of the microbial load was noticed from one enterprises to another. With the exception of a few enterprises, about 18% of pineapple juices are microbiologically non-compliant. These pineapple juices contain mycotoxigenic yeasts and moulds, lactic acid bacteria, thermotolerant coliforms and *S. aureus*. The consumption of these juices poses a real public health problem, as it exposes consumers to the severe food poisoning risks.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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