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Formulation of a Flavored Drink Based on Sapodilla Puree, Pineapple Juice and Lemongrass Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All the authors have identified and validated the theme of the study. Authors CTRK, MVA, YAK and GMB led the production and analysis activities in the laboratory. Authors CTRK and MVA wrote and reviewed the article draft. Authors EDA, DCKS supervised all activities. All the authors read and approved the final version of the manuscript.

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ABSTRACT

Aims: Neglected or underutilized plant species, such as sapodilla (*Manilkara zapota*), which play a substantial role in the livelihoods of populations but whose potential remains still under-exploited, deserve particular attention from researchers to a better knowledge of its food applications. This

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study aims to develop a novel drink based on sapodilla fruit and other known ingredients with acceptable sensory, microbiological and physicochemical characteristics.
Place and Duration of Study: The study was carried out at *Ecole des Sciences et Techniques de Conservation et de Transformation des Produits Agricoles* of the *Université Nationale d'Agriculture* (UNA) in Benin between June and September 2021.
Methodology: Flavored beverages with the essential oil of *Cymbopogon citratus* were formulated from sapodilla puree and pineapple juice following a mixing plan. The microbiological, organoleptic and physicochemical analyzes of formulated cocktail were then carried out by standard methods.
Results: All the formulated drinks presented a satisfactory microbiological quality. The E5 formula containing 10% of the sapodilla puree and 90% of the juice of pineapple and the essential oil of *C. citratus* was the most accepted by tasters. This beverage had a pH of 5.46±0.06, a total soluble solid of 17.25±0.35 ºBrix and a specific gravity of 1.08±0.01. The titratable acidity, total ash and dry matter content were 0.80±0.01 g/100g, 0.19±00% and 15.28±1.07 respectively.
Conclusion: The fruit of sapodilla can be valued through good quality drinks which can be popularized after a nutritional characterization.

Keywords: Food development; Manilkara zapota; Ananas comosus; organic flavoring; valorization.

1. INTRODUCTION

Hunger and malnutrition remain global scourges affecting thousands of people living mainly in developing countries where food security remains almost a luxury. To address the problem of food insecurity, agricultural production should be increased and local products enhanced by improving their nutritional quality and shelf life [1]. Increasing the production and valorization of natural resources is therefore a major challenge for developing countries.

In recent years, the place of diversification in farming strategies has been widely recognized, particularly in sub-Saharan Africa [2,3]. The continent contains thousands of food plants used in traditional medicine, agriculture and food, but only a small number are very well investigated by scientific research and valued. Yet many farmers, especially in marginal areas, rely on neglected and underutilized species like sapodilla (*Manilkara zapota*) for their livelihoods. These plants therefore have considerable potential for increasing food production, guaranteeing food security for populations, ensuring coverage of primary health care needs, and contributing to the effort to reduce poverty [4]. But various species of this group of plants are threatened with extinction due to the lack of investigation by the scientific community, lack of interest of decision-makers and agro-industrial groups, and the disappearance of their habitats. However, there is growing international recognition that neglected species play a vital role in traditional medicine, food and nutrition security, and provide income opportunities for small-scale farmers. Unfortunately, their expansion and marketing are limited by their lack of knowledge and the lack of a related value chain.

The sapodilla tree (*Manilkara zapota*) is reported to be the most widely cultivated fruit from Sapotaceae family and a rich source of nutrients, minerals and bioactive phenolic compounds [5]. In Benin, the species is involved in food habits and other endogenous practices. However, the species faces serious threats, both natural (climate variations) and anthropogenic (land use changes and overexploitation), which have negatively affected their natural populations. Local communities would have widely reported the rarity of the species in unprotected ecosystems and their near total absence in protected areas. To remedy this situation with the aim of protecting biodiversity, it is urgent to resort the ways of valuing this species in order to allow its contribution to the resolution of nutritional problems. The main way of adding value to the species is through the processing of its fruits into agri-food products (juice, nectar, jam and other derivatives) with added value. The development of other value added sapodilla products will be useful to promote its utilization. Thus, a functional juice with a good microbial stability from sapodilla fruit was previously developed by Lim et al. [6]. In the same context, the current study proposed to investigate the possibilities of formulation of a drink based on sapodilla fruits and pineapple in the perspective to use sapodilla as a novel source of food ingredient and to propose new recipes. This is an innovation because to our knowledge such a product does not yet exist on the market.

2. MATERIALS AND METHODS

2.1 Plant material Collection

The plant material used in the study were pineapple (*Ananas comosus*) fruits, leaves of lemongrass (*Cymbopogon citratus*) bought in Sakété (6° 44′ 11″ North, 2° 39′ 29″ East) as well as sapodilla fruits (*Manilkara zapota*) bought at the Takon market in the same commune. All the chosen fruits were ripe and without any default. The study took place between June and September 2021.

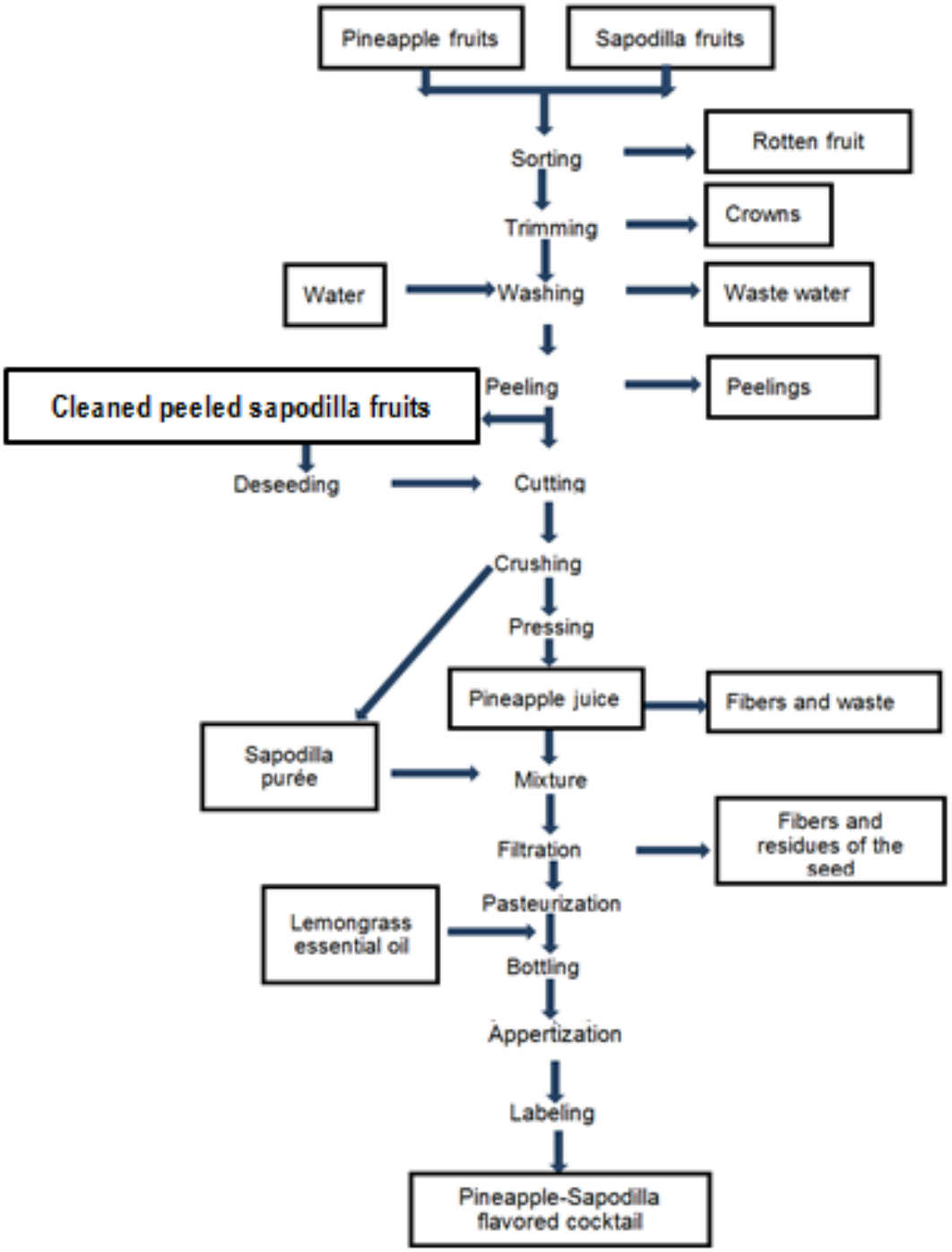

Fig. 1. Flow chart for production of beverages from the pineapple and sapodilla fruits

2.2 Methods

2.2.1 Essential oil and drink production processes

The essential oil from the leaves of lemongrass (*Cymbopogon citratus*) was produced by a hydro distillation using a Clevenger apparatus. To produce the drink, the pineapple and sapodilla fruits were sorted and washed. The pineapple fruits were peeled, cut up while the sapodilla seeds were removed from the fruit pulp. The fruits pulp of pineapple and sapodilla were crushed to obtain purees. The pineapple puree was pressed then mixed with the sapodilla puree and the whole was filtered and pasteurized. The essential oil of *C. citratus* was added and the packaged drink was apertized (Fig. 1).

2.2.2 Experimental setup

For the drink formulation, the mixing plan (Table 1) was implemented. This mixing plan was selected after a preliminary taste test which eliminated the formulas rejected by the primary tasters. According to microbiological and sensory analyses, the most appreciated formula by the tasters was selected. This drink was subsequently produced and the effect of adding two drops of essential oil from *C. citratus* per liter to flavor was evaluated by complementary sensory analyses. It should be noted that control samples (formula retained without addition of essential oil were also considered.

Table 1. Mixing plan for the drink formulation from sapodilla puree and pineapple juice (%)

	Proportions of ingredients	
Formula code	**Sapodilla puree (%)**	**Pineapple juice (%)**
E1	50	50
E2	40	60
E3	30	70
E4	20	80
E5	10	90

2.2.3 Microbiological characterization of drinks

The microbiological quality of samples of drinks was evaluated using standard methods. The total bacteria were counted by inoculation on the Plate Count Agar (PCA) medium and incubation at 30°C for 24-48 hours [7]. Total and thermotolerant coliforms were sought on the Violet Red Bile Lactose (VRBL) medium with incubation at 30 and 44°C respectively for 24 hours [8]. As for *E. coli*, one or two loops of VRBL medium are inoculated on Eosin Methylene Blue (EMB) medium and incubation was carried out for 24 hours at 37°C [9]. *Staphylococcus aureus* was tested on Baird Parker (BP) medium with incubation at 37°C for 24-48 hours [10], while yeasts and molds were tested on Sabouraud medium with chloramphenicol, then incubated at 25°C for 3 to 5 days [11]. Sulfite-Reducing Anaerobic Bacteria were sought on Tryptone Sulfite Neomycin (TSN) agar with incubation at 46°C for 20 hours [12].

2.2.4 Organoleptic analyzes of drinks

The sensory characteristics of the various drinks were evaluated using a pre-established tasting sheet. The sensory panel was 58 untrained people selected for their availability and having no aversion to the product. The coded samples were served randomly to each taster in blind distribution. Sour taste, sweet taste, fluidity and overall appreciation were evaluated. These analyzes served to determine the drink formula most appreciated by the tasters on which the physico-chemical analyzes were performed.

2.2.5 Physico-chemical characterization of drinks

The pH and total soluble solide were measured with a pH meter (HANNA HI 98129) and portable refractometer (ATAGO, Japan) respectively. Regarding the acidity (citric acid percentage per unit volume), it was determined with 0.1 mol/L sodium hydroxide. Phenolphthalein was used as a color indicator [13]. On the other hand the dry matter content was determined according to the described method [14]. For the determination of ash content, the drinks were incinerated at (550 ± 15) °C in a muffle furnace. Finally, their relative density at 20°C was determined by the method reported by Konfo [15].

2.2.6 Statistical analysis

The mixing plan for the formulation of the drink was generated with the software Minitab 16. The data from the sensory characterization of the samples was organized in tabular and graphical form using the Microsoft spreadsheet Excel 2010. The data from this study was organized in tables and figures using the Microsoft Excel 2010. Means comparison was performed using Analysis of Variance (ANOVA) in Minitab 16. Significance of difference was recorded at 0.05 levels.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Microbiological quality of formulated beverages from the pineapple and sapodilla fruits

The results of the microbiological analyzes (Table 2) revealed a total absence of coliforms, *Escherichia coli* β-glucuronidase positive, coagulase positive *staphylococci*, and sulphite-reducing bacteria. On the other hand, the total flora was expressed as colony forming units (CFU) with values ranging from $1.2x10^4$ to $7.7x10^4$ CFU/mL of juice. Yeasts were absent from the two samples (E4 and E5) whereas they were counted between 20 and 40 CFU/mL of juice for the other samples. Finally, the molds were numbered in all the samples at 20 and $1.2x10^2$ CFU/mL. All the cocktails formulated therefore had a very satisfactory microbiological quality.

3.1.2 Sensory quality of formulated beverages from the pineapple and sapodilla fruits

The appreciation of the acidic taste level of the formulated drinks from pineapple and sapodilla fruits (Fig. 2) revealed that 69.23%, 51.92% and 55.76% of the tasters found that the corresponding drinks to the formulas E1, E2 and E3 respectively had a slightly acid taste. However, 59.61% and 69.23% of the tasters found that drinks from the formulas E4 and E5 respectively were not acid.

Table 2. Microbiological characteristics of formulated cocktails from the pineapple and sapodilla fruits

Samples / Germs	E1	E2	E3	E4	E5	Criteria
Total flora (CFU/mL)	$7.7x10^4$	$2.2x10^4$	$1.2x10^4$	$6.7x10^4$	$5.6x10^4$	NS
Total coliforms (CFU/mL)	<1	<1	<1	<1	<1	NS
Thermo-tolerant coliforms (CFU/mL)	<1	<1	<1	<1	<1	NS
Escherichia coli (CFU/mL)	<1	<1	<1	<1	<1	NS
Coagulase positive staphylococci (CFU/mL)	<1	<1	<1	<1	<1	NS
Sulphite-Reducing Bacteria (CFU/mL)	<1	<1	<1	<1	<1	NS
Yeasts (CFU/mL)	30	20	40	< 10	< 10	$10 - 10^4$
Molds (CFU/mL)	$1.1x10^2$	90	$1.2x10^2$	50	20	$10 - 10^4$

NS: Not specified, colony forming units: CFU, E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice

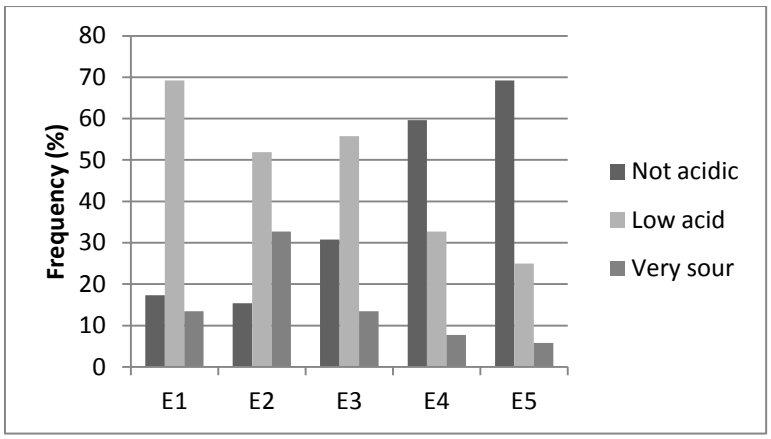

Fig. 2. Appreciation of the acidic taste of formulated beverages

E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice

On the other hand, the drinks corresponding to the E5 formula were though very sweet by 69.23% of tasters while the other had a slightly sweet taste (Fig. 3).

The fluidity of drinks from the formulas E3, E4 and E5 was liked by the tasters while that of drinks from formulas E1 and E2 was disapproved by them (Fig. 4).

The drink from E5 formula (10% of the sapodilla puree and 90% of the pineapple juice) was the most accepted of the five drinks from the five tested formulas (Fig. 5).

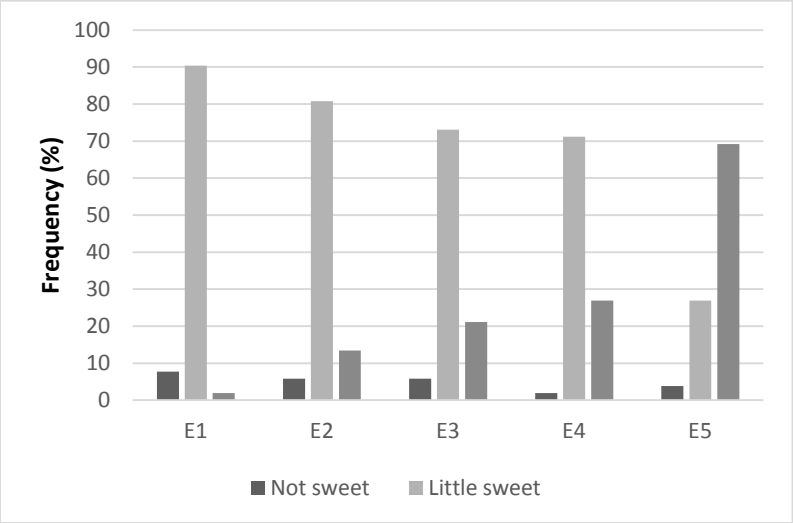

Fig. 3. Appreciation of sweet taste of formulated beverages

E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice

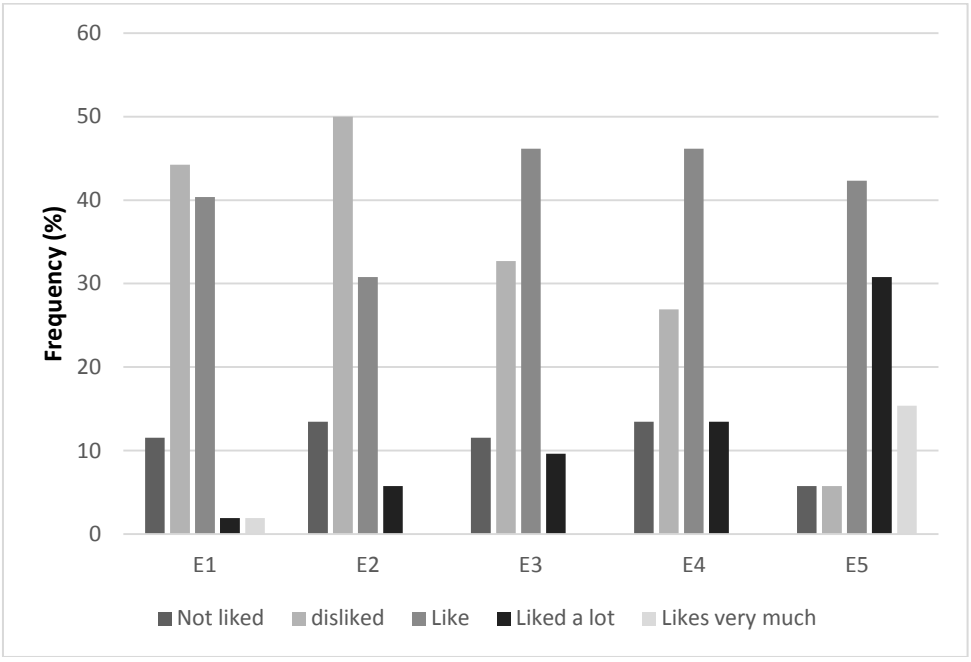

Fig. 4. Appreciation of the fluidity of formulated beverages

E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice

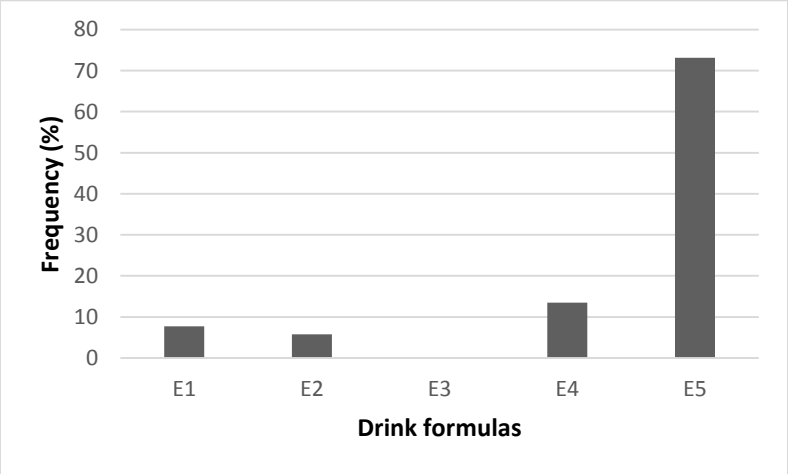

Fig. 5. Overall acceptability of formulated beverages

E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice

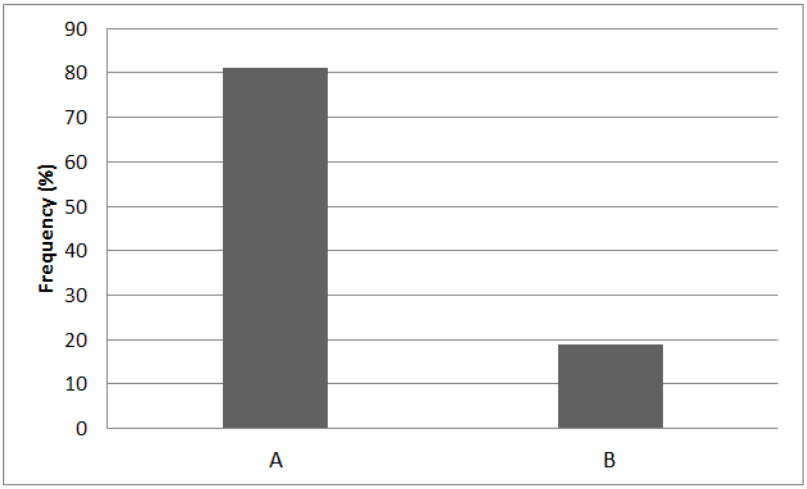

Fig. 6. Appreciation of the sensory characteristic of the drink from E5 formula flavored or not with the essential oil of *C. citratus*

A: E5 formula flavored with lemongrass essential oil, B: E5 formula: (cocktail containing 10% of sapodilla puree and 90% of pineapple juice.)

Two variants of drinks from this formula 5 were subsequently evaluated: a variant was flavored with two drops of lemongrass essential oil while the second was not flavoring. The results showed that the flavored drink was better appreciated (81.03%) (Fig. 6).

3.1.3 Physico-chemical quality of formulated beverages

The pH, total soluble solid, titratable acidity and density of the most appreciated drink from the formula 5, containing 10% of the sapodilla puree and 90% of the pineapple juice flavored or not with lemongrass essential oil were compared to those of pineapple juice (Table 3). A significant difference between pineapple juice and the different variants of the formulated drink were noted. On the other hand, no difference between the favored and non-flavored drinks was observed for the abovementioned physico-chemical parameters determined. All the samples had an acid pH which varied between 5.42±00 and 5.66±0.01. The total soluble solid, the titratable acidity and the density varied respectively from 14.5±0.10% to 17.45±0.21%; 0.64±00 g/100g to 0.80±0.01 g/100g and from 1.06±0.00 to 1.08±0.01. The pineapple juice presented ash content (0.2±0.01%) similar to that of the flavored cocktail (0.19±00%). These two

drinks had ash content significantly different from that of the flavored drink (0.38±00%). No significant difference was observed for the dry matter content which varied from 12.21±0.06% to 15.28±1.07%.

3.2 Discussion

Many farmers, especially in marginal areas, rely on neglected and underutilized species (NUS) for their livelihoods [16]. But the expansion and commercialization of NUS is constrained by a low knowledge base, weak value chains and inadequate capacity and policies [17]. Sapodilla is one of these species that merit more investigation to improve the knowledge about how it can be used as a food product. In this study, a processing method for producing a drink from sapodilla fruits, pineapple fruits and lemongrass essential oil was tested. The developed processing method had common and specific operations to each type of fruits. For instance, the deseeding operation was specific to sapodilla fruits while pressing was specific to the pineapple fruits. The processing method led to a beverage and showed that it was possible to obtain a drink by crushing the pulp of sapodilla and by pressing the pineapple fruit and mixing them in the respective proportions of one and nine volumes. The tasters' preference for the formula that contains the lowest sapodilla puree content could be explained by the fact that they are accustomed to pineapple juice. Indeed, the survey results among Beninese consumers of pineapple juice show that juices and soft drinks are consumed in order of importance and the consumption of pineapple juice amounts on average to about 34 liters per inhabitant per year [18].

Microbiological analyzes revealed absence of coliforms, *E. coli*, *S. aureus* and sulphite-reducing bacteria. The total flora counted consists mainly of yeasts and molds. Their presence can be explained by their osmophilic character. Indeed, an osmophile is a microorganism adapted to environments with high osmotic pressures, such as the high concentrations of sugar found in pineapple and sapodilla [19]. This high sugar content was confirmed by the values noted for total soluble solid which varied between 14.5±0.10 and 17.45±0.21 °Brix. In addition, the drinks had presented acidic pH; which could further justify the absence of pathogenic microorganisms [20]. The microbiological quality of all the formulated cocktails was acceptable. The potential acidity of the fruits used could justify the fact that the formulated beverages were less susceptible to microorganisms growth. It is also known that the microbial spoilage of fruits products are usually caused by molds, yeasts and some bacteria. The number of colony forming units (CFU) in formulated beverages was similar to those found by Lim et al. [6] in a sapodilla juice stored in ambient temperature of 28°C. This gives some information that can be correlated to the quality of raw material, the conditions of processing and storage, level of contaminants and the organoleptic attributes [21].

Table 3. Physicochemical characteristics of the drink produced

	pH	Total soluble solid (ºBrix)	Titratable acidity (g/100g	Density	Ash content (%)	Dry matter content (%)
Pineapple juice	5.66±0.01a	14.5±0.10a	0.64±0.00a	1.06±0.00a	0.2±0.01a	12.21±0.06a
Non flavored Cocktail pineapple-sapodilla	5,42±00b	17.45±0.21b	0.76±0.00b	1.08±0.00b	0.38±00b	15.13±1.03a
Cocktail pineapple-sapodilla flavored with lemongrass essential oil	5.46±0.06b	17.25±0.35b	0.80±0.01b	1.08±0.01b	0.19±0.00a	15.28±1.07a
P-value	0.010	0.002	0.017	0.020	0.000	0.000

In the same column, the averages not sharing any letter are significantly different at 5%

The five formulations of the beverage from both of the fruits were appreciated through a sensory evaluation. The results revealed that the taste acceptability were different according to the proportions of blended sapodilla and pineapple juices. The less sapodilla juice mixed with the pineapple juice, the higher the acceptability of the organoleptic qualities. Formulation E5 was the most preferred, followed by formulation E4 and E3. The formulation E1 containing 50% sapodilla puree and 50% pineapple juice and E2 containing 40% sapodilla puree and 60% pineapple juice were not appreciated. The taster was influenced by the sweet taste of formulated beverages. The taste of the foods products depends on its composition. According to Saliba-Colombani et al. [22], sugar and organic acid were the major chemical compounds that influence consumer preference. The addition of lemongrass essential oil to the most preferred formulation E5 was also appreciated by the tasters. This also demonstrated that this natural product can be valorized in the beverages production to embellish the sensory characteristics of the formulated products.

The formulated beverages containing 10% of fresh sapodilla puree and pineapple juice were analyzed for pH, total soluble solid, titratable acidity and density. The obtained values for non-flavored and flavored beverages with lemongrass essential oil were quite comparable except for the ash content. These values were higher than those of pure pineapple juice analyzed in this study. The pH value of formulated beverage in this study was similar to 5.36 reported by Kulkarni et al. [23] and to 5.51 found by Lim et al. [6] for sapodilla juice. The titrable acidity and total soluble solid of formulated beverage in this study were higher than those of pure pineapple juice analyzed in this study and values reported by Lim et al. [6] for pure sapodilla juice.

For the continuation of the study, the nutritional characteristics of the formulated drink will be determined. However, it is well known in the literature that the very good fiber content of the sapodilla makes this fruit a very good ally in the fight against constipation but also against colon cancer. It also contains quantities of tannins, antioxidants known for their antibacterial, antiparasitic, antiviral and anti-inflammatory properties. It has also been reported that several centuries ago, Arab doctors used sapodilla to treat certain ailments such as coughs. The sapodilla also contains good levels of vitamin A beneficial to vision, and vitamin C to resist infections. Finally, when the fruit is fully ripe, it becomes an interesting source of iron, copper and potassium, all essential elements for the proper functioning of the body [24,25,26]. These previous information glimpsed the potential of food products based on Sapodilla fruits.

4. CONCLUSION

In this study, a drink from sapodilla and pineapple fruit flavored or not with *C. citratus* essential oil was formulated. The formula appreciated by the panel of tasters contained nine volumes of pineapple juice for one volume of sapodilla puree. This work holds its originality in the valorization of the fruit of the sapodilla tree, a neglected and underused species. It also shows a way to valorize the sapodilla fruits as a novel source of ingredient for drink products development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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