

Formulation of a flavored drink based on sapodilla puree, pineapple juice and lemongrass essential oil

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 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 °Brix 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, $0.19 \pm 0.00\%$ 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

27 production, guaranteeing food security for populations, ensuring coverage of primary health
28 care needs, and contributing to the effort to reduce poverty [4]. But various species of this
29 group of plants are threatened with extinction due to the lack of investigation by the scientific
30 community, lack of interest of decision-makers and agro-industrial groups, and the
31 disappearance of their habitats. However, there is growing international recognition that
32 neglected species play a vital role in traditional medicine, food and nutrition security, and
33 provide income opportunities for small-scale farmers. Unfortunately, their expansion and
34 marketing are limited by their lack of knowledge and the lack of a related value chain.

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

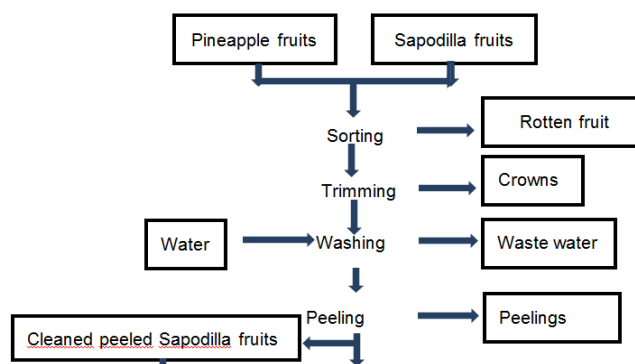
55 2.1 Plant material collection

56 The plant material used in the study were pineapple (*Ananas comosus*) fruits, leaves of
57 lemongrass (*Cymbopogon citratus*) bought in Sakété (6° 44' 11" North, 2° 39' 29" East) as
58 well as sapodilla fruits (*Manilkara zapota*) bought at the Takon market in the same
59 commune. All the chosen fruits were ripe and without any default. The study took place
60 between June and September 2021
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64 2.2 Methods

65 2.2.1 Essential oil and drink production processes

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

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

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2.2.3 Microbiological Characterization of drinks

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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].

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2.2.4 Organoleptic analyzes of drinks

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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.

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2.2.5 Physico-chemical Characterization of drinks

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The pH and total soluble solids 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].

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2.2.4 Statistical analysis

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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.

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3. RESULTS AND DISCUSSION

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3.1 Results

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

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The results of the microbiological analyzes (Table 2) revealed a total absence of coliforms, *Escherichia coli* β-glucuronidase positive, coagulase positive *staphylococci*, and sulphite-

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175 reducing bacteria. On the other hand, the total flora was expressed as colony forming units
 176 (CFU) with values ranging from 1.2×10^4 to 7.7×10^4 CFU/mL of juice. Yeasts were absent
 177 from the two samples (E4 and E5) whereas they were counted between 20 and 40 CFU/mL
 178 of juice for the other samples. Finally, the molds were numbered in all the samples at 20 and
 179 1.2×10^2 CFU/mL. All the cocktails formulated therefore had a very satisfactory
 180 microbiological quality.

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

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Germes	Samples					Criteria
	E1	E2	E3	E4	E5	
Total flora (CFU/mL)	7.7×10^4	2.2×10^4	1.2×10^4	6.7×10^4	5.6×10^4	NS
Total coliforms (CFU/mL)	<1	<1	<1	<1	<1	NS
Thermo-tolerant coliforms (CFU/mL)	<1	<1	<1	<1	<1	NS
<i>Escherichia coli</i> (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)	1.1×10^2	90	1.2×10^2	50	20	$10 - 10^4$

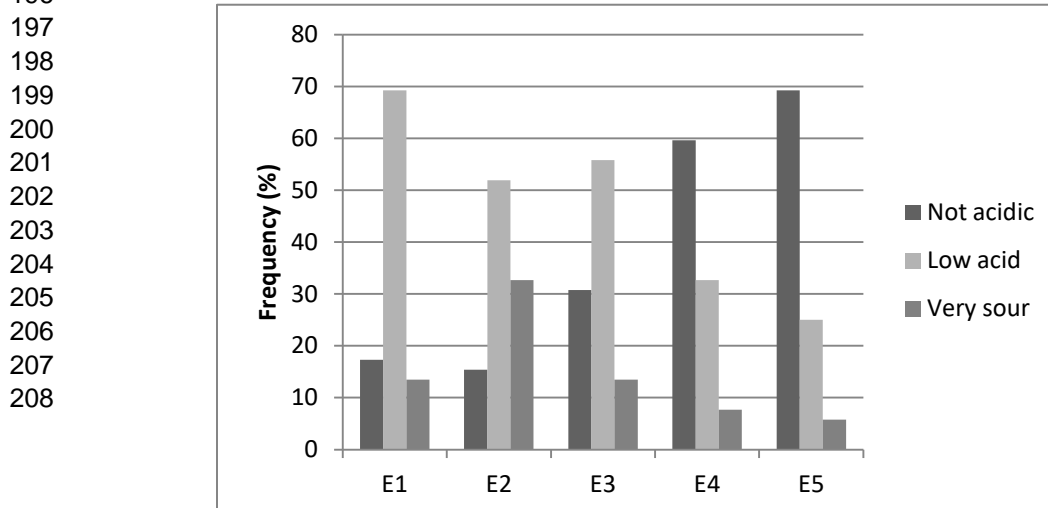
186 NS: Not specified, colony forming units: CFU, E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60%
 187 pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla
 188 puree - 90% pineapple juice.

189 **3.1.3 Sensory quality of formulated beverages from the pineapple and sapodilla fruits**

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

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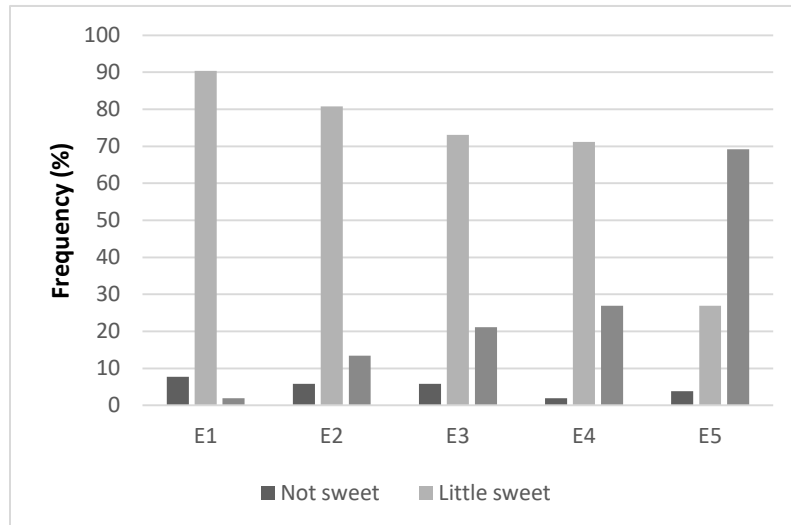


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E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice.

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

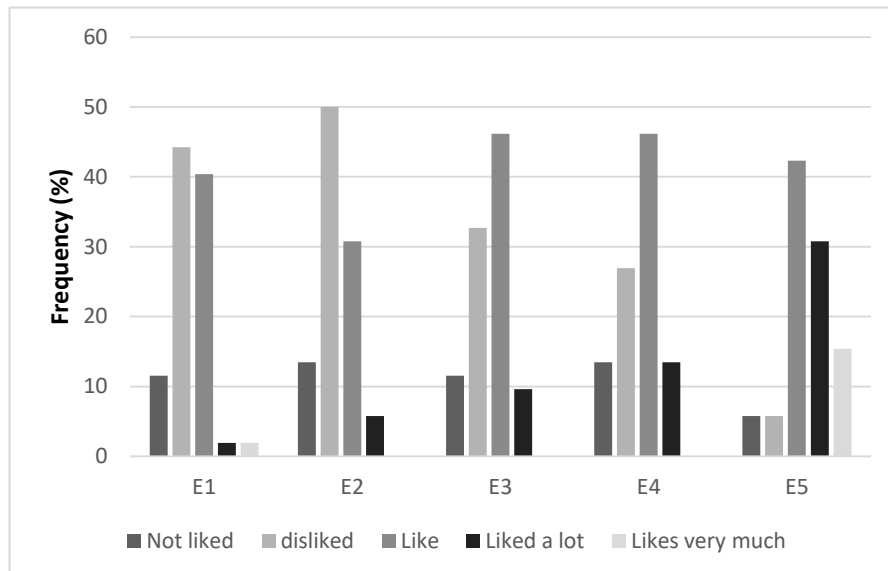
On the other hand, the drinks corresponding to the E5 formula were though very sweet by 69.23% of tasters while the other had a slightly sweet taste (Figure 3).



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. 3. Appreciation of sweet taste of formulated beverages

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 (Figure 4).

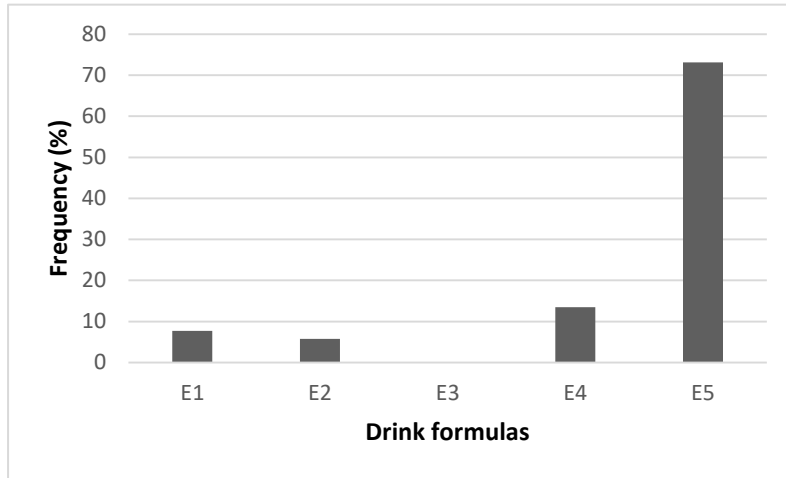


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E1: 50% sapodilla puree - 50% pineapple juice, E2: 40% sapodilla puree - 60% pineapple juice, E3: 30% sapodilla puree - 70% pineapple juice, E4: 20% sapodilla puree - 80% pineapple juice, E5: 10% sapodilla puree - 90% pineapple juice.

Fig. 4. Appreciation of the fluidity of formulated beverages

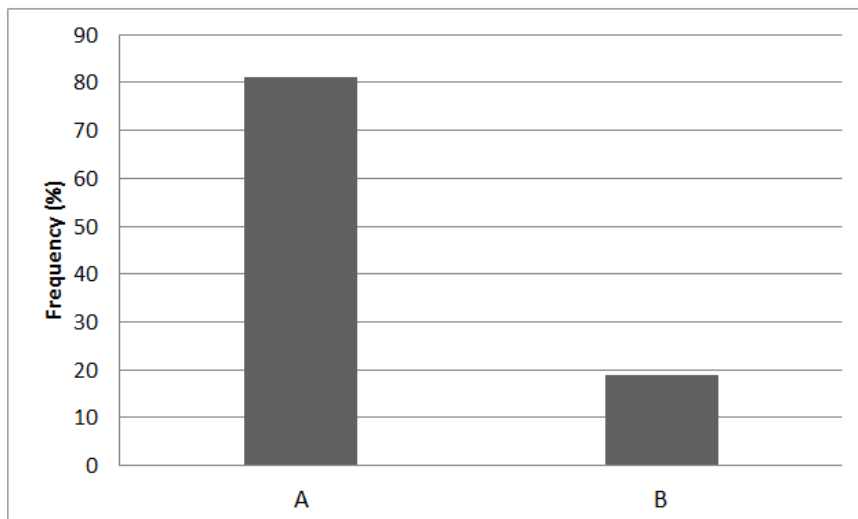
The drink from E5 formula (10% of the sapodilla puree and 90% of the pineapple juice) was the most accepted of the five drinks from the five tested formulas (Figure 5).



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

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%) (Figure 6).



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

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

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3.1.4 Physico-chemical quality of formulated beverages

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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 ± 0.00 and 5.66 ± 0.01 . The total soluble solid, the titratable acidity and the density varied respectively from $14.5 \pm 0.10\%$ to $17.45 \pm 0.21\%$; 0.64 ± 0.00 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 ($0.2 \pm 0.01\%$) similar to that of the flavored cocktail ($0.19 \pm 0.00\%$). These two drinks had ash content significantly different from that of the flavored drink ($0.38 \pm 0.00\%$). No significant difference was observed for the dry matter content which varied from $12.21 \pm 0.06\%$ to $15.28 \pm 1.07\%$.

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Table 3. Physicochemical characteristics of the drink produced

	pH	Total soluble solid (°Brix)	Titratable acidity (g/100g)	Density	Ash content (%)	Dry matter content (%)
Pineapple juice	$5.66 \pm 0.01a$	$14.5 \pm 0.10a$	$0.64 \pm 0.00a$	$1.06 \pm 0.00a$	$0.2 \pm 0.01a$	$12.21 \pm 0.06a$
Non flavored Cocktail pineapple-sapodilla	$5.42 \pm 0.00b$	$17.45 \pm 0.21b$	$0.76 \pm 0.00b$	$1.08 \pm 0.00b$	$0.38 \pm 0.00b$	$15.13 \pm 1.03a$
Cocktail pineapple-	$5.46 \pm 0.06b$	$17.25 \pm 0.35b$	$0.80 \pm 0.01b$	$1.08 \pm 0.01b$	$0.19 \pm 0.00a$	$15.28 \pm 1.07a$

sapodilla flavored with lemongrass essential oil						
P-value	0.010	0.002	0.017	0.020	0.000	0.000

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

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356 **3.2 Discussion**

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

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375 Microbiological analyzes revealed absence of coliforms, *E. coli*, *S. aureus* and sulphite-
376 reducing bacteria. The total flora counted consists mainly of yeasts and molds. Their
377 presence can be explained by their osmophilic character. Indeed, an osmophile is a
378 microorganism adapted to environments with high osmotic pressures, such as the high
379 concentrations of sugar found in pineapple and sapodilla [19]. This high sugar content was
380 confirmed by the values noted for total soluble solid which varied between 14.5±0.10 and
381 17.45±0.21 °Brix. In addition, the drinks had presented acidic pH; which could further justify
382 the absence of pathogenic microorganisms [20]. The microbiological quality of all the
383 formulated cocktails was acceptable. The potential acidity of the fruits used could justify the
384 fact that the formulated beverages were less susceptible to microorganisms growth. It is also
385 known that the microbial spoilage of fruits products are usually caused by molds, yeasts and
386 some bacteria. The number of colony forming units (CFU) in formulated beverages was
387 similar to those found by Lim et al. [6] in a sapodilla juice stored in ambient temperature of
388 28° C. This gives some information that can be correlated to the quality of raw material, the
389 conditions of processing and storage, level of contaminants and the organoleptic attributes
390 [21].

391 The five formulations of the beverage from both of the fruits were appreciated through a
392 sensory evaluation. The results revealed that the taste acceptability were different according
393 to the proportions of blended sapodilla and pineapple juices. The less sapodilla juice mixed
394 with the pineapple juice, the higher the acceptability of the organoleptic qualities.
395 Formulation E5 was the most preferred, followed by formulation E4 and E3. The formulation
396 E1 containing 50% sapodilla puree and 50% pineapple juice and E2 containing 40%
397 sapodilla puree and 60% pineapple juice were not appreciated. The taster was influenced by
398 the sweet taste of formulated beverages. The taste of the foods products depends on its
399 composition. According to Saliba-Colombani *et al.* [22], sugar and organic acid were the

400 major chemical compounds that influence consumer preference. The addition of lemongrass
401 essential oil to the most preferred formulation E5 was also appreciated by the tasters. This
402 also demonstrated that this natural product can be valorized in the beverages production to
403 embellish the sensory characteristics of the formulated products.

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

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

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425 **4. CONCLUSION**

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

433

434 **ACKNOWLEDGEMENTS**

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436 The authors thank their respective institutions for financial and technical support.

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440 **COMPETING INTERESTS**

441

442 The authors agree no conflict of interest.

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444 **AUTHORS' CONTRIBUTIONS**

445

446 All the authors have identified and validated the theme of the study. KONFO T. R. Christian,
447 AISSI M. Vahid, KOUDORO A. Yaya and BOCOGA M. Gérard led the production and
448 analysis activities in the laboratory. KONFO T. R. Christian and AISSI M. Vahid wrote and

449 reviewed the article draft. DAHOUENON-AHOUSSE Edwige, SOHOUNHLOUE C.K.
450 Dominique supervised all activities. All the authors read and approved the final version of the
451 manuscript.

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454 REFERENCES

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