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Sustainability of the Cocoa Industry: Cocoa Waste Mucilage Use to Produce Fermented Beverages. Case Study in Los Ríos Province

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ABSTRACT

Los Ríos province is eminently agricultural since the main activity of its population is the product cultivation such as cocoa, coffee, soy, passion fruit and others. Two cacao varieties are cultivated: the Nacional (delicate aroma) and the CCN51. The increase in cocoa production in the Los Ríos province has generated a large amount of waste (mucilage). Much of the cocoa mucilage is eliminated in the drainage systems, discarding a product with a high content of sugars and beneficial yeasts for fermentation processes. Due to this, the work aims to analyse the use of cocoa mucilage CCN-51 or Nacional in the production of fermented beverages that allow obtaining an added value of the surpluses of cocoa production. The methodology consisted first of the approach of design combinations (with eight experimental units and three repetitions) for fermented beverage elaboration. Then, these combinations were analysed with physicochemical parameters (pH, °Brix, °GL, transmittance, absorbance, and suspended solids) and with organoleptic analyses (product tasting). As a result, the fermented carambola beverage showed better physicochemical characteristics. Of the eight experimental units, number 8 (carambola + mucilage concentration 15% + national variety) presented better results; with transmittance values of 38.40%, suspended solids 0.0061%, density 0.978 kg/m^3, alcoholic degrees 16.67 °GL, acidity 0.48 g/L and pH 3.66. Also, the organoleptic results obtained an excellent acceptance thanks to the good colour, smell, taste, sweetness, and low astringency. This allowed having another alternative/solution for discarded mucilage use in Los Rios province.

1. INTRODUCTION

Within the components of cocoa are fermented and dry cocoa almonds (or unfermented). With these, by-products and final products are obtained through industrial processes. Cocoa by-products are cocoa paste or liquor, butter, cake, and cocoa powder. Cocoa final products are mainly chocolates and other items such as toppings, candies, chocolate bars of bitter, milk, white, fruit, nuts, or bonbons [1]. Other components of cocoa are yeasts, lactic acid and acetic acid bacteria involved in cocoa fermentation [2, 3].

Cocoa husk (*Theobroma cacao L.*) is a residue from the cocoa production process. Recent studies have shown that this product has medicinal properties, enhancing its attractiveness as an infusion [4]. According to the study carried out by Arteaga Estrella [5], he states that farmers from Quevedo-Ecuador, discard the mucilage that emerges from the cocoa bean. This is not used, causing waste of raw material (mucilage). Mucilage is a product of plant origin, and it is made up of cellulosic polysaccharides with the same number of sugars as gums and pectins [6].

One of the factors/causes that give rise to cocoa mucilage waste is the lack of farmers' knowledge to optimise and give added value to this cocoa component. The mucilaginous pulp is composed of spongy parenchymal cells, which contain sap cells rich in sugars (10-13%), pentoses (2-3%), citric acid (1-2%), and salts (8-10%). Approximately 40 litres of pulp can be obtained from 800 kilos of fresh seeds. The pulp, which has a delicious tropical flavour, has been used to make various products such as cocoa jelly, alcohol, vinegar and cream [5, 7].

In fermented beverages, elaboration, fruit components and a particular acidity are needed for an adequate fermentation. Due to its excess production, at some times of the year in Ecuador, the fruits to consider are carambola (Averrhoa carambola L.) and orange. Carambola stands out for its characteristic star shape and its solid golden colour. Its composition and physicochemical characteristics vary widely during its maturation [8]. Oranges' physicochemical characteristics are total acidity between 3.5-10, °Brix greater than six and pH between 2.8-3.8 [9]. These two fruits allow, suitably, to combine with the cocoa mucilage and start the alcoholic fermentation.

Alcoholic fermentation is an anaerobic metabolic process by which some microorganisms, mainly yeasts, acquire energy from sugars, which are transformed into ethanol (C_2H_5OH) and CO_2 as the leading products, with S. cerevisiae (unicellular fungus) being the species most used in the alcoholic beverage production industry [10].

In the world, cocoa production is found in a higher percentage in Africa with 61%, while in America, 21% is produced [11]. Ecuador is one of the most important cocoa exporters for European and North American countries due to the excellent quality of aroma and flavour [12]. Cocoa production in the province of Los Ríos corresponds mainly to small and medium-sized farmers. The cocoa production trend, in Los Ríos province, during the period 2015-2018 was increasing. In 2015, a production of 36,185 tons was obtained, while in 2018, it was 70,374 tons. As for the planted area, a maximum of 130,585 hectares was reached in 2017 [13].

A considerable mucilage volume has been generated from this production, a source of yeast and easily fermentable sugars. Approximately 40 litres of pulp can be obtained from 800 kilos of fresh seeds [12]. A study in Ecuador showed that 81% of farmers in the cocoa sector do not enhance technical development in the value chain of this product, presenting a waste of cocoa mucilage of the order of 72% due to a lack of interest in agricultural innovation [14]. Many studies have investigated utilising the cacao mucilage to produce juices, jams, and other processed products [15, 16].

This work aims to analyse the use of cocoa mucilage CCN-51 or Nacional in the production of fermented beverages that allow obtaining an added value of the surpluses of cocoa production.

2. METHODOLOGY

The following work was carried out in the State Technical University of Quevedo (UTEQ) laboratories, located in Quevedo, Los Ríos province, Ecuador. The carambola was acquired in Quevedo, Los Ríos province, to elaborate the fermented cocoa mucilage beverage. The oranges and cocoa mucilage were acquired in Valencia, Los Ríos province (see Figure 1).

Quevedo is located within a subtropical zone at 1° 20' 30" South Latitude and 79° 28' 30" West Longitude. Its average annual temperature is 25.2°C, with an average elevation of 74 meters above sea level (m.a.s.l.). Valencia is in a tropical zone at 0° 57' 09" South Latitude and 79° 21' 11" West Longitude. Its average annual temperature is 26°C, with an average elevation of 105 meters above sea level (m.a.s.l.).

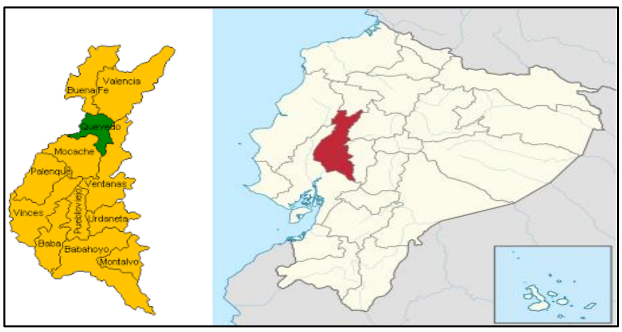

Figure 1. The geographical location of the study area (Los Ríos province)

The methodology used to carry out this research consisted of three stages (see Figure 2).

Figure 2. Investigation methodology

2.1 Research combinations design

A randomised design was used, with a factorial arrangement of A*B*C, whose factor:

(1) A= fruit types (Orange and carambola);

(2) B= cocoa mucilage concentrations (10% and 15%);

(3) C= mucilage type (CCN-51 and National).

Corresponds to 8 experimental units (EU) with three replicates, a total of 24 measurements, as described in Table 1.

The Tukey test determined the effects between levels and treatments ($p<0.05$). Tukey's test is a method that aims to compare the individual means from an analysis of the variance of several samples subjected to different treatments [17].

Table 1. Description of each experimental unit

Experimental unit	Description
1	Orange + Mucilage Concentration 10% + CCN-51
2	Orange + Mucilage Concentration 10% + National
3	Orange + Mucilage Concentration 15% + CCN-51
4	Orange + Mucilage Concentration 15% + National
5	Carambola + Mucilage Concentration 10% + CCN-51
6	Carambola + Mucilage Concentration 10% + National
7	Carambola + Mucilage Concentration 15% + CCN-51
8	Carambola + Mucilage Concentration 15% + National

2.2 Description of the fermented beverage elaboration process

The process of making the fermented cocoa mucilage beverage is described in the following steps. In Figure 3, we can see images of some processes carried out. These processes apply to all the experimental units (see Table 1).

Figure 3. Images of some processes of elaboration of the fermented beverage

(1) Selection: It consists of selecting the fruit free of contaminants and without mechanical damage (cuts, blows or others), whose purpose is that the fruits do not interfere in the fermentation process, which can contaminate the product.

(2) Cleaning: It removes impurities such as land, twigs, and leaves that the fruits may contain.

(3) Peel: This operation was carried out in oranges to prevent the essential oil (from the orange peel) from acidifying the juice. This can block the fermentation process, leading to an astringent taste.

(4) Fruit conditioning: 25 kg of carambola were chopped into small slices to obtain the carambola extract. Then, it is immersed in citric acid for 15 minutes to prevent the oxidation of the fruit.

(5) Pasteurisation: The orange and carambola extract are pasteurised at 65°C for 15 minutes. Then, its temperature is lowered to eliminate all kinds of microorganisms in the fruits.

(6) Must preparation: The mixture was made to obtain the must, which consists of 2 litres of carambola extract, then to correct the Brix degrees (°Brix) to 21.

(7) Fermentation: The must obtain placed in a conditioned container. For the fermentation process, it is sealed correctly, and a valve (Air lock CO_2) is placed to eliminate the CO_2 produced during the oxidative process of the sugars.

(8) Maturation: The maturation process is the stage in which it acquires a greater intensity of its aromas and flavours. This process took about 40 days. The longer the fermented beverages have matured, the greater the intensity of their flavours, aromas and colour.

(9) Storage: In glass bottles and suitable places. They can be refrigerated at 4°C or on hangers at room temperature with low light intensity.

2.3 Physicochemical parameter analysis

INEN 374-2015 (Alcoholic beverages or Fruit wine) standards were considered to identify the fermented beverage's physicochemical properties. For this, the following parameters were determined:

(1) pH measurement: A pH meter was measured at 25°C (room temperature), which was calibrated before using pH 4.0 and pH 7.0 buffers.

(2) Soluble solids measurement: Measurements were performed at 25°C using an electronic refractometer. This equipment has a scale from 0 to 100° Brix. The method used was 932.12 of the Official Association of Analytical Chemistry [18].

(3) Titratable acidity determination: Titratable acidity (TA), as a percentage of lactic acid, was determined according to researches of ref. [19] and [18]. Eq. (1) shows how the TA is determined.

$$\%A = \frac{\left(V \times N \times \text{mleq (lactic acid)}\right)}{PM \times 100} \tag{1}$$

(4) Transmittance and Absorbance: A spectrophotometer with a 420-nanometer wavelength was used. A 2 mL sample was taken and placed in the spectrophotometer for analysis. A commercial white wine was used as a reference standard. The results obtained are in percentage (%) [20, 21].

(5) Suspended solids: 1 mL of sample was taken in the Eppendorf tube, and then the tubes were placed in the centrifuge for 5 min at 13,000 rpm. Before centrifugation, the sample (1 mL) was weighed on an analytical balance. Then, it is weighed again to obtain the value consumed during centrifugation [22].

(6) Density: It was determined by the INEN 349 standard [23].

2.4 Organoleptic analysis

The characteristics and organoleptic analysis of the fermented beverage were determined using a 5-point scale for colour, smell, taste, sweetness, and astringency. (1=dislike very much, 2=dislike, 3=neutral, 4=like, 5=like very much).

The product testing was carried out by a selection of 10 tasters between the ages of 20-25, and each person received three different samples, with a volume of 30 mL.

3. RESULTS

3.1 Physicochemical parameter analysis

Table 2 shows the means of the physicochemical analyses performed on the Experimental Units (from Table 1).

Acidity has a minimum average of 0.48 in EU 5 (Carambola + Mucilage Concentration 10% + CCN-51), while a higher average of 0.93 in EU 4 (Orange + Mucilage Concentration 15% +National). Furthermore, it is observed that the use of orange gives values between 0.79-0.93 and using carambola between 0.48-0.59.

In °Brix, the maximum value was 21.27 in EU 1 (Orange + Mucilage Concentration 10% + CCN-51), while EU 5 (Carambola + Mucilage Concentration 10% + CCN-51) had the lowest value of 15.20.

In pH, a maximum value of 3.83 is observed in EU 3 (Orange + Mucilage Concentration 15% + CCN-51), and a minimum of 3.66 in EU 5-6.

Table 2. Means of the A*B*C interaction (fruit types, cocoa mucilage concentrations and mucilage type) of the experimental units

Physico-chemical parameter	Experimental units (EU)							
	1	2	3	4	5	6	7	8
Acidity	0.92	0.79	0.86	0.93	0.48	0.56	0.59	0.58
°Brix	21.27	20.10	19.10	20.00	15.20	16.33	15.27	16.37
pH	3.72	3.79	3.83	3.77	3.66	3.66	3.71	3.71
Absorbance	1.50	0.90	1.24	1.28	0.43	0.68	0.54	0.55
Transmittance	4.07	12.17	6.20	6.70	38.40	27.63	30.77	28.90
Suspended solids	0.017	0.018	0.013	0.013	0.009	0.007	0.012	0.007
Density	0.989	0.990	0.988	0.998	0.982	0.983	0.980	0.978
Alcohol content (°GL)	8.33	7.33	9.00	8.00	14.33	13.00	15.33	16.67

1: Orange + Mucilage Concentration 10% + CCN-51, **2:** Orange + Mucilage Concentration 10% + National, **3:** Orange + Mucilage Concentration 15% + CCN-51, **4:** Orange + Mucilage Concentration 15% + National, **5:** Carambola + Mucilage Concentration 10% + CCN-51, **6:** Carambola + Mucilage Concentration 10% + National, **7:** Carambola + Mucilage Concentration 15% + CCN-51, **8:** Carambola + Mucilage Concentration 15% + National.

Table 3. Organoleptic analysis results

Organoleptic analysis	Experimental units (EU)							
	1	2	3	4	5	6	7	8
Color	3.00	3.67	3.67	4.33	4.33	3.67	4.00	4.00
Smell	3.33	3.67	4.00	3.67	3.00	3.00	3.33	4.33
Taste	4.00	3.33	3.33	2.67	2.67	3.33	4.00	5.00
Sweetness	3.33	3.33	4.00	2.33	3.33	3.67	4.00	4.00
Astringency	3.00	3.33	3.33	2.33	3.00	3.33	3.67	4.33

In the results in the absorbance parameter, a maximum average of 1.50 is observed in EU 1 (Orange + Mucilage Concentration 10% + CCN-51). While there is a minimum value of 0.43 in EU 5 (Carambola + Mucilage Concentration 10% + CCN-51).

Regarding transmittance, a minimum value of 4.07 is observed in EU 1 (Orange + Mucilage Concentration 10% + CCN-51), while EU 5 (Carambola + Mucilage Concentration 10% + CCN-51) shows the largest value of 38.40. Furthermore, it is observed that the use of orange gives values between 4.07-12.17 and using carambola between 27.63-38.40.

In the suspended solids parameter, a maximum average of 0.018 is obtained in EU 2 (Orange + Mucilage Concentration 10% + National), while the minimum value was 0.007 in EU 6-8.

In the mean density values, there is a minimum of 0.978 in EU 8 (Carambola + Mucilage Concentration 15% + National), while in EU 4 (Orange + Mucilage Concentration 15% + National) it is observed a minimum of 0.998.

Finally, in °GL, a maximum value of 16.67 is observed in EU 8 (Carambola + Mucilage Concentration 15% + National), while the minimum value of 7.33 can be seen in EU 2 (Orange + Mucilage Concentration 10% + National). Furthermore, it is observed that the use of orange gives values between 7.33-9.00 and using carambola between 13.00-16.67.

3.2 Organoleptic analysis

Table 3 shows the results obtained from organoleptic analyses. It represents the qualification provided by the tasters of the fermented beverages. It was obtained that the EU 8 (Carambola + Mucilage Concentration 15% + National) is the experimental unit with the best qualifications in the tasting.

EU 8 beverage presented the following characteristics: in colour with a 4 (like), smell obtained a rating between 4-5 (like very much), taste showed a 5 (like very much), the sweetness was of 4 (like) and astringency obtained between 4-5 (like very much).

4. RESULT ANALYSIS

4.1 pH

The results show a mean pH of 3.78 with orange (EU 1-4) and 3.68 with carambola (EU 5-8). These values are like those obtained by García Zapateiro et al. [24] in investigating the elaboration and physicochemical characterisation of a young wine made from borojó fruit (*B. Patinoi Cuatrec*) with a pH of 3.50. [24] showed that it was done with commercial yeast (2 g/l) for the must preparation, which acted correctly with borojó.

The commercial yeast was replaced using the CCN-51 or Nacional cocoa mucilage in this research, presenting favourable results. The results obtained are within the regulations. The concentrations were according to the cocoa variety.

4.2 Acidity

According to Tukey's test, in factor A (fruit type), the acidity with orange presented a significant difference compared to carambola. Carambola has a better value (0.55 g/L) than orange (0.875 g/L), expressed in tartaric acid. There was a variation in the fermentation process depending on the cocoa mucilage. These acidity values are within the range established by the NTE INEN 374-2015 standard for alcoholic beverages and fruit wines [20]. It does not establish a minimum value but a maximum value of 1.5 g/L. This acidity guarantees that the wines are preserved [9]. This research on the production of sweet orange wine (*citrus sinensis osbeck*), by induced fermentation comparing two strains of *Saccharomyces cerevisiae*, presented an acidity value of 0.4% (g/L). [9] comments that the acidity in wines is essential as a preservative agent to prevent the development of various agents that cause diseases or wine alterations. Also, it helps to preserve the flavour and provides the smell and pleasing appearance.

Due to the mucilage acidity (for its amino acids) and the fruits, the acidity was stabilised with sugar. Therefore, 100 g of sugar per litre of fermented beverage was added after maturation.

4.3 °Brix

Regarding factor A (fruit type), a value in °Brix was determined with the orange of 20.12 and carambola of 15.79. These are close to the 18 °Brix data reported by [9]. Wine yeast is reported to perform better during fermentation and maturation. In this research, cocoa mucilage was used as yeast, and the values obtained are like the results obtained by Robles Calderón et al. [25]. Robles Calderón et al. [25] obtained 22°Brix during the alcoholic fermentation of Italia grape must to obtain white wine.

Factor C (mucilage type) presented 17.70°Brix in CCN-51 and 18.20°Brix in the National. These results comply with the provisions of the INEN 372-2016 [20] and INEN 374-2016 [26] standards for alcoholic beverages, in which the minimum value (5-8) and maximum (18-23). Also, these results are similar to the values presented by Vera-Loor et al. [27] in the study of orange juices intended for winemaking. (13°Brix).

4.4 Density and Alcohol content (°GL)

A density of 0.980 g/cm^3 was obtained at a mucilage concentration of 15% and 0.988 g/cm^3 at a mucilage concentration of 10%. These values are related to the results obtained by (García et al., 2016), in producing a young borojó fruit wine, with a density of 1,033 g/cm^3.

Regarding °GL, 10.75 was obtained in mucilage concentration at 10% and 12.25 in mucilage at 15%. These results are assimilated to that stated by Bedoya et al. [9] in the production of sweet orange wine, obtaining 13.5°GL. Therefore, the results comply with the regulations of the alcoholic beverage since the ranges are established at 8% and 18%.

4.5 Suspended solids

In the results in solids in suspension, values of 0.015 are presented with orange, while a value of 0.008 with carambola. Due to the composition of each fruit, in the case of suspended solids produced in wines, the must enrichment in unwanted substances is generated, such as tannins and unwanted organic acids [28]. The skin breaking can release unwanted tannins due to its hardness and bitterness, as well as fatty acids that, by oxidation form hexanol and cis-3-hexenal that cause herbaceous odours. Suspended solids, mainly fibrous residues resulting from the grinding of the cane that is generated in ethanol production, increase the value of solids [29].

4.6 Organoleptic Analysis

The results of the organoleptic analysis, for the fermented beverage, in the experimental units (EU) with A*B*C interaction (fruit types, cocoa mucilage concentrations and mucilage type) are observed in the attributes such as colour, smell, taste, sweetness and astringency. EU 8 (Carambola + Mucilage Concentration 15% + National) has the best rating. Each component of the organoleptic analysis is a quality indicator. It can be interpreted individually to broaden the scope of the organoleptic characteristics of an alcoholic beverage, as shown by Fajardo de Andara [30].

5. CONCLUSIONS

This research represents a contribution since it allows cocoa mucilage to work in producing fermented beverages as a fermentation agent without the addition of another type of yeast. Also, cocoa mucilage is used for its content of sugars and amino acids, preventing it from being wasted and polluting the environment.

This research presents the broadest use of cocoa mucilage that can be combined with different fruits. We worked with acid and easily oxidised fruits that can affect the fermentation process and the colour change. There will be no significant change when working with other fruits with a higher pH, and the initial pH must be corrected to enter the fermentative phase.

The fermented carambola beverage showed better physicochemical characteristics (°Brix, acidity, pH, density, °GL, transmittance, absorbance, and suspended solids) within what is established by the INEN 374-2016 and INEN 372-2016 standards on alcoholic beverages.

According to the cocoa mucilage concentrations, the mucilage concentration is 15%. This ensures a good fermentation process of the beverage. This generated favourable results in °Brix, pH, density and °GL, the same ones in the ranges established in the INEN 372/374 standards.

Of the eight experimental units, in the organoleptic analysis, EU 8 (carambola + mucilage concentration 15% + national variety) presented better colour, smell, flavour, sweetness and astringency, providing acceptable properties by tasters. Therefore, it can be indicated that carambola (*Averrhoa Carambola*) interacts better with cocoa mucilage.

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