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Production Process and Physicochemical Characterization of the Guatemalan Artisanal Fermented Beverages Boj and Suchiles

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ABSTRACT

This study provides the manufacturing process and the main physicochemical characteristics to describe the Guatemalan artisanal beverages Boj and Suchiles. Flow charts were established for the manufacturing processes of these two indigenous fermented beverages of Guatemala. Physicochemical characteristics were analyzed to determine the degree of action of the microorganisms responsible for the alcoholic fermentations. Boj and Suchiles are produced throughout the year using regional ingredients such as sugarcane and pineapple. Boj is especially popular in the northern city of Coban and Suchiles in Guatemala City and Antigua, Guatemala. Although they have cultural relevance, few scientific studies about these beverages exist. The process and raw materials used to elaborate Boj and Suchiles were documented, and samples were taken to conduct the physicochemical characterization of both beverages. Boj and Suchiles showed the following results: pH, 3.3 ± 0.1 and 3.0 ± 0.1 units; alcohol, 6.9 ± 0.9 and $0.79 \pm 0.4\%$ (v/v); total soluble solids (TSS), 3.1 ± 0.3 and 15.4 ± 1.2 (g/100g); reducing sugars 8.6 and 85.2g/L; lactic acid, 8.7 ± 1.6 and 10.7 ± 3.0 g/L; acetic acid, 2.8 ± 0.6 and 6.9 ± 2.9 g/L. Essential and nonessential amino acids, sugars, protein, organic acids, and B vitamins were analyzed.

KEYWORDS

Boj; fermented beverages; indigenous beverages; pineapple; spices; spontaneous fermentation; Suchiles; sugarcane

Introduction

Throughout history, humanity has discovered and developed methods to predigest food prolonging its life. One of these methods is fermentation, which leads to changes in the base food characteristics, resulting in a longer shelf life.^[1–3] Apart from the modification in foods' organoleptic characteristics, such as the intensity of flavor and texture, lactic or alcoholic fermentation converts the carbohydrates present into alcohol and organic acids and reduces the pH. The degradation and solubilization of carbohydrates connected to a low pH creates an unsuitable environment for harmful microorganisms. In addition, some bacteria found in fermented and unspiced products can benefit the digestive system.^[4–6]

The microorganisms used for the fermentation process may originate from a defined culture—careful cultivation of a defined and identified number of microorganisms, or they may come from the environment in which the fermentation takes place.^[7] Spontaneous fermentation uses natural starters, either from the environment, which includes surroundings, the containers and utensils, or from the raw materials being used. The fundamental ingredients for Boj and Suchiles, sugarcane and pineapple, respectively, can be found in local markets throughout the year and already contain the base microorganisms responsible for fermentation. Isolates from

sugarcane stalks show a rich microbiota such as bacteria and fungi, and yeast of the order Saccharomycetales.^[8–11] Different types of bacteria: bacilli and cocci, fungi, and yeast have also been identified from pineapple.^[12–14]

Yeast has been identified in sugarcane and pineapple and its different strains are responsible for alcoholic fermentation. For yeast to grow, reproduce and perform this function, sources of carbon, nitrogen, vitamins, and some minerals are required.^[15] To examine the effect of the presence of yeast in the studied beverages, several parameters were analyzed: pH, alcohol, organic acids, amino acids, and sugars. During fermentation, yeast consumes carbohydrates and primarily transforms them into alcohol and carbon dioxide. Yeast also metabolizes amino acids and produces organic acids, which consequently alter the pH.^[16]

Many fermented beverages known to date were the result of casual events such as the production of beer through a spontaneous fermentation of barley. In indigenous villages in various parts of the world, fermented beverages have been developed using readily available cereals or fruits. Some indigenous fermented beverage examples include, Sake in Japan, Tchoukoutou and Yabra in Africa, and Pulque and the different versions of Chicha in Latin America.^[17–19] Guatemala, the cradle of the Mayan civilization, is no exception. As a country rich in traditions inherited from its

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ancestors and influenced by Spanish conquerors, it has a diversity of preserves, food, and some fermented drinks as part of its culture.^[20,21]

Boj and Suchiles are two of the most popular handcrafted fermented beverages in Guatemala with indigenous roots; nevertheless, there is a great deal of mystery, secrecy, and many myths surrounding these beverages due to the scarce information available. There is a lack information about production processes or characteristics regarding traditional beverages from Guatemala and other countries.^[22]

Other beverages that fall into the category of artisanal fermented beverages in Guatemala are Chica and Caldo de Frutas. These together with Boj and Suchiles are the most recognized and mentioned in the culinary, anthropological, and, to some extent, scientific fields.^[20,21,23–25] Only Boj has had some deeper studies in terms of chemical analyses.^[24]

Boj and Suchiles are beverages with important religious and cultural roles, as is the case with many other traditional fermented beverages in Latin America.^[26,27] Herein lies the importance of this study, which is to describe the physicochemical properties and manufacturing processes for future generations, so that the heritage of the ancestors is not lost, and so that these beverages can be reproduced later.

Interviews were conducted with producers of Boj and Suchiles. In all cases, these were people with low schooling and who did not have a knowledge of microbiology. According to the information collected from them, the producers learned the process from their ancestors and do not consider adding a starter culture per se to the initial substrate. An analogy would be the artisanal manufacture of bread, where it is clear to the producers that without the addition of yeast, the desired bread would not be produced. In the case of Boj and Suchiles, the producers have no knowledge as to how the alcohol is generated.

Many other traditional fermented beverages worldwide are produced with spontaneous fermentation methods, and Latin America is no exception.^[26,28–30] The preparation of Boj and Suchiles is simple, as there is no heating or boiling process. These beverages are characterized by their mix of sweetness and acidity. Consumers look to these regularly as a refreshing product and one that can produce “feelings of joy.”^[20,23]

In the interviews, the artisanal way of producing these beverages was noted and it was found that for producers, quality is noticeable when their product is purchased in order for consumers to return on future occasions and thus continue their business. The consumer expects an economically viable, refreshing product with a certain alcohol content.

Ethanol was analyzed according to its percentage by volume in the different samples to establish its presence. The pH was measured as its importance lies in the fact that an alcoholic fermentation carried out for longer periods, and in the presence of air and acetobacteria, can result in ethanol being converted into acetic acid and then the pleasant perception of the beverage can decrease.^[15]

In the current manufacturing process of both Boj and Suchiles, there is a possibility of microbiological contamination that could affect or deviate the flavor (Appendix A). To stabilize the process, conditions must be improved in

terms of good manufacturing practices, keeping low temperatures to minimize the activity of microorganisms to maintain the flavor of these beverages for a longer period, and, preferably, knowing the microorganisms involved in order to produce these beverages in an industrial and controlled manner.

With this study, it was possible to document for the first time the processing schemes of these two indigenous Guatemalan beverages, Boj and Suchiles. A future study will analyze the microorganisms used in these fermentations and their sensory peculiarities.

Experimental

Boj and Suchiles samples

The samples of the traditional spontaneous fermented beverages Boj and Suchiles, indigenous to Guatemala, were taken from regions where they are very popular, commercially available, and have high production (Figure 1). Producers of Boj and Suchiles samples were chosen based on whether the producers could speak Spanish, had an accessible location and whether they were willing to share information on their raw materials and manufacturing procedures.

The sample collection process was repeated four times (January to August 2019) to obtain samples from four different production batches. The samples were treated and held at a stable low-temperature to avoid over-fermentation and then were sent to Germany for analysis. The first three samples were analyzed for physicochemical and sensory aspects. The fourth sample was used for microbe isolation and the microorganisms' identification (microbiological and sensory characterization are the focus of an upcoming paper). This paper only describes the physicochemical results using three batches of each beverage.

Boj samples were collected in Cobán, the most influential city of Alta Verapaz, located 219 km away from Guatemala City at 1,320 m above sea level. In Cobán, there are many Bojerías (a place where Boj is prepared and sold); however, many of these are in the mountains, in areas that are difficult to access. The owners and producers of these Bojerías usually only speak Q'eqchi' (Mayan language native to Cobán).^[20]



Figure 1. Sampling locations (1) Boj and (2) Suchiles.

The Suchiles samples were collected in Guatemala City, in the city's historical center in zone 1, on 10th Avenue and 5th Street, since there are only two famous producers of Suchiles.

Handling of the samples

Boj and Suchiles were obtained in one-gallon plastic containers, placed in a cooler box with frozen cool-packs to keep the samples cold during transfer to the bottling facility. Transfer time to the bottling facility was 5 h for Boj and 20 min for Suchiles. Before bottling, the samples were kept no longer than 24 h in a refrigerated room at 10 °C.

When taking the samples, the Boj had been fermenting for 8 days and Suchiles for 5 days prior to shipment to Germany. Prior to transfer, preservatives (0.1 g/L NisinZ and 0.04 g/L Natap Ultrapure) were added to prevent gram-positive bacteria or fungal growth in the samples for the physicochemical analysis. After adding the preservatives, the samples were mixed vigorously by hand and then poured into 350-mL sterile glass bottles and closed using a hand-operated crowner. The bottles were previously sanitized with a 2% Vortexx solution.

After capping, the bottles were placed into plastic crates for pasteurization. A plastic barrel drum with water at 65 °C was used. Bottles were inside the barrel for 12 min to obtain 62 Pasteurization Units (PU). The PU were calculated using the formula described by Kunze.^[16] The remaining samples obtained from the producers were not pasteurized to preserve the microflora, which were isolated afterward.

Prior to shipment to Germany, the crates were placed into Styrofoam coolers packed in cardboard boxes, using aluminum foil as insulation. Frozen gel-packs surrounded the plastic bins before closing the coolers (Figure 2). The transit time was approximately 10 days. During transit, the samples were kept below 8 °C to prevent the untreated samples from over fermenting.

Juices taken as references

The core beverage ingredients for Boj and Suchiles, sugarcane and pineapple juice, respectively, were chosen as a reference. This provided a basis against which to compare

the relative reduction of amino acids during fermentation. Sugarcane juice was purchased in 300-mL transparent glass bottles, covered with a shrink-wrap sleeve, produced by Sustên Indústria e Comércio de Produtos Alimentícios Ltda. in Brazil (expiry date June 2020). A pasteurized organic pineapple juice was purchased in 700-mL transparent glass bottles, and it was produced by Voelkel GmbH in Germany (expiry date March 23, 2020).^[31]

Analysis

Both for the Boj and Suchiles samples, as well as for sugarcane and pineapple juices, physicochemical analyzes were conducted by the accredited laboratory (DIN EN ISO 17025) Research Center Weihenstephan for Brewing and Food Quality, Freising, Germany according to the MEBAK method collection described in the Central European Commission for Brewing Analysis (*Mitteleuropäische Brautechnische Analysenkommission* e. V. or MEBAK) 2012.^[32] Methods are evaluated with proficiency testing and reference standards. MEBAK is similar to European Brewing Convention (EBC) or American Society of Brewing Chemist (ASBC) methods. The samples were analyzed in Germany two months after they were taken in Guatemala.

Sugars: MEBAK method WBBM 2.7.1, HPLC Thermo Fischer Scientific, Ultimate 3000 with light scattering detector Varian ELSD 385-LC. Reagents and chemicals: acetonitrile (Merck 114291), fructose (Merck 105323), glucose (Merck 108337), and saccharose (Merck 107653). The calibration was conducted as an external standard method using a one-point calibration.

Amino acids: MEBAK method WBBM 2.6.4.1.2, HPLC Thermo Fischer Scientific, Ultimate 3000 with fluorescence detector. Reagents and chemicals: acetonitrile (HPLC grade), DL-norvalin, tetraethylammonium hydroxide 20% (Merck 8.22149.1000), OPA-Reagent (Alltech Grom GmbH, 980.0600), methanol (HPLC grade), acetic acid 10%, sodium acetate, di-sodium hydrogen phosphate (Merck 1.06586.0500), sodium azide (Roth K305.1), hydrochloric acid 10%, 5-sulfosalicylic acid, boric acid, potassium dihydrogen phosphate (Merck 4873), L-aspartic acid, aminobutyric acid, L-glutamic acid, L-tyrosine, DL-asparagine monohydrate, L-valine, L-serine, L-methionine, L-glutamine, L-tryptophan, L-histidine, L-phenylalanine, glycine, L-isoleucine, L-threonine, L-leucine, L-alanine, L-lysine, L-arginine. The internal standard used was norvaline-based.

Organic acids: MEBAK method WBBM 2.21.7.2, IC HPLC, Dionex IC 2000 with eluent generation (KOH), autosampler AS-40, and separation column AS-18. Reagents and chemicals: KOH as gradient and H₂O double-distilled; from Alfa Aesar: sodium pyruvate, sodium acetate, sodium succinate, sodium citrate dihydrate, and sodium oxalate; from VWR: maleic acid, sodium formate and lactic acid; from Fluka: butyric acid; from Riedel-de-Haen: propionic acid. The calculation of the peak areas and the resulting concentrations of the calibrated substances was obtained by means of software. Standard solutions were used to calibrate the equipment prior to taking measurements.

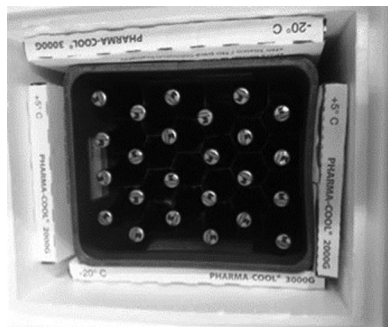


Figure 2. Plastic bottle crates packed in Styrofoam coolers for shipping.

Other analysis: Suspended solids and alcohol (MEBAK method WBBM 2.9.6.3), pH (MEBAK method WBBM 2.13), caloric value (MEBAK method WBBM 2.10.3.7), and total acidity as lactic acid (method described by Krüger and Bielig K-11.3).^[33]

Support was obtained from the SGS Institut Fresenius for the following analysis: protein (method according to SOP M 1559), riboflavin (CIN EN 14152), niacin (AOAC 944.13), pantothenic acid (AOAC 945.74), biotin (SOP M 3532), and total folate (according to DIN EN 14131:2003).

Results and discussion

Description of Boj and Suchiles process and sociocultural background

Crucial information was obtained from the interviewed producer of Boj. The substrate is juice from sugarcane that grows in nearby areas (Appendix Figure S1). The juice is either enriched with Boj from an older production batch, similar to cachaça starters used in Brazil,^[26,34] or a mixture of damaged corn (*Zea mays*) fermented in water is used (process characterized in Figure 3). If a small portion of the previous batch is reused, the microorganisms become specialized in this specific substrate, resulting in less microbial stress and better fermentations.^[26] The use of damaged corn confers essential microflora to the beverage since microorganisms are present on the surface of insects and plants.^[35–38] With this practice, the microbes from the surface and beyond encounter the substrate that the corn offers. The beverage's substrate is enhanced with other flora in addition to those present in the environment, atmosphere, containers, and utensils.^[23]

The use of damaged corn in the manufacture of Boj draws attention to possible mycotoxin issues as the soaking of the grain produces a suitable environment for the proliferation of molds and fungi, where some may have the ability to produce mycotoxins. Examples of mycotoxins in cereals include ZEA (zearalenone) and DON (deoxynivalenol).^[16] In the present work, Boj was not analyzed for this and the extent to which mycotoxins might be transferred through the different generations of production is not known, or whether producers are even aware of the effects of using soaked corn on the production and possible consumption of mycotoxins. More research and sharing of information with producers are needed on this topic.

Another area of concern during the production of Boj is the absence of hygiene or good manufacturing practices, a factor common to many traditional fermented beverages in Latin America.^[26,28] During Boj production, none of the utensils used were previously washed or protected from insects or other contaminants, and the production process was usually conducted in the open or in areas without basic hygiene requirements. Only when Boj is destined to be used in religious ceremonies was there a cleaning process and that only consisted of rinsing the sugarcane with water prior to the pressing step.

According to Sandoval and Ortiz^[23] and confirmed by the interviewed producer, Boj is consumed both every day and in religious rituals and festivities. Due to the reverence

and purity associated with religious ceremonies, in the tradition of the Boj producers, they try to employ better techniques when producing ceremonial Boj. For this purpose, the sugarcane is washed, and the drink is sanctified by leaving it in front of a worshiped saint, prior to consumption.^[20]

For Suchiles production (Figure 4), among the most relevant information gathered from the interviewed producer was that its production is very similar to the manufacture of Mexican Tepache,^[39–41] not only in the ingredients used but also in the processing times. Suchiles is a fermented beverage made with various ingredients that can be found in local markets, such as barley (*Hordeum vulgare*), yellow corn (*Zea mays*), cassia fistula (*Cassia fistula*), ginger (*Zingiber officinale*), cinnamon (*Cinnamomum verum*), tamarind (*Tamarindus indica*), star anise (*Illicium verum*), clove (*Syzygium aromaticum*), allspice (*Pimenta dioica*), pineapple (*Ananas comosus*) juice and peels, and water. The microbial culture used for this beverage comes from its raw materials and the environment. Similar to Tepache, Suchiles can also contain small portions of fruits other than pineapples, which is why the beverage can have slightly different characteristics depending on the season in which it is produced.^[30,40] A record of ratios and proportions of other fruits was not available.

In the preparation of Suchiles, a comal is used to brown or roast some ingredients to give them a brown color and a light toasted appearance (Figure 4). It prevents them from burning and turning very dark or black and the practice confers special flavor tones. A comal is a round and flat clay utensil that is generally placed over a wood fire to cook or roast. The use of the comal began in the Preclassic (or Formative Period) of the Mayan civilization and although it has undergone some changes through history, its use and materials are similar. The comal is widely used in the country and in some other regions of Latin America, such as Mexico and Central America. The most frequent use for a comal in Guatemala and Mexico is in the elaboration of corn-based tortillas, other uses include cooking and roasting of cereals, coffee (*Coffea*), and cacao (*Theobroma cacao*).^[24,42,43]

Panela is also mentioned as an ingredient of Suchiles. Panela is an unrefined whole cane sugar used as a sweetener. Its refining process is not as extensive as that of commercial sugar. Sugarcane stalks are passed through a mill, the juice is extracted and concentrated. At the end of the process, it appears crystalline and brown in color.^[44,45]

Clay pots are used to ferment Suchiles, and these are cured once and then reused without an actual cleaning process between uses. Therefore, it can be expected that the microflora of a new batch is enriched with the microflora of the previous batch.^[24,26] Other factors that contribute to the microflora of a Suchiles fermentation include pineapple peels, corn husks, and the use of porous materials such as wooden spoons and clay jars.^[26,46]

A short fermenting period is another essential characteristic of Suchiles production. Suchiles is usually fermented for three days (in comparison to the eight days that Boj is fermented). According to the producer, more extended

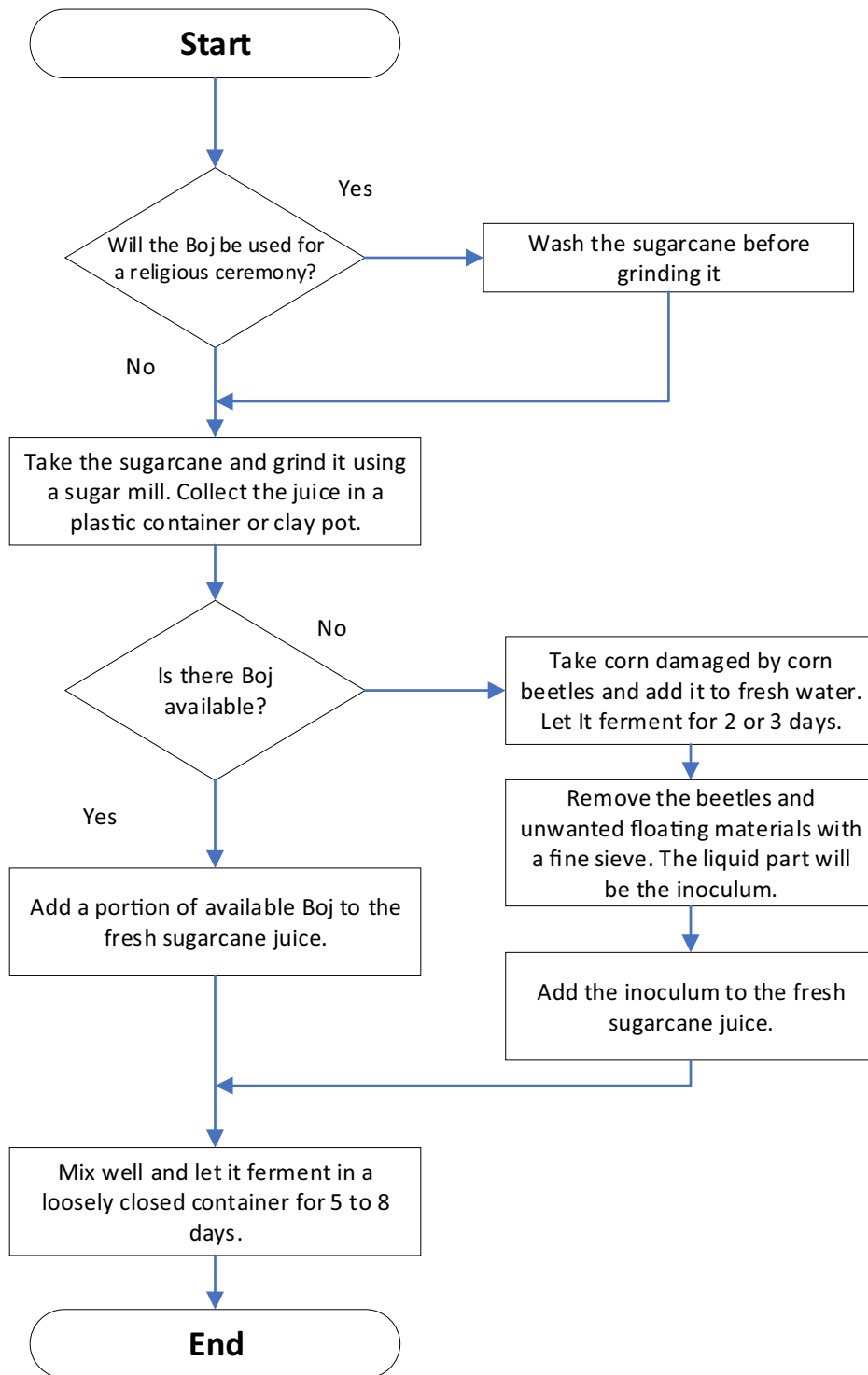


Figure 3. Boj production process in Cobán, Guatemala.

fermentation periods will produce an increased acidic taste. This can probably be attributed to lactic acid bacteria.^[40,41] A sour taste is usually undesirable and represents higher costs to the producer as more sugar must be added at the end to balance the sweet and sour taste, a similar process to Tepache.^[30]

The Suchiles producer also relayed the old custom of burying the fermenting jar. According to the producer, this custom is no longer frequently practiced, but it can still be performed on very hot days to avoid the fermentation jar

becoming too hot. A comparable practice to regulate the temperature of fermented beverages occurs in different Latin American areas.^[30]

Physicochemical analysis

The physicochemical analyses performed on Boj and Suchiles made it possible to compare the two beverages. Different parameters that could show the activity of the yeast present were chosen. When starved of oxygen, the yeast is able to

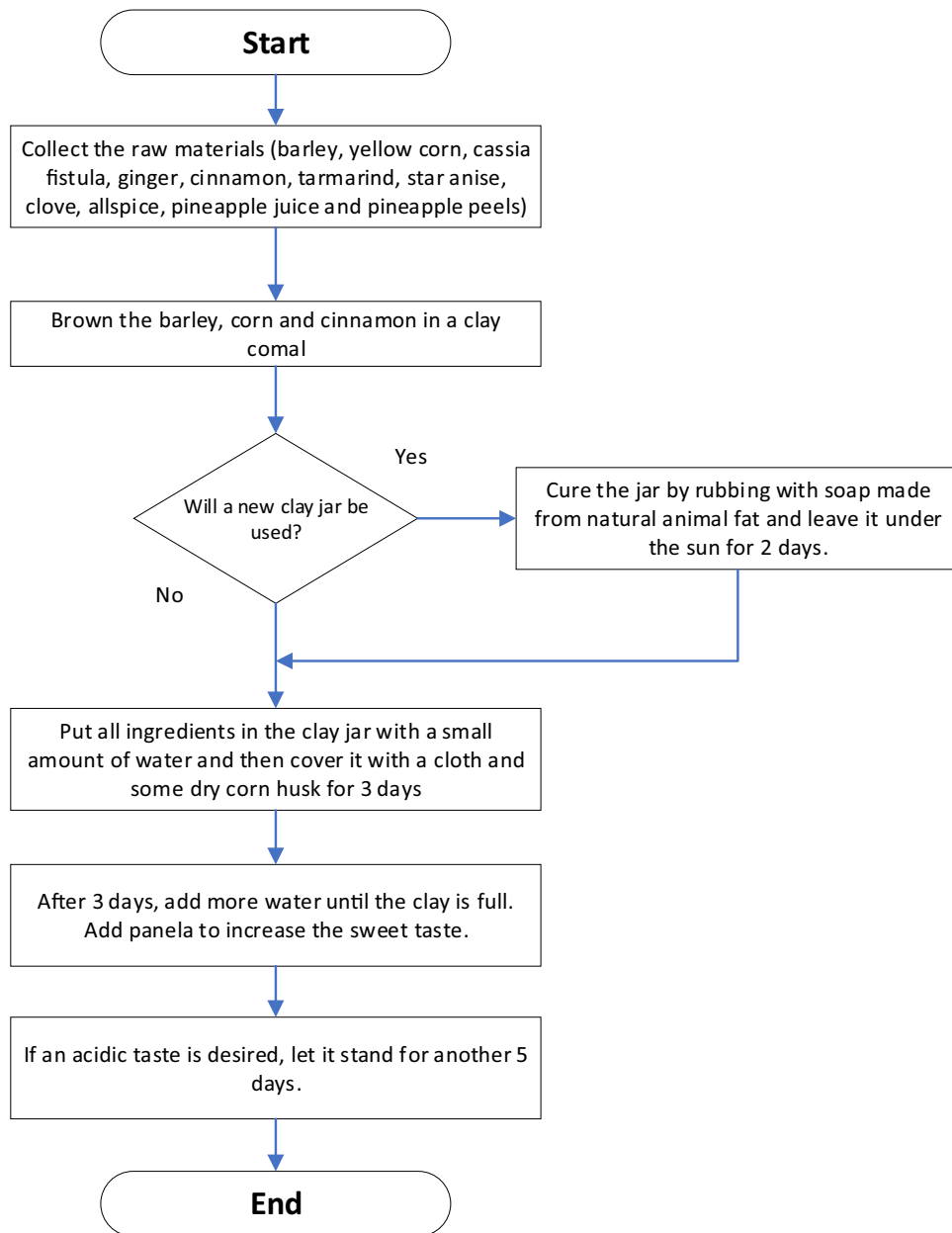


Figure 4. Suchiles production process in Guatemala City, Guatemala.

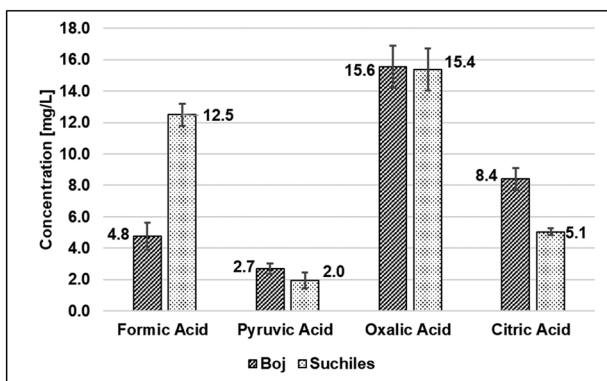


Figure 5. Organic acid concentration in Boj and Suchiles collected from artisan producers in the northern and central regions of Guatemala in 2019.

ingest sugars present in its medium and then transform them mainly into ethanol, carbon dioxide, and energy.^[16] The substrates of the two beverages studied, Boj (sugarcane juice) and Suchiles (pineapple juice), contained dissolved sugars, the basis of yeast nutrition.

When analyzing the amount of ethanol (% v/v) present in these beverages, it was found that Boj had 6.9 and Suchiles 0.79 mL/100 mL (Tables 1 and 2). For Boj, the yeast present produced much more ethanol, probably due to the substrate, since it is a simpler base containing only sugarcane juice. With Suchiles, the matrix is more complex with many more ingredients that could interfere with the yeast's performance. Another factor that could contribute to this is the amount and type of microorganism present. Regarding these results, Boj is within the range of alcohol generated by other sugarcane-based fermented beverages, and it is also

Table 1. Comparison of the characteristics of Boj and different sugarcane-based fermented juices.

Fermentation Characteristics		Boj-2019	Boj-1988 [23]	Sugarcane wine-like ^{[47]a,b}	Sugarcane wine-like ^{[47]a,b}	Sugarcane fermented ^{[48]c}
Duration, Temperature	d, °C	8 d, ND	ND	6 d, 30°C	6 d, 30°C	7 d, ND
Microorganisms involve		<i>yeast, LAB, AAB</i>	<i>yeast, AAB, others?</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae var. ellipsoideus</i>	<i>S. cerevisiae</i>
Total soluble solids, substrate	g/100 g	13.5 ± 1.3	ND	20.0	20.0	20 ± 0.0
Total soluble solids, end product	g/100 g	3.1 ± 0.3	3.3 ± 0.2	18.0	3.0	4.8
Reducing sugars end product	g/100 g	0.86	0.86 ± 0.2	ND	ND	ND
Total sugars end product	g/100 g	0.94	1.46 ± 0.3	ND	ND	ND
Alcohol	mL/100 mL	6.9 ± 0.9	6.6 ± 1.1	1.6	10.0	4.5 ± 0.4
Degree of attenuation	%	77.1 ± 2.6	ND	10.0	85.0	76.0
pH	Units	3.3 ± 0.1	3.3 ± 0.1	ND	3.2	4.0 ± 0.0

^aSelf-made calculation for TSS end product using data from Rivera-Espinoza et al. [47]

^bAdjustments in this study: TSS-substrate from 19.7 to 20 g/100 g; pH-substrate 5.1 to 3.5–4.0.

^cSelf-made calculations for TSS end product, Alcohol and pH using data from Resende Oliveira et al. [48]

Abbreviations: LAB, lactic acid bacteria; AAB, acetic acid bacteria; ND, no data.

Table 2. Comparison of the characteristics of Suchiles and different pineapple-based fermented juices.

Fermentation characteristics		Suchiles-2019	Pineapple wine ^{[49]a}	Fermented pineapple juice ^{[50]b}	Fermented pineapple juice ^{[50]b}	Tepache ^{[50]c}
Duration, Temperature	d, °C	5 d, ND	5 d, 26°C	10 d, 25°C	10 d, 25°C	2.5 d, ND
Microorganisms involve		<i>yeast, LAB</i>	<i>bakery yeast</i>	<i>S. cerevisiae</i>	<i>S. cerevisiae, H., SI</i>	<i>yeast, LAB, AAB</i>
Total soluble solids, substrate	g/100 g	16.5 ± 1.5	ND	22.0	22.0	ND
Total soluble solids, end product	g/100 g	15.4 ± 1.2	ND	8.4	6.6	11.0
Sugars	g/L	155.90	ND			
Alcohol	mL/100 mL	0.79 ± 0.4	1.6	12.3	13.0	0.24
Degree of attenuation	%	6.6 ± 2.7	10.0	61.8 ³	70.0 ³	ND
pH	Units	3.0 ± 0.1	ND	ND	ND	3.1

^aBefore fermentation, brown sugar was also added to the juice.

^bBefore fermentation, sucrose was also added to the juice

^cSelf-made calculations using data from Chanprasartsuk et al. [50]

Abbreviations: LAB, lactic acid bacteria; AAB, acetic acid bacteria; ND, no data; H, Hanseniaspora; SI, *Sacharomyces ludwigii*.

Table 3. Characteristics of different sugarcane and pineapple juices.

Sample	Total soluble solids, substrate g/100g	pH units
Sugarcane juice [Kanai]	17.35	ND
Sugarcane varieties ^{[35]a}	18.76	±3.07
Sugarcane juice ^[48]	20.00	±0.05
Sugarcane juice ^[52]	19.54	±2.21
average	18.91	5.73
Pineapple juice [bio Voelker]	13.30	5.20
Pineapple juice ^[53]	14.0	3.74
Pineapple juice ^{[54]b}	12.00	ND
Pineapple juice ^[50]	18.10	3.70
average	14.35	±0.38
		±0.00
		3.69

^aSelf-made calculations using data from Martini et al. [35]

^bSelf-made calculations using data from Cárnara et al. [54]

very similar to the alcohol content of some beers.^[16,51] Suchiles however exhibits a low alcohol content by volume, and this drink can be classified as a “low alcohol” product.^[16]

The alcohol contained in beverages provides a positive sensation in terms of organoleptic aspects, influencing taste and odor because many substances can be dissolved by this compound. It also provides body; a sensation of thickness that can be perceived in beverages.^[51]

However, from the point of view of health, alcohol can be harmful,^[51] but there is also another danger that occurs in the types of beverages such as those analyzed here. In

the absence of controls, they can be altered with methanol, which is toxic to humans, causing blindness and eventually death. Both Boj and Suchiles have been previously studied for their methanol content.^[25] In this study, the samples analyzed by gas chromatography all had levels below the permitted limit for both beverages; however, ongoing public health monitoring is required for these beverages to be considered safe for the consumer.

A relevant aspect is the change in pH during the spontaneous fermentation of Boj and Suchiles. The pH decreases due to the formation of organic acids during fermentation,

and this brings with it the inhibition of the growth of microorganisms.^[16] Taking into consideration the standard pH values of sugarcane juice and pineapple juice (i.e., 5.2 and 3.7 units, respectively) (Table 3), Boj exposes a further reduction in pH reaching a value of 3.3 and Suchiles as far as 3.0 pH units.

Most organic acids are formed during fermentation as the yeast utilizes the amino group (-NH₂) for formation of necessary nitrogenous compounds and results in organic acids that do not contain the amino group. The variety and quantity of these compounds influences the flavor and odor of the beverage.^[16] Although Boj and Suchiles had a similar pH, the total acidity expressed as lactic acid presented a higher value in Suchiles (13.40 ± 1.68 g/L) in comparison to Boj (8.81 ± 1.35 g/L), which suggests that the concentration of organic acids would be higher for Suchiles,^[49] and this was then confirmed (Figure 5 and 6). Both the mixture of organic acids and the total amount of suspended solids have an impact on the sensory aspects of the beverages.

Analyzing Boj-2019 with other sugarcane fermented beverages (Table 1) allowed comparison to the results of a previous study of Boj (Boj-1988) by Sandoval and Ortiz in 1988,^[23] and it suggests that these characteristics of Boj are consistent over time. Boj has a distinctive character given by spontaneous fermentation, while the other sugarcane wines mentioned in Table 1 were made under specific and controlled conditions, producing beverages with different physicochemical and probably different sensory characteristics. Similar observations were made with Suchiles (Table 2). There are many pineapple-based fermented beverages, but they do not correlate with the particularities of Suchiles.

Boj and Suchiles are spontaneously fermented beverages, meaning that the microorganisms involved in their production are taken from the environment, the raw material or the utensils;^[7,23,26,28] due to this, one reason why no similarity is found with other artisanal beverages with similar substrates could be the existence of other microorganisms that enter during the manufacturing process in an uncontrolled manner and that are part of the environmental factors of the region.

To compare Boj and Suchiles with simpler unfermented substrates, commercially available sugarcane juice and

pineapple juice were purchased and analyzed. The selection was based on the primary raw material used to produce Boj (sugarcane) and Suchiles (pineapple). The juices were bought in Germany in 2019 to avoid the shipping delay and the biological risks of bringing the original bases of the beverages directly from Guatemala. In a later stage of this research (data not shown; follow-up paper will focus on the microbiology and sensory of Boj and Suchiles), the juices were also used as the substrate for various small-scale fermentation processes using microorganisms isolated from Boj and Suchiles. The reference chosen for Boj was Kanai juice. The total soluble solids (TSS) in this juice correlated with other studies on sugarcane juices,^[35,48,52] it was assumed that the pH was modified to prevent biological contamination. The pineapple juice, bio Voelker presented similar TSS and pH values to other pineapple juices used in different studies (Table 3).

The analyses included amino acids for Boj, Suchiles, the unfermented sugarcane juices, and the pineapple juices bought in Germany. Comparing the concentration of amino acids in Boj against the amino acids in the reference sugarcane juice, a relative average reduction was 74%. A similar calculation for Suchiles gave a higher average value of 96% (Table 4), suggesting that the microorganisms in Suchiles assimilated more of the amino acids. Only two amino acids showed a different trend: glutamine in Boj and serine in Suchiles. This can probably be attributed to the original raw materials' characteristics, but more investigation is required.

Both beverages presented high levels of acetic and lactic acid (Figure 6); however, Boj presented lower values than Suchiles, suggesting that acetic acid bacteria (AAB) and lactic acid bacteria (LAB) are higher in Suchiles.^[16,55] The higher alcohol level, lower TSS, and lower composition of sugars in Boj (Tables 1 and 2, and Figure 7) harmonize with the more extended fermentation period of Boj. Identifying the microorganisms present in these spontaneously fermented beverages is recommended and will be performed by the authors for publication in a follow-up paper.

The beverages were fermented for 8 days (Boj) and 5 days (Suchiles) prior to shipment to Germany and were then processed with an antibacterial and an antifungal added to prevent further fermentation. Samples were analyzed approximately 2 months after the samples were originally collected; therefore, it was not possible to analyze for changes in the beverage over time.

The TSS in the substrates for Boj and Suchiles (Tables 1 and 2) were 13.5 and 16.5 g/100 g, respectively, and in the end product, the TSS values were 3.1 and 15.4 g/100 g, respectively. When analyzing this information together with the total sugar content in the finished beverages (Figure 7), it can be seen that Suchiles has a higher amount than Boj. In the case of Suchiles, the amount of total sugars present is high, and the value of total sugars in the finished product is even higher than the TSS present in the substrate. The producer indicated that it is common to add a certain amount of sugar before selling the product to improve the flavor; this could be the reason for the results seen in the analysis.

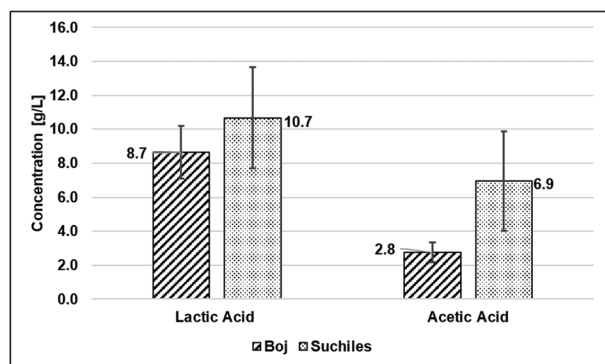


Figure 6. Concentration of lactic and acetic acid in Boj and Suchiles collected from artisan producers in the northern and central regions of Guatemala in 2019.

Table 4. Amino acid composition of sugarcane juice, Boj, pineapple juice, and Suchiles.

Amino acids, mg/ 100 mL ^a	Sugarcane juice	Boj	Reduction %	Pineapple juice	Suchiles	Reduction %
Aspartic acid	4.50	0.35	92	6.10	0.17	97
Glutamic acid	1.90	0.53	72	3.30	0.13	96
Asparagine	16.80	0.10	99	29.50	0.03	100
Serine	1.40	0.17	88	<0.50	0.10	NC
Glutamine	<0.50	0.24	NC	<0.50	<0.50	NC
Histidine	0.40	0.05	88	15.90	<0.50	100
Glycin	0.30	0.29	3	1.20	0.07	94
Threonine	0.50	0.14	72	1.50	0.03	98
Alanine	1.50	0.61	59	3.50	0.60	83
Arginine	0.70	0.49	30	2.00	0.07	97
G-Amino butanoic Acid	0.90	0.11	88	1.80	<0.50	NC
Tyrosine	0.60	0.09	85	4.60	<0.50	NC
Valine	1.00	0.13	87	1.30	0.03	98
Methionine	0.20	0.06	70	2.80	<0.50	NC
Tryptophan	0.50	0.03	94	0.40	<0.50	NC
Isoleucine	0.50	0.07	86	0.90	0.03	97
Phenylalanine	0.60	0.12	80	1.10	<0.50	NC
Leucine	0.90	0.18	80	1.80	<0.50	NC
Lysine	0.60	0.26	57	2.00	0.10	95
		Relative Average reduction	74%			96

^aThe detection limit for all amino acids is 0.5 mg/100 mL.
Abbreviations: NC, not calculable.

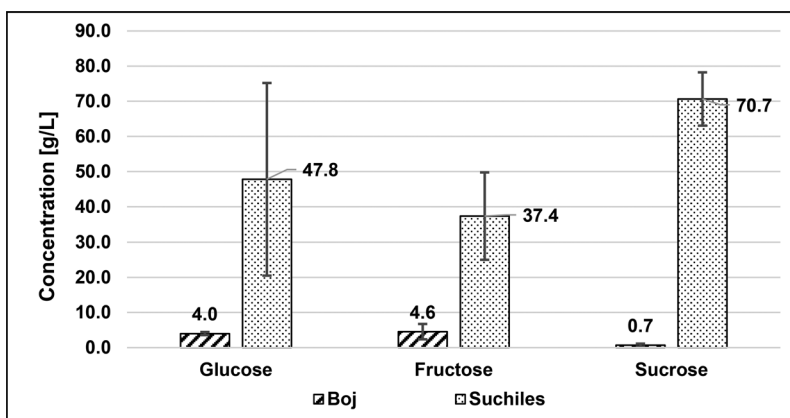


Figure 7. Sugar composition of Boj and Suchiles collected from artisanal producers in the northern and central regions of Guatemala in 2019. Boj reducing sugars 4.0 + 4.6 = 8.6 g/L; Suchiles reducing sugars 47.8 + 37.4 = 85.2.

Table 5. Comparison of the vitamin content in a liter of Boj, Suchiles, and Pilsner beer, along with compliance of Recommended Daily Intake (RDI).

Sample	Vitamin B2 riboflavin mg	Vitamin B3 niacin mg	Vitamin B5 pantothenic acid mg	Vitamin B7 biotin ug	Vitamin B9 folic acid ug
Boj-2019	0.12 ± 0.01	1.37 ± 0.03	1.26 ± 0.34	23.25 ± 1.12	55.63 ± 13.63
Suchiles-2019	ND	1.17 ± 0.19	0.40 ± 0.11	3.81 ± 1.20	54.33 ± 20.25
Pilsner Beer ^[51]	0.34	7.73	1.49	12	86
RDI ^[56]	1.40	16.00	6.00	60	400
Boj-2019, %	8	9	21	39	14
Suchiles-2019, %	ND	7	7	6	14
Pilsner Beer, %	24	48	25	20	22

Abbreviation: ND, not detectable.

For Boj, no sugar is added at the end of the production process; when comparing the information in Table 1 and Figure 7, the TSS of the final product (3.1 g/100 g) and the total sugar content (9.3 g/L), there is a noticeable difference between these parameters. The composition of TSS refers to all soluble solids found in the beverage, but it is not limited to soluble sugars. The remaining soluble solids in

the beverage may contain unfermentable sugars, dextrans, nitrogenous substances, gums, and minerals,^[16] which could explain the difference noted in Boj.

A previous study of Boj^[23] recommended the evaluation of nutritional factors for this beverage. Within this research, the following nutritional analyses were observed: protein content, caloric intake, and some vitamins. The latter are

shown in Table 5. The results showed that Boj and Suchiles had less than 0.35 g of protein per mL, which is the lower limit detected with the analysis used. The caloric intake in kcal/100 mL was 36.7 ± 6.7 for Boj and 57.0 ± 19.2 for Suchiles, and these values are close to the ones found in beer and wine (i.e., 43 and 83 kcal/100 mL, respectively).^[57]

An analysis of the vitamin contribution of Boj and Suchiles due to the consumption of these beverages instead of solid food is highly relevant. They were compared with a liter of Pilsner beer, which is also a commonly ingested beverage. For Suchiles, only folic acid had a relevant value. For Boj, the content of folic acid was similar to Suchiles. For vitamins assessed, the biotin content for Boj was double that of Pilsner beer. However, consuming one liter of any of these beverages would still fail to meet established Recommended Daily Intake (RDI) values.

Conclusions

In the present study, samples of Boj and Suchiles were collected in 2019. The process flow charts for both indigenous fermented beverage productions were created in cooperation with the artisanal producers. For Boj-2019, when compared to a previous study published in 1988,^[23] in which samples of Boj from different manufacturers were analyzed, Boj-2019 confirmed their results for TSS, reducing sugars, alcohol, and pH.

Boj and Suchiles presented similar pH values, but total acidity was higher in Suchiles, consistent with a higher concentration of organic acids and a lower pH value for Suchiles.

According to essential and non-essential amino acid results, Boj presented higher concentrations than Suchiles. The case is the opposite when reviewing the analysis of sugars and organic acids present in the finished beverages, where Suchiles presented higher values for lactic and acetic acid, suggesting that the presence of AAB and LAB are higher in Suchiles.

Although different fermented beverages use similar raw materials to Boj and Suchiles, there are no physicochemical similarities with the other fermented beverages using sugarcane and pineapple juice as a base that were compared in this study.

Both Boj and Suchiles are deficient in protein and B vitamins, and they are not an alternative to meet the RDI.

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Author contributions

C. Ronquillo Blau was responsible for conceptualization of this research. C. Ronquillo Blau, M. Zarnkow, and M. Hutzler were responsible for methodology of this paper. D. Escoto was responsible for the

formal analysis performed for this study. C. Ronquillo and D. Escoto were responsible for investigation and writing of the manuscript. M. Zarnkow, M. Hutzler, J.P. Sampaio, and F. Jacob were responsible for supervision. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Disclosure statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A



Figure S1. Sugarcane juice extraction during the artisanal production of Boj in Cobán, Guatemala.