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Production and Characterization of a Distilled Alcoholic Beverage Obtained by Fermentation of Banana Waste (*Musa cavendishii*) from Selected Yeast

Mara Eli de Matos, Adriane Bianchi Pedroni Medeiros *, Gilberto Vinicius de Melo Pereira, Vanete Thomaz Soccol and Carlos Ricardo Soccol

Department of Bioprocess Engineering and Biotechnology, Universidade Federal do Paraná, Rua Coronel Francisco Heráclito dos Santos, Curitiba 81531-990, Brazil; maraelimatos@gmail.com (M.E.d.M.); gilbertovinicius@gmail.com (G.V.d.M.P.); vanetesoccol@gmail.com (V.T.S.); soccol@ufpr.br (C.R.S.)

* Correspondence: adrianebpm@ufpr.br; Tel.: +55-41-3361-3697

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Abstract: Banana is one of the most important fruits in the Brazilian diet and is mainly consumed naturally. Losses from crop to final consumer are high and estimated in about 30%. The aim of this work was to elaborate a distilled alcoholic beverage from discarded banana and to compare with commercial trademarks. Initially, yeast strains were isolated from banana fruit and characterized by their production of volatile aroma compounds. The highest aroma-producing yeast isolate was identified by ITS-rRNA gene sequencing as *Pichia kluyveri*. Pasteurized banana pulp and peel was fermented by the selected *P. kluyveri* at approximately 10^7 cells/mL. The sugars were converted quickly, and a high ethanol concentration (413 mg/L) was achieved after 24 h of fermentation. The fermented banana must was distilled in a Femel Alambic, and the head, heart and tail fractions were collected. The banana brandy produced had highest concentration of volatile compounds compared to trademarks, such as isoamyl acetate (13.5 mg/L), ethyl hexanoate (0.8 mg/L) and others. The results showed that whole banana must could be a good substrate for fermentation and distillation, and the sensory analysis performed revealed that the produced beverage had good acceptance by the tasters. This study demonstrates the potential of banana as a possible alternative to reduce waste and increase income to farmers.

Keywords: banana brandy; *Pichia kluyveri*; aroma

1. Introduction

Banana is widely consumed throughout the world and has a significant presence in international trade. World banana production in 2014 was 144.8 million tons [1], which corresponds to 17.4% of the total volume of world fruit growing. Brazil is the third largest producer, losing only to India and China. Production in 2016 was 6.9 million tons [2]. This fruit of small added value and high nutritive constitution has a fast process of deterioration, which makes the fruit commercialization in nature quite difficult after maturation. The discarded fruit can be used in the manufacture of jellies, juices or fermented beverages.

Fruit brandy is an alcoholic beverage from 36% to 54% volume alcohol obtained from fermented fruit must and distillation [3]. Banana brandy consists mainly of ethanol, water and secondary compounds present in small quantities that characterize it sensorially. In this case, the characteristic aroma of ripe bananas is mainly due to isoamyl acetate [4]. The aroma is among the main attributes that determine the choice and consumption of food and beverages. The final aroma is a result of the combination of several volatile molecules, one of which usually predominates over the others [5].

In the manufacturing process of distilled alcoholic beverages, the choice of a yeast suitable for the production of brandy will depend primarily on the nature of the must, the industrial conditions and the desirable characteristics for the final product [6]. However, depending on how this transformation is carried out, a higher or lower quantity and quality of brandy can be obtained [7]. Besides, the composition of the beverages largely depends also on the conduction of the distillation step [8]. The distillation must be carried out aiming to preserve the aroma and the taste of the natural volatile compounds contained in the raw materials that formed during the fermentation [3].

The objectives of this study were to (i) evaluate the potential of banana waste in the production of an alcoholic beverage fermented using selected yeast; (ii) analyze the volatile compounds present in distinctive stages of brandy production; and (iii) evaluate the sensory quality of the final beverage produced.

2. Material and Methods

2.1. Must Preparation

The banana (*Musa cavendishii*) was purchased at a local market in Curitiba/Paraná–Brazil. The fruits were analyzed in relation to the carbohydrate, lipid, fiber, mineral and ash contents according to analytical guides of Instituto Adolfo Lutz [9]. The analyzes were done in duplicate.

Ripe bananas with peel with maturation of degrees 5 (more yellow than green peel), 6 (totally yellow) and, 7 (yellow/a few brown spots) were washed and ground in a blender. The banana must was prepared in a ratio of 1:1 (*v*/*v*) with water. Microbial inactivation of the must was performed at 70 °C for 40 min [10]. The pH of the prepared must was about 4.5 and the reducing sugars content was 143 g/L.

2.2. Yeast Isolation

For the isolation of wild yeasts, tenfold dilutions from ripe banana with peel were prepared and 0.1 mL of each dilution was spread onto Yeast extract peptone glucose (YEPG) agar (yeast extract 10 g, peptone 20 g, glucose 20 g, agar 15 g per liter of water) containing 100 mg/L chloramphenicol to inhibit bacterial growth. According to the macroscopic observations, two yeast colonies were purified by repetitive streaking. For the identification of the isolated yeasts, DNA was extracted according to [11] and the 5.8S ITS rRNA gene region of yeast isolates was amplified using the primers ITS4 and ITS5. The 55 µL volume reaction consisted of 5.5 µL of 10× PCR buffer (Invitrogen, Carlsbad, CA, USA), 2 µL of $MgCl_2$ (50 mM), 1.21 µL of dNTP Mix (10 mM), 4 µL of the combined forward and reverse primers (ITS1 and ITS4), 0.4 µL of 5 U/µL Platinum® Taq DNA polymerase (Invitrogen, Waltham, MA, USA). The PCR amplicons were purified according to the methods described by [12]. The 5.8S ITS rRNA gene region was sequenced by the company ACTGene Análise Moleculares Ltda. (Center of Biotechnology, UFRGS, Porto Alegre, RS, Brazil) using the ABI-PRISM 3100 Genetic Analyzer automatic sequencer armed with 50 cm capillaries and POP6 polymer (Applied Biosystems, Foster City, CA, USA).

2.3. Inoculum Preparation and Banana Brandy Production

Pichia kluyveri was selected for brandy production due to its high production of flavor-active esters [10]. The inoculum was prepared in 50 mL of YM broth in a 250 mL Erlenmeyer flask. The cultivation was carried out in shaker at 28 °C, 120 rpm, for 24 h. The cell count was performed in a Neubauer chamber.

The banana must was inoculated with 1×10^7 cells/ mL of the selected yeast. The fermentation was conducted in 2 L and 6 L erlenmeyers at 28 °C for 24 h. After fermentation the fermented must was filtered and distilled in a Femel still. The distillate was collected and separated into three fractions: head, heart and tail. The fractions were separated according to the observation of the temperature of the vapors measured at the inlet of the condenser. The first fraction was collected below 70 °C (for the "head"), the "heart" was distilled between 71 and 75 °C and above, the "tail" was collected.

2.4. Analytical Procedure

Aroma compounds were measured by headspace analysis of the samples on a gas chromatograph Shimadzu GC 17A equipped with a flame ionization detector at 230 °C. The operating conditions were: HP-DB 5 (30 m × 0.32 mm) column, oven temperature from 40 °C (hold 5 min) to 150 °C (hold 4 min) at a rate of 20 °C/ min, injector temperature 230 °C. Nitrogen was the carrier gas at 1.5 mL/min and the split ratio was 1:5. Five mL of the material to be analyzed was transferred into 30 mL vials and exposed at 30 °C for 10 min. With a gas-tight syringe, 1 mL of the headspace was injected into the GC injection port.

Volatile compounds were expressed as mg/L, as ethanol equivalent. The compounds were identified by comparing their retention times with those of standard analytical compounds (Sigma-Aldrich, St. Louis, MO, USA). Headspace analyzes were carried out at time zero, in the fermented and filtered must and in the distillate (head, heart and tail).

The alcohol content of the spirits was determined with an alcoholmeter (Gay-Lussac Alcoholmeter, mod. 5683, Incoterm, Porto Alegre, RS, Brazil). The alcoholic content was expressed in Gay-Lussac grade (°GL), at 20 °C.

2.5. Comparison between Banana Brandy and Trademarks

Eight brands of alcoholic beverages containing "banana" on the label were purchased for comparison with the banana brandy produced in this study. Table 1 shows the coding of commercial alcoholic beverages, as well as the characteristics, °GL and origins. Samples of the beverages were submitted to the headspace analysis by gas chromatography as previously indicated.

Table 1. Characteristics and commercially produced banana alcoholic beverages from Minas Gerais (MG), Rio Grande do Sul (RS) and Santa Catarina (SC).

Code	Characteristics	°GL	Origin
C-1	Banana brandy "prata"	40	Itajubá—MG
C-2	Banana brandy "ouro"	40	Itajubá—MG
C-3	Brandy with banana	38	Ivoti—RS
C-4	Banana brandy	36	Passa Quatro—MG
C-5	Brandy with banana	39	Luis Alves—SC
C-6	Brandy with banana	38	Luis Alves—SC
C-7	Brandy with banana	42	Luiz Alves—SC
C-8	Artisanal cachaça with banana	45	MG

2.6. Sensory Analysis

The sensory analysis of the brandy was carried out according to [13]. Samples containing 5 mL of the beverage were dispensed in transparent glasses with a capacity of 10 mL and offered water and biscuit for after tasting. The convocation of the volunteers requested over 18 years for sensorial analysis of spirits, without mentioning the origin of the raw material and without distinction of the public, that is, people approached randomly and not trained.

The evaluators analyzed the brandy by assigning it grades according to the hedonic scale from 1 (one) to 10 (ten) for the attributes: aroma, aroma intensity and taste. The aroma was evaluated in three categories and scores: Excellent/Very good (7 to 10), Good (4 to 6) and Fair/Poor (0 to 3). The aroma intensity as follows: weak (0 to 3), moderate (4 to 6) and strong (7 to 10). The taste was evaluated and classified in disgust (0 to 3), indifferent (4 to 6) and liked (7 to 10). The evaluator was also asked to identify the aroma of the beverage.

3. Results and Discussion

3.1. Yeast Isolation and Identification

Wild yeasts were initially recovered from banana fruits in order to obtain strains well adapted for must fermentation. Two representative isolates were selected based on the phenotypic appearance on YEPG medium and identified by ITS-rRNA gene sequencing as *Pichia kluyveri* and *Hanseniaspora* sp. (sequences with 99% similarity to *Hanseniaspora uvarum*, *Hanseniaspora opuntiae* and *Hanseniaspora guilliermondii*). These yeast species are reported to be fermentative and have been found in soil, fruits and trees [14].

Sequencing data were submitted to GenBank and registered under the following codes: *Pichia kluyveri* (LPBII-A) BankIt1841595 KT309129 and *Hanseniaspora* sp. (LPBII-B) BankIt1841595 KT309130.

3.2. Banana Brandy Production

The banana with peel was chemically characterized before initiating the fermentation process. The results indicated in Table 2 show that banana with peel constitutes a good medium for microbial growth (high concentrations of sugars and proteins and presence of essential minerals) and supplementation was not performed.

Table 2. Physico-chemical composition of banana with peel.

Parameter	Content (%)
Protein	5.70
Carbohydrates	12.2
Lipids	4.03
Crude Fiber	1.89
Ash	3.98
Ca	0.18
P	0.10
Na	0.02
K	1.67
Mg	0.08

Pichia kluyveri LPBII-A was selected for banana brandy production due to its high production of flavor-active esters (viz., isoamyl acetate, ethyl acetate, propyl acetate and ethyl hexanoate) in relation to *Hanseniaspora* sp. [10].

The volatile compounds produced in distinctive stages of brandy production is shown in Table 3. The major compounds in the distilled beverage were ethanol (413.5), ethyl acetate (76.5), isoamyl acetate (13.5) and 3-methyl-1-butanol (8.2).

Table 3. Volatile compounds identified in the headspace during different stages of the process to obtain banana brandy fermented by *Pichia kluyveri*.

Volatile Compound	Initial Must (mg/L)	Fermented Must (mg/L)	Head (mg/L)	Heart (mg/L)	Tail (mg/L)
Methanol	nd	0.9	1.1	nd	nd
Acetaldehyde	0.4	0.5	0.6	0.2	nd
Ethanol	1.6	70.6	0.2	413.5	2.7
Ethyl acetate	0.6	15.0	nd	76.5	nd
2-entanone	0.4	0.1	nd	2.9	nd
Propyl acetate	nd	0.2	nd	nd	nd
3-Methyl-1-butanol	0.2	0.3	nd	8.2	nd
isobutyl acetate	1.0	0.4	nd	2.2	nd
2-hexanol	nd	0.3	nd	0.7	nd

Table 3. *Cont.*

Volatile Compound	Initial Must (mg/L)	Fermented Must (mg/L)	Head (mg/L)	Heart (mg/L)	Tail (mg/L)
Not identified	0.7	0.4	nd	0.8	nd
Isoamyl acetate	1.8	8.8	nd	13.5	nd
2-octanone	nd	1.1	nd	nd	nd
Ethyl hexanoate	nd	0.3	nd	0.8	nd
Hexyl acetate	0.2	0.2	nd	nd	0.2
Not identified	1.5	0.7	nd	0.5	nd
Not identified	0.5	0.2	nd	nd	nd
Ethyl octanoate	nd	0.3	nd	2.7	nd
1-Decanol	nd	0.7	nd	nd	0.3
Caprylic acid	nd	0.3	nd	0.3	0.2
Ethyl decanoate	0.2	0.3	nd	0.6	0.3

"nd" means compound not detected.

It could be observed that in the initial must, constituents of the ripe banana were detected and identified such as 2-pentanone, isobutyl acetate and isoamyl acetate. During maturation, the fatty acids and amino acids present in the fruits are converted into esters, ketones and alcohols. The esters contribute most of the impact aroma of the fruits. In the ripe banana (*Musa cavendishii*), [15] identified that 2-heptanol, isoamyl alcohol (3-methyl-1-butanol), 2-pentanone, isoamyl acetate, butyl butanoate, isobutyl acetate and 2-methylpropyl butanoate are common compounds detected. These are important volatile compounds that characterize the banana like aroma [16,17].

3.3. Comparing the Banana Brandy with Trademarks

The brandy (heart) produced with banana must and *Pichia kluyveri* were compared with commercial beverages (Table 4). The alcohol content was 36 °GL, which is lower when compared to commercial brandies. According to the Brazilian current legislation [3], the brandy can be graded between 36 and 54 °GL, therefore, the brandy produced is in the established range.

Table 4. Comparison of the identified volatile compounds in the headspace of the banana brandy and those of commercially purchased alcoholic beverages.

Beverage	Banana Brandy	C1	C2	C3	C4	C5	C6	C7	C8
Alcoholic Content (°GL)	36	40	40	38	36	39	38	42	45
Volatile Compounds (mg/L)									
Methanol	nd	nd	nd	0.2	0.3	0.3	0.7	0.4	nd
Acetaldehyde	1.0	nd	nd	nd	0.3	0.4	0.7	0.4	nd
Ethanol	413.9	623.7	624.9	504.5	412.0	560.5	517.1	719.5	832.5
2,3-butanodione	nd	nd	nd	2.0	1.9	nd	nd	nd	nd
Ethyl acetate	76.5	nd	0.6	0.9	3.7	1.4	5.4	3.4	34.9
Acetic acid	nd	nd	0.4	nd	nd	nd	nd	nd	nd
2-pentanone	2.8	nd	nd	nd	nd	nd	nd	nd	nd
3-methyl-1-butanol	8.2	nd	0.3	0.7	1.2	0.6	1.2	1.6	3.4
Isobutyl acetate	2.2	nd	nd	nd	0.8	0.2	nd	nd	nd
2-hexanol	0.7	nd	nd	nd	nd	nd	0.4	nd	nd
Non identified	0.8	nd	nd	nd	nd	nd	nd	nd	0.8
1-hexanol	nd	nd	0.4	nd	nd	nd	nd	nd	nd
Isoamyl acetate	13.5	2.6	0.2	1.3	0.3	0.3	0.7	5.9	4.1
Ethyl hexanoate	0.8	nd	nd	nd	nd	nd	nd	nd	nd
R-(+)-Limonene	nd	nd	nd	0.4	nd	nd	nd	nd	nd

Table 4. *Cont.*

Beverage	Banana Brandy	C1	C2	C3	C4	C5	C6	C7	C8
Non identified	0.5	nd	nd	0.3	0.2	nd	0.3	1.5	0.5
Ethyl octanoate	2.7	nd	nd	nd	nd	nd	nd	nd	2.0
Caprilic acid	0.3	nd	nd	0.2	0.3	0.2	nd	nd	6.0
Ethyl decanoate	0.6	nd	nd	nd	nd	nd	nd	nd	nd

The codes C1–C8 are according to Table 1. "nd" means compound not detected.

Greater variety of compounds were found, some already present in the original banana must and detected in higher concentration. In the headspace of the brandy produced with *P. kluyvery* were detected 2-pentanone, isobutyl acetate, ethyl hexanoate and ethyl octanoate. These compounds were almost exclusively detected in the beverage produced. Isoamyl acetate was detected in concentrations of 2 to 6 times higher than in commercial beverages.

The compounds detected in our banana brandy resemble spirits obtained with other fruits and plays important roles in the brandy flavor. Thirty-three volatile compounds were detected by [18] in the distillate of raspberry (*Rubus ideaus* L.) and arbutus berry (*Arbutus unedo*), especially the (Z)-3-hexene-1-ol, 1-hexanol, hexanal, (E)-2-hexenal, esters (ethyl 3-hexenoate, ethyl dodecanoate) and hexadecanoic acid, higher alcohols (1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and 1-hexanol), esters methyl acetate, ethyl acetate, ethyl decanoate, and ethyl-2-trans-4-cis-decadienoate); aldehyde (acetaldehyde) and the presence of methanol and furfural [19].

3.4. Sensory Analysis

In the acceptability test of the brandy, 148 evaluations of the banana brandy were carried out. Of this population, 44% were female and 56% male. The age range was distributed between 18 and 66 years, with the distribution being 25.7% (18–19 years), 37.8% (20–29 years), 14.2% (30–39 years), 6.7% (40–49 years), 12.2% (50–59 years) and 3.4% (60–66 years).

The results of the assessments for the attributes were summarized in Table 5.

Table 5. Sensory evaluation of banana brandy.

Attributes	Distribution (%)					
Aroma	Excellent/very good	60.8	Good	37.8	Fair/Poor	1.4
Aroma Intensity	Weak	7.4	Moderate	33.8	Strong	58.8
Taste	Disgust	8.1	Indifferent	17.6	Liked	74.3

Almost 98% of the evaluators considered the aroma between good and excellent. Concerning the intensity, about 93% of the evaluators found the aroma between moderate and strong.

The aroma is the perception of the volatile compounds present in the sample, representing the sensation provoked by the smell and taste combined. The sensory analysis of the banana brandy is consistent with the profile of volatiles identified by chromatography. The total concentration of volatile compounds impacting the overall flavor of the brandy was 802 mg/L and the alcoholic content was 36 °GL. The high alcohol content also influences the sensorial sensations. Considering that the evaluators were not trained to identify the aroma compounds, the concentrations of the fruit esters and mainly of the isoamyl acetate were enough, once 85% of the tasters had identified the banana aroma. Up to 74% of evaluators approved the taste of the banana brandy.

In the free choice question, 85% of the evaluators identified the banana aroma (Figure 1). Among them, 6% identified the banana aroma with another aroma or characteristic. It is important to point out that the source of the brandy produced was not mentioned during the sensory analysis. Different aromas were suggested by 13% of the evaluators, such as vanilla, champagne, fruit, medicine, alcohol,

cereal bar, rice, apple, sugarcane, gin, sweet and citrus fruits. Of the total evaluations, only 2% did not identify any aroma, answering "I do not know".

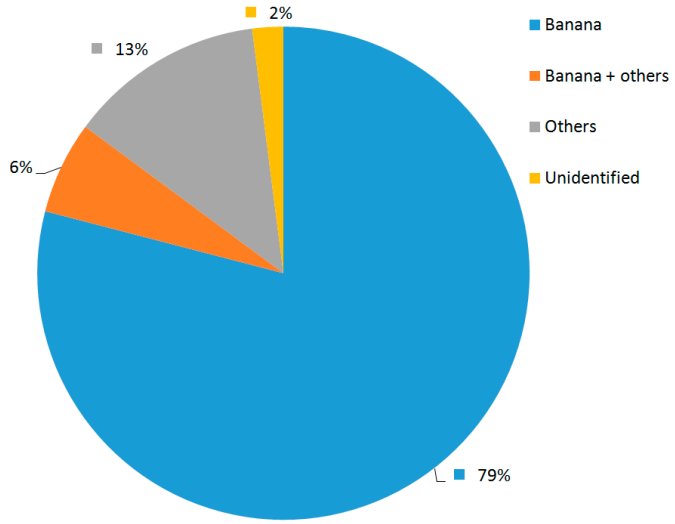

Figure 1. Identification of the aroma from banana brandy according to sensory evaluation.

4. Conclusions

The banana brandy produced by selected *P. kluyveri* has pleasant characteristics of the fruit under the standards established in the current legislation. Some of the volatile compounds characteristics of the banana aroma were identified in the brandy, viz., isoamyl acetate, ethyl acetate, 2-pentanone, 3-methyl-1-butanol, ethyl hexanoate, isobutyl acetate, ethyl octanoate and ethyl decanoate. Many of these compounds have not been identified in commercial beverages, which also proves its best aroma quality. Thus, based on the characteristics of the substrate and acceptance in the sensory analysis, banana with peel showed good potential for use in the production of fermented beverage, which can be considered as a new waste valorization strategy.

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Conflicts of Interest: The authors declare no conflict of interest.

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