Vol.63: e20190181, 2020
http://dx.doi.org/10.1590/1678-4324-2020190181
ISSN 1678-4324 Online Edition

Article - Food/Feed Science and Technology

Alternative Process for Production of Sweet Potato Distilled Beverage

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Received: 2019.03.26; Accepted: 2020.02.13.

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HIGHLIGHTS

- Sweet potato waste can be used for distilled beverages production.
- Alternative process to produce a beverage similar to shochu.
- Use of enzymes instead of koji, reducing production time from 14 to 1 day.
- Addition of pectinase in fermentation causes higher formation of methanol.

Abstract: Shochu is the most widely consumed spirit in Japan. In its manufacture is used koji, a solid fungus culture traditional of the Asian countries, but that makes the production process slow. Shochu can be produced from a variety of starchy sources, including sweet potato. About 7% of the world's sweet potato production is wasted due to imperfections that make it unsuitable for consumption. However, this material can be used in ethanol production. Considering the high productivity of sweet potato in Brazil, an opportunity to add value to this raw material is perceived. An alternative process for the production of sweet potato distillate similar to shochu was proposed. Koji was replaced by a mixture of alpha-amylase and glucoamylase. Process time was reduced from 14 to only 1 day. Composition analyses were performed by HPLC and GC. The experimental yield of alcoholic fermentation using pectinase enzyme reached 67.31-73.65%, but methanol was above the limits of the legislation. Without the addition of pectinase, no methanol was formed. However, there was a decrease in yield (51.65-54.75%), due to the incomplete disintegration of sweet potatoes. The distillate produced and the commercial shochu presented the same absorption bands in FTIR analysis, identifying the similarity between them.

Keywords: tuber crops; ethanol; alcoholic beverage; shochu; enzymatic process.

INTRODUCTION

In Brazil, the majority of bioethanol is produced by fermentation of sugarcane. However, other raw materials such as the ones rich in cellulosic fibers and starch might be used as well [1]. The sweet potato crop, a human food with good nutritional content, has great importance in animal feed and industrial production of flour, starch, and alcohol. It occupies the sixth place among the most planted vegetables in Brazil, being cultivated in all the regions of the country, with emphasis on the South. Although in its cultivation little technology is used, the productivity of sweet potato has been increasing in the last years [2], reaching 60 tons per hectare [3].

About 53% of the sweet potato production is used in animal feed and 40% goes for human consumption. If there are cuts in the sweet potato, fungi, and bacteria will lodge in the food, making it unfit for consumption. The global amount of sweet potato waste in 2011 accounted for about 7% of the entire crop [4]. However, this imperfect material could be used for other purposes, such as ethanol production. This fact, together with the knowledge of the enzymatic hydrolysis process of starch in fermentable sugars, allows the transformation of this raw material into alcoholic beverages, making it possible to diversify the production and to use sweet potatoes unfit for commercialization, adding value to it.

The beverage industry is an important industrial sector, being responsible for 3% of the Brazilian manufacturing industry production in 2014 [5]. Consistent products sale has been one of the main drivers of the global alcoholic beverage market growth. Factors such as globalization, technological advancement, and deregulation of the most diverse economic sectors have profoundly altered the beverage market in Brazil in recent years, causing per capita consumption of beverages to increase, providing a series of business opportunities to companies and strong competition in the sector. These changes require increasing professionalization, particularly about the distribution of products and the optimization of production processes [6].

Shochu is a typical Japanese distillate with an alcoholic strength of about 25% $v.v^{-1}$ that can be obtained from the fermentation of various raw materials such as rice, barley, and sweet potato, and subsequent distillation [7]. The consumption of shochu has a significant share of the Japanese market (10.6%), being more consumed than sake (6.8%) and whiskey (1.3%) [8].

The traditional process for making sweet potato shochu is described by Yoshizaki and coauthors [9]. The first fermentation lasts five days and the second fermentation nine days, totalizing 14 days of processing. In the production of shochu, a solid culture of fungi called koji is used. Although maintaining the tradition of the Asian countries, the use of yellow koji (made with *Aspergillus oryzae*) ends up making the productive process slow and with a high risk of contamination.

In order to ensure distillate identity and quality standards as well as consumer safety, the limits of shochu components are outlined in Brazilian legislation, regulated by *Ministério da Agricultura, Pecuária e Abastecimento* (MAPA) [10]. These values are shown in Table 1.

Table 1. Brazilian legislation for shochu.

Component	Minimum	Maximum
Methyl alcohol (mg/100 mL anhydrous alcohol)	-	20
Volatile acidity, in acetic acid (mg/100 mL anhydrous alcohol)	-	100
Higher alcohols (*n*-propyl alcohol + *iso*-butyl alcohol + isoamyl alcohol) (mg/100 mL anhydrous alcohol)	-	200
Aldehydes, in acetic aldehyde (mg/100 mL anhydrous alcohol)	-	20
Coefficient of congeners* (mg/100 mL anhydrous alcohol)	200	500
Esters, in ethyl acetate (mg/100 mL anhydrous alcohol)	-	200
Alcoholic graduation (% $v.v^{-1}$ at 20°C)	15	35
Furfural + hydroxymethylfurfural (HMF) (mg/100 mL anhydrous alcohol)	-	5
Sugar content (g/L)	-	6

*The coefficient of congeners is the sum of volatile acidity, aldehydes, total esters, higher alcohols, furfural and hydroxymethylfurfural.

There are reports of new alternatives aiming the improvement of the alcoholic beverage production process, such as the use of yeast cells immobilized in wine production and genetic modifications in yeasts to

improve the fermentative properties of beer [11,12]. Also, studies are looking for more economical alternatives of malt adjuvants for the beer production process and the production of new alcoholic beverages, such as gluten-free beer [13,14], brandy of orange liquor using brewer's yeast from the brewing industry, and fermented and distilled beverages from whey protein [6].

In the case of amylaceous alcoholic beverages, recent reports of alternative processes of Chinese rice wine production have been reported, with new pretreatment techniques replacing the rice cooking stage [15,16], use of different microorganisms, immobilized or not, and optimization of the fermentative stage and reforms in sterilization technology [15]. Wei and coauthors [16] concluded that, compared to traditional manufacturing technology, pre-treatment techniques that replace the cooking step of rice save water resources and reduce environmental pollution. Jiao and coauthors [15] states that innovation brings challenges and opportunities to the Chinese rice wine market, not only because it is associated with health, nutrition, safety, and palatability, but also because of the possibility of large-scale production due to energy and time savings, convenience, high efficiency, sanitation, and hygiene.

In this way, it is possible to notice the importance of alternative processes for producing alcoholic beverages with higher applied technology and the elimination of rudimentary steps. It can lead to a greater yield of alcoholic fermentation, reduction of costs and production time, besides providing differentiated consumption options, bringing benefits for both the industry and the final consumer.

Previous studies in our group (GIMSCOP) have improved the process of using sweet potato on ethanol production [17,18]. Schweinberger [19] showed that it is better to let sweet potatoes ripen for a specified time than to process them soon after harvest, with a maximum value of ethanol production and conversion efficiency achieved at 25 days after harvest. Thus, rotting sweet potatoes, a market residue, can be used for ethanol production. Besides, there are still the sweet potato harvest residues, which are crops with imperfections considered unsuitable for sale, but also suitable for producing ethanol.

In this context, considering the high productivity of sweet potato in Brazil, there is a great opportunity to add value to this waste raw material through the production and commercialization of a sweet potato distillate similar to shochu in the country, but with a production process that is faster and with less contamination risk, aiming to reduce costs and make the final product more competitive in the market.

The economic analysis of the proposed process has already been evaluated [20], bringing an important result not only as a technological alternative but also as a reduction of production costs. The main objective of this work is to study the technical viability of the alternative process, based on the enzymatic process of fuel ethanol production from sweet potato studied in our research group, with some modifications, aiming the production of distilled beverage from sweet potato waste. Also, the influence of the use of enzymes on product composition will be investigated.

MATERIAL AND METHODS

The experimental procedures were carried out at *Laboratório de Controle e Integração de Processos* (LACIP) of Chemical Engineering Department from Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. All experiments and analyses were performed in triplicate.

Material

The laboratory glassware used was previously autoclaved at 1 bar and 121 °C for 15 minutes.

Sweet potato

Sweet potatoes with cream peel and cream pulp were purchased in a local market in the city of Porto Alegre, RS, Brazil.

Reagents

The reagents used in the experiments were the following: anhydrous dextrose P.A., glycerin P.A., ethyl acetate UV/HPLC, and n-propyl alcohol P.A. from Dinâmica Química Contemporânea Ltda; D-fructose (levulose), hydrochloric acid P.A., dry methyl alcohol P.A. (methanol) from Vetec Química Fina; absolute ethyl alcohol P.A., and potassium metabisulfite P.A. from Synth; glacial acetic acid 99.8% P.A., iso-butyl alcohol P.A., isoamyl alcohol P.A., pure anhydrous acetaldehyde P.A., and furfural P.A. from Neon; 5-(Hydroxymethyl) furfural from Sigma-Aldrich.

Enzymes

It was studied to replace koji by the enzyme Stargen 002, a commercial mixture of the Genencor brand manufactured by DuPont, containing *Aspergillus kawachi* alpha-amylase expressed in *Trichoderma reesei* and *T. reesei* glucoamylase. Instead of koji, these enzymes were used to perform the hydrolysis of sweet potato starch, aiming to reduce the time required for hydrolysis and fermentation, reducing the risk of contamination and increasing the effectiveness. Pectinex Ultra AFP pectinase enzyme, supplied by LNF Latin America, was used to reduce the viscosity of the medium.

Yeasts

Three types of yeast were tested: *Saccharomyces cerevisiae* Angel Thermal Resistance Alcohol Yeast, *S. cerevisiae* var. *Bayanus* Lalvin DV10, and *S. cerevisiae* var. *bayanus* Lalvin EC1118, provided by LNF Latin America.

Physicochemical analysis

Moisture and total reducing sugars content were determined for all the sweet potato samples before their use. The moisture analysis was performed by oven drying at 105°C to constant weight [21]. The quantification of total reducing sugars was done through acid hydrolysis of the sweet potato starch, according to Schweinberger [17], followed by HPLC analysis. Briefly, 2 g of fresh sweet potato was crushed and homogenized in a 2 mm sieve. 25 mL of distilled water and 1 mL of hydrochloric acid were added. The solution was autoclaved at 1 bar and 121°C for 2 h. The mixture was neutralized with 10% (v/v) sodium hydroxide solution to pH 3.5-4.0, diluted and filtered. The collected samples were frozen for later chromatographic analysis.

Fermentation

The fermentation process was performed according to Schweinberger [22], with some changes (Figure 1). The sweet potato was steamed until it reached 76 °C, cooled and crushed. pH 4 buffer solution of citric acid and sodium citrate and potassium metabisulfite 0.15 $g.L^{-1}$ solution were prepared. To 240 g of ground sweet potato were added 140 mL of buffer solution, 20 mL of potassium metabisulfite solution, 240 µl of Stargen 002, 24 µL of pectinase and 0.8 g of yeast. The mash was fermented in a shaker at 30 °C and 160 rpm for 24 hours. Broth samples were centrifuged at 1372 G-force during 10 minutes. The supernatants were collected, filtered with 0.20 µm nylon membrane and frozen.

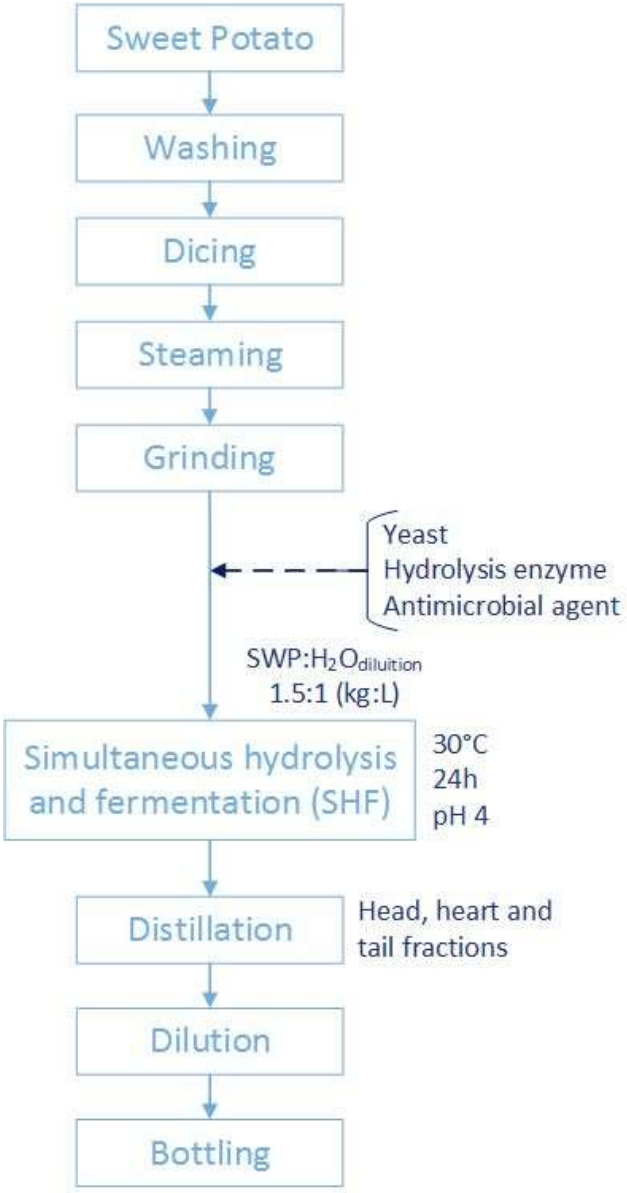

Figure 1. Distilled beverage alternative production process.

The theoretical ethanol content formed in the fermentation ($x_{et,theor}$) can be calculated by Equation 1, as deduced in Schweinberger [23], as follows:

$$x_{et,theor}(\%, v.v^{-1}) = \frac{92.\left(TRS_{swp}.x_{c\_swp}\right)}{142.02 + 1.4202.\left(x_{m\_swp}.x_{c\_swp}\right) + 0.778.\left(TRS_{swp}.x_{swp}\right)}, \quad (1)$$

where: TRS is the total reducing sugars; x_c is the concentration; x_m is the moisture content; subscript "swp" corresponds to sweet potato.

Since $x_{et,exp}$ is the experimental ethanol content formed in the fermentation, the experimental yield of the fermentation (Y_{exp}) is calculated by Equation 2:

$$Y_{exp} \quad (\%) = \frac{x_{et,exp}}{x_{et,theor}} \times 100. \quad (2)$$

Distillation

The distillation was carried out using a batch distillation apparatus with a 1 L reservoir, equipped with a 24x600 mm column packed with 7x15 mm Raschig rings (Figure 2).

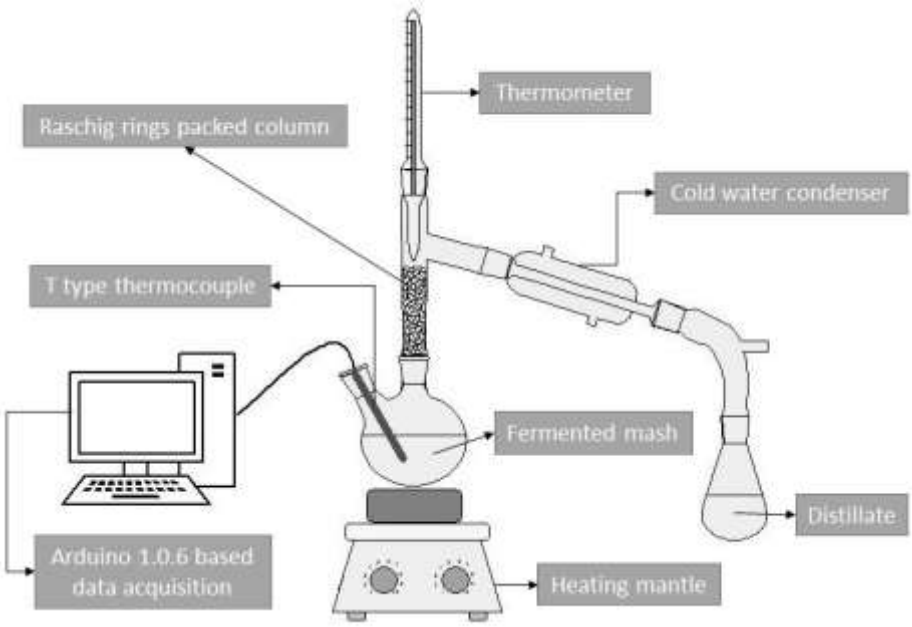

Figure 2. Distillation apparatus with Raschig rings packing column.

The temperature in the flask was monitored by T-type thermocouple with mineral insulation threaded to the side of the flask and monitored during the distillation in the Arduino 1.0.6 software. Also, the temperature was monitored at the top of the column using a thermometer. The distillate was condensed in a straight condenser with tap water circulation at room temperature and collected at the end of the system.

The alcohol content of the distillate was monitored using a portable digital refractometer, and checked by a previously constructed ethanol calibration curve with concentration ranging from 0 to 45% ($v.v^{-1}$) ethanol solution at room temperature.

It is known that for cachaça, a distilled spirit made from fermented sugarcane juice typical of Brazil, the head fraction corresponds to the collection of 5%, the heart represents 80% and the tail corresponds to 15% of the total volume of the distillate [24]. Three distillate fractions were separated according to their alcohol content: head (up to 50% $v.v^{-1}$), heart (50-38% $v.v^{-1}$) and tail (38-10% $v.v^{-1}$) [25]. The samples were diluted and filtered with a 0.20 µm nylon membrane for further analysis. Before the chromatographic analysis, all samples remained for 10 minutes in an ultrasonic bath (Unique USC 1600A 40 kHz) to eliminate bubbles.

Chromatographic Methods

High-Performance Liquid Chromatography (HPLC)

The determination of glucose, fructose, ethanol, glycerol, acetic acid, acetaldehyde, methanol, furfural, and hydroxymethylfurfural was performed by high-performance liquid chromatography (HPLC) using Agilent Technologies 1260 Infinity II chromatograph equipped with Agilent Hi-Plex H column, according to methods applied by Ball and coauthors [26]. Isocratic milli-Q water was used as mobile phase at a flow rate of 0.6 $mL.min^{-1}$; the column temperature was 60°C; the detector used was RID (Refractive Index Detector) at 55°C and the volume of sample injected was 20 µL.

Gas Chromatography (GC)

The determination of *n*-propyl alcohol, *iso*-butyl alcohol, isoamyl alcohol, and ethyl acetate was performed by gas chromatography on a SHIMADZU GC-2014 chromatograph, with manual injection, flame ionization detector (FID), Elite-WAX column with stationary phase polyethylene glycol (30 m x 0.25 mm x 0.25 mm), according to the method employed by Vilela and coauthors [27]. The temperature was 150°C for the injector and the detector. The temperature program of the column was 60 °C for 2.5 minutes, increasing at a rate of $2°C.min^{-1}$ to 80°C, where it remained for 2 minutes. The volume of the sample injected was 1 µL, and the split rate was 1:30. Nitrogen was used as a carrier gas; hydrogen and synthetic air were used for the formation of the flame. The pressure was 103.4 kPa, and the linear velocity was 33.5 $cm.s^{-1}$.

Fourier-Transform Infrared Spectroscopy (FTIR)

Infrared analysis was performed to identify similarities and differences between the distillate produced and the commercial shochu. The infrared region of the electromagnetic spectrum is divided by convenience into three sub-regions according to the wavelength range: NIR (near-infrared region, ~14000-4000 cm^{-1}), MIR (mid-infrared region, ~4000-400 cm^{-1}), and FIR (far-infrared region, ~400-10 cm^{-1}). MIR is used to study fundamental vibrations and associated rotational-vibrational structures. This region is the most widely used to study organic functional groups, such as the component molecules of alcoholic beverages [28]. MIR spectrum was collected in the mid-infrared region of 4000-650 cm^{-1} using a PerkinElmer Frontier FT-NIR spectrometer, equipped with Universal Attenuated Total Reflectance (UATR) analysis module. The transmittance spectra were recorded at a spectral resolution of 16 cm^{-1}. Each spectrum was an average of 16 scans.

Principal Component Analysis (PCA)

For a better interpretation of the results obtained for the compounds analyzed in the samples of sweet potato distillate, chemometric treatment using principal component analysis (PCA) with the aid of the Python 2.7 software was performed using the scikit-learn package. Both tools are completely open-source. The PCA method projects multivariate data into a smaller space, reducing the dimensionality of the original space in a new axis system called principal components (PC) [29,30]. The data matrix is decomposed into a product of two matrices, called scores and weights or loadings. The scores indicate the relationship between the samples and the weights the relationship between the variables [29]. The purpose of PCA is to find relationships between different parameters and detection of clusters in samples or variables [31]. The PCA was applied to separate the sweet potato distillate samples according to the results of FTIR spectra.

Statistical analysis

Statistical analysis was carried out with Statistica 64 software (academic license). The results were presented as means ± SD (standard deviations). Differences in the compounds of the beverage fractions (head, heart, and tail) produced by three types of yeast were analyzed by a One-Way Analysis of Variance (ANOVA) together with the Tukey's test. Differences reaching a minimum confidence level of 95% ($p<0.05$) were considered as being statistically significant.

RESULTS AND DISCUSSION

The total sugar content of the sweet potato with cream peel and cream pulp was 26.93±0.86% and moisture content was 68.16±0.38%. The theoretical ethanol content that should be formed (11.66%) is calculated using equation (1), and then the experimental yield of the fermentation (Y_{exp}) is calculated by equation (2). The experimental ethanol content formed in the fermentation process was 8.59±0.10 (% $v.v^{-1}$), 8.21±0.11 (% $v.v^{-1}$), and 7.85±0.09 (% $v.v^{-1}$) for Angel Thermal Resistance Alcohol, Lalvin DV10, and Lalvin EC1118 yeasts, respectively.

The experimental yield of alcoholic fermentation was higher using Angel Thermal Resistance Alcohol (73.65%), followed by Lalvin DV10 (70.39%), and Lalvin EC1118 (67.31%). Due to the formation of fewer byproducts of the fermentation, such as glycerol (not detected by HPLC), and to the non-occurrence of contamination by acetic bacteria, verified by the absence of acetic acid in the fermented, a great yield of alcoholic fermentation was reached. Similar results were achieved by Swain and coauthors [32], that reported a fermentation yield of 72% using sweet potato flour as biomass.

Additionally, the process requires fewer steps and is faster, taking only 1 day, while the traditional method of shochu production takes 14 days.

Using the distillation system installed, shown in Figure 2, a temperature gradient was observed in the column, which reveals the steam enrichment mechanism in the more volatile components. When reaching a certain point in the column, the steam coming from lower positions, warmer, undergoes partial condensation. The liquid flows into lower positions and the steam permeates the packing to higher points; the process is repeated several times. In this way, it is possible to carry out a better separation of the components of the distillate.

The desired distillate fraction cuts, with head (up to 50% $v.v^{-1}$), heart (50 to 38% $v.v^{-1}$), and tail (38 to 10% $v.v^{-1}$) were successfully performed. Santiago and coauthors [33] also performed fraction cuts at similar

ethanol concentrations. The results of the chromatographic analysis of these three fractions are shown in Table 2.

Table 2. Results of the chromatographic analysis of the sweet potato distillate fractions produced by the new route (with pectinase addition).

Component*	Head			Heart			Tail		
	TH[1]	DV[2]	EC[3]	TH[1]	DV[2]	EC[3]	TH[1]	DV[2]	EC[3]
Methyl alcohol[4]	239.88[b] ±13.75	347.00[ac] ±19.82	293.63[bc] ±15.56	384.40[a] ±20.37	302.64[bc] ±17.25	380.89[a] ±18.36	579.32[e] ±28.54	396.38[a] ±26.73	485.15[d] ±32.96
Volatile acidity[4] (in acetic acid)	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]
N-propyl alcohol[4]	24.71[b] ±2.28	34.42[a] ±1.83	33.46[a] ±2.61	27.48[bc] ±1.47	31.14[ac] ±2.04	33.70[a] ±1.75	25.54[bc] ±1.59	36.05[a] ±1.98	35.80[a] ±1.96
Iso-butyl alcohol[4]	20.49[ab] ±1.42	22.33[b] ±1.18	15.53[a] ±1.07	40.25[c] ±2.19	44.88[cd] ±2.51	18.76[ab] ±1.12	46.16[d] ±2.86	42.46[cd] ±2.29	29.70[e] ±2.09
Isoamyl alcohol[4]	102.67[a] ±5.75	119.55[a] ±8.07	102.24[a] ±7.54	112.13[a] ±6.22	108.42[a] ±6.68	113.52[a] ±6.11	118.84[a] ±7.93	111.23[a] ±6.96	108.56[a] ±6.53
Higher alcohols[4 5]	147.87[a] ±11.89	176.30[ab] ±14.63	151.23[ab] ±12.70	179.86[ab] ±13.57	184.44[ab] ±16.31	165.98[ab] ±14.95	190.54[b] ±15.82	189.74[ab] ±15.76	174.06[ab] ±15.49
Aldehydes[4] (in acetic aldehyde)	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]
Coefficient of congeners[4]	275.94[a] ±21.49	297.49[a] ±27.37	288.47[a] ±26.83	314.57[a] ±28.94	330.51[a] ±32.35	305.10[a] ±28.67	341.94[a] ±31.80	344.29[a] ±33.39	318.03[a] ±30.48
Esters[4] (in ethyl acetate)	128.07[ac] ±7.43	121.19[c] ±7.88	137.24[abc] ±8.78	134.71[abc] ±8.02	146.07[ab] ±7.45	139.12[abc] ±8.24	151.40[ab] ±9.69	154.55[b] ±8.96	143.97[abc] ±9.71
Alcoholic graduation (% v.v^{-1} at 20°C)	66.99[de] ±3.75	60.14[d] ±3.11	71.63[e] ±3.87	38.88[a] ±2.02	40.77[a] ±2.13	39.31[a] ±2.72	22.83[bc] ±1.28	23.78[c] ±1.24	16.26[b] ±1.09
Furfural + HMF[4]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]
Sugar content (g/L)	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]

* Mean values that do not share the same letter in each component's line are significantly different, according to Tukey test with 95 % confidence ($p<0.05$).
[1] Yeast Angel Thermal Resistance Alcohol.
[2] Yeast Lalvin DV10.
[3] Yeast Lalvin EC1118.
[4] In mg/100 mL anhydrous alcohol.
[5] Higher alcohols = n-propyl alcohol + iso-butyl alcohol + isoamyl alcohol.
[6] ND = not detected.

The methyl alcohol component presented difficult separation, being distributed throughout the fractions of the distillate. Small amounts of methanol may be present in alcoholic beverages, formed as a by-product of the fermentative process. Since the limit of the Brazilian legislation for methanol equals to twenty milligrams per hundred milliliters of anhydrous alcohol, it is observed that the methanol content is above the limit for all the fractions of distillates, in the results of all yeasts. It makes the distillate unfit for consumption because of the high toxicity of methanol, which can be harmful to health. Blinder and coauthors [34] state that the amount of methanol may increase due to inadequate storage conditions and also by the presence of pectinases and other enzymes. Badolato and coauthors [35] report that, in small amounts, methanol can cause a headache, dizziness, nausea, and vomiting. Consumption of 20 mL can cause blindness while 60 mL is usually lethal if left untreated. All other compounds are within limits set by legislation.

In the head fraction, the first to leave the distiller, the more volatile components such as esters and aldehydes are expected to be present in higher concentration. The acetic aldehyde compound was below the detection limit of the equipment, while for the esters the opposite behavior was observed. Higher concentrations of ethyl acetate were recorded in the tail fraction, followed by the heart, and finally the head. Thus, no efficient separation of the compounds occurred. In the case of a complex mixture, deviations from ideality occur, and the conditions necessary to obtain adequate separation of the compounds go beyond the evaluation of the boiling points and the resolution that the distillation apparatus offers.

In the tail fraction, the last to leave the pot, the compounds that remain are less volatile than ethyl alcohol such as organic acids and higher alcohols. Acetic acid was below the equipment limit of detection, whereas higher alcohols presented behavior as expected, being in higher concentrations in the tail fractions. Among the higher alcohols, the highest amount was isoamyl alcohol. In the sensory analysis performed in Yuan and coauthors [36], it was reported that samples of shochu with higher amounts of isoamyl alcohol presented a more bitter taste.

The reflux occurs due to the condensation of steam inside the column, albeit less efficiently than the external reflux. The height of the column and the packing provide a temperature gradient and flow resistance required for condensation and then reflux of distillate. Also, the packing allows a high contact area between the liquid and the vapor, facilitating the transfer of heat and mass.

To identify the possible causes for the high content of methanol, and knowing that the amount of methanol of wine is higher when pectinolytic enzymes are applied [25], it was decided to suppress the pectinase.

Sweet potatoes from different crops show differences in composition. Kolbe and coauthors [37] state that the size of sweet potatoes affects their content of organic and inorganic components, including water, starch, sugars, and organic acids. For this reason, moisture analysis and acid hydrolysis of sweet potatoes were repeated in all experiments. In this one, acid hydrolysis of sweet potato resulted in a reducing sugar content of 31.66±0.24% and a moisture content of 66.25±0.08%. Through equation (1), the theoretical ethanol content (x_{et}) that should be produced is 12.58% and, afterward, the experimental yield of the fermentation (Y_{exp}) is calculated by equation (2). For the yeasts Angel Thermal Resistance Alcohol, Lalvin DV10, and Lalvin EC1118, the experimental ethanol content formed in the fermentation process was 6.78±0.03 (% $v.v^{-1}$), 6.50±0.04 (% $v.v^{-1}$), and 6.89±0.05 (% $v.v^{-1}$), respectively.

The alcoholic fermentation yield was similar using the three types of yeast, being slightly higher for Lalvin EC1118 (54.75%), followed by Angel Thermal Resistance Alcohol (53.88%), and finally Lalvin DV10 (51.65%). These values were about 20% lower than those obtained in the experiment performed with the addition of pectinase. Leonel and coauthors and Schweinberger [19,38] concluded that the use of pectinase as a complementary enzyme to the amylases in the hydrolysis-saccharification process provides better yields to the process. These results indicate that the high viscosity of the medium and the incomplete disintegration of the sweet potato pieces, caused by the absence of pectinase, impair the progress of the fermentation process, causing a decrease in the yield of the alcoholic fermentation.

The separation of head, heart, and tail was performed as described in the previous section. The results of the chromatographic analysis are presented in Table 3.

Table 3. Results of the chromatographic analysis of the sweet potato distillate fractions produced by the new route (without pectinase addition).

Component*	Head			Heart			Tail		
	TH[1]	DV[2]	EC[3]	TH[1]	DV[2]	EC[3]	TH[1]	DV[2]	EC[3]
Methyl alcohol[4]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]
Volatile acidity[4] (in acetic acid)	ND[6]	0.76^{d} ±0.03	1.13^{e} ±0.05	1.00^{a} ±0.04	1.03^{a} ±0.04	ND[6]	0.61^{c} ±0.02	ND[6]	0.17^{b} ±0.01
N-propyl alcohol[4]	37.08^{e} ±1.67	75.92^{b} ±4.25	89.44^{c} ±5.72	23.38^{d} ±1.40	50.48^{a} ±2.62	53.13^{a} ±3.35	54.62^{a} ±2.79	78.41^{b} ±4.49	91.56^{c} ±5.16
Iso-butyl alcohol[4]	24.01^{a} ±1.25	19.10^{d} ±1.31	19.09^{d} ±1.01	14.88^{c} ±0.86	10.33^{b} ±0.52	13.05^{bc} ±0.70	27.52^{ae} ±1.58	30.65^{e} ±1.66	25.78^{a} ±1.73
Isoamyl alcohol[4]	83.45^{e} ±4.91	51.83^{ac} ±3.11	57.07^{a} ±3.20	56.24^{a} ±3.27	36.44^{b} ±2.33	41.81^{bc} ±2.46	97.87^{f} ±5.97	81.84^{de} ±4.62	71.67^{d} ±4.73
Higher alcohols[4 5]	144.54^{c} ±8.67	146.85^{c} ±9.52	165.60^{ac} ±9.77	94.50^{b} ±5.86	97.25^{b} ±5.64	107.99^{b} ±6.80	180.01^{a} ±11.34	190.90^{a} ±10.45	189.01^{a} ±12.14
Aldehydes[4] (in acetic aldehyde)	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]	ND[6]
Coefficient of congeners[4]	280.85^{b} ±16.85	339.58^{a} ±15.29	340.90^{a} ±18.61	244.11^{b} ±14.06	277.44^{b} ±14.37	272.16^{b} ±13.84	378.83^{a} ±19.46	388.14^{a} ±20.15	387.97^{a} ±19.06
Esters[4] (in ethyl acetate)	135.38^{c} ±8.19	191.97^{ab} ±11.90	173.67^{abd} ±9.52	148.33^{cd} ±8.31	178.81^{ab} ±9.66	163.83^{bcd} ±9.15	198.15^{a} ±10.48	197.24^{a} ±10.24	198.59^{a} ±12.03
Alcoholic graduation (% v.v^{-1} at 20°C)	54.62^{b} ±2.89	58.60^{b} ±3.28	54.84^{b} ±3.64	36.61^{d} ±1.94	29.99^{c} ±1.71	32.65^{cd} ±1.95	12.56^{a} ±0.68	13.52^{a} ±0.74	14.03^{a} ±0.83
Furfural + HMF[4]	0.93^{f} ±0.04	ND[6]	0.50^{e} ±0.03	0.28^{a} ±0.01	0.35^{b} ±0.02	0.34^{ab} ±0.02	0.06^{c} ±0.03	ND[6]	0.20^{d} ±0.01
Sugar content (g/L)	0.01^{a} ±0.00	0.01^{a} ±0.00	0.03^{b} ±0.01	0.01^{a} ±0.00	0.01^{a} ±0.00	0.05^{c} ±0.01	0.01^{a} ±0.00	ND[6]	ND[6]

* Mean values that do not share the same letter in each component's line are significantly different, according to Tukey test with 95 % confidence ($p<0.05$).
[1] Yeast Angel Thermal Resistance Alcohol.
[2] Yeast Lalvin DV10.
[3] Yeast Lalvin EC1118.
[4] In mg/100 mL anhydrous alcohol.
[5] Higher alcohols = n-propyl alcohol + iso-butyl alcohol + isoamyl alcohol.
[6] ND = not detected.

All compounds analyzed were within the limits of the current legislation. The methyl alcohol component was below the detection limit of the equipment. As the experimental conditions were identical to the previous item, except for the addition of pectinase, it is concluded that this enzyme is responsible for the high concentration of methanol formed in the previous results, caused by the hydrolysis of pectin. The results found are in agreement with those presented by Zhang and coauthors [39], that found that the use of pectinase enzyme significantly increased the methanol concentrations of apple distillates.

In order to identify the similarities and differences between the composition of the produced distilled (heart fraction) and the commercial shochu, FTIR analyses were performed. The result of the overlap of the MIR spectrum of the two beverages can be seen in Figure 3.

The FTIR spectrum of sweet potato distillates was dominated by the absorption bands of alcohol and water. The O-H stretch bands can be observed in the ranges of 3550-3100 cm^{-1} and 1750-1550 cm^{-1}. The C-O stretch band is in the range of 1100-1000 cm^{-1}, and in the range 2980-2850 cm^{-1} there is C-H stretch band.

The bands of C-O, C-C, CH_2, CH_3, C-OH, C-H and C≡N can be observed in the range 1450-1150 cm^{-1}. The band at 860 cm^{-1} is attributed to off-plane C-H folds [40].

It is observed that all samples overlap showing peaks in the same absorption bands, only with a variation of intensity. Thus, it can be concluded that the composition of the sweet potato distillate produced by the alternative route without the addition of pectinase resembles the commercial shochu composition, concerning the components identified by the FTIR analysis.

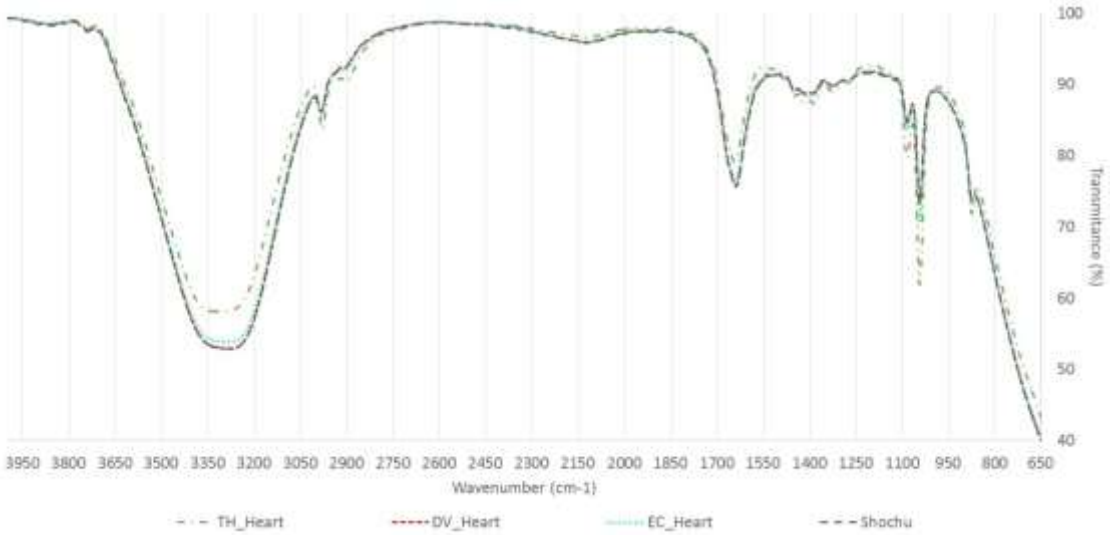

Figure 3. FTIR spectrum of the heart fraction of sweet potato distillates and shochu. TH, DV and EC refer to the yeasts Thermal Resistance Alcohol, Lalvin DV10 and Lalvin EC1118, respectively.

The resulting FTIR spectra were analyzed by means of PCA analysis, which allowed the identification of the clusters and the outliers. The normalized graph of principal components analysis can be visualized in Figure 4.

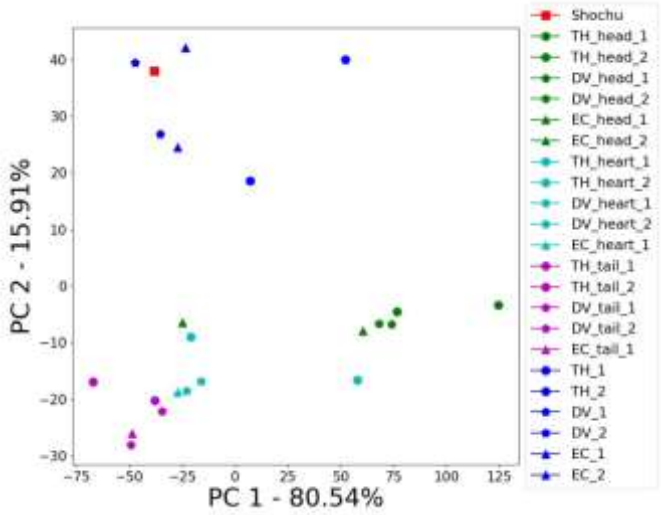

Figure 4. PC1 vs. PC2 normalized PCA of MIR spectrum. TH, DV and EC refer to the yeasts Thermal Resistance Alcohol, Lalvin DV10 and Lalvin EC1118, respectively.

From PCA it can be seen that 80.54% of the data variability can be described with only one main component, PC1, which is associated with ethanol (correlated) and water (uncorrelated), with samples containing higher ethanol contents with higher PC1 values. The PC2 component, which describes 15.91% of the data variation, is associated with variations in peaks due to changes in ethanol-water composition and hydrogen bonding. PC1 and PC2 together describe 96.45% of the variability of the spectra. Similar results were reported by Nordon and coauthors [41]. With the graphical analysis, it was also possible to verify some groupings of samples relative to the fractions of distillate collected in the process. Besides, it is found that the heart samples of the final product that used the yeasts Lalvin DV10 and Lalvin EC1118 were the closest to commercial shochu.

CONCLUSION

The proposal to produce a sweet potato distillate similar to shochu by an alternative process was achieved, allowing a reduction in production time and reducing the risk of contamination.

The fermentation process time was reduced from 14 days to only one day and, in addition, there were no contamination problems.

The distillate produced and the commercial shochu presented the same absorption bands in FTIR analysis, identifying the similarity between them.

The use of pectinase in the production process resulted in a fermentation efficiency of approximately 70%, but the presence of methanol was found to be above the limits of the legislation, which makes the distillate produced using this enzyme unfit for consumption. Without pectinase addition, there was no significant methanol formation, but the yield fell to about 50%, due to the incomplete degradation of the sweet potatoes. Even with a lower yield, this is not an economic problem for production, since the profit margin in the production of the beverage is higher than, for example, in the production of fuel ethanol.

Funding: This research was funded by National Council for Scientific and Technological Development (CNPq) and the Federal University of Rio Grande do Sul (UFRGS).

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

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