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Vinegar Production from Jabuticaba (*Myrciaria jaboticaba*) Fruit Using Immobilized Acetic Acid Bacteria

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Summary

Cell immobilization comprises the retention of metabolically active cells inside a polymeric matrix. In this study, the production of jabuticaba (*Myrciaria jaboticaba*) vinegar using immobilized *Acetobacter aceti* and *Gluconobacter oxydans* cells is proposed as a new method to prevent losses of jabuticaba fruit surplus. The pulp of jabuticaba was processed and *Saccharomyces cerevisiae* CCMA 0200 was used to ferment the must for jabuticaba wine production. Sugars, alcohols (ethanol and glycerol) and organic acids were assayed by high-performance liquid chromatography. Volatile compounds were determined by gas chromatography-flame ionization detector. The ethanol content of the produced jabuticaba wine was approx. 74.8 g/L (9.5 % by volume) after 168 h of fermentation. Acetic acid fermentation for vinegar production was performed using a mixed culture of immobilized *A. aceti* CCT 0190 and *G. oxydans* CCMA 0350 cells. The acetic acid yield was 74.4 % and productivity was 0.29 g/(L·h). The vinegar had particularly high concentrations of citric (6.67 g/L), malic (7.02 g/L) and succinic (5.60 g/L) acids. These organic acids give a suitable taste and flavour to the vinegar. Seventeen compounds (aldehydes, higher alcohols, terpene, acetate, diether, furans, acids, ketones and ethyl esters) were identified in the jabuticaba vinegar. In conclusion, vinegar was successfully produced from jabuticaba fruits using yeast and immobilized mixed cultures of *A. aceti* and *G. oxydans*. To the best of our knowledge, this is the first study to use mixed culture of immobilized cells for the production of jabuticaba vinegar.

Key words: fruit vinegar, jabuticaba, alcoholic fermentation, acetic acid bacteria, immobilized cells

Introduction

The jabuticaba tree (*Myrciaria jaboticaba* Berg.), belonging to the family Myrtaceae, is native to Brazil. It grows spontaneously over the country, from north to south regions. The jabuticaba fruit looks like a berry, with a smooth black-purple skin when ripe. It has an average diameter of 1.9 cm and contains one to four seeds. The skin of the jabuticaba fruit is thin and fragile and its pulp is viscous and whitish with a sweet and slightly acid taste (*1–4*).

The fruit's grape-like character allows its use in the production of food products such as fermented beverages, juices, jam, liqueur, and potentially, vinegar (*5*). *M. jaboticaba* fruits are considered a natural source of functional

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antioxidant pigments (phenolic compounds) and vitamin C and have been used for the treatment of various diseases, including asthma, diarrhoea and hemoptysis (*6*,*7*). In Brazil, the local population consumes only a small amount of this fruit because approx. 40 to 50 % of the produced fruits are lost due to their short shelf life (*7*).

Although the number of studies on wine (*8–12*) and vinegar production from fruits has increased (*13–18*), very few studies are available on the production of jabuticaba vinegar. The industrial production of vinegar varies according to the used method, the raw materials, and the type of vinegar to be produced. Vinegar is produced by a two-step process: alcoholic fermentation of the must followed by the acetification. Starter cultures are commonly used in the food fermentation industry to predict and safeguard the product quality (*19*). These microbial starters have a significant role in the fermentation process (*20*). Yeast inoculation has been used extensively in the food industry to obtain a product with a predictable quality, including in the production of beverages such as wine (*19*,*21*) and beer (*22*,*23*). In contrast, the use of acetic acid bacteria (AAB) as inoculum in the vinegar industry has traditionally been limited to the use of the mother of vinegar or back slopping. In such cases, the produced vinegar is a result of competition between microorganisms present in the base wine, particularly, wild AAB.

The taxonomy of AAB has changed significantly in the last years. Currently, apart from *Acetobacter* and *Gluconobacter*, 17 other genera are classified as AAB: *Acidomonas*, *Ameyamaea*, *Asaia*, *Bombella*, *Commensalibacter*, *Endobacter*, *Gluconacetobacter*, *Granulibacter*, *Komagataeibacter*, *Kozakia*, *Neoasaia*, *Neokomagataea*, *Nguyenibacter*, *Saccharibacter*, *Swaminathania*, *Swingsia* and *Tanticharoenia* (*24*). Different vinegars show different AAB profiles; nevertheless, species of the genera *Acetobacter* (*25–28*), *Gluconobacter* (*29*,*30*) and *Gluconacetobacter* (*31–33*) (several of them have been recently renamed under the *Komagataeibacter* genus) (*34*) have been reportedly used in vinegar production.

Acetobacter aceti is a bacterium frequently used in the vinegar industry (*35*) because it immediately starts the fermentation process; however, when acetic acid concentrations exceed 5 %, other bacterial species take over the process (*36*). On the other hand, *Gluconobacter* gives a distinctive flavour to vinegar and can oxidize ethanol to acetic acid under acidic conditions (*29*). Thus, the use of mixed cultures ensures the best quality production of vinegar because of a rapid start and a good end to the fermentation process (*36*).

Cell immobilization can be defined as the physical confinement of intact cells to a defined space to preserve the metabolic or catalytic activity. Immobilization mimics the enclosure or cell aggregation that normally occurs when microorganisms grow in natural environments, with the benefit of compartmentalizing the immobilized cell. In this regard, the use of various substances as a support for cell immobilization has been studied (*37–39*).

In this study, a new method is developed to use and process surpluses of jabuticaba fruits in order to generate income to the post-harvest processing of jabuticaba. First, alcoholic fermentation was performed using lyophilized *Saccharomyces cerevisiae* CCMA 0200 cells for jabuticaba wine production, followed by vinegar production using mixed immobilized cells of *A. aceti* and *G. oxydans*. To the best of our knowledge, this is the first study to use Ca-alginate bead-immobilized AAB for the production of jabuticaba vinegar.

Materials and Methods

Jabuticaba pulp processing

Jabuticaba (*Myrciaria jaboticaba* Berg.) fruits were obtained from a farm in the state of Minas Gerais, southeastern Brazil. Ripe jabuticaba fruits were collected by hand from stems and branches of the jabuticaba tree and were washed with water to remove dirt. The fruit pulp was extracted by mechanical pressure and stored in polystyrene bags (2.0 L) at –20 °C. The pulp samples were then characterized for total soluble solids and pH (*40*,*41*).

The must was prepared by defrosting the jabuticaba pulp at 22 °C. The pulp had an average sugar content of 9.2 degree Brix (°Bx) and pH=3.8. The soluble solids were adjusted to 16 °Bx using a sucrose solution. Dipotassium disulphite (0.3 g) was added to the final jabuticaba must (3.0 L) as an antibacterial and antioxidant agent. To improve the sedimentation of the nonfermentable solids, 1 g/L of bentonite was added to the jabuticaba must (*8*).

Microorganisms

Yeast

Lyophilized *Saccharomyces cerevisiae* CCMA 0200 cells (Culture Collection of Agricultural Microbiology (CCMA), Federal University of Lavras, Lavras, MG, Brazil) at an initial count of 10^7 cells/mL were used for the production of jabuticaba wine. The yeast cells were rehydrated in sterile water at 38 °C for 30 min and then inoculated into the jabuticaba must for alcoholic fermentation.

Acetic acid bacteria

Vinegar was produced using a mixture of immobilized *Acetobacter aceti* CCT 0190 cells (André Tosello Tropical Culture Collection, Campinas, SP, Brazil) and *Gluconobacter oxydans* CCMA 0350 cells (CCMA).

Two culture media with different compositions were used for cell culture. The bacteria were separately cultivated in YEPD medium containing (in %): yeast extract 1, bacteriological peptone 2 and D-glucose 2 (HiMedia, Mumbai, India) at 30 °C and 100 rpm in an orbital shaker (Excella® E25; New Brunswick Scientific, Hamburg, Germany) to reach a cell count of 10^6 CFU/mL. Subsequently, the cells were transferred to YEP ethanol medium containing (in %): yeast extract 1, bacteriological peptone 2 (HiMedia) and ethanol 6 (Synth, Diadema, Brazil) to adapt to the ethanol present in the jabuticaba wine. The cells were then incubated for 18 h at 30 °C and 100 rpm in an orbital shaker and then immobilized in calcium alginate.

Immobilized bacterial cells

Bacterial cells were immobilized in calcium alginate following the method of Oliveira *et al.* (*42*), with modifications. A volume of 300 mL of each cell suspension (*A. aceti* and *G. oxydans*) at a count of 10^6 CFU/mL was mixed and 6.0 g of sodium alginate (final mass per volume ratio of 2 % alginate; Merck, Darmstadt, Germany) were added to the mixed cell suspension. To obtain immobilized cells

(Fig. 1), the sodium alginate cell suspension mixture was transferred to Mariotte bottles and homogenized, and beads containing the bacterial cells were prepared by dropping the suspension into a 0.1 M $CaCl_2$ (Merck) solution. The prepared beads were then used as inoculum for the jabuticaba wine during acetification.

Vinegar making

Alcoholic fermentation

Fermentation was performed in a 5-litre Biostat APlus glass fermentor (Sartorius Stedim Biotech GmbH, Goettingen, Germany) at 22 °C. The must was inoculated with 10 mL of yeast cell suspension, corresponding to a final cell count of 10^7 cells/mL in 3.0 L of must. Fermentation was considered finished when the sugar level (°Bx) was stable, which was observed after 168 h. The production of CO_2 was observed during the fermentation process. Samples were taken at the beginning and the end of fermentation for microbiological and chemical analyses. At the end of fermentation, the glass fermentor was placed in an incubator (Eletrolab® EL101; Eletrolab, São Paulo, Brazil) at 10 °C to facilitate sedimentation of the solid material in the jabuticaba wine. After 10 days of incubation, the beverage was transferred to an Erlenmeyer flask to provide aeration and was incubated at 10 °C for another 30 days. After this, the jabuticaba wine was filtered through diatomaceous earth and cellulose filters under vacuum (*8*) and used for the production of jabuticaba vinegar during acetification.

Acetic acid fermentation

Immobilized bacterial cells (*A. aceti* and *G. oxydans*) at a count of 10^6 CFU/mL were added to 1.2 L of jabuticaba wine in a 2-litre Inceltech LH.SGI Discovery series 100 bioreactor (Inceltech, Toulouse, France). Approximately 10 980 beads were inoculated into the bioreactor. The experiment was performed under controlled conditions at 28 °C, 0.05 L/min of oxygen and an initial pH of 5.0, without stirring. Acetification was considered finished after ethanol consumption and acetic acid production were stabilized, which was observed after 264 h. During acetification, samples were taken every 24 h for subsequent physical and chemical analyses.

Viable cells

The viable cells (CFU/mL) from the total number of cells of AAB inside the beads were counted at time zero (when the jabuticaba wine was inoculated) and every 24 h until the end of vinegar production (after 264 h). Ten beads (approx. 0.5 mL of carrier beads) were placed on a glass filter to drain the solution. The beads were then transferred to a 5-mL burette containing 3.5 mL of sterile distilled water. The liquid height was recorded to determine the increase in volume due to the beads. The beads were then crushed in sterilized water using a glass stick to recover the immobilized cells. The total number of AAB cells (CFU/mL) was determined by inoculating this cell suspension on GYC agar containing (in %): yeast extract 1, D-glucose 10, calcium carbonate 2 and agar 2 (Merck) for 24–48 h at 30 °C. This culture medium is commonly used for the isolation of AAB because it enables the observation of the clear halo formed around their colonies after incubation (*43*). The differentiation of *Acetobacter* from *Gluconobacter* was done by acetate overoxidation using the chalk-ethanol test, plating samples on the Carr and Passmore medium containing (in %): glucose 0.05, yeast extract 0.5, peptone 0.3 (HiMedia), calcium carbonate 1.5, agar 1.2 and ethanol 1.5 (99.8 %; Merck) filter-sterilized and added after sterilization of the basal medium for 24–48 h at 30 °C (*44*). The overoxidation of acetic acid by *Acetobacter* results in the reprecipitation of $CaCO_3$. The clear halo was observed around the colonies of the *Gluconobacter* strains.

Physicochemical and chromatographic analyses

The pH values of the alcoholic and acetic acid fermentations were measured at room temperature using a digital pH meter (B474; Micronal, São Paulo, Brazil). The total soluble solid content was determined using a digital refractometer (PAL-1; Atago, Tokyo, Japan) and the results are expressed in °Brix. The concentration of total reducing sugars was determined by the dinitrosalicylic acid (DNS) method following the method of Miller (*45*).

The yield was calculated as the acetic acid produced in relation to the theoretical yield. The theoretical yield was calculated by measuring the amount of ethanol con-

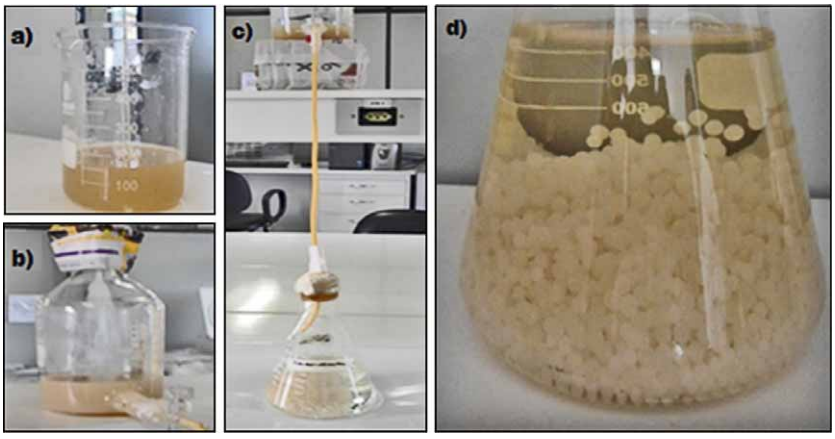

Fig. 1. Cell immobilization of acetic acid bacteria: a) hydrated sodium alginate, b) cell suspension in sodium alginate, c) immobilization system: cells suspended in the sodium alginate solution that were added dropwise to the calcium chloride solution to prepare beads, and d) prepared beads (for colour version see: www.ftb.com.hr)

verted to acetic acid, in which 1.0 g of ethanol yields 1.304 g of acetic acid (*46*). Productivity was calculated from the quantity of acetic acid produced per unit of volume over time (g/(L·h)).

The concentration of organic acids (lactic, acetic, tartaric, malic and succinic acids), glycerol, ethanol and carbohydrates (glucose, fructose and sucrose) was measured by high-performance liquid chromatograph (LC-10AI; Shimadzu Corp., Tokyo, Japan) equipped with a refractive index detector (RID, 10A; Shimadzu) and an ultraviolet (UV) detector (SPD-10AI; Shimadzu). A cation exchange column (Shim-pack SCR-101H, 7.9 mm×30 cm; Shimadzu) was used at 30 °C to measure the concentration of sugars and ethanol and at 50 °C for the determination of organic acids, using 100 mM perchloric acid as the eluent at a flow rate of 0.6 mL/min. The acids were detected by UV absorbance at 210 nm using a spectrophotometer (SPD--10AI; Shimadzu), whereas the sugars and ethanol were detected by RID. Individual sugars, acids and alcohols were identified by comparing their retention times with those of certified standards. Alcohols, sugars and acids were quantified by applying calibration curves obtained using standard compounds (Sigma-Aldrich, Steinheim, Germany). All samples were examined in triplicate.

Volatile compounds in the jabuticaba vinegar were analyzed directly, according to Duarte *et al.* (*47*). Analyses were performed using a gas chromatography system (GC-17A; Shimadzu) with a flame ionization detector (GC–FID). A DB Wax silica capillary column was used (30 m×0.25 μm×0.25 mm i.d.; J&W Scientific, Folsom, CA, USA). The GC-FID was operated using a gradient temperature program (50 °C for 5 min, increased to 190 °C by increments of 3 °C/min, and then kept at 190 °C for 10 min). Injector and detector temperatures were kept at 240 °C, and the carrier gas (N_2) was kept at a flow rate of 1.2 mL/min. The split mode (1:10) was defined for the 1-μL sample injections. Volatile compounds were identified by comparing the retention times of the samples with those of the standards under the same conditions. The quantification of volatile compounds was expressed as 4-nonanol (125 mg/L) internal standard equivalents. To calculate the linear retention index (LRI) of each compound in the sample, a standard mixture of *n*-alkanes (C8–C40 alkanes calibration standard; Sigma-Aldrich, St. Louis, MO, USA) was used. One microlitre of the *n*-alkane standard was injected into the GC-FID under the same conditions as those described above and its retention times were used as an external reference for calculating the LRI of the compounds, according to van Den Dool and Kratz (*48*).

Statistical analyses

Each fermentation process (alcoholic and acetic acid) was conducted in duplicate and the mean values±standard deviations are reported. The Tukey's test using Statgraphics Plus for Windows v. 4.1 software (Statistical Graphics Corp. Rockville, MD, USA) was performed to evaluate the statistical significance ($p<0.05$).

Results and Discussion

Jabuticaba wine production

Table 1 shows the concentrations of the compounds detected in the jabuticaba wine. The concentration of ethanol increased during the fermentation of the jabuticaba must, reaching a maximum of 74.80 g/L (9.5 % by volume) at 168 h of fermentation. These results are different from those obtained by Duarte *et al.* (*10*), who reported a value of 57.0 g/L when fermenting jabuticaba fruits (*Myrciaria jaboticaba*) with an initial 16 °Bx. Small concentrations of sucrose, fructose and reducing sugars were detected in the jabuticaba wine (0.09, 0.06 and 0.04 g/L, respectively). Glucose was not detected in the jabuticaba wine (Table 1).

A rapid decrease in the sugar content and increase in the ethanol concentration (74.80 g/L) during an inoculated fermentation was also observed by Domizio *et al.* (*49*) during the fermentation of grape must under controlled temperature conditions. This result confirmed that the selected yeast promoted a rapid increase in the concentration of ethanol. Nurgel *et al.* (*50*) found that the fermentation of nonpasteurized grapes using the selected yeast (approx. $5.0 \cdot 10^6$ CFU/mL) was completed in 6 days, whereas indigenous fermentation lasted 10 days. These studies also reported that the pH values of the wines were similar (approx. 3.08).

Table 1. Physicochemical characteristics of the jabuticaba wine

t(fermentation)/h	γ(acid)/(g/L)				
	Acetic	Citric	Succinic	Malic	Oxalic
0	(0.11±0.01)^a	(1.99±0.01)^a	(0.25±0.01)^a	(0.11±0.01)^a	(0.09±0.01)^a
168	(0.92±0.01)^b	(1.37±0.01)^b	(1.15±0.01)^b	(1.00±0.02)^b	(0.29±0.01)^b
	γ(sugar)/(g/L)				
	Sucrose	Glucose	Fructose	Reducing sugars	
0	(92.6±0.11)^a	(12.60±0.08)^a	(23.40±0.21)^a	(36.4±0.31)^a	
168	(0.09±0.01)^b	n.d.	(0.06±0.01)^b	(0.04±0.01)^b	
	γ(alcohol)/(g/L)				
	Ethanol	Glycerol	pH	TTS/°Bx	
0	n.d.	n.d.	(3.05±0.02)^a	(15.5±0.02)^a	
168	(74.80±0.41)^b	(5.10±0.01)^b	(3.02±0.02)^a	(5.37±0.02)^b	

The data are the mean values of duplicate measurements±standard deviation. Different letters in the same column indicate statistically significant differences ($p<0.05$). n.d.=not detected, TTS=total soluble solids

Lactic, tartaric, propionic and butyric acids were not detected in the jabuticaba wine. Acetic acid was formed during the fermentation of the jabuticaba must, reaching a maximum concentration of 0.92 g/L at 168 h (Table 1). Acetic acid at low concentrations (<1.0 g/L) provides a pleasant taste and inhibits the development of undesirable or pathogenic microorganisms.

Malic and citric acids were also detected in the jabuticaba wine (Table 1). The concentration of malic acid was 1.00 g/L at 168 h of fermentation. During fermentation, the concentration of citric acid decreased from 1.99 (at 0 h) to 1.37 g/L at the end of fermentation (168 h). It is possible that citric acid was metabolized as a carbon and energy source by *S. cerevisiae*, which has the ability to ferment or assimilate this organic acid, resulting in an increase in pH (*51*), as observed in the present study. Citric and malic acids are commonly found in fermented fruit beverages, where they act as preservatives with antimicrobial activity (*12*,*50*).

Oxalic acid was detected in the jabuticaba wine before the aerobic phase of acetic fermentation (Table 1). The organic acids produced by the yeast and bacterial species contribute to the refreshing flavour, unique aroma and texture, in addition to controlling the growth of food spoilage microorganisms (*10*). The concentration of glycerol in the jabuticaba wine was low (5.10 g/L). This value was consistent with that suggested by Dias *et al.* (*8*) (<10.0 g/L) to confer the characteristic body and texture of the beverage. Glycerol is the main secondary product of alcoholic fermentation by *S. cerevisiae*, which was used as the inoculum in this study. The glycerol concentration of approx. 5.1 g/L was close to the minimum concentration of 5 g/L in grape wine suggested by Ribéreau-Gayon *et al.* (*52*).

Jabuticaba vinegar

In the present study, a new method for vinegar production was applied using a mixture of immobilized *A. aceti* and *G. oxydans* cells in submerged culture fermentation in a bioreactor.

The analysis of the AAB population showed that the count of the mixed immobilized cell population was $5.2 \cdot 10^6$ CFU/mL after the end of vinegar fermentation (Fig. 2). The population of *A. aceti* was higher than that of *G. oxydans* during acetic acid fermentation. The ratio of *A. aceti*/*G. oxydans* population was approx. 1.20 at the end of fermentation (Fig. 2). The concentration of acetic acid in the jabuticaba vinegar reached 77.8 g/L after 264 h. Therefore, the mixed starter culture efficiently fermented the jabuticaba wine to produce vinegar.

The assessment of the experimental data revealed that the immobilized cell model allows an apparently proper use of the substrate and proper production of acetic acid. Encapsulation might protect the microorganism from its environment, and a link between the microbial environment and the production of acetic acid was demonstrated by the model. A slight increase in the cell density was observed during the making of jabuticaba vinegar (Fig. 2). The model system might have resulted in widespread surface growth and thus, cells were continuously released from the gel beads into the fermentation medium, leading to a decrease in the cell population in the beads (*39*). However, the results showed that the release from the immobilized cell beads was negligible (data not shown).

Fig. 2 shows the ethanol consumption and acetic acid production during vinegar making. With regard to industries, the conversion of 1.0 g ethanol to 1.0 g of acetic acid can be considered economic (*53*). Fig. 3 shows the yield and productivity of the acetic fermentation. The production was favourable, reaching a yield of 77.4 % and productivity of 0.29 g/(L·h). Therefore, we can conclude that the evaporation of volatile compounds was low, which might be attributed to the use of appropriate aeration (0.05 L/min) and thermal conditions (28 °C).

Several organic acids in vinegar are important for imparting a suitable taste and flavour. Table 2 shows the contents of various organic acids in the jabuticaba vinegar. The total acetic acid concentration in the jabuticaba vinegar produced using a mixed culture of immobilized *A. aceti* and *G. oxydans* cells was 77.8 g/L (Fig. 2). A similar observation had previously been made by Kocher *et al.* (*54*), who compared the production of vinegar from sugarcane juice using different inert materials for the immobilization of *A. aceti* cells; however, their fermentation time of 28 days was considered too long. The mixed culture of immobilized *A. aceti* and *G. oxydans* cells, an unusual condition for vinegar production, shows satisfactory

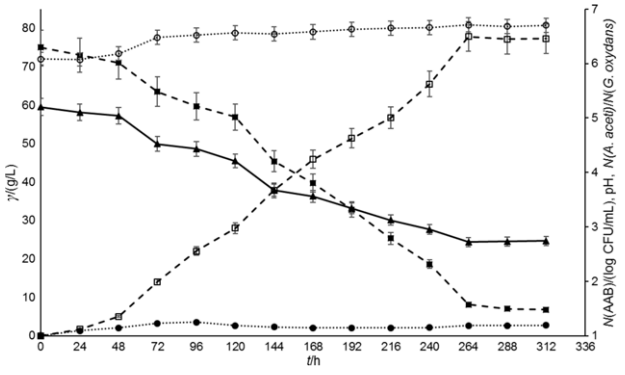

Fig. 2. Ethanol concentration (–■–), acetic acid concentration (–□–), count of acetic acid bacteria (log CFU/mL) (····○····), pH value (—▲—), and ratio of *Acetobacter aceti*/*Gluconobacter oxydans* counts (····●····) in the jabuticaba alcoholic must during acetic acid fermentation. The bars indicate standard deviation. AAB= acetic acid bacteria

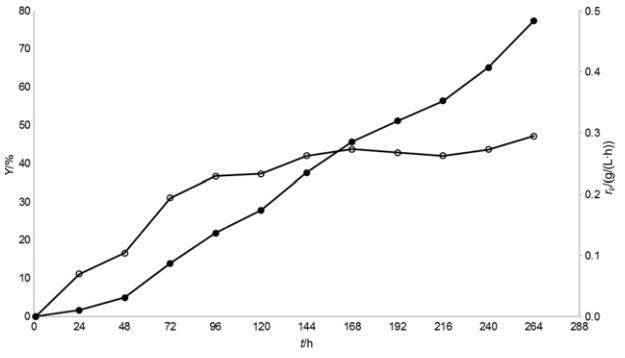

Fig. 3. Yield (*Y*; —●—) and productivity (r_p; —⊖—) of acetic acid in the jabuticaba vinegar

Table 2. Chemical characteristics of the jabuticaba vinegar

t(fermentation)/h	γ(acid)/(g/L)				γ(glycerol)/(g/L)
	Citric	Succinic	Malic	Oxalic	
0	(1.39±0.01)[a]	(1.52±0.01)[a]	(1.07±0.01)[a]	(0.22±0.01)[a]	(5.32±0.01)[a]
264	(6.67±0.01)[b]	(5.60±0.01)[a]	(7.02±0.01)[b]	(0.18±0.01)[a]	(5.36±0.01)[a]

The data are the mean values of duplicate measurements±standard deviation. Different letters in the same column indicate statistically significant differences ($p<0.05$)

results (*i.e.* the production of acetic acid along with other organic acids; Table 2) for acetic acid fermentation using jabuticaba wine. According to FAO/WHO (*55*), the residual ethanol content in vinegars produced by different substrates (those different from wine) must be less than 1 %. Further, it is recommended to have a minimum titratable acidity of 4 % (*56*).

Table 2 shows the concentrations of citric, malic and succinic acids. The contents of these three acids increased by approx. five times compared with their initial values in the jabuticaba wine. These acids were also observed in commercial and traditional vinegars from Korea by Jang *et al*. (*57*). The lactic, tartaric, butyric and propionic acid concentrations in the jabuticaba wine were negligible and remained negligible in the vinegar. The final pH of the jabuticaba vinegar was 2.74, which is attributed to the high production of acetic acid during fermentation (Fig. 2).

Seventeen compounds were identified and quantified in the jabuticaba vinegar: aldehydes, higher alcohols, terpene, acetate, diether, furans, acids, ketone and ethyl esters (Table 3, *58–61*). Callejón *et al.* (*62*) identified 96 compounds in sherry vinegar: 26 esters, 23 carbonyl compounds, 20 alcohols, 14 acids, 6 volatile phenols, 3 lactones, 2 ethers, 1 acetal and 1 terpene. The flavour of vinegars is determined by a series of volatile constituents of three different origins: substrate, acetification and ageing. Although several major volatile compounds such as acetic acid, ethyl acetate and acetaldehyde contribute to the final aroma of vinegar, many other minor compounds with a wide range of polarities, solubilities and volatilities could help explain the complexity of the overall sensation, especially if the vinegar is produced from fruits (*63,64*). Acetaldehyde (3.72 mg/L) was the only aldehyde found in the jabuticaba vinegar. It is a very volatile compound; its content tends to decrease during acetification because it is an intermediary metabolite during the conversion of ethanol to acetic acid and is thus converted to acetic acid by the same metabolic pathway (*62*). At low levels, acetaldehyde gives a pleasant fruity aroma to wines; however, at high concentrations, it has a pungent, irritating odour (*65*). Four higher alcohols were identified in the jabuticaba vinegar. Among them, 2-phenylethanol was present at high levels (Table 3). Its presence may result in flowery and sweet notes (*66*), which could be considered as a positive feature in the jabuticaba vinegar. Acetoin (3-hydroxy-2-butanone) was also a unique ketone that was identified in the jabuticaba vinegar (149.64 mg/L). It is a characteristic product of acetification and its concentration is very high in traditional vinegars (*64,67*). Natera *et al.* (*68*) reported that the content of acetoin ranged from 18 mg/L in malt vinegar to 227 mg/L in apple vine-

Table 3. Concentrations of volatile compounds identified in the jabuticaba vinegar by GC-FID

No.	Compound	LRI	γ / mg/L	Odour quality
	Aldehyde (1)			
1	Acetaldehyde	709	3.72	Fresh, green[a]
	Higher alcohols (4)			
2	2-Heptanol	1329	11.48	Coconut-like, ketonic solvent-like, unpleasant[b]
3	2-Methyl-1-propanol	1079	7.35	Malty[a]
4	2-Methyl-1-butanol and 3-methyl-1-butanol	1240	22.21	Malty, solvent-like[a]
5	2-Phenylethanol	1896	31.40	Flowery, honey-like[a]
	Terpene (1)			
6	α-Terpineol	1682	4.17	Pine-like, terpenoid-like[b]
	Acetate (1)			
7	Phenylethyl acetate	1826	1.38	Apple-like, honey-like, rosy, sweet[b]; flowery[c]
	Diether (1)			
8	1,1-diethoxyethane	755	1.63	n.d.
	Furan (1)			
9	Furfuryl alcohol	1639	14.81	n.d.
	Acids (5)			
10	Decanoic	2287	2.61	Waxy, tallow, rancid, soapy[b]; fatty[c]
11	Isobutyric	1546	6.38	Sweet, bitter[b]; cheesy, rancid[c]
12	Hexanoic	1850	1.20	Fatty acid-like, vegetable oil-like[b]; cheesy, sweet[c]
13	Propionic	1523	2.77	Vinegar-like[c]
14	Octanoic	2061	1.77	Fatty acid-like, vegetable oil-like[b]; rancid, harsh[c]
	Ketone (1)			
15	Acetoin	1309	149.64	Buttery, creamy, cheesy[d]
	Ethyl esters (2)			
16	Ethyl acetate	816	179.38	Solvent-like, fruity[b]
17	Ethyl octanoate	1398	148.23	Apple-like, fruity[b]; sweet[a]

LRI=linear retention index; [a]Czerny *et al.* (*58*), [b]Meilgaard (*59*), [c]Siebert *et al.* (*60*), [d]Romano and Suzzi (*61*); n.d.=not determined

gar. High concentrations of ethyl acetate and ethyl octanoate were also found in the jabuticaba vinegar (179.38 and 148.23 mg/L, respectively; Table 3). The quantitatively most important volatile ester is ethyl acetate. It is present especially in vinegars produced by slow acetification processes, where high amounts of ethanol in conjunction with acetic acid are present; but in industrial vinegar, these amounts are lower. The initial ethanol content determines the formation of certain compounds such as ethyl acetate (*69*,*70*). Ethyl acetate was also the major volatile compound reported by Callejón *et al.* (*71*), who described the aroma profile of different categories of sherry vinegar and reported ethyl acetate concentrations between 132 and 3955 mg/L, followed by considerable concentrations of acetoin (between 194 and 1020 mg/L) in all samples.

The results of the jabuticaba vinegar analyses show that the final product has an acceptable acetic acid mass per volume ratio of approx. 7.78 % and an ethanol volume fraction lower than 1.0 %. Fig. 4 shows the clear appearance of the jabuticaba vinegar. The final product had a good colour (pale yellow). The vinegar had a strong flavour of jabuticaba, which compensated for the pungent smell due to volatile acids, and proved to be a very promising product.

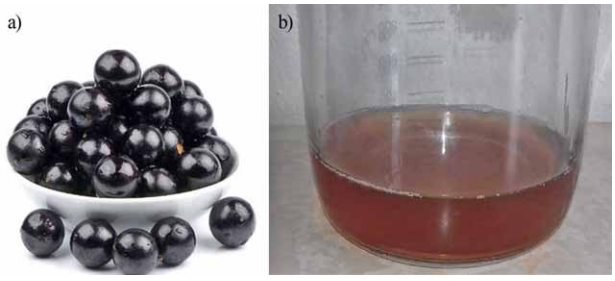

Fig. 4. The jabuticaba fruit (a) and jabuticaba vinegar (b) (for colour version see: www.ftb.com.hr)

Conclusion

In conclusion, vinegar was successfully produced from jabuticaba must using yeast and immobilized cells of mixed cultures of *Acetobacter aceti* and *Gluconobacter oxydans*. To the best of our knowledge, this is the first study to produce jabuticaba vinegar using mixed culture of immobilized cells. The chemical analyses revealed that the jabuticaba vinegar had a high content of organic acids and volatile compounds, which add functional value and aroma to the vinegar. The technology proposed in this study is important and proved to be a viable technique for using harvest surpluses and obtaining products with market value.

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