

Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production

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Abstract The objectives of this study were to evaluate the potential of gabiroba *Campomanesia pubescens* (DC) O. Berg in the production of a beverage fermented using selected and wild yeasts from indigenous fermentation, analyze the volatile compounds profile present during the process of fermentation, and evaluate the sensory quality of the final beverage produced. Throughout the process of fermentation, when *Saccharomyces cerevisiae* UFLA CA 1162 was inoculated, there were stable viable populations around $9 \log \text{ cells ml}^{-1}$. During indigenous fermentation, yeast population increased from $3.7 \log \text{ CFU ml}^{-1}$ to $8.1 \log \text{ CFU ml}^{-1}$ after 14 days. The diversity and dynamics of the yeast population during indigenous fermentation observed by PFGE analysis showed five different karyotyping profiles in the first days of fermentation. After the seventh day, there was a higher frequency of a similar *S. cerevisiae* profile. The yeast non-*Saccharomyces* were identified by sequencing of the ITS region as *Candida quercitrusa* and *Issatchenkia terricola*. Inoculated fermentations yielded a higher amount of alcohol than indigenous ones, indicating the efficiency of selected strains. There was also a greater concentration of higher alcohols, which are usually responsible for the flavor found in alcoholic beverages. Based on the characteristics of the pulp and acceptance in the sensory analysis, gabiroba fruits showed good potential for use in the production of fermented beverage.

Introduction

The specie *Campomanesia pubescens* (DC) O. Berg, popularly known as gabiroba or guabi-roba, belongs to the family Myrtaceae. The genus *Campomanesia*, represented by shrubs and trees, can be found from northern Argentina to Trinidad as well as from the Brazilian Coast to the Andes and Peru, Ecuador, and Colombia [26, 41]. The gabi-roba fruits are commonly consumed in the producing regions and are highlighted as a potential food resource for both avifauna and man [41]; people use gabi-roba in the production of homemade ice cream, jam, juices, and sweets. However, not all of the harvest finds its destination in the market, because most of the fruits are damage during post-harvesting. New forms of use or processing of the production must be developed, in order to prevent losses, generate profits, and favor sustainable exploitation of the resources of the cerrado biome by encouraging conservation. One of the alternatives is its use in fermented beverage production.

Grapes have been used as the main raw material in the production of wine. However, a number of researches found other suitable fruits for wine production. Over the years, fruit wine has been prepared from several different fruits, such as cajá [13], banana [1], pupunha [3], mango [33], acerola [37] and cocoa [12].

The fermentation process for elaboration of the beverage depends on the performance of yeast to convert the sugars into alcohol and esters. Different species of yeast that develop during fermentation determine the characteristics of the flavor and aroma of the final product. Due to the differences in fruit composition, yeasts strains used for fermentation have to adapt to different environments (e.g., sugar composition and concentrations, presence of organic acids, etc.). In addition, the applied yeast has to compete for sugar utilization with other micro organisms present in the

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mashes, e.g., other yeast species or bacteria, depending on the fruit of choice and varying climatic conditions. Natural grape fermentation involves a succession of yeasts, with *Saccharomyces cerevisiae* as the dominant species [20]. The presence and permanence of different yeast species throughout fruit fermentation, and consequently, their influence on the final product, is determined by the fermentation conditions, such as inoculum of *S. cerevisiae* starter culture, the temperature of the fermentation, and the fruit juice composition [12]. This is the first report of the use of gabi-roba in fermented beverage. In the present study, we have investigated the yeast population and the fermentation kinetics during indigenous and inoculated fermentation of gabi-roba juice and their influences on the analytical and sensory properties of the final beverage.

Materials and methods

Fruits

The gabi-roba fruits, *C. pubescens* (DC) O. Berg, were collected in November and December of 2006 and 2007 in the cerrado areas present in the cities of Ijaci, Ingai and Lavras, Minas Gerais, Brazil. The fruits were washed in clean water to remove the residues of calyx. The fruit pulp was extracted manually by mechanical pressure and stored in polystyrene bags at -20°C . The fruits were then analyzed in relation to total weight, size, diameter, and seed:pulp weight ratios. Samples were taken from the gabi-roba pulp and were characterized in relation to soluble solids [4], total [43] and reducing sugars [29], total titratable acidity and pH [4], pectins [28] and phenolics, pectinametilsterase [7], peroxidase [27], polyphenoloxidase [42] and polygalacturonase [7].

Gabi-roba must

The gabi-roba must was prepared according to Dias [12, 13], with minor modifications. To prepare the fermenting must, gabi-roba pulp was defrosted at room temperature. The initial Brix value was, on average, 14 and the pH was 4.1. The gabi-roba pulp was diluted with a sucrose solution to adjust the sugar concentration to 10%. CaCO_3 was added to increase the pH value to 4.5. Due to the viscous aspects of the gabi-roba pulp, pectinolytic enzymes were also added to aid the clarification of the juice. Ultrazym[®] AFP-L (Novozymes, Novo Nordisk Ferment Ltd, Fuglebakken, Denmark) was added to 0.7 ml l^{-1} . Sulfur dioxide was added up to 200 mg of $\text{K}_2\text{S}_2\text{O}_5$ per liter, to inhibit bacteria growth. One percent bentonite was added to the must to facilitate sedimentation of the non-fermentable solids. The bentonite was suspended in water at a 10%

concentration to facilitate the dispersion of the clay in the must.

Fermentation assays

Four batch fermentations were performed: two of them were inoculated with $10^8\text{ cells ml}^{-1}$ (9 log CFU ml^{-1}) of *S. cerevisiae* UFLA CA1162 (IN CA1162 A e IN CA1162 B) and the other two (SPON 1A e SPON 1B) were allowed to ferment with indigenous micro organisms the gabi-roba pulp. The inoculated strains were grown in gabi-roba pulp and inoculated at maximum 8% of total volume of fermentation must. After the pulp Brix decreased to 3° Brix in either inoculated or indigenous fermentation, there was an addition of gabi-roba pulp plus sucrose until the Brix in fermentation flasks reached 10° Brix. All vinifications were carried out in 5 l flasks in a cold room at 22°C and the fermentation was monitored by the daily measurement of Brix value, CO_2 , and temperature. The maximal fermentation rate was determined by the maximum ethanol production and decrease in sugar content. The fermentation was considered complete when the Brix level was stable. Samples were taken at the indicated points for microbiological and chemical analysis. At the end of fermentation, the vats were transferred to a 10°C incubator to aid the sedimentation of solid material from the gabi-roba pulp. After 10 days at this temperature, the wine transfer was carried out with some aeration and the beverage was incubated at 10°C for another 30 days. After that period, another transfer without aeration was carried out and the fruit wine was left for another 10 days at 10°C , prior to filtration. The gabi-roba wine was then filtered using cellulose filters and stored at 10°C in glass bottles fully filled to avoid oxygen entrance.

Isolation and identification yeast from indigenous fermentation

Samples were taken from each fermentation flask during fermentation and the dilution series was plated out onto Petri dishes using two different media: YPD–agar medium (1% yeast extract; 2% peptone, 2% glucose and 2% agar) supplemented with 100 mg L^{-1} of chloramphenicol and 50 mg L^{-1} of chlortetracycline to inhibit bacterial growth for yeast counts and nutrient agar (0.3% meat extract, 0.5% peptone and 1.5% agar) supplemented with 4 mg l^{-1} of cycloheximide for bacterial counts. Plates were incubated at 28°C for 2 days to allow colony development. Following incubation, the number of colony-forming units (CFU) was recorded, the morphological characteristics of each colony type were recorded, and counts were made for each type. For each colony type, a number of colonies corresponding to the square-root of the number of colonies of each type were re-streaked and purified on YPD. The purified isolates

were stored at -80°C in YPD broth containing 20% (v/v) glycerol.

Electrophoretic karyotype analysis yeast from indigenous fermentation

Analysis of yeast chromosome polymorphism was performed as described by Bernardi et al. [5]. After electrophoresis, gels were stained with 1% ethidium bromide for 1 h and rinsed twice with MilliQ (Millipore, Jaffrey, USA) water for 15 min. The gels were visualized in UV transillumination and documented with a Polaroid (Concord, USA) camera. The yeast *S. cerevisiae* UFLA CA1162 was used as reference strain. Cluster analysis of the band profile obtained by electrophoretic karyotyping analysis was performed using the software NTSYS 2.11f[®] (Exeter software, Setauket, USA).

Molecular identification of yeast from indigenous fermentation

The yeast DNA from the pure cultures was extracted according to the method described by Bernardi et al. [5]. Sequencing of portions of the ITS region was used for identification of representative yeast isolates to species level. For amplification of the ITS region, the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') were used. PCR was performed according to the method described by Naumova et al. [31] and was conducted in a model Thermo PCYL220 thermal cycler (Thermo Fisher Scientific Inc., Waltham, USA). The amplification products were separated by electrophoresis on a 0.5% agarose gel at 60–65 V in $0.5 \times \text{TAE}$ for 1 h. The sequencing of portions of the ITS region was accomplished by Macrogen, Seoul, Korea. Sequence similarity searches were performed using the BLAST database from GenBank (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Substrates and metabolites

Alcohols (ethanol and glycerol), organic acids (acetic acid, lactic acid, malic acid, succinic acid, tartaric acid, and citric acid), and carbohydrates (glucose, sucrose, and fructose) were obtained from pulp extracts and analyzed [36]. Analyses were carried out with a high-performance liquid chromatography system (HPLC) (Shimadzu, model LC-10Ai (Shimadzu Corp., Tokyo, Japan), equipped with a dual detection system consisting of a UV detector and a refractive index detector (RID—10A SPD-10Ai). A Shimadzu ion exclusion column (Shim-pack SCR-101H, 7.9 mm \times 30 cm) operated at a temperature of 50°C was used to achieve chromatographic separation. Water-soluble acids, sugars (fructose, glucose, and sucrose), and ethanol were eluted with 100 mM of perchloric acid at a flow rate of 0.8 ml min^{-1} .

The acids were detected via UV absorbance (210 nm) and RI, while sugars and ethanol were only detected via an RID. Individual sugars, acids, and ethanol were identified and their concentrations were determined by comparison with retention times and amounts of authentic standards. All samples were examined in triplicate. The coefficient of variation was less than 5% in each case. Higher alcohols (1-propanol, isobutanol, 1-butanol, isoamyl alcohol, amyl alcohol, and hexanol), acetaldehyde, methanol, and esters (ethyl acetate and methyl acetate) were analyzed by gas chromatography (GC) using a Shimadzu model 17A, equipped with a flame ionization detector (FID) and using a capillary column of silica HP FFAP (30 m \times 0.25 mm i.d. \times 0.25 μm) (J&W Scientific, Folsom, USA). For GC analysis, 100 μl of each sample (non-distilled) was diluted 20-fold in milli-Q water and filtered using a nitrate-cellulose membrane (0.20 μm pores) before injection in the GC. Operating conditions were as follows: oven temperature was kept at 60°C for 3 min, programmed to 75°C at $2^{\circ}\text{C min}^{-1}$, kept at 100°C for 3 min, programmed to 184°C increasing $3^{\circ}\text{C min}^{-1}$, then kept at 184°C for 30 min and then programmed to 220°C in 15 min. Injector and detector temperature were kept at 240°C and the carrier gas (N_2) was kept at a flow rate of 1.2 ml min^{-1} . The identification of volatile compounds was done by comparison of their retention times with those of standards. One sample, which contained the internal standard and the standard compounds at concentrations similar to those found in the wine, was also treated in the same way as the wine samples and the final calculations are described on the basis of the concentration of this reference solution. Evaluation of the different compounds was performed in triplicate.

Sensory evaluation

The final beverage was evaluated by 50 panellists, males and females, 18–55 years of age (staff and students of the Universities UNILAVRAS and UFLA). The panellists were selected for participation on the basis of their preference for dry (less than 5 g l^{-1} of sugar) wines, interest, and availability. Randomized, refrigerated (10°C) samples of 20–25 ml were served in clear, tulip-shaped glasses with a volume of 100 ml; these were marked with three digit random numbers and covered with plastic Petri dishes. Distilled water was provided for rinsing of the palate during the testing. Evaluations took place in the mornings between 9:00 and 10:00 a.m. and were conducted at room temperature (20 – 22°C) under white light.

The gabiropa wines were evaluated for taste, clarity, colour, and general acceptability according to the hedonic scale [12]. This scale consists of the comparison, punctuation, and classification of foods and beverages of the same class or origin according to their qualities and defects.

A card containing six parameters (visual examination, smelling intensity, smelling quality, taste intensity, taste quality, and harmony) was provided, accompanied by a scale of nine categories: dislike extremely = 1, dislike much = 2; dislike moderately = 3; dislike slightly = 4, neither dislike nor like = 5, like slightly = 6; like moderately = 7; like much = 8, like extremely = 9. The sensory analysis was performed in two sensory sessions, each lasting 1 h. The wines were evaluated in duplicate in each session and the mean score of wines for each quality attribute was computed.

Statistical analysis

The statistical analysis was performed using SISVAR[®] (Lavras, Brazil) software version 4.5. Data from gabirola must fermentation underwent statistical analysis (principal component analysis, PCA) using The Unscrambler[®] 9.7 (CAMO, Oslo, Norway) software.

Results and discussion

The gabirola fruits and pulp were chemically characterized before initiating the fermentation process. The averages of the results in triplicate are found in Table 1. The gabirola pulp had on average pH of 4.1, which characterized it as an acidic fruit. The total and reducing sugars concentration was 12.5 and 7.6%, respectively. With these amounts of sugars, the gabirola pulp could be compared with other tropical fruits, such as cocoa, with potential for use in the beverage industry [2]. The total soluble solid concentration in the pulp of gabirola, determined as its Brix value, was 14.1 (Table 1). As the determination of °Brix by refractometry indicates total soluble solids, which are not necessarily constituted of only sugars, the final alcohol yield might appear to be low if based on the Brix value. When pH, water content, and total sugars are considered, the results of gabirola characterization in this study were similar to those described for the genus *Campomanesia* [41]. The concentration of ascorbic acid (vitamin C) found in the species *C. pubescens* (1,000 mg/100 g), when compared with other species of the family Myrtaceae, was equivalent to that found in acerola (984 mg/100 g) and was superior to those described for red guava (60 mg/100 g), Pitanga (24 mg/100 g), and jaboticaba (16 mg/100 g) [39] as well as those observed in two other species of *Campomanesia*, both *C. adamantium* (234 mg/100 g) [41].

The bentonite introduced into the gabirola must during the pre-fermentative phase provided better clarification of the beverage by facilitating sedimentation of the must solid, thereby improving decanting and filtering in later stages [8]. Similar results have described the improvement of the

Table 1 Physico-chemical characterization of mature gabirola pulp

Characteristics	Mean	±SD
Total weight (g)	47.00	2.51
Rind (%)	43.69	0.88
Seed (%)	6.43	0.50
Pulp (%)	51.95	1.59
Moisture (%)	79.73	0.64
Dry matter (%)	20.27	0.64
pH	4.14	0.10
Total titratable acid (%)	0.16	0.01
Reducing Sugars (%)	7.61	0.34
Total sugars (%)	12.52	0.63
Sucrose (%)	4.66	0.29
Total soluble solids (°Brix)	14.07	0.12
Soluble pectin (%)	2.27	0.17
Total pectin (%)	3.38	0.27
Solubilization (%)	67.20	0.31
Starch (%)	1.18	0.05
Vitamin C (mg/100 g)	1090.0	10
Phenolics soluble in water (%)	0.70	0.03
Phenolics soluble in methanol 50% (%)	0.68	0.02
Phenolics soluble in methanol (%)	0.96	0.05
Crude fiber (%)	1.94	0.10
Neutral detergent fiber (%)	3.32	0.16
Acid detergent fiber (%)	3.00	0.03
Cellulose (%)	0.32	0.14
Hemicellulose (%)	1.40	0.06
Lignin (%)	1.68	0.04
Polyphenoloxidase (mmol/g min)	272.91	6.00
Peroxidase (mmol/g min)	116.51	2.64
Pectin methyl esterase (mmol/g min)	208.00	6.93
Polygalacturonase (mmol/g min)	23.85	1.00

appearance of fruit wine produced from caja and cocoa pulp [12, 13]. The presence of endogenous pectinases is relatively common in fruits; however, the natural activity of pectinases was low (28.8 mmol/g min, Table 1) in the gabirola pulp. Therefore, addition of the enzymatic complex Ultrazym AFP-L (Novo Nordisk, Bagsvaerd, Denmark) was necessary to reduce the viscosity of the pulp and aid the clarification of the beverage [12]. The addition of exogenous enzymes, such as protease, pectinases, and glucanases, is a common practice in the production of wine to reduce problems during filtration and improve the aroma (glucosidases). The pH of the must was adjusted slightly to 4.5 to encourage the activity of the enzyme complex. The acidic pH is unfavorable for the growth of bacteria and promotes the growth of yeast. The addition of K₂S₂O₅ in the must (200 mg l⁻¹) of gabirola before indigenous microbiota fermentation contributed to the reduction of the

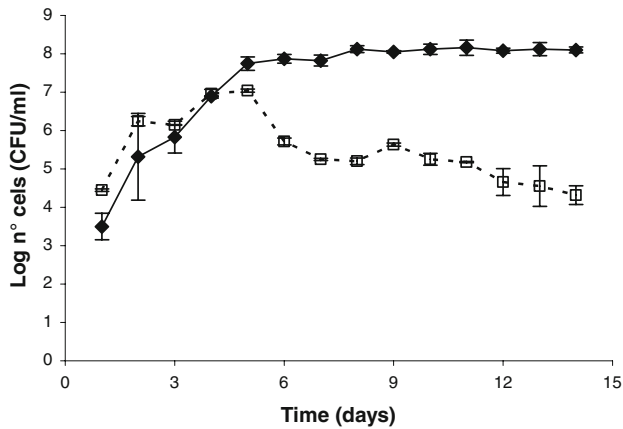


Fig. 1 Viable counts of (solid line with filled diamond) yeasts and (dotted line with open circle) bacteria during indigenous gabirola pulp fermentation

bacterial population (Fig. 1). When counting the viable cell population during the period of inoculated fermentation, the presence of bacteria was not observed, indicating that metabisulphite was efficient in the control of the bacterial growth during the inoculated fermentative process (data not shown). Dias et al. [12] found that the use of SO₂ in the elaboration of fruit wine was effective for inhibition of undesirable bacteria during fermentation. It was also observed that once the fermentation had started, its course was not affected by sulphite, as also described by Gerbaux and Meurgues [22].

Yeast fermentation dynamics

The viable counts of yeasts and bacteria during indigenous fermentation of gabirola pulp are shown in Fig. 1. At the beginning of the indigenous fermentation, the fresh gabirola pulp exhibited a total yeast count on YPD medium of 3.5 log CFU ml⁻¹, while aerobic mesophilic bacteria populations were almost three times higher (Fig. 1). Yeast counts increased during the indigenous process and after 5 days of fermentation, they were greater in number than bacteria. At this time, the maximum number of yeasts, 8 log CFU ml⁻¹ was reached. The bacterial population showed a small increase during at the first days of fermentation and then decreased to 3.2 log CFU ml⁻¹ in the late phases. The increase in yeast population and decrease in bacterial population could be explained by ethanol concentrations found in the fermenting must (Fig. 2). The increase in the Brix value observed after 2 days in inoculated fermentation and after 9 days in indigenous fermentation was due to an addition of gabirola must at 10 Brix to increase the ethanol production and enhance the fruit flavor (Fig. 2).

Throughout the process of fermentation, when *S. cerevisiae* UFLA CA 1162 (IN CA 1162 A and CA1162 B) was inoculated, there were stable viable populations around

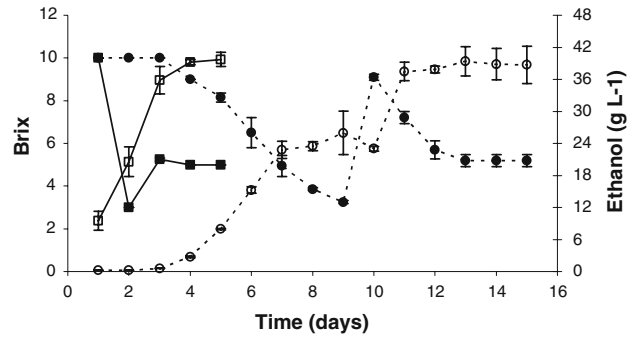


Fig. 2 Consumption of soluble solids and ethanol production in gabirola pulp fermentation. (dotted line with filled circle) Brix (SPON); (solid line with filled square) Brix (IN UFLA CA1162); (dotted line with open circle) Ethanol (SPON); (solid line with open square) Ethanol (IN UFLA CA1162). Inoculated fermentation finished after 5 days; indigenous fermentation finished after 14 days

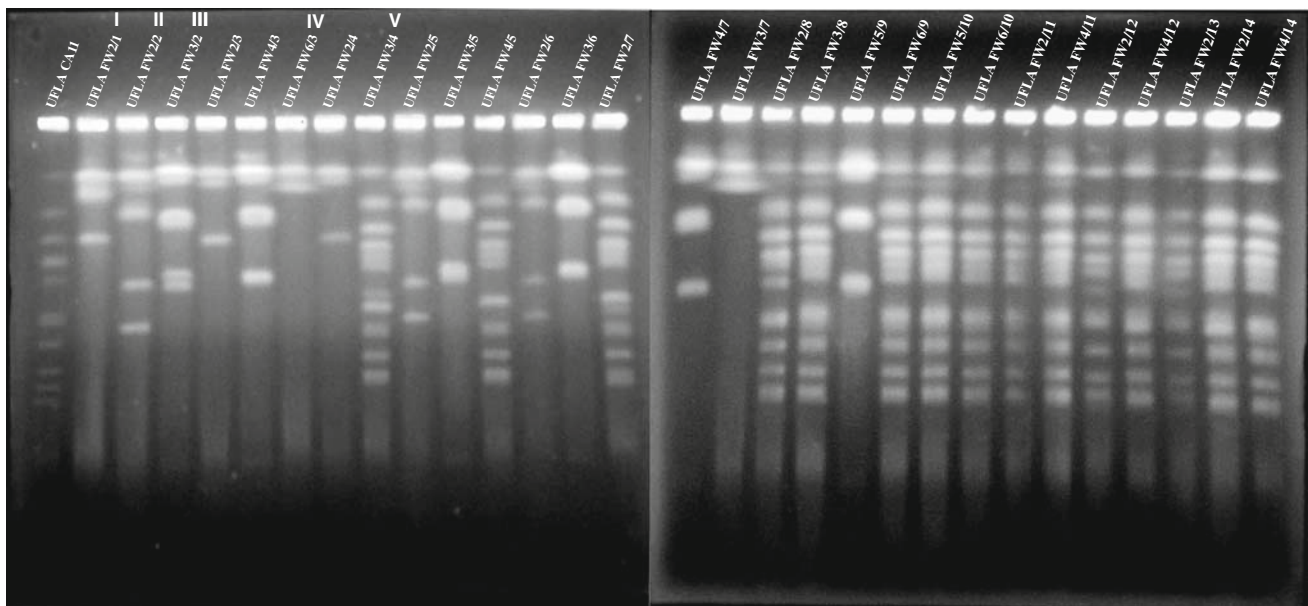
9 log CFU ml⁻¹. The cell viability was high throughout the fermentation process, showing slight decreases at the end of 2–4%. It is known that the inoculation of selected yeasts reduces the growth of native yeasts [19]. A reduction in yeast diversity in the middle and end of fermentation was observed when yeast was used as starter culture [16].

According to the morphological characterization of colonies during indigenous fermentation (SPON 1A and 1B), six morphotypes were found during the 14 days of fermentation. These isolates were evaluated microscopically (data not shown), it was found that the same colony morphotype could be a different cell type. According to Kuthan et al. [25], colonies of *S. cerevisiae* may change their morphology under different environmental conditions. Each yeast morphotype presented variable distribution during the fermentation; however, morphotype 2 was present during the entire period of fermentation. The distribution of morphotypes found during indigenous fermentation is presented in Table 2. Morphotype 1 was only detected in the first day of fermentation and morphotype 5 was found on the ninth and tenth days, while the morphotypes 2 and 4 were found all through the fermentation.

All yeasts isolates were analyzed by PFGE. The diversity and dynamics of the yeast population during indigenous fermentation observed by PFGE analysis showed five different karyotyping profiles in the first days of fermentation (Fig. 3). During the first days of fermentation, it was observed a diversity of karyotyping, however, from the seventh day, the profile V dominated until the end of fermentation (Table 2; Fig. 3). The profile V was similar to yeast reference used, indicating they were strains of *S. cerevisiae* (Fig. 3). The other profiles were found to be different, since it had fewer bands and may be considered species (or genus) distant to *S. cerevisiae* [5]. In the early stages of wine fermentation, *Kloeckera*, *Hanseniaspora*, *Candida*, *Metschnikowia*, *Pichia*, and *Kluyveromyces* are frequently

Table 2 Viable counts of yeast population, profile obtained by PFGE and species identification during the indigenous fermentation of gabirola pulp

Morphotype	Days of fermentation	Mean of CFU ml ⁻¹	Profile PFGE	Sequencing identification	Similarity (%), accession number
1	1	5.50×10^3	V	<i>S. cerevisiae</i>	99 AY235811.1
2	1–14	4.75×10^8	I II V	<i>Candida quercitrusa</i> Unidentified <i>S. cerevisiae</i>	99 AM158924.1 99 AM900394.1
3	2, 4–7 and 8	1.85×10^8	III V	<i>Issatchenkia terricola</i> <i>S. cerevisiae</i>	99 AY235808.1 99 AY235811.1
4	3–14	1.46×10^8	III V	<i>Issatchenkia terricola</i> <i>S. cerevisiae</i>	99 AY235808.1 99 AY235811.1
5	9 and 10	1.10×10^8	III V	<i>Issatchenkia terricola</i> <i>S. cerevisiae</i>	99 AY235808.1 99 AY235811.1
6	3, 9, 10, 13 and 14	2.50×10^8	IV V	<i>Candida quercitrusa</i> <i>S. cerevisiae</i>	100 AM160627.1 99 AY235811.1

**Fig. 3** Electrophoretic karyotypes (profiles) of isolates during indigenous fermentation gabirola pulp. Lines 1 *S. cerevisiae* UFLA CA 11; 2–30 yeasts isolated at 24 h intervals during 14 days of fermentation; 2–4 isolates from 2nd day; 5–7 isolates from 3rd day; 8–9 isolates from 4th day; 10–12 isolates from 5th day; 13–14 isolates from 6th day;

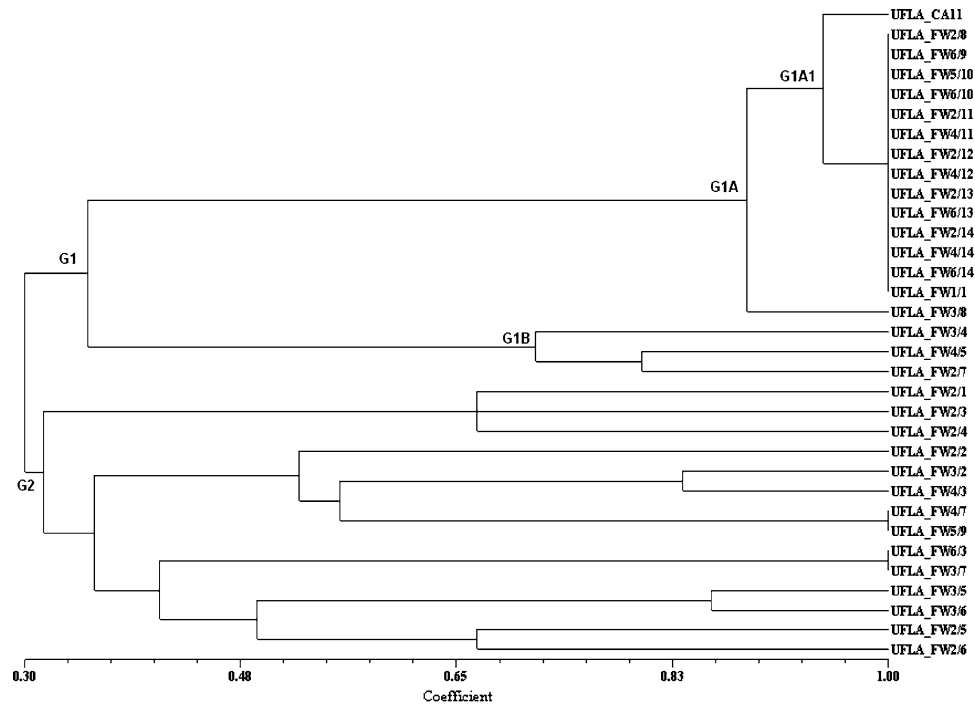
15–17 isolates from 7th day; 18–19 isolates from 8th day; 20–21 isolates from 9th day; 22–23 isolates from 10th day; 24–25 isolates from 11th day; 26–27 isolates from 12th day, 28 isolates from 13th day; 29–30 isolates from 14th day of fermentation. Numbers I, II, III, IV and V refer to different karyotyping profiles

found, but they will gradually die, which is dependent on their tolerance to low ethanol concentrations [19]. These yeasts are replaced by *S. cerevisiae*, which tolerates high ethanol concentrations. Schwan et al. [36] reported that the yeast strains of *S. cerevisiae* were the only ones in the final phase of fermentation of sugar cane and that the conditions of low pH, concentration of sugars, and increasing concentrations of ethanol favor their dominance in the fermentation must. The yeast isolated during the inoculated fermentations showed the same karyotyping profile as UFLA CA 1162 showing the dominance of the selected strain (data not shown).

The dendrogram (Fig. 4) that was developed based on coefficient dice, shows a group of 32 strains, which were

representatives of those found in indigenous fermentation. Two large groups were observed (G1 and G2) based on PFGE profiles. The G1 group had a similarity of >36% and the profiles were similar to the one of *S. cerevisiae* UFLA CA11 [5]. The G1 group was subdivided into two subgroups showing a similarity above 71% (G1A e G1B). The subgroup G1A was comprised of all isolates with 95% similarity. These isolates dominated the fermentation process after the ninth day of fermentation. The G2 was a group of 14 isolates that demonstrated less similarity with UFLA CA11. These isolates were present in the indigenous fermentation until the seventh day, except UFLA FW5/9, which was also detected on the ninth day of fermentation. The isolates UFLA FW4/7 and UFLA FW3/7 showed

Fig. 4 Cluster analysis of the profiles obtained by PFGE of the yeast isolated from the indigenous fermentation gabirola pulp



100% similarity with UFLA FW5/9 and UFLA FW6/3, respectively.

The construction of dendrograms based on the presence and intensity of bands has been used to evaluate the similarity among yeasts [38]. The high incidence of chromosomal differences between yeast isolates makes the electrophoretic karyotyping technique an efficient technique to distinguish different strains [24]. Isolates of *S. cerevisiae* evaluated by PFGE have been found to have 13 chromosomes with a size ranging between 200 and 2000 kb [6]. The main source of variation in yeast chromosomal structure growing under stressful conditions may be due the transposons [15]. Chromosomal rearrangements due to reciprocal translocation during meiosis may be the reason for an association of more than two chromosomes counterparts [18].

A representative strain was chosen from the five electrophoretic profiles for sequencing. The results obtained from sequencing of the ITS region (ITS1 and ITS2 5.8S rRNA) allowed for the identification of different yeasts species. The isolated UFLA FW1/1 was identified as *S. cerevisiae* with 99% identity with the closest relative accession number AY235811.1. The isolates UFLA FW2/8, UFLA FW3/8, UFLA FW6/9, UFLA FW5/10, UFLA FW6/10, UFLA FW2/11, UFLA FW2/12, UFLA FW2/13, UFLA FW2/14, and UFLA FW4/14 were also identified as *S. cerevisiae*. The isolate UFLA FW2/1 (profile I) showed 99% similarity with *Candida quercitrusa* and was present during the first days of indigenous fermentation. The isolate UFLA FW4/3 (profile III) was identified as *Issatchenkia terricola* and this species was found from the second until the ninth day of

fermentation. The isolates UFLA FW2/2, UFLA FW2/5, and UFLA FW2/6 found in the 2nd, 50th and 60th days, of fermentation, respectively, could not be identified by either traditional methods or sequencing. Non-*Saccharomyces* yeasts were often found in the first 10 days of indigenous fermentation; however, *Saccharomyces* isolates dominated after this period of fermentation. Our results were similar to those reported by Fleet [20], who observed that species of *Candida*, *Issatchenkia* and *Pichia* persisted well into the middle stage of grape fermentation.

The non-*Saccharomyces* yeasts isolated showed low ethanol tolerance (data not shown). These results are in agreement with those reported by Jemec et al. [23]. *I. terricola* demonstrated a slow ability to ferment glucose and sucrose; this strain has not been used in mixed fermentations due to the high production of ethyl acetate [11]. *C. quercitrusa* is usually associated with insects [30] and fruits, such as mango, banana, pineapple, longan, and rambutan [9].

Chemical analyses during fermentation

The chemical compounds produced by microbial activity were analyzed throughout the process of fermentation. The decrease in soluble solids and the increase the content of ethanol are shown in Fig. 2. The final values for ethanol concentration and soluble solids (Brix) either indigenous (SPON) or inoculated (IN CA1162) fermentation process was similar (Fig. 2). The fermentation performed with the yeast *S. cerevisiae* UFLA CA1162 was 30% faster than the indigenous fermentation. The final pH value was

Table 3 Organic compounds determined by HPLC and GC during fermentation and in the wine elaborated by indigenous fermentation

Compound	1	3	5	7	9	11	14	TRA ^a	Wine ^b
Acetaldehyde (mg L ⁻¹)	ND	ND	ND	34.87 ± 6.65	50.07 ± 9.89	42.48 ± 11.28	44.41 ± 7.77	37.26 ± 5.15	33.74 ± 6.14
2-Furaldehyde (mg L ⁻¹)	ND	170.89 ± 10.53	192.35 ± 1.70	250.00 ± 38.29	329.62 ± 42.99	376.27 ± 59.86	369.72 ± 31.97	308.66 ± 11.96	290.45 ± 31.17
Ethyl acetate (mg L ⁻¹)	135.79 ± 1.68	140.82 ± 1.80	144.22 ± 2.39	158.87 ± 1.63	144.07 ± 1.87	161.36 ± 41.88	130.23 ± 2.19	114.30 ± 12.37	141.77 ± 16.08
Phenyl acetate (mg L ⁻¹)	ND	4.51 ± 5.21	16.02 ± 2.17	7.25 ± 0.35	6.52 ± 8.42	18.60 ± 1.49	6.06 ± 2.51	8.95 ± 1.40	8.67 ± 1.42
Diethyl succinate (mg L ⁻¹)	ND	287.31 ± 16.06	271.67 ± 7.24	46.13 ± 11.33	ND	32.18 ± 3.99	ND	ND	ND
2,3-butanediol (mg L ⁻¹)	ND	ND	267.88 ± 1.24	283.66 ± 4.73	322.47 ± 8.14	334.25 ± 7.37	337.11 ± 4.88	354.75 ± 3.56	341.66 ± 7.71
1,2-propanediol (mg L ⁻¹)	ND	ND	ND	4.90 ± 0.14	5.02 ± 0.25	10.95 ± 1.50	6.99 ± 0.17	19.59 ± 6.94	20.66 ± 4.05
2-phenylethanol (mg L ⁻¹)	ND	ND	ND	26.55 ± 2.19	40.18 ± 3.56	50.35 ± 3.93	60.11 ± 4.47	61.55 ± 16.18	63.79 ± 3.80
Isoamyl alcohol (mg L ⁻¹)	ND	ND	26.17 ± 2.19	85.62 ± 10.14	122.33 ± 22.57	132.80 ± 4.63	159.93 ± 4.75	159.04 ± 5.62	136.73 ± 2.39
Hexanoic acid (mg L ⁻¹)	ND	ND	ND	ND	ND	79.79 ± 5.34	ND	ND	ND
Octanoic acid (mg L ⁻¹)	ND	ND	ND	ND	148.18 ± 1.22	125.09 ± 2.72	50.91 ± 0.93	ND	ND
2-ethylcaproic acid (mg L ⁻¹)	ND	ND	ND	ND	29.89 ± 1.11	33.79 ± 0.43	ND	ND	ND
Propionic acid (mg L ⁻¹)	ND	6.42 ± 0.28	11.21 ± 2.14	35.72 ± 3.22	44.40 ± 6.94	83.59 ± 11.97	95.80 ± 10.28	73.60 ± 11.76	74.61 ± 12.48
Acetic acid (g L ⁻¹)	0.41 ± 0.11	0.43 ± 0.08	0.52 ± 0.01	1.41 ± 0.62	3.41 ± 0.19	2.93 ± 0.80	2.04 ± 0.40	1.49 ± 0.07	1.64 ± 0.14
Lactic acid (g L ⁻¹)	ND	0.13 ± 0.15	0.69 ± 0.04	0.82 ± 0.02	1.07 ± 0.02	0.83 ± 0.05	0.90 ± 0.09	1.89 ± 0.23	1.14 ± 0.41
Citric acid (g L ⁻¹)	1.39 ± 0.22	2.54 ± 1.23	2.47 ± 0.28	2.03 ± 0.07	3.63 ± 0.25	3.11 ± 0.35	2.60 ± 0.22	2.03 ± 0.40	4.04 ± 0.22
Malic acid (g L ⁻¹)	2.13 ± 0.12	0.87 ± 1.00	2.93 ± 0.18	2.51 ± 0.03	ND	2.57 ± 0.03	ND	ND	ND
Tartaric acid (g L ⁻¹)	0.50 ± 0.05	0.68 ± 0.11	5.67 ± 0.06	5.46 ± 0.44	0.98 ± 0.04	0.79 ± 0.00	0.73 ± 0.00	1.19 ± 0.34	1.25 ± 0.40
Succinic acid (g L ⁻¹)	5.15 ± 0.16	4.74 ± 0.22	7.59 ± 1.45	5.47 ± 0.55	9.09 ± 0.38	6.24 ± 0.02	6.01 ± 1.10	4.90 ± 0.45	6.20 ± 0.34
Oxalic acid (g L ⁻¹)	ND	ND	ND	ND	0.16 ± 0.03	ND	0.03 ± 0.00	ND	ND
Acetoin (mg L ⁻¹)	ND	ND	ND	ND	ND	ND	ND	ND	10.35 ± 3.35
Glucose (g L ⁻¹)	13.75 ± 1.11	22.85 ± 2.34	27.77 ± 4.16	8.11 ± 1.07	3.39 ± 0.64	12.63 ± 1.89	3.37 ± 0.44	2.01 ± 0.16	1.94 ± 0.26
Sucrose (g L ⁻¹)	50.01 ± 2.51	28.28 ± 0.60	ND	ND	ND	ND	ND	ND	ND
Fructose (g L ⁻¹)	17.36 ± 3.30	22.95 ± 2.16	36.95 ± 3.42	16.93 ± 1.80	1.09 ± 0.10	22.64 ± 2.79	1.46 ± 0.12	0.88 ± 0.01	0.85 ± 0.12
Glycerol (g L ⁻¹)	0.08 ± 0.02	0.15 ± 0.02	0.87 ± 0.03	1.56 ± 0.20	1.63 ± 0.19	1.76 ± 0.14	1.88 ± 0.09	2.06 ± 0.31	2.05 ± 0.18
Ethanol (g L ⁻¹)	0.28 ± 0.00	0.58 ± 0.04	7.90 ± 0.13	22.77 ± 1.60	25.96 ± 4.06	37.46 ± 1.74	38.85 ± 2.94	42.32 ± 1.80	39.08 ± 1.13

ND not detected, ± standard deviation

^a TRA: gabirola wine 10 days after end of fermentation^b Gabirola wine 40 days after end of fermentation

Table 4 Organic compounds determined by HPLC and GC during fermentation and in the final product of gabiropa must inoculated with *Saccharomyces cerevisiae* UFLA CA1162

Compound	Days of fermentation					TRA ^a	Wine ^b
	1	2	3	4	5		
Acetaldehyde (mg L ⁻¹)	43.61 ± 1.03	23.23 ± 1.59	39.94 ± 2.64	81.21 ± 2.70	89.98 ± 0.82	119.44 ± 1.42	71.31 ± 2.33
2-Furaldehyde (mg L ⁻¹)	248.83 ± 3.21	256.75 ± 0.83	221.43 ± 0.95	269.84 ± 2.06	238.62 ± 4.12	167.29 ± 3.76	151.48 ± 2.33
Ethyl acetate (mg L ⁻¹)	181.95 ± 4.03	180.82 ± 0.03	151.33 ± 8.59	207.45 ± 1.77	190.81 ± 4.12	110.61 ± 1.97	115.55 ± 2.60
Phenyl acetate (mg L ⁻¹)	ND	ND	ND	ND	ND	ND	ND
Diethyl succinate (mg L ⁻¹)	191.69 ± 4.66	ND	ND	12.34 ± 0.52	18.65 ± 0.56	ND	ND
2,3-butanediol (mg L ⁻¹)	300.85 ± 5.15	293.78 ± 5.76	365.13 ± 4.94	384.43 ± 6.61	315.54 ± 6.12	311.74 ± 3.42	308.42 ± 0.13
1,2-propanediol (mg L ⁻¹)	6.75 ± 0.71	25.10 ± 0.45	71.19 ± 0.37	50.32 ± 1.43	47.77 ± 2.18		
2-phenylethanol (mg L ⁻¹)	34.00 ± 0.84	30.42 ± 2.28	76.59 ± 3.44	92.18 ± 0.21	36.95 ± 3.85	36.19 ± 4.11	39.87 ± 6.05
Isoamyl alcohol (mg L ⁻¹)	36.53 ± 1.86	141.15 ± 1.48	244.14 ± 0.98	291.14 ± 3.20	296.48 ± 3.15	248.04 ± 1.80	214.28 ± 13.02
Hexanoic acid (mg L ⁻¹)	90.23 ± 1.94	72.74 ± 2.38	149.53 ± 2.06	102.92 ± 0.04	96.64 ± 2.50	ND	ND
Octanoic acid (mg L ⁻¹)	166.38 ± 2.09	110.19 ± 1.74	247.38 ± 2.19	188.30 ± 0.14	111.06 ± 2.07	ND	ND
2-ethylcaproic acid (mg L ⁻¹)	33.43 ± 3.14	20.79 ± 0.30	22.06 ± 1.35	11.24 ± 0.52	ND	ND	ND
Isobutyric acid (mg L ⁻¹)	ND	ND	2.55 ± 0.32	2.31 ± 0.29	1.92 ± 0.07	ND	ND
1-hexanol (mg L ⁻¹)	1.37 ± 0.01	ND	ND	1.22 ± 0.02	1.64 ± 0.02	ND	ND
Butyric acid (mg L ⁻¹)	23.61 ± 1.44	ND	27.36 ± 0.02	28.06 ± 3.68	29.62 ± 0.91	ND	ND
Propionic acid (mg L ⁻¹)	5.96 ± 0.048	5.50 ± 0.01	10.44 ± 0.03	16.61 ± 0.01	7.51 ± 0.01	7.46 ± 0.05	7.45 ± 0.01
Acetic acid (g L ⁻¹)	1.29 ± 0.22	1.78 ± 0.07	2.34 ± 0.39	2.35 ± 0.48	2.10 ± 0.40	1.36 ± 0.23	1.21 ± 0.09
Lactic acid (g L ⁻¹)	ND	ND	0.61 ± 0.10	0.59 ± 0.08	0.51 ± 0.03	ND	ND
Citric acid (g L ⁻¹)	1.72 ± 0.40	1.62 ± 0.16	2.46 ± 0.18	2.39 ± 0.51	2.19 ± 0.26	2.71 ± 0.07	3.13 ± 0.25
Malic acid (g L ⁻¹)	2.79 ± 0.42	1.95 ± 0.24	2.08 ± 0.01	2.22 ± 0.22	1.80 ± 0.29	3.46 ± 0.39	2.70 ± 0.13
Tartaric acid (g L ⁻¹)	0.78 ± 0.05	0.74 ± 0.01	0.85 ± 0.02	0.84 ± 0.01	0.72 ± 0.03	1.37 ± 0.30	1.02 ± 0.11
Succinic acid (g L ⁻¹)	7.32 ± 2.62	5.28 ± 0.50	7.78 ± 1.68	8.44 ± 2.43	6.98 ± 2.76	10.32 ± 2.15	8.73 ± 0.41
Oxalic acid (g L ⁻¹)	ND	ND	ND	ND	ND	ND	0.26 ± 0.06
Acetoin (mg L ⁻¹)	ND	ND	ND	ND	ND	ND	ND
Glucose (g L ⁻¹)	15.24 ± 1.42	0.60 ± 0.06	0.08 ± 0.03	0.06 ± 0.00	0.10 ± 0.03	0.22 ± 0.08	0.49 ± 0.02
Sucrose (g L ⁻¹)	0.00 ± 0.00	ND	ND	ND	ND	ND	ND
Fructose (g L ⁻¹)	29.01 ± 0.61	ND	0.67 ± 0.01	ND	0.56 ± 0.03	1.01 ± 0.12	0.85 ± 0.01
Glycerol (g L ⁻¹)	0.69 ± 0.11	1.18 ± 0.01	1.45 ± 0.10	1.58 ± 0.02	1.40 ± 0.28	1.72 ± 0.12	1.65 ± 0.08
Ethanol (g L ⁻¹)	9.50 ± 1.77	20.55 ± 2.78	35.83 ± 2.59	39.24 ± 0.47	38.72 ± 1.32	43.81 ± 2.65	37.16 ± 0.64

ND not detected, ± standard deviation

^a TRA: gabiropa wine 10 days after end of fermentation

^b Gabiropa wine 40 days after end of fermentation

approximately 3.7 for both fermentative processes. The rapid decrease in sugar content and increase the concentration of ethanol during the inoculated fermentation (Fig. 2) was also observed by Domizio [14] during fermentation of grape must under controlled temperature conditions. This confirms that the use of selected yeast promotes a rapid increase in the concentration of ethanol, besides these strains dominate the fermentation process. Nurgel et al. [32] found that using selected yeast (6.7 log CFU ml⁻¹) in the fermentation of non pasteurized grape was completed in 6 days, while indigenous fermentation lasted 10 days. These authors also found that the values of pH were similar (approximately 3.8) for both beverages produced by indigenous or inoculated fermentation. Similar results were shown in this study where the pH value was 3.73 in the indigenous and 3.77 in the inoculated fermentations.

The results of chemical analysis showed variations of the different compounds during the fermentation processes (SPON and IN CA1162) and in the final beverages. Small differences in the composition of fruit wine were also found between the first period of fruit wine elaboration (10 days after the end of fermentation) and the final beverages (Tables 3, 4). Inoculated fermentations yielded a higher amount of alcohol than indigenous ones, indicating the efficiency of selected strains.

The highest ethanol concentration was achieved after 4 days of fermentation in the inoculated process and after more than 14 days during indigenous fermentation (Fig. 2). For the final beverages, the results shown in Tables 3 and 4 were grouped according to the chemical compounds group and data were subjected to analysis of variance 5% Tukey's test. When the chemical compounds

groups were compared, the produced gabiropa wines (IN CA1162 and SPON) showed similar values, regardless of the type of fermentation, with the exception of the total concentration of aldehydes (324.19 mg L^{-1} for SPON and 222.79 mg L^{-1} for IN CA1162), ketones (10.76 mg L^{-1} for SPON), and sugars (2.79 g L^{-1} for SPON and 1.36 g L^{-1} for IN CA1162) (Table 5). However, when the components isolated (via HPLC and GC) from the final beverage and during the fermentation process are considered, there were differences between the beverages depending on the method used to conduct the fermentation: the use of selected yeast or indigenous process (Table 6). This difference in the composition of the beverage depending on the type of inoculum was also reported by Garde-Cerdán and Ancín-Azpilicueta [21], which evaluated the contribution of wild and selected yeasts to the formation of volatile compounds in the fermentation of grapes. These authors found that the use of a selected strain led to the production of different concentrations of total acids and esters than the ones found in indigenous fermentation. PCA was carried out with organic compounds produced during indigenous and inoculated fermentations. Figure 5 shows the similarities between the two fermentations processes according to the variables analyzed. The formation of two groups could be observed in relation to the type of yeast inoculum. The beverage produced by indigenous fermentation was characterized mainly by the presence of lactic, citric, and acetic acid as well as glucose, while the fruit wine produced using *S. cerevisiae* UFLA CA1162 was characterized by concentrations of malic and succinic acids.

The presence of acetoin was detected in the gabiropa wine produced by indigenous fermentation. This compound is formed during fermentation by microbial activity, such as lactic acid bacteria and yeast, and this compound is important for formation of the wine flavor. Acetoin is also related to the biosynthesis of 2,3-butanediol and diacetyl

Table 5 Averages of compounds by chemical group present in gabiropa wine

Compound	SPON	UFLA CA1162
Esteres (mg L^{-1})	150.44 ^a	115.55 ^a
Alcohols (mg L^{-1})	562.84 ^a	562.57 ^a
Aldehyde (mg L^{-1})	324.19 ^b	222.79 ^a
Ketones (mg L^{-1})	10.76 ^b	0.00 ^a
Acids (g L^{-1})	15.03 ^a	17.79 ^a
Sugars (g L^{-1})	2.79 ^b	1.36 ^a
Glycerol (g L^{-1})	2.10 ^a	1.65 ^a
Ethanol (g L^{-1})	38.71 ^a	38.72 ^a
pH	3.73 ^a	3.77 ^a

Values identified by the same letters are not significantly different at the 0.1 level (Tukey test)

Table 6 Averages of chemical compounds present in gabiropa wine

Compound	SPON	UFLA CA1162
Acetaldehyde (mg L^{-1})	33.74 ^a	71.31 ^b
2-furaldehyde (mg L^{-1})	290.46 ^b	151.48 ^a
Ethyl acetate (mg L^{-1})	141.77 ^a	115.65 ^a
Phenyl acetate (mg L^{-1})	8.67 ^b	0.00 ^a
2,3-butanediol (mg L^{-1})	341.67 ^b	308.42 ^a
1,2-propanediol (mg L^{-1})	20.66 ^b	0.00 ^a
2-phenylethanol (mg L^{-1})	63.79 ^b	39.87 ^a
Isoamyl alcohol (mg L^{-1})	136.74 ^a	214.28 ^b
Propionic acid (mg L^{-1})	74.61 ^a	74.51 ^a
Acetic acid (g L^{-1})	1.64 ^a	1.22 ^a
Lactic acid (g L^{-1})	1.14 ^b	0.00 ^a
Citric acid (g L^{-1})	4.05 ^b	3.13 ^a
Malic acid (g L^{-1})	0.00 ^a	2.70 ^b
Tartaric acid (g L^{-1})	1.26 ^a	1.02 ^a
Succinic acid (g L^{-1})	6.21 ^a	8.73 ^b
Oxalic acid (g L^{-1})	0.00 ^a	0.26 ^a
Glucose (g L^{-1})	1.94 ^b	0.49 ^a
Fructose (g L^{-1})	0.85 ^a	0.85 ^a
Acetoin (mg L^{-1})	10.76 ^b	0.00 ^a
Glycerol (g L^{-1})	2.05 ^a	1.65 ^a
Ethanol (g L^{-1})	38.71 ^a	38.72 ^a

Values identified by the same letters are not significantly different at the 0.1 level (Tukey test)

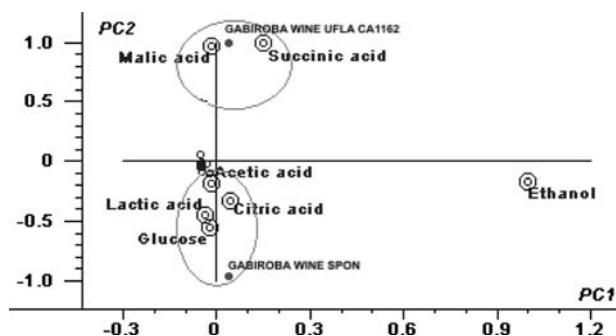


Fig. 5 Principal components analysis (PCA) of organics chemical compounds in gabiropa wine

[35]. *Saccharomyces* strains generally do not produce significant amounts of acetoin, which is a characteristic of *Kloeckera* and *Hanseniaspora* yeasts [10, 36]. Similar results to these were published by Valles et al. [40], who found higher concentrations of acetoin in cider that was fermented indigenously.

In the beverage produced with UFLA CA1162, there was a decrease in the acetaldehyde concentration. The oxidation reactions that occur during aging can contribute to conversion of acetaldehyde into 5-hydroxy-2-methyl-1,3-dioxane. During the aging of wine, the concentration of

Table 7 Percentage of acceptance of the gabirola wine produced by indigenous and inoculated fermentation

Attribute	SPON	UFLA CA1162
Appearance	69.57	63.04
Aroma	71.74	65.22
Taste	54.35	43.48
Overall	65.22	47.83

cis and *trans* isomers of 5-hydroxy-2-methyl-1,3-dioxane increased, unlike the acetaldehyde concentration. Around 30–40% of acetaldehyde is converted into *cis* and *trans* isomers 5-hydroxy-2-methyl-1,3-dioxane by acetylation in a proportion of 75 and 25%, respectively [17].

Sensory analysis of the beverage

After the chemical analyses, the beverage was subjected to sensory analysis to assess its acceptance among the consumers. Table 7 presents percentage of acceptance attributed to the beverage by 50 untrained tasters, designated based upon the nine-point hedonic scale. For all attributes assessed, the beverage produced by indigenous fermentation showed greater acceptance when compared with the beverage produced with a selected strain of *S. cerevisiae* UFLA CA1162.

The distribution of individual notes for each point on the hedonic scale for different attributes that were evaluated is illustrated in Fig. 6. It was possible to observe that a greater

average number of panelists chose values above five in the hedonic scale for the beverage fermented indigenously. According to Nurgel et al. [32], specific compounds present in wine are responsible for the typical characteristics of smell and taste. The main source of these compounds is the metabolism of yeast during fermentation; however, some compounds in wine are from the grapes. In general, the gabirola wine produced by indigenous fermentation showed higher concentrations of certain compounds, when compared to that produced by inoculation of yeast UFLA CA1162 (Table 3). The differences in sensory analysis found for these two beverages analyzed here may be the result of the different compositions of these final products. The beverages produced in this study had high concentrations of 2,3-butanediol, and according to Romano et al. [34], this high concentration can influence the bouquet of wine, because of its slightly bitter taste and can also change the body of the beverage because of its viscosity.

From the data reported in this experiment, comparing indigenous and inoculated fermentation, it can be concluded that the processes normally used in fermented beverage manufacturing could be adapted for the elaboration of the fermented gabirola pulp. The dilution of gabirola pulp with sucrose solution, to improve the fluidity of must, diminished the concentration of marker compounds of the beverage’s aroma, but did not interfere with the production of ethanol, since the formation of ethanol follows a different pathway. The use of the enzymatic complex and the solution of bentonite during all the fermentative process brought satisfactory results for the clarification of the

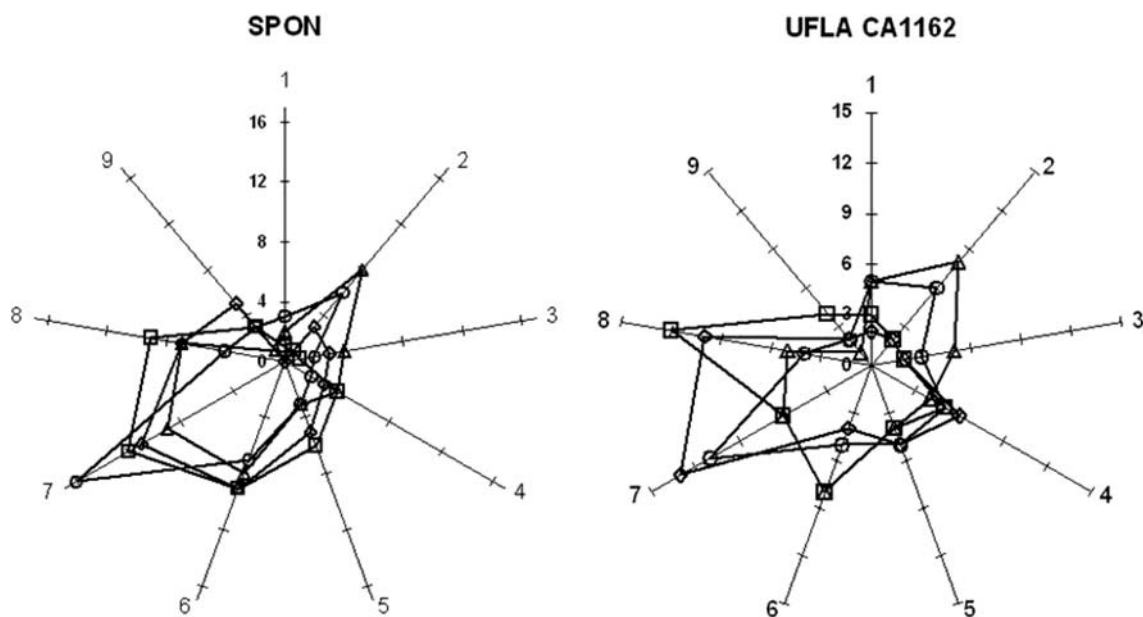


Fig. 6 Distribution of number of panellists in sensory analysis. Numbers 1 to 9 range from the disliked extremely (1) to liked extremely (9). Solid line with open diamond appearance; solid line with open square

aroma; solid line with open triangle taste; solid line with open circle overall

beverage as well as the SO₂, which in the applied concentration, inhibited the growth of bacteria and did not interfere with the quality of the final beverage. The sensory analysis revealed good acceptance, mainly when taking into account the fact that the tasters were not familiar with the gabirola-derived beverage. The methodology described here is simple and can be applied in a relatively small space without high cost. Based on the characteristics of the pulp and acceptance in the sensory analysis, gabirola fruits showed good potential for use in the production of fermented beverage. It was observed that, from the acceptability of the beverage, this technology can be an alternative for the use of the tropical fruit pulp, and may provide a new industrial outlet for this fruit.

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