Alan Mario Zuffo
(Organizador)

A produção do Conhecimento nas Ciências Agrárias e Ambientais 3

Atena Editora
Ano 2019

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Atena Editora
2019

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Editora Chefe: Profª Drª Antonella Carvalho de Oliveira
Diagramação e Edição de Arte: Lorena Prestes e Geraldo Alves
Revisão: Os autores

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

P964 A produção do conhecimento nas ciências agrárias e ambientais 3 [recurso eletrônico] / Organizador Alan Mario Zuffo. – Ponta Grossa (PR): Atena Editora, 2019. – (A Produção do Conhecimento nas Ciências Agrárias e Ambientais; v. 3)

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-85-7247-286-9
DOI 10.22533/at.ed.869192604

1. Agronomia – Pesquisa – Brasil. 2. Meio ambiente – Pesquisa – Brasil. I. Zuffo, Alan Mario. II. Série.

CDD 630

Elaborado por Maurício Amormino Júnior – CRB6/2422

APRESENTAÇÃO

A obra “A produção do Conhecimento nas Ciências Agrárias e Ambientais” aborda uma série de livros de publicação da Atena Editora, em seu III volume, apresenta, em seus 28 capítulos, com conhecimentos científicos nas áreas agrárias e ambientais.

Os conhecimentos nas ciências estão em constante avanços. E, as áreas das ciências agrárias e ambientais são importantes para garantir a produtividade das culturas de forma sustentável. O desenvolvimento econômico sustentável é conseguido por meio de novos conhecimentos tecnológicos. Esses campos de conhecimento são importantes no âmbito das pesquisas científicas atuais, gerando uma crescente demanda por profissionais atuantes nessas áreas.

Para alimentar as futuras gerações são necessários que aumente à quantidade da produção de alimentos, bem como a intensificação sustentável da produção de acordo como o uso mais eficiente dos recursos existentes na biodiversidade.

Este volume dedicado às áreas de conhecimento nas ciências agrárias e ambientais. As transformações tecnológicas dessas áreas são possíveis devido o aprimoramento constante, com base na produção de novos conhecimentos científicos.

Aos autores dos diversos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos, os agradecimentos do Organizador e da Atena Editora.

Por fim, esperamos que este livro possa colaborar e instigar mais estudantes, pesquisadores e entusiastas na constante busca de novas tecnologias para as ciências agrárias e ambientais, assim, garantir perspectivas de solução para a produção de alimentos para as futuras gerações de forma sustentável.

Alan Mario Zuffo

SUMÁRIO

CAPÍTULO 6

EFFECT OF SOIL NUTRIENTS ON POLYPHENOL COMPOSITION OF JABUTICABA WINE

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RESUMO: A textura e os nutrientes do solo podem afetar diretamente a composição química dos frutos e seus produtos processados. Os vinhos de jabuticaba foram preparados a partir de frutos cultivados em solo arenoso com baixa fertilidade e solos argilosos sob cultivo convencional ou orgânico. As variações químicas foram analisadas por meio de métodos estatísticos multivariados. A origem do solo contribuiu com 22% da variação das propriedades químicas dos vinhos, as quais foram relacionadas aos níveis de Fe^{3+} e P do solo. Os vinhos diferiram quanto aos teores de antocianinas, fenóis totais, taninos, ácido acético, açúcares residuais e acidez. Os melhores parâmetros de cor e composição em polifenóis foram obtidos em vinhos produzidos com frutos cultivados com manejo orgânico.
PALAVRAS-CHAVE: *Myrciaria cauliflora*, RMNq, elagitaninos, antocianinas, fermentação.

ABSTRACT: Soil texture and nutrients may directly affect fruits' chemical composition and their processed products. Jabuticaba wines were prepared from fruits grown in sandy soil with low fertility and clayey soils under conventional or organic cultivation. Chemical variations were analyzed via multivariate statistical methods. The soil origin contributed with 22% of variation in wines' chemical properties, which were related to Fe^{3+} and P levels in soil. The wines differed regarding contents of anthocyanins, total phenols, tannins, acetic acid, residual sugars, and acidity. Wines with better color parameters and polyphenol composition were produced with fruits grown on organic management.
KEYWORDS: *Myrciaria cauliflora*, qNMR, ellagitannin, anthocyanin, fermentation.

1 I INTRODUCTION

Jabuticaba (*Myrciaria cauliflora* (Mart.) O. Berg, Myrtaceae) is a very popular edible fruit in Brazil due to its exotic flavor and sweet, slightly acid taste. This dark purple berry is highly perishable and must be processed in the form of jellies, juices, and wines to enhance post-harvest use (WU; LONG; KENNELLY, 2013). Jabuticaba wine production has grown in recent years and has become an option for small producers to add commercial value to the fruit. Recent studies have shown that this fermented beverage is a rich source of nutraceuticals with high antioxidant and vasodilatory activity (BARROS; CAMPOS; MOREIRA, 2010; MARTINS DE SÁ et al., 2014). Polyphenols e.g. ellagitannins and flavonoids, present mainly in the seeds and peel of jabuticaba berries, are responsible for the beverage's biological activities (PEREIRA et al., 2017; WU et al., 2012; YOSHIDA; AMAKURA; YOSHIMURA, 2010). In addition to being functional ingredients, polyphenols contribute, together with terpenoids, sugars, and organic acids, to organoleptic characteristics of fruits and processed products. In the case of fermented beverages, such compounds are responsible for color, aroma, body, and structure, with wine quality depending on the perfect balance of these chemicals. However, factors such as soil composition, water availability, and climate may change the concentration of various chemical components in fruits (VAN LEEUWEN et al., 2004; COHEN; KENNEDY, 2010). Environmental factors may thus influence the composition of fruits and the quality of their products, as has been widely reported for wines of different grape varieties (VAN LEEUWEN; SEGUIN, 2006). Edaphic factors strongly affect the content of phenolic compounds, organic acids, and sugars in jabuticaba berries (DUARTE et al., 2012). However, the effect of nutrients and soil texture on the chemical composition of the fermented jabuticaba beverage is not yet known. Therefore, the aim of this study was to analyze contents of phenolic compounds and color parameters via spectrophotometric methods, as well as of organic acids and sugars via quantitative ^{1}H NMR in jabuticaba musts and wines prepared with fruits from three orchards with different soils. Sensorial and chemical data and soil parameters from each site were assessed using multivariate statistical methods. Principal Response Curves (PRC), a time-dependent multivariate analysis, was used to evaluate changes during the fermentation process. Canonical redundancy analysis (RDA) was also applied to determine the environmental influence on the chemical variability of jabuticaba wines, with soil parameters as environmental variables.

2 I MATERIALS AND METHODS

2.1 Chemicals

Tannic acid, gallic acid, and iron (III) chloride were purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu's phenol reagent, Bovine Serum Albumin

(BSA) and TMSP-2,2,3,3-D_4 (sodium 3-trimethylsilylpropionate) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Fruit samples and beverage processing

Fermentation of *M. cauliflora* fruits took place at the Jabuticabal Winery, Hidrolândia city, Goiás State, Brazil. Ripe fruits were harvested in October 2012 from trees grown in three different soils (sampling sites): S1 (S 16° 55' 23'', W 49° 21' 50", 728 m), S4 (S 16° 55' 24", W 49° 21' 36", 735 m), and S5 (S 16° 54' 41", W 49° 21' 26", 758 m) (DUARTE et al., 2012). Fruit samples from each site were washed, crushed, and divided into three 200 L stainless steel tanks. Sodium metabisulfite was added (16.2 g 100 kg^{-1} of jabuticabas) and sugar concentration was adjusted to 22ºBrix with sucrose. The same amount of wild (indigenous) yeasts, previously prepared with jabuticaba fruits, was inoculated in each tank. Fermentation was conducted at 25-35ºC, pH 3.4-3.5, and caps were immersed five times a day. Seed and skin contact lasted for four days, after which musts were pressed at 1.5 bar, transferred to oak wood barrels, stored at room temperature for six months, and bottled. Samples were collected prior to inoculation (time zero) and following it at days 1, 3, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, and 180. Samples were kept frozen at –18ºC and, prior to analyses, were defrosted and centrifuged at 2000 g for 10 min.

2.3 Determination of total acidity

Total acidity was measured by titrating an aliquot (1.0 mL) of the must or wine with 0.1 mol L^{-1} of NaOH to pH 8.2. Results were expressed as g acetic acid per liter of must or wine. Measurements were performed in triplicate.

2.4 Determination of phenolic compounds

Total phenolic analysis was performed via the Folin-Ciocalteu method adapted from Escarpa and González (2001). An aliquot (0.2 mL) of the diluted sample (5 fold) and 0.5 mL of 2 mol L^{-1} Folin-Ciocalteu reagent were mixed in a 25 mL volumetric flask. After 5 min, 4.0 mL of 20% Na_2CO_3 solution were added and the volume adjusted to 25 mL of distilled water. This mixture was then allowed to stand for 30 min at room temperature and the absorbance was determined at 750 nm. The standard curve was constructed with gallic acid at the following dilutions: 0.02, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg mL^{-1}. The correlation coefficient was r = 0.999. Total phenolic content was calculated as g gallic acid equivalents (GAE) per liter.

Tannin content was quantified by protein precipitation assay (WATERMAN; MOLE, 1994). Samples (1.0 mL) were precipitated with 2.0 mL of BSA solution (1.0 mg mL^{-1}) in 0.2 mol L^{-1} acetate buffer (pH 4.9). Following centrifugation, the precipitate was dissolved in sodium dodecyl sulphate/triethanolamine/isopropanol solution (4.0

mL) and tannins were complexed with 1.0 mL of $FeCl_3$ solution. The colored complex was then read at 510 nm. Measurements were made in the $0.2 < A < 0.9$ range. The standard curve was constructed with tannic acid at the following dilutions: 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 mg mL^{-1}. The linearity range went from 0.2 to 0.6 mg mL^{-1} and the correlation coefficient for this range was r = 0.999. Tannin content was calculated as g tannic acid equivalents (TAE) per liter.

Anthocyanin content was determined by the pH differential method (WROLSTAD; DURST; LEE, 2005). Pigment concentration was calculated and expressed as mg cyanidin 3-glucoside equivalents per liter of must or wine using the following equation: anthocyanins (mg L^{-1}) = A . MW. DF. 1000 / ε . l, with A (absorbance) = $(A_{520\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{520\ nm} - A_{700\ nm})_{pH\ 4.5}$, MW (molecular weight) = 449 g mol^{-1}, DF (dilution factor) = 50, l (cuvette path length) = 1.0 cm and ε (molar attenuation coefficient for cyanidin 3-glucoside) = 26900 L mol^{-1} cm^{-1}. All measurements were performed in triplicate.

2.5 Color evaluation

Color measurements were made with a Beckman DU-70 spectrophotometer (Beckman Instruments, Inc., CA, USA) with a 1.0 mm optical path length glass cell. Must or wine color intensity was determined as the sum of absorbances at 420, 520, and 620 nm and hue was the ratio of A_{420}/A_{520} (IVANOVA et al., 2011). All measurements were performed in triplicate.

2.6 ^{1}H NMR quantification

Prior to analyses, the pH of centrifuged must and wine samples was adjusted at 1.00 with 0.10 mol L^{-1} HCl (DEL CAMPO et al., 2006). Samples (0.6 mL) were placed in a 5 mm NMR tube, and 0.1 mL of TMSP solution (1.0 g L^{-1} of TMSP and 70% v/v D_2O) was added as internal standard for the quantitative analysis and internal chemical shift reference (δ = 0 ppm). NMR spectra were recorded on a Bruker Avance III 500 spectrometer operating at 500.13 MHz for ^{1}H. The following parameters were applied to quantitative ^{1}H NMR spectra: the spectral window was 10 ppm and data were collected into 65 k data points after 48 scans; the recycle delay was 5 s and had a flip angle of 90º, with an acquisition time of 4.06 s at a fixed temperature of 25ºC. Ten metabolites were quantified by measuring the peak area ratio of their signals in the ^{1}H NMR spectrum relative to TMSP. Data was analyzed by TopSpin version 2.1 (Bruker BioSpin Corp., MA, USA). All measurements were performed in triplicate.

2.7 Statistical analysis

Multiple average comparisons were performed using conjoint analysis in which fruits' sites and sampling days were factors. Degrees of freedom (df) for fructose, *β*-glucose, acetic acid, ethanol, alcohols, and tannins were corrected according to Satterthwaite (1946). Differences were determined with Tukey's *post hoc* test ($P <$

0.05) performed in SAS (1996).

Principal response curves (PRC) were applied to investigate the effects of sensorial and chemical variables and their alterations over time (VAN DEN BRICK; TER BRAAK, 1999; TER BRAAK; ŠMILAUER, 2012). In PRC, sampling days were used as a categorical covariable and the interaction between sampling time and treatments (beverages from S4 and S5 sites) was used as an explanatory variable. The analytical diagram shows the time gradient and the first or second PRC axis of variable differences of treatments in relation to a control, here attributed to the beverage prepared from fruits belonging to the S1 site. Monte Carlo permutation tests were performed to assess whether the PRC accounts for a significant part of treatments' variance in relation to control in all time series (999 permutations), as well as to verify whether treatment results in one variable changed significantly in each sampling time (499 permutations).

To assess the influence of soil origin on jabuticaba wines, sensorial and chemical variables on days 120 to 180 (response matrix, 27 samples × 16 variables), as well as the measured texture and nutritional soil parameters from different sites (explanatory matrix, 27 × 12), were submitted to redundancy analysis (RDA). The latter showed an ordination of response data constrained by explanatory variables, which accounts for the patterns of the only explained variation between data sets. Unrestricted Monte Carlo permutation tests (999 permutations) were performed to assess the significance of canonical axes. In all analyses, variance inflation factor of variables (VIF) were used to determine the selection of explanatory variables (LEPŠ; ŠMILAUER, 2003).

Multivariate analyses were performed with response data log(x + 1)-transformed and centered, while explanatory data were centered and standardized to obtain similar weight. Analyses were conducted in CANOCO (2012) and R (R CORE TEAM, 2014).

3 I RESULTS

Fruit collection for producing jabuticaba wines was based on a previous study in which phenolics, including total phenols, tannins, and anthocyanins, sugars, organic acids, and fruit acidity contents exhibited strong edaphic influence at a local scale (DUARTE et al., 2012). High chemical divergence among sampling sites, especially in orchards S1, S4, and S5, was correlated with geographical distance, hence suggesting different ecotypes. Unlike phenolics, fruits' essential oil chemovariations were genetically determined. The S1 sampling site has a low-nutrient sandy soil, whereas S4 and S5 sites are composed of high-nutrient sandy loam soils, on which only organic (S4) or chemical (S5) fertilization is applied. Soil characteristics in sampling sites may be obtained in Duarte et al. (2010).

3.1 Sensorial and chemical variation during fermented jabuticaba beverages

production

The evolution of sensorial parameters (color intensity, hue, and acidity) and the contents of phenolics, sugars, organic acids, and alcohols were monitored by colorimetric methods and quantitative ^{1}H NMR from time zero, prior to the must inoculation of up to 180 days, when the fermented beverage was bottled.

Analysis of variance (two-way ANOVA), with sampling site and different sampling time as factors, indicated that most chemical contents and color parameters varied according to the same trends observed in a previous study on jabuticaba fermentation (FORTES et al., 2012). Fructose and β-glucose of samples from S4 and S5 sites were totally consumed in the first ten days, while the concentration of ethanol increased and reached a maximum of 10.8% and 9.8%, respectively. However, S1 samples did not show complete fermentation, with sugar residues remaining at the end of the process and maximum ethanol content only reaching 8.2%. Other alcohols such as methanol, higher alcohols, and glycerol were produced in reduced amounts in all musts.

Citric acid content increased up to days 3 and 5 in all musts and then slowed down towards the end of the process, a trend also observed for succinic and lactic acids, which are products of fermentation. The S1 must/wine was distinguished by its high amount of acetic acid, mainly from day 5 (2.80 g L^{-1}), which contributed to its higher total acidity (6.45 g L^{-1}) in comparison to the others (S4 = 4.73 and S5 = 4.46 g L^{-1}).

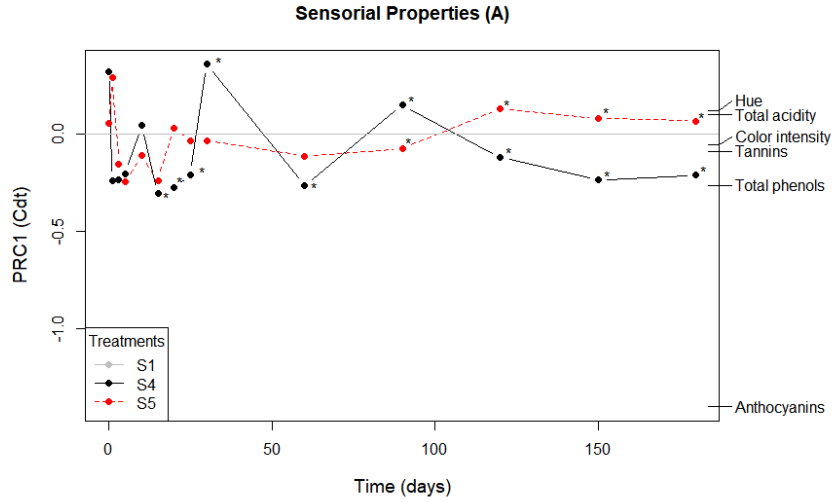
Phenolic compounds were extracted from skins and seeds during the four-day maceration phase, in which contents of total phenols, tannins, and anthocyanins increased and then decreased in all musts. A similar variation was observed for color intensity. The only difference concerns the fermented beverage of the S4 site, which showed an increase in total phenols and tannins at the end of the process (120 to 180 days). Beverages' hue from all orchards increased slowly throughout the fermentation process.

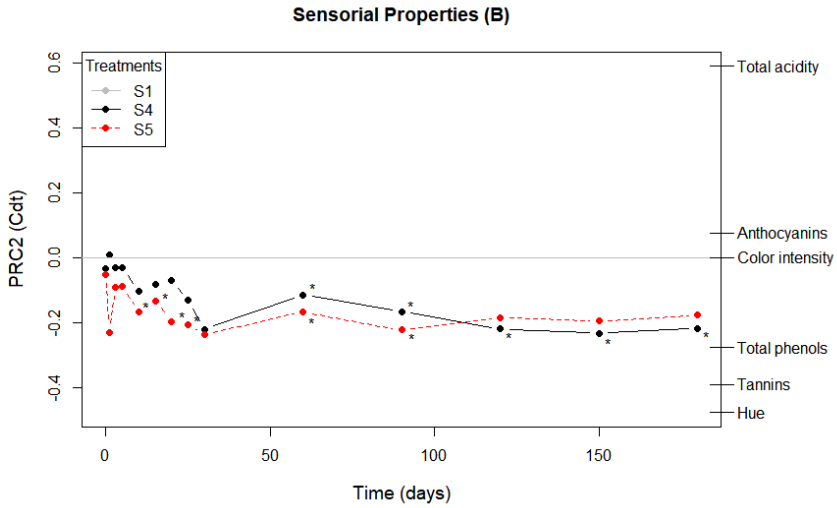
To obtain an overview of the whole process and to detect which variables were the most important for differentiating each site's must/wine over time, PRCs were performed on sensorial and chemical data sets. Results indicated that the interaction sampling site × time (predictor variables) explained 90.5% of total variance in phenolics (total phenols, tannins, and anthocyanins), total acidity, color intensity, and hue. The effect of fruit sampling sites (treatments) contributed with 10.9% of total variance, of which 96.6% were represented by the first three significant ($P \leq 0.002$) PRCs (PRC1: 77.9%, $F = 201$; PRC2: 12.0%, $F = 48.7$; PRC3: 6.7%, $F = 40.4$). Differences among sampling days contributed to the majority of the total variance (87.9%).

The PRC1 diagram (Fig. 1A) shows that anthocyanin content was mainly responsible for the distinction between musts/wines from S4 and S5 sites in relation to the control (S1), and even between S4 and S5 along the whole process. When data were restricted to each time period, differences occurred in most of S4's sampling time

($P \leq 0.035$), while S5's only differed ($P \leq 0.030$) in the final period (90-180 days).

In PRC2 (Fig. 1B), the differentiation of musts from S4 and S5, in relation to the control, was attributed to lower total acidity values. However, S4 samples differed ($P \leq 0.025$) from control after 90 days, while those from S5 differed during the initial periods (10-90 days, $P \leq 0.040$). On the other hand, in PRC3 (Fig. 1C), total phenol contents distinguished S4 samples from the others in the 60-180 days period ($P \leq 0.020$).





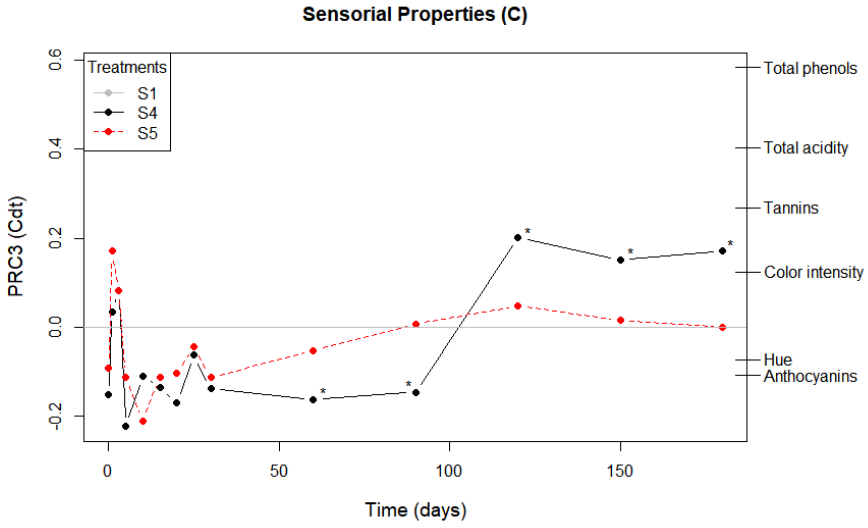


Figure 1. First (A), second (B) and third (C) PRC diagrams of chemical contents between must/wines samples from S1 (control), S4 and S5 along sampling times (days). Significant differences in S4 and S5 samples scores compared to S1 are represented by an asterisk.

When applied to the content of alcohols (methanol, ethanol, glycerol, and higher alcohols), carboxylic acids (acetic, citric, lactic, and succinic acids), and sugars (fructose and β-glucose), the PRC shows that predictor variables (interaction site × time) explained 97.2% of total variance of the must/wine. In this analysis, the treatment effect (site) contributed to 22.4% of total variance, of which 93.5% were represented by PRC1 ($F = 838$, $P = 0.002$). The time (days) taken to process the fermented beverage contributed with 77.7% of total variance. The PRC diagram (Fig. 2) indicates that lower sugar and acetic acid contents in S4 and S5 must/wine samples were responsible for their differentiation in relation to control (S1), mainly from day 10. However, S4 samples differed ($P \leq 0.025$) in the final period (60-180 days), whereas those from S5 significantly differed ($P \leq 0.035$) only at the start of the process (0-25 days).

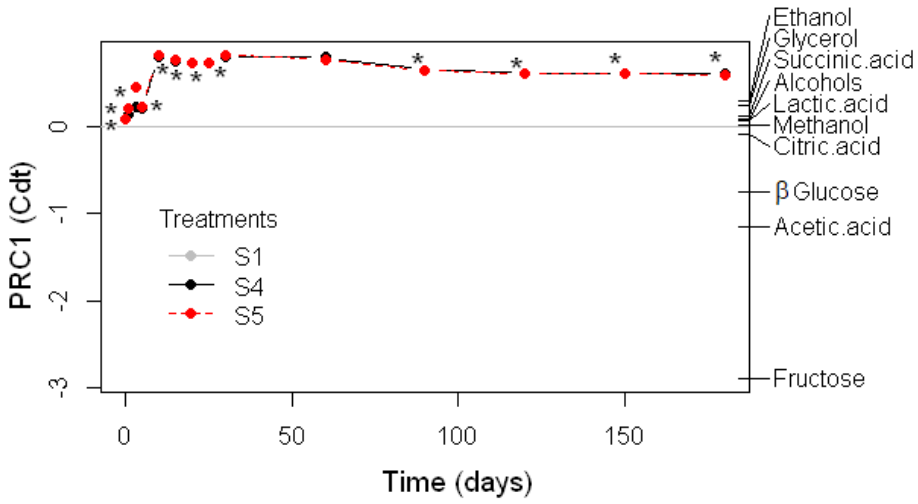


Figure 2. First PRC diagram indicating the difference in chemical contents between must/wines samples from S1 (control), S4 and S5 along sampling times (days). Significant differences in S4 and S5 samples scores compared to S1 are represented by an asterisk.

All PRC results showed that the production process of jabuticaba wines stabilized in the last three months (120-180 days), hence not revealing significant changes with regard to sensorial and chemical contents.

3.2 Soil influence on the chemical composition of fermented jabuticaba beverages

Association patterns of jabuticaba wines and environmental variables were assessed by the RDA of sensorial and chemical contents on days 120 to 180 (response matrix) conditioned to texture and nutritional soil parameters from different sites (explanatory matrix). Response data modeling by RDAs yielded Fe^{3+} and phosphorus as explanatory variables.

RDA results (Fig. 3) indicate that correlations between both data matrices were

higher in the first two canonical axes (R1 = 0.9998 and R2 = 0.9486) and VIFs were considered low (VIF < 1.02), hence suggesting no multicollinearity among variables in multivariate regression models (LEPŠ; ŠMILAUER, 2007). Monte Carlo permutation tests (999 permutations) showed highly significant (P = 0.001) results for the first two canonical axes (RDA1: 94.5%, F = 410; RDA2: 3.6%, F = 44.2), indicating that variation patterns of the original matrices did not arise by chance. The sum of canonical axes was also significant (sum = 0.9805, F = 604, P = 0.001), so that 98.1% of the total variance of fermented jabuticaba beverages was retained by selected explanatory variables (TER BRAAK; ŠMILAUER, 2012; LEGENDRE; LEGENDRE, 2003; LEPŠ; ŠMILAUER, 2007).

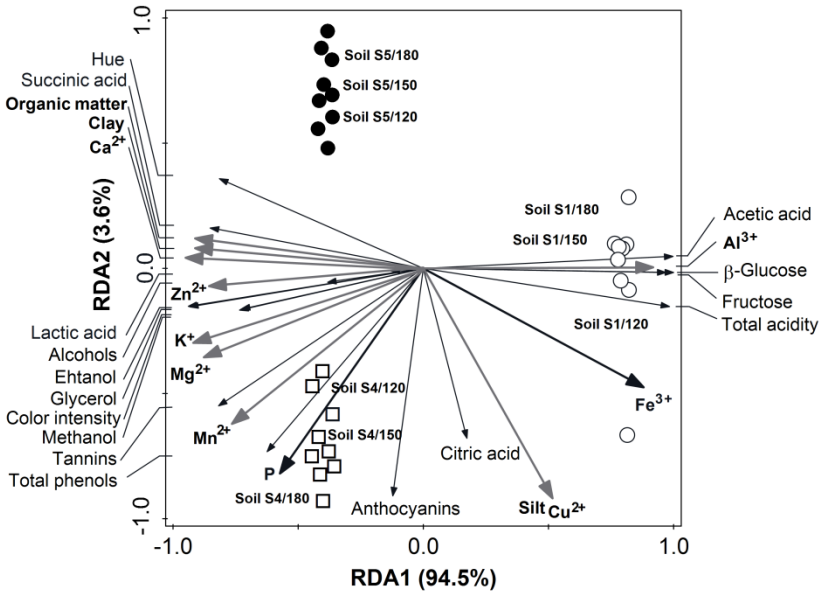


Figure 3. RDA triplot with chemical compounds and color parameters of jabuticaba wines explained by soil nutrients (Fe3+ and P), in addition to supplemental edaphic factors (organic matter, clay, Ca2+, Zn2+, K+, Mg2+, Mn2+, Cu2+, silt, Al3+), represented by gray arrows. Sample wines from 120-180 days were prepared with fruits grown in the soils: S1 (○), S4 (○) and S5 (●).

According to the RDA triplot, the increase in the RDA1 axis is especially associated with an increase in fructose and β-glucose residues, in addition to high acetic acid and total acidity contents in wine made with fruits from S1's sandy soil, poor in organic matter and mineral nutrients (except Fe^{3+}). On the other hand, a decrease in RDA2 is related to higher P levels in the S4 soil, whose samples accumulated mostly anthocyanins, total phenols, and tannins, while an increase in RDA2 is associated with wine samples of higher hue and lower anthocyanin contents, made with fruits from the S5 soil, poor in P and Fe^{3+}. Thus, whereas RDA1 suggests the influence of stuck fermentation, RDA2 mainly describes differential changes in sensorial and chemical properties of jabuticaba wines from different sites.

4 I DISCUSSION

Fruit wines are composed of a complex mixture of substances which provide them with characteristic flavor, astringency, structure, color, and aroma. Some of these substances come from the fruit, such as phenolic compounds, volatile constituents, and certain organic acids e.g. citric, malic, and tartaric acid. However, a considerable part of wines' chemical components is generated in reactions that occur during fermentation, such as ethanol, glycerol, and succinic acid (HORNSEY, 2007). Even substances extracted from fruits undergo reactions of decomposition and/or formation of new compounds, such as lactic acid produced from malic acid in malolactic fermentation and reactions between anthocyanins, acetaldehyde, and co-pigments that modify red wine coloring over time (HORNSEY, 2007; WROLSTAD; DURST; LEE, 2005).

Several studies have already shown that soil fertility and water retention capacity may influence phenolic contents in grapes, and more stressful conditions such as water restriction and nutrient deficiency generally lead to the formation of fruits that are richer in anthocyanins and tannins (VAN LEEUWEN; SEGUIN, 2006). This has also been observed for the jabuticaba, whose fruits grown in the S1 site, poorer and with greater water drainage, presented higher levels of total phenols, tannins, and anthocyanins than those from the S4 and S5 sites (DUARTE et al., 2012). Soil fertility also plays an important role in the nutritional level of musts. The lack of assimilable nitrogen sources and of some ionic nutrients may limit the growth of Saccharomyces cerevisiae yeasts, thus reducing fermentation and ethanol production (ALEXANDRE; CHARPENTIER, 1998; UDEH; KGATLA; JIDEANI, 2014).

Jabuticaba wines prepared with S1 fruit samples differed from the others in terms of low concentrations of anthocyanins, total phenols, and tannins (Fig. 3), although in a previous study the highest phenolic contents were quantified in whole fruits at the same site (DUARTE et al., 2012). The fermented beverage's chemical composition is not only a consequence of the constituents of the whole fresh fruit, since several factors contribute to the extraction and stabilization of these compounds during the winemaking process. The PRC analysis of musts/wines (Fig. 2) showed that from day 5 there was an interruption in the must fermentation at the S1 site before sugars' complete consumption, therefore low ethanol production and contamination with acetic acid bacteria strains took place.

Fermentation is interrupted when there are not enough nutrients in the must to support the development of S. cerevisiae yeasts. Generally, fruits of plants grown on low fertility soils exhibit nitrogen deficiency in the form of amino acids and ammonia, and various ionic nutrients may be below the limits required to promote complete fermentation (ALEXANDRE; CHARPENTIER, 1998; MALHERBE; BAUER; DU TOIT, 2007; HORNSEY, 2007). In fact, the RDA triplot (Fig. 3) indicates that the S1 site has edaphic conditions which favor fermentation interruption, that is, a combination of sandy soil with low organic matter, Ca^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}, K^{+} and P contents, in addition

to Al^{3+} residues and higher Fe^{3+}levels.

Nitrogen deficiency inhibits the synthesis of sugar-carrying proteins into yeast cells, limiting growth and biomass formation (ALEXANDRE; CHARPENTIER, 1998). Mono and divalent cations are essential for fungal growth and metabolism (JONES; GADD, 1990), and Mg^{2+} and K^{+} are enzymatic activators in glycolysis reactions and stimulate phosphate uptake by increasing fermentation (JONES; GREENFIELD, 1984; ALEXANDRE; CHARPENTIER, 1998). Cations such as Mg^{2+} and Ca^{2+} have a protective effect against ethanol stress and along with Zn^{2+} are required as cofactors in several metabolic pathways (BIRCH; WALKER, 2000; PEREIRA et al., 2010). The synthesis of proteins and thiamine is stimulated by Mn^{2+}, and Fe^{3+} participates in the active site of several enzymes (hemoenzymes), but at concentrations of 10 to 15 µM it inhibits growth and fermentation (JONES; GREENFIELD, 1984). Phosphorus plays a central role both in energetic metabolism and in the biosynthesis of phospholipid membranes, its limitation affecting cell growth, biomass formation, and consequently fermentation speed (JONES; GADD, 1990).

Two other factors may have contributed to the interrupted fermentation in S1 musts. The first was the competition of non-Saccharomyces wild yeasts by nutrients, aggravating the nutritional deficiency of the S1 must (MEDINA et al., 2012); the second was the presence of high acetic acid levels, which increase ethanol's toxic effect and may inhibit Saccharomyces activity, resulting in the cessation of fermentation (ALEXANDRE; CHARPENTIER, 1998).

The combination of lower ethanol contents, high acetic acid concentrations, and higher total acidity negatively influenced the extraction and stabilization of anthocyanins and ellagitannins of the jabuticaba cultivated at the S1 site. Montes et al. (2005) have reported that the efficiency of extracting anthocyanins from jabuticaba skins was reduced when acetic acid was used instead of citric acid, and that acetic acid extracts had higher lightness values and were less colorful. Ellagitannins are mainly found in jabuticaba seeds and therefore require higher ethanol contents to be extracted more efficiently (PEREIRA et al., 2017). In addition, a higher amount of acetic acid may lead to the hydrolysis of these ellagitannins to yield ellagic acid that is insoluble in the aqueous medium (QUIDEAU, 2009). Excessive acidity may also promote other reactions such as oxidation, polymerization, and condensation, all responsible for ellagitannin reduction in the must/wine (QUIDEAU et al., 2005).

Fermentation was complete in S4 and S5 musts, as evidenced by total sugar consumption, but the S4 site showed greater efficiency in ethanol production, probably due to its higher content of phosphates and other nutrients such as Mn^{2+}, Mg^{2+} and K^{+}. Although the two sites had more fertile soils (DUARTE et al., 2010), significant differences were observed in samples' phenolic contents during and after fermentation (Fig. 1). Therefore, these samples formed two distinct groups in the RDA (Fig. 3). These differences followed the same trend observed in the previous study, i.e. organically cultivated jabuticabas (S4) had higher levels of anthocyanins, tannins, and total phenols

when compared to conventional (S5) cultivation (DUARTE et al., 2012). Similar results were obtained with organic wines from wine grapes cultivated in two regions of Croatia, which presented higher levels of total phenols, flavonoids, catechins, and phenolic acids and higher antioxidant activity when compared to wines from the same grape varieties cultivated in a conventional way (VRČEK et al., 2011).

In another study, wines produced with organic grapes initially contained higher levels of anthocyanins and resveratrol than conventional wine grapes, but after six months of storage differences were no longer detected (MULERO; PARDO; ZAFRILLA, 2009). Higher amounts of polyphenols in organically grown fruits can be explained by the biotic stresses that the plant suffers, since no pesticides are applied, as well as by the use of animal manure involving slow nutrient release and higher phosphorus uptake (WEIBEL et al., 2000). Plants at the S5 site which were treated with fertilizers containing soluble inorganic nitrogen have higher nitrogen availability, which directly influences the balance between the syntheses of proteins and phenolic compounds (RAPISARDA et al., 2005).

5 I CONCLUSIONS

The texture and availability of soil nutrients not only influenced fruits' chemical composition, as seen in a previous study, but also musts' nutritional composition, which negatively affected the fermentation and composition of the S1 wine. The wine produced with organically grown fruits at the S4 site yielded the best color parameters and polyphenol composition, showing that the balance between nutrients and soil organic matter was a determinant factor in ethanol production and, consequently, enhanced efficiency in the extraction of fruit compounds.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, #470655/2012-7), and received scholarship for L.D.P. from Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG).

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Agência Brasileira do ISBN
ISBN 978-85-7247-286-9