Vol. 22, No. 3(2023) Alim2377
Revista Mexicana de Ingeniería Química

Fermented beverage of *Ardisia compressa* fruits based on commercial and native yeasts: evaluation of kinetic changes

Bebida fermentada de frutos de *Ardisia compressa* a base de levaduras comerciales y nativas: evaluación de cambios cinéticos

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Received: June 28, 2023; Accepted: September 15, 2023

Abstract

The fruits of the genus *Ardisia* Sw. (Primulaceae) are resources scarcely exploited along the world. An alternative to add value is the preparation of a fermented beverage but, to enhance properties, the use of native yeasts should be evaluated. The objective was to evaluate the use of a commercial strain of *Saccharomyces cerevisiae* (SC) and a native yeast (NY) during the preparation of a fermented beverage from *Ardisia compressa* fruits through monitoring the compounds variation. Fermentation processes were conducted and rates of sugar consumption and alcohol production were higher with NY than with SC. The fermented product contained 73.6 and 95.9 g L^{-1} of alcohol with SC and NY, while total soluble phenols, anthocyanins, and antioxidant activity were 2,367.0 mg L^{-1}, 215.5 mg L^{-1}, and 16,832.0 μmol L^{-1} with SC, and 2,213.0 mg L^{-1}, 287.4 mg L^{-1}, and 18,614.0 μmol L^{-1} with NY, respectively. The better performance of the native yeast was confirmed through modeling, which explained the variation of yeast concentration, the logarithmic soluble phenols increase, and anthocyanins depletion. Besides, the fermentation with native yeast allowed obtaining better color and better antioxidant properties. The native yeast was a better option to prepare a fermented beverage from *A. compressa* fruits.

Keywords: *Ardisia compressa*, *Saccharomyces cerevisiae*, fermentation, native yeast.

Resumen

Los frutos de *Ardisia* Sw. (Primulaceae) son escasamente explotados a nivel mundial. Una alternativa para agregar valor es preparar una bebida fermentada, pero con levadura nativa para enfatizar propiedades distintivas. El objetivo fue evaluar el uso de una cepa comercial de *Saccharomyces cerevisiae* (SC) y una levadura nativa (LN) para preparar una bebida fermentada con frutos de *Ardisia compressa* mediante el monitoreo de la variación de compuestos. Se condujo fermentación, con tasas de consumo de azúcar y producción de alcohol mayores con LN que con SC. El producto fermentado contuvo 73.6 y 95.9 g L^{-1} de alcohol con SC y LN, respectivamente, con contenidos de fenoles solubles totales, antocianinas y actividad antioxidante de 2,367.0 mg L^{-1}, 215.5 mg L^{-1} y 16,832.0 μmol L^{-1} con SC y 2,213.0 mg L^{-1}, 287.4 mg L^{-1} y 18,614.0 μmol L^{-1} con LN. El mejor desempeño de LN se confirmó con modelado, que permitió explicar la variación de concentración de levadura, el aumento logarítmico de fenoles solubles y el agotamiento de antocianinas. La fermentación con LN permitió obtener mejor color y mejores propiedades antioxidantes. La levadura nativa fue mejor opción para preparar una bebida fermentada con frutos de *A. compressa*.

Palabras clave: *Ardisia compressa*, *Saccharomyces cerevisiae*, fermentación, levadura nativa.

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https://doi.org/10.24275/rmiq/Alim2377
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

The genus *Ardisia* Sw. (Primulaceae) includes species that are present in tropical regions of America, Asia, and Africa (POWO, 2023). They generally develop as wild plants (Manjato *et al*., 2020), with little or no cultivation practices (Wang *et al*., 2014). However, based on local uses, their potential to reduce different diseases has been demonstrated (Blin *et al*., 2021; Islam *et al*., 2019). *Ardisia compressa* is a deciduous shrub, which produces globular fruits, with a thin skin, deep red in color, with a bittersweet flavor, and high antioxidant activity due to the presence of anthocyanins and other flavonoids, diterpenes, gallotannins, phenol-conjugated glycosides, and chlorogenic acids (Joaquín-Cruz *et al*., 2015; Vázquez-Sánchez *et al*., 2019). The production is seasonal, with a harvest period of only two or three months. Fruits are highly perishable, which limits marketing to production regions, so their processing has been proposed to take advantage of their chemical content.

Fermentation is an alternative to add or recover value of materials of low economic importance (Antonio-Narcizo *et al*., 2023). In this regard, Flores-García *et al*. (2019) showed that the production of a fermented beverage from the juice of *A. compressa* can be an alternative to use the fruit and evaluated a process based on a commercial strain of *Saccharomyces cerevisiae* that produced a moderate alcohol content (6.0-6.4%), which suggests the need to look for another yeast to improve the process. According to Kumsa (2020), the incomplete and slow consumption of sugars during a fermentation process is an aspect that must be addressed in each specific case. Besides, the preparation of fermented beverages oriented towards a typification by product or by region is focused on the characterization and use of native yeasts. With this purpose, Alcazar-Valle *et al*. (2019) demonstrated the fermentative capabilities of native yeast strains grown on juices from different Agave species; Berbegal *et al*. (2023) studied the variability of the *Saccharomyces cerevisiae* strains present during the spontaneous fermentation of Grenache grape musts; Álvarez-Ainza *et al*. (2015) studied the genomic diversity of yeasts associated with the artisanal production of Bacanora, a fermented beverage obtained from *Agave angustifolia*, and Nuñez-Guerrero *et al*. (2016) studied the behavior of *Saccharomyces* and non-*Saccharomyces* native yeasts during fermentation of *Agave duranguensis* juice.

Although these studies have focused on products with a high commercial development, there is a growing interest in the study of autochthonous yeasts to favor the obtaining of other fermented products to diversify this marketing area. With this point of view, Rai and Appaiah (2014) used native yeast from *Garcinia xanthochumus* to prepare a fermented beverage; Dellacassa *et al*. (2017) conducted a vinification process from pineapple and demonstrated the suitability for commercial fermentation based on native yeasts; Bae *et al*. (2022) prepared a fermented beverage from coffee cherries using autochthonous yeasts, and Portugal *et al*. (2017) determined that the use of native yeast can favor a distinctive chemical quality and flavor of cachaça obtained from the fermentation of sugarcane juice.

On the other hand, one way to evaluate the fermentation process is to monitor the kinetics of alcohol production in relation to the substrate consumption (Setford *et al*., 2019). Besides, in red fermented beverages as those as wines, the color is determined by anthocyanins that are extracted from solids during fermentation (Bimpilas *et al*., 2016; Setford *et al*., 2018), thus the evaluation of the process can be improved through the kinetics monitoring of compounds such as phenolics and anthocyanins. In this context, the objective was to evaluate the use of a commercial strain of *Saccharomyces cerevisiae* and a native yeast in terms of alcohol production, sugar consumption, and changes in bioactive compounds, during the preparation of a fermented beverage from *A. compressa* fruits, in order to achieve add value to this phytogenetic resource.

2 Materials and methods

2.1 Plant material

Ripe fruits of *Ardisia compressa* were collected in Xicotepec de Juárez, Mexico (20°16'33" N, 97°57'39" W, 1167 m above sea level) and evaluated in terms of moisture, contents of total soluble solids, acidity, total soluble phenols, and total anthocyanins, as well as pH and antioxidant activity. Since the harvest index has not yet been determined, the collection of fruits was carried out based on the experience of the producers, which consists in the observation of the appearance and size acquired by the fruits, which were purple in color, turgid in appearance, with average weight of 0.71 (±0.12) g, and with average polar and equatorial diameters of 8.98 (±0.74) and 11.40 (±1.17) mm, respectively.

2.2 Fermentation process

Batches of 300 g of macerated fruits were mixed with sucrose to adjust total soluble solids (TSS) to 20 °Brix. Batches were placed at 25 °C for 5 d to induce natural fermentation and produce must from their native yeast (NY). Also, batches of 250 g of juice, with 50 g of epidermis and seeds, with 20 °Brix, were added with

15 ppm (w/v) of potassium metabisulfite and 60 mg of a commercial yeast (*Saccharomyces cerevisiae*; SC; Fermex S.A. de C.V.). The mixture was placed at 25 °C for 3 d to activate the yeast and produce must from SC.

Twelve kilograms of fruits were manually crushed and pressed to separate the epidermis, the seed, and the juice. Six experimental units (Eu) with 1.08 kg of juice and 0.2 kg of pressed solids (epidermis and seeds) were prepared. TSS were adjusted to 24 °Brix with sucrose and potassium metabisulfite was added at 15 ppm (w/v). Three Eu were incorporated with 0.07 kg of NY inoculum and other three Eu with 0.07 kg of SC inoculum. Units were placed at 30 °C for 10 d and manual agitation was applied every 24 h to mix the cap. On days zero (beginning of the fermentation), 2, 4, 6, 8, and 10, samples of 10 mL were obtained from experimental units, which were submitted to centrifugation at 6,000 rpm for 10 min and measurement of color, antioxidant activity, and contents of TSS, total sugars, ethyl alcohol, total soluble phenols, and total anthocyanins.

2.3 Response variables

The moisture content was determined with an Ohaus analyzer (MB45, Germany). The total soluble solids (TSS) were determined in the juice with a digital refractometer (ATAGORM, PAL−1, USA) and they were expressed in °Brix. A dissolution obtained with 0.5 g of pulp in 15 mL of distilled water was prepared to measured pH with a potentiometer (Hanna Instruments, model HI-8420, Italy). The titratable acidity was measured in the same liquid with 0.01 N NaOH and phenolphthalein as indicator. The total sugar content was measured with the method of Dubois *et al*. (1956), in 300 μL of sample diluted with 300 μL of 5% phenol solution, where 1.5 mL of H_2SO_4 were added, followed by agitation and rest for 25 min. Thereafter, absorbance at 490 nm was measured in a microplate reader with integrated UV/Vis spectrophotometer (software Gen5, Biotek Instruments Inc., Winooski, Vermont, USA) and the total sugar content was calculated in grams per liter (g L^{-1}), based on a curve of glucose in the range of 6.4 to 30.0 mg L^{-1}.

The alcohol content was determined with the method described by Sayyad *et al*. (2015), with 1 mL of sample mixed with 1 mL of tri-n-butyl phosphate (TBP). Vigorous stirring was applied and two phases were formed. From the upper phase, 500 μL were taken and mixed with 500 μL of potassium dichromate dissolved at 10% (w/v) in H_2SO_4 5 M. Agitation was applied for 10 min until two phases were formed again. The lower phase was diluted and absorbance was read at 595 nm with an UV/Vis spectrophotometer. Ethanol content was expressed in grams per liter (g L^{-1}), with a standard curve of ethyl alcohol in the range of 7.5 to 150.0 g L^{-1}.

The phytochemical composition of fruits was evaluated in extracts prepared at 25 °C with the procedure described by Hernández-Rodríguez *et al*. (2019). One gram of fruit without seeds was mixed with 10 mL of 80% methanol and the pH was adjusted to 3.0 with 5% HCl. Agitation in vortex (Symphony VWR, China) was applied at 2,500 rpm for 3 min, followed by sonication in the dark with a Cole-Parmer 8890 bath (USA) for 15 min, agitation in an incubator (INO-650M, Prendo ®, Mexico) at 150 rpm and 30 °C for 30 min, and centrifugation with J-600 SolBat equipment (Mexico) at 3,500 rpm for 15 min. Supernatants were made up to 10 mL with 80% methanol. In the case of the fermentation kinetics, samples consisted on liquid taken from the experimental units.

The total soluble phenols (TSP) content was quantified with the Folin-Ciocalteu (F-C) method (Singleton and Rossi, 1965) adapted to a microplates reader (Salgado-Escobar *et al*., 2020). Based on this, 25 μL of extract, 125 μL of distilled water, 20 μL of F-C reagent diluted at 1:10 ratio, and 30 μL of 20% sodium carbonate were mixed; agitation and rest were applied for 30 min in the dark, and absorbance was measured at 760 nm with an UV/Vis spectrophotometer. Results were expressed as milligrams equivalent of gallic acid per gram of fresh sample (mg g^{-1}) and per liter (mg L^{-1}) in the fermented product, based on a standard curve prepared in the range of 2.5 to 29.0 mg L^{-1}. The total anthocyanin content was determined with the pH differential method (Lee *et al*., 2005) in aliquots of 100 μL of extract mixed with 900 μL of buffers pH 1 (0.025 M KCl) and pH 4.5. Absorbance was measured at 510 and 700 nm in each case with an UV/Vis spectrophotometer. The total anthocyanins content (*Ant*) was determined with Equation (1), where *A* was obtained with Equation (2), *M* is the molecular weight of cyanidin-3-O-glucoside (449.2 g moL^{-1}), ε is the molar extinction coefficient of this compound (26,900 L moL^{-1} cm^{-1}), and the constant 0.38 is the path length of the light beam. Results were expressed as milligrams equivalent of cyanidin-3-O-glucoside per gram of fresh sample (mg g^{-1}) and per liter (mg L^{-1}) in the fermented product.

$$Ant = \frac{AM}{0.38\varepsilon} \tag{1}$$

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5} \tag{2}$$

Color density was measured with absorbances at 420 (A_{420}), 520 (A_{520}), and 620 nm (A_{620}), measured with the Biotek UV/Vis spectrophotometer. Color intensity (*CI*) was calculated with Equation (3), tone (*T*) with Equation (4), and percentages of yellow (*Ye*) and red (*Re*) colors with Equations (5) and (6), respectively

(Glories, 1984).

$$CI = A_{420} + A_{520} + A_{620} \quad (3)$$

$$T = A_{420}/A_{520} \quad (4)$$

$$Ye = 100A_{420}/CI \quad (5)$$

$$Re = 100A_{520}/CI \quad (6)$$

The antioxidant activity (Aox) was measured with the FRAP (ferric reducing antioxidant power; Benzie & Strain, 1996) and ABTS (2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid; Re *et al*., 1999) assays adapted to a microplates reader (Hernández-Rodríguez *et al*. 2019). The FRAP reagent was prepared with acetate buffer (300 mM, pH 3.6) mixed with a 10 mM solution of 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM iron (III) chloride solution, in a 10:1:1 ratio. Aliquots of 20 μL reacted with 180 μL of FRAP reagent and 60 μL of distilled water. Absorbance at 595 nm was measured with an UV/Vis spectrophotometer and antioxidant activity (Aox, FRAP) was quantified with the aid of a standard curve of Trolox ranging from 3.8 to 46.0 μM. On the other hand, the $ABTS^+$ free radical was generated with the reaction of ABTS 7.4 mM and sodium persulfate 2.6 mM, mixed in a 1:1 (v/v) ratio. Six hundred microliters of $ABTS^+$ were taken and made up to 10 mL with methanol. Twenty microliters of extract were mixed with 180 μL of the $ABTS^+$ solution and the decrease of absorbance at 734 nm was measured in an UV/Vis spectrophotometer. A Trolox standard curve ranging from 4.99 to 59.93 μM was prepared and Aox (ABTS) was calculated. Both methods expressed results in micromoles equivalent of Trolox per gram of fresh sample (μmol g^{-1}) and per liter (μmol L^{-1}) in the fermented product.

2.4 *Mathematical modeling of the fermentation process*

The fermentation process was modeled as described by Setford *et al*. (2019) and Miller *et al*. (2019) through Equations (7) to (10), which described the behavior of total yeast, active yeast, alcohol production, and sugars consumption, respectively.

$$\frac{dc_{Ty}}{dt} = \mu c_{Ay} \quad (7)$$

$$\frac{dc_{Ay}}{dt} = \mu c_{Ay} - k_d c_{Ay} \quad (8)$$

$$\frac{dc_{Et}}{dt} = B c_{Ay} \quad (9)$$

$$\frac{dc_S}{dt} = \frac{B c_{Ay}}{Y_{Et/S}} \quad (10)$$

In these models, c_{Ty} is total yeast concentration (g L^{-1}), c_{Ay} is active yeast concentration (g L^{-1}), c_{Et} is ethanol concentration (g L^{-1}), c_S is sugar concentration (g L^{-1}), μ (d^{-1}) is the specific rate of biomass production, expressed through Equation (11) in mass of total yeast per unit of mass of active yeast per unit of time (d^{-1}), k_d is the rate of biomass inactivation, described by Equation (12) in grams of inactivated yeast per liter of fermentation medium per day (g L^{-1} d^{-1}), k'_d is the yeast sensitivity to ethanol, expressed in grams of inactivated yeast per grams of ethanol per day (d^{-1}), B is the mass of alcohol produced per unit of mass of active yeast and per unit of time (d^{-1}), described by Equation (13), $Y_{Et/S}$ is the ratio of alcohol production rate to sugar consumption rate (dimensionless), $\mu_{\max}$ is the maximum specific rate of biomass or yeast growth (d^{-1}), $B_{\max}$ is the maximum rate of alcohol produced, expressed in grams of ethanol produced per grams of active yeast per day (d^{-1}), and k_s is the Monod constant expressed in grams of sugar per liter of fermentation medium (g L^{-1}).

$$\frac{dc_{Ty}}{dt} = \mu c_{Ay} \quad (11)$$

$$\frac{dc_{Ay}}{dt} = \mu c_{Ay} - k_d c_{Ay} \quad (12)$$

$$\frac{dc_{Et}}{dt} = B c_{Ay} \quad (13)$$

$$\frac{dc_S}{dt} = \frac{B c_{Ay}}{Y_{Et/S}} \quad (14)$$

$$\mu = \frac{\mu_{\max} * c_S}{k_s + c_S} \quad (15)$$

$$k_d = k'_d c_{Et} \quad (16)$$

$$B = \frac{B_{\max} * c_S}{k_s + c_S} \quad (17)$$

In addition, Equation (14) was used to describe the rate of solubilization of phenols in the fermentation medium (Chan *et al*., 2014), where k_1 is an extraction constant, expressed in grams of soluble phenols extracted per grams of ethanol per day (d^{-1}), c_{TSP} is total soluble phenol concentration (g L^{-1}), and $c^{\max}_{TSP}$ is the maximum soluble phenols concentration (g L^{-1}).

$$\frac{dc_{TSP}}{dt} = c_{Et} * k_1 (c_{TSP} - c^{\max}_{TSP})^2 \quad (18)$$

Similarly, Equation (15) described the modification of anthocyanins content (Fernandes *et al*., 2020), where k_A is the constant of anthocyanins liberation (g L^{-1} d^{-1}), $c^{\max}_{Ant}$ is the maximum anthocyanins concentration (g L^{-1}), k_{dA} is a constant of anthocyanins degradation, in grams of anthocyanins degraded per gram of alcohol per day (d^{-1}), and c_{Ant} is anthocyanins concentration (g L^{-1}).

$$\frac{dc_{Ant}}{dt} = k_A \left(\frac{c^{\max}_{Ant} - c_{Ant}}{c^{\max}_{Ant}} \right) - k_{dA} c_{Et} \quad (19)$$

The fourth order Runge-Kutta method was applied to solve the differential equations together with the nonlinear least squares algorithm developed through

the lsqnonlin.m function, available in the Optimization Toolbox of Matlab® (The Mathworks Inc., 2008), to calibrate $B_{\max}$, k_s, $Y_{Et/S}$, $\mu_{\max}$, k'_d, k_1, $c^{\max}_{TSP}$, k_A, k_{dA}, and $c^{\max}_{Ant}$. The default values for the iterations number (200) and the tolerance function (1×10^{-6}) were used.

2.5 Data analysis

The experimental organization was conducted as a completely randomized design to evaluate the effect of the type of yeast used (NY and SC). Also, the characteristics of the fermented products were compared with a commercial wine (CW) that was obtained with 'Tempranillo' grapes. Routines of analysis of variance and comparison of means were performed with the Tukey's test, with significance level of 0.05. Three repetitions were used in all experimental routines. In addition, the simulated behavior corresponding to changes in concentrations of alcohol, sugars, soluble phenols, and anthocyanins, was compared with experimental data. In this regard, the goodness of fit was evaluated with the determination coefficient (r^2), defined in the form of Equation (16) (Madadi *et al*., 2013), where x_{exp} represents experimental data, x_{mod} data estimated by equations, and x_{mean} the mean value, under the consideration that as r^2 results closer to unity the fitting of data to the model is considered adequate.

$$r^2 = \frac{\sum_1^n (x_{exp} - x_{mod})^2}{\sum_1^n (x_{exp} - x_{mean})^2} \tag{20}$$

3 Results and discussion

3.1 Plant material

The fruits presented the characteristics shown in Table 1. pH and contents of TSS, soluble phenols and anthocyanins were lower than those reported by Flores-García *et al*. (2019) and Joaquín-Cruz *et al*. (2015), who evaluated fruits from Aguascalientes and Veracruz, México, respectively, which suggested that the composition of *A. compressa* can vary between regions. In this regard, the influence of environmental factors on the physicochemical characteristics of *Ardisia compressa* fruits has not yet been studied; however, the reported works refer to areas with different altitude conditions. In other fruits, such as those of *Prunus avium* L., it has already been reported that altitude has an effect on quality characteristics and antioxidant activity (Faniadis *et al*., 2010).

3.2 Must and fermented products

SC and NY produced must with pH of 3.013 (±0.012) and 3.007 (±0.090), acidity of 15.83 (±0.017) and 23.27 (±0.47) g L^{-1}, TSP of 3.48 (±0.15) and 4.45 (±0.37) mg L^{-1}, and total anthocyanins of 0.81 (±0.08) and 1.22 (±0.09) mg L^{-1}, respectively. The fermented products had alcohol content of 73.63 and 95.88 g L^{-1}, respectively (Table 2), with significant difference between them ($p \leq 0.05$), but the latter was similar ($p > 0.05$) to the alcohol content of the CW (101.7 g L^{-1}). Besides, the alcohol content of the NY-product was higher than that reported between 60 and 64 g L^{-1} by Flores-García *et al*. (2019).

The sugar content varied between 3.8 and 4.5 g L^{-1}. No significant difference ($p > 0.05$) was found between products of SC and NY, thus the latter had more capacity to produce alcohol from similar sugars quantities. This content was lower than that of the CW, where the value was 6.6 g L^{-1} and was similar to the value between 3.2 and 3.8 g L^{-1} obtained by Flores-García *et al*. (2019). The acidity varied between 13.8 and 14.9 g L^{-1} in the fermented products obtained with SC and NY, without difference ($p > 0.05$) between them, but it was 2.5 times higher than that of CW (Table 2), due to the acid character of *A. compressa* fruits. In this regard, Flores-García *et al*. (2019) obtained values between 8.6 and 9.2 g L^{-1} with less acid fruits. Based on criteria of the Organisation für Rebe und Wein, Navrátilová *et al*. (2020) indicated that a dry wine have sugar content of up to 4 g L^{-1}, but may have up to 9 g L^{-1} if the difference between sugars and acid contents is less than 2 g L^{-1}. In addition, authors indicated that a semi-dry wine has sugar content that exceeds the sugar content of the labeled "dry" up to 12 g L^{-1}, but it may have up to 18 g L^{-1} if the difference between sugars and acid contents is higher than that for the labeled as "dry". In this regard, the fermented products obtained with SC and NY showed similar characteristics to those of a semi-dry wine, while the CW had the characteristics of a dry wine.

Table 1. Physicochemical and phytochemical characteristics of *Ardisia compressa* fruits.

Parameter	Value	Parameter	Value
TSS (°Brix)	6.38 (±0.29)	TSP (mg/g)	9.62 (±0.72)
pH	3.15 (±0.18)	Ant (mg/g)	4.42 (±0.073)
Total acidity (g/kg)	18.77 (±0.22)	Aox FRAP (μmol/g)	53.30 (±2.43)
Moisture (%)	87.00 (±1.61)	Aox ABTS (μmol/g)	73.84 (±8.03)

TSS: total soluble solids; TSP: total soluble phenols; Ant: total anthocyanins; Aox: antioxidant activity. Values in parentheses are standard deviation.

Table 2. Phytochemical characteristics of the fermented product obtained with *Ardisia compressa* fruits using *Saccharomyces cerevisiae* and native yeast.

Parameter	*S. cerevisiae*	Native yeast	Commercial wine*
Alcohol content (g/L)	73.63 (±5.90) b	95.88 (±3.34) a	101.70 (±2.45) a
Total sugars (g/L)	4.48 (±0.37) b	3.83 (±0.015) b	6.56 (±0.02) a
Total acidity (g/L)	13.85 (±0.63) a	14.83 (±0.02) a	5.38 (±0.28) b
TSP (mg/L)	2367.00 (±75.25) a	2213.00 (±172.06) a	244.96 (±7.20) b
Ant (mg/L)	215.46 (±50.94) b	287.40 (±3.23) a	28.87 (±1.10) c
Aox (ABTS, μmol/L)	16832.0 (±343.12) b	18614.00 (±386.56) a	1168.22 (±15.08) c

*Commercial wine (CW) produced with 'Tempranillo' grapes. Equal letters indicate non-significant difference (Tukey, 0.05).

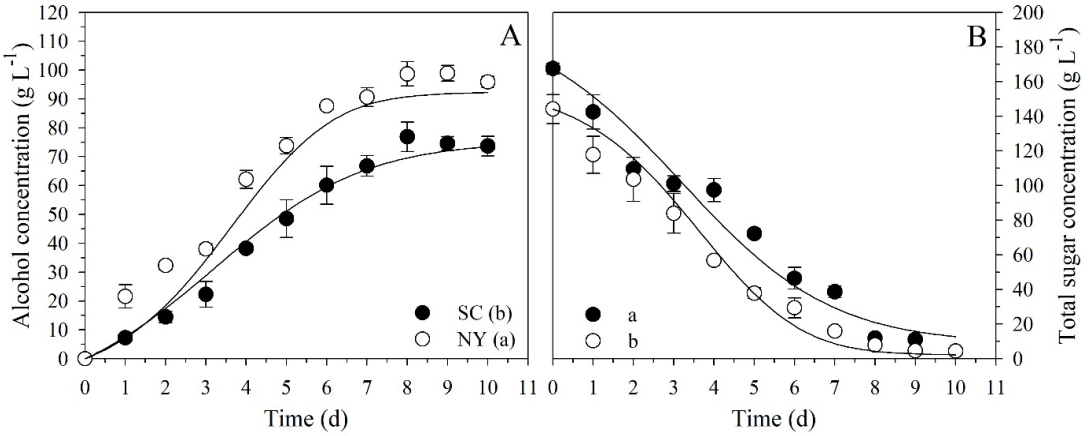

Fig. 1. Changes in alcohol (A) and sugar (B) contents during the alcoholic fermentation of *Ardisia compressa* fruits using *S. cerevisiae* (SC) and native yeast (NY). Symbols are experimental data. Lines correspond to simulated behavior. Equal letters in legends indicate non-significant difference (Tukey, 0.05). Error bars correspond to standard deviation.

The TSP content varied between 2,200.0 and 2,370.0 mg L^{-1} and was similar ($p > 0.05$) in the products of both yeasts, but these values were higher ($p \leq 0.05$) to the concentration of 245.0 mg L^{-1} of CW (Table 2). In addition, the TSP content was similar to values between 1,110.0 and 2,860.0 mg L^{-1} reported by Flores-García *et al*. (2019), which suggested that the fermented product of *A. compressa* fruits can contain higher quantities of antioxidants. The concentration of anthocyanins varied between 215.0 and 288.0 mg L^{-1}, which was found higher ($p \leq 0.05$) in the product obtained with NY than in that obtained with SC (Table 2) and in the CW, with ratios of 1.33/1.00 and 9.95/1.00, respectively. The color in fermented beverages such as wines is associated with anthocyanin content (Ribéreau-Gayon *et al*., 2021). Similarly, based on the high anthocyanin content found in the material obtained from *A. compressa* fruits, it was accepted that the observed color was derived from the presence of these compounds. On the other hand, the content of phenolic compounds can be important in the antioxidant activity of a material (Adebo and Medina-Meza, 2020). In this regard, Joaquín-Cruz *et al*. (2015) found that the antioxidant activity of *A. compressa* fruits is higher than that of fruits such as strawberry, blueberry, and blackberry, and explained that this was due to a higher concentration of phenolic compounds. Therefore, the higher content of soluble phenols and anthocyanins obtained with NY may be determinant to allow higher antioxidant activity in relation with SC and the CW (Table 2), with ratios of 1.11/1.00 and 15.93/1.00, respectively. Romero *et al*. (2016) demonstrated that the presence of anthocyanins acts synergistically with polyphenols, increasing the antioxidant activity of the fruits of *Syzygium cumini* L. In wines made from blueberry and grape fruits using NY and SC, Martín-Gómez *et al*. (2021) reported that the beverages obtained with spontaneous fermentation had higher total phenolic content and antioxidant activity.

3.3 Kinetics of changes in the fermentation process

There was a constant decrease in sugar content during the first eight days of the fermentation process, at a rate of 19.45 g d^{-1} with SC and 17.23 g d^{-1} with NY. Subsequently, the sugar content became approximately constant, with similar values between 4.5 and 3.8 g L^{-1} (Fig. 1). Consistently, the alcohol

content increased in the first stage, at 9.61 g d^{-1} and 12.33 g d^{-1} with SC and NY, respectively. Thereafter, it tended to 73.63 g L^{-1} with SC, which was close to the alcohol content obtained by Flores-García *et al.* (2019) with *S. cerevisiae*. However, the concentration was 95.88 g L^{-1} with native yeast. Experimental data of alcohol production fitted well Equation (9), with determination coefficients (r^2) of 0.979 and 0.912 for SC and NY, respectively (Fig. 1A). According to such model, the variation of alcohol production (dc_{Et}/dt) is a function of the active yeast concentration (c_{Ay}). The ratio between them corresponds to the rate of alcohol produced per unit of mass of active yeast per unit of time (B; d^{-1}), which was expressed through a Monod model, as a function of sugar concentration (c_S), that constituted the main fermentation substrate (Equation 11). In this regard, the maximum production of alcohol per unit of mass of active yeast and per unit of time ($B_{\max}$; Equation 13) was 1.20 and 2.31 d^{-1} in processes with SC and NY, respectively, which explained that the concentration of alcohol was higher with the latter. Similarly to a Michaelis-Menten kinetics, the Monod constant (k_s) expresses the concentration of substrate at which the reaction rate reduces to 50% relative to the maximum rate (Liu, 2013). The modeling process indicated that k_s was of 3.40 and 1.72 g L^{-1} with SC and NY, respectively, which indicated that NY maintained higher alcohol production rates than SC, even at lower sugar concentrations.

The rate of alcohol production was used to express the rate of sugar consumption (dc_s/dt) through dividing the former (dc_{Et}/dt) by the ratio of both ($Y_{Et/S}$). Data fitted well Equation (10), where r^2 and $Y_{Et/s}$ were 0.976 and 0.50 with SC and 0.990 and 0.59 with NY, indicating better performance of NY (Fig. 1B). Besides, the sugar concentration (c_S) determined the rate of biomass production (μ; Equation 11). In this regard, the simultaneous analysis of this model and Equations (7) and (8) indicated that the maximum variation of total yeast per unit of mass of active yeast and unit of time ($\mu_{\max}$; d^{-1}) was 0.10 and 0.08 for SC and NY, respectively, which suggested that there was higher concentration of active native yeast than active *S. cerevisiae*. In fact, the model solution indicated that there was higher concentration of total native yeast than *S. cerevisiae* (Fig. 2), and also that the ratio of total yeast to active yeast was higher with NY than with SC.

In the case of total yeast, three stages were observed: an initial adaptation period, an accelerated growth stage, and a stationary stage. In this regard, the alcohol exerts an inhibitory effect on yeasts (Zhang *et al.*, 2018), which explained the last stage. However, according to Setford *et al.* (2019), a reduction in the concentration of active yeast occurs during the last stage, as described by Equation (8) and Fig. 2,

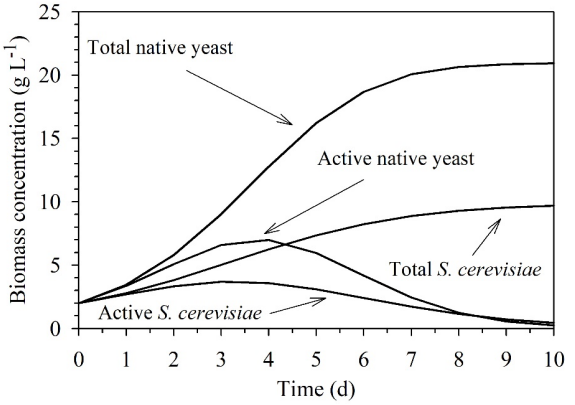

Fig. 2. Variation of total and active yeast during the fermentation process.

where yeast sensitivity to ethanol (k'_d) was 0.0103 and 0.0110 d^{-1} for processes based on SC and NY, respectively, which pointed out that the native yeast experienced higher sensitivity to the increment of alcohol concentration and, around 60 g L^{-1}, the accelerated stage ended and began the inactivation of the yeast. However, during most of the fermentation time, the active native yeast was maintained at higher concentration than *S. cerevisiae*, as indicated by k'_d, which again explained the better behavior of the former.

A fermented beverage can exhibit high quality and unique flavors when it is obtained with native or wild yeasts, but these can also cause deterioration of the product, since their behavior is unpredictable, so a careful evaluation of the process should be performed (Kumsa, 2020). In the present work, the native yeast showed a better performance than *S. cerevisiae*, but more investigation must be conducted to purify and identify the specific strain, which would allow to standardize the fermented product (Dellacassa *et al.*, 2017). Besides, the evaluation of conditions at which the yeast can be propagated should be taken into account (Estrada-Martínez *et al.*, 2023). In this regard, according to Agbenorhevi *et al.* (2019), the changes in pH, titratable acidity, alcohol production, specific growth rate, polyphenol concentration, antioxidant activity, and quality of the final product are influenced by the type and amount of initial inoculum.

TSP varied between 1,504.0 (±29.8) and 1,566.0 (±20.0) μg mL^{-1} at the beginning and increased rapidly during the first two days of fermentation. After, the trend was logarithmic towards values between 2,100 and 2,300 μg mL^{-1} at the end of 10 d, with no significant difference between yeasts (Fig. 3A). Experimental data fitted well Equation (14), where the extraction constant (k_1) was 0.110 and 0.485 d^{-1}, for processes with SC and NY, respectively. The raw material of the process was juice mixed with epidermis and seeds of fruits.

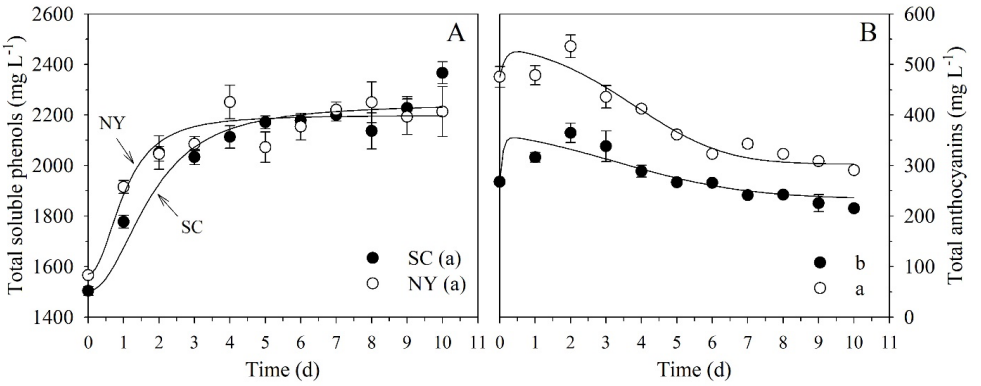

Fig. 3. Changes in the content of total soluble phenols (A) and total anthocyanins (B) during the alcoholic fermentation of *Ardisia compressa* fruits using *S. cerevisiae* and native yeast. Symbols are experimental data. Lines correspond to simulated behavior. Equal letters in legends indicate non-significant difference (Tukey, 0.05). Error bars are standard deviation.

As the fermentation progressed, the native yeast allowed higher rate of alcohol production than *S. cerevisiae*, which favored greater extraction of soluble phenols from the solid phase, thus the extraction coefficient was higher. However, as time passed, the extraction capacity of soluble phenols was homogenized, which caused that the maximum concentration of soluble phenols ($c_{TSP}^{\max}$) reached the similar values of 2.25 and 2.20 g L^{-1} for SC and NY, respectively.

The increment in phenolic content with fermentation has been reported before (Leonarski *et al*., 2022; Mahmoudi *et al*., 2021). According to Adebo and Medina-Meza (2020), this is due to breakdown of cell walls and enzymes activity that leads to compounds liberation. The initial content of soluble phenols corresponded to that of the liquid phase, while the rapid increase in concentration corresponded to compounds that came from the suspended solid phase, incorporated through a solid-liquid extraction phenomenon. Besides, the continuous upward trend suggested that phenolic compounds are in general not part of the fermentation substrate.

The raw material color largely determines the fermented product color. The *A. compressa* fruits contain high anthocyanin concentration, thus the appearance of the fermented beverage exhibited an intense red color. In particular, delphinidin-3-O-galactoside, petunidin-3-O-galactoside, and malvidin-3-O-galactoside have been found in greater proportion in these fruits (Joaquín-Cruz *et al*., 2015). The total anthocyanin content was different between systems from the beginning (Fig. 3B), with 268.00 (±4.68) mg L^{-1} with SC and 465.00 (±35.60) mg L^{-1} with NY. Although both processes were prepared under similar conditions, they differed in the content of anthocyanins in the added must. In this regard, NY production was based on macerated whole fruits and the natural fermentation caused extraction of anthocyanins from the solid fraction. However, clarified juice and a portion of already pressed solid material were used with SC, which caused lower anthocyanin content.

The anthocyanin concentration increased from day zero to day two in both fermentations, but a rapid decrease occurred between days two and six, and such trend was maintained in the final stage, although at a lower rate. This behavior was coherent with Equation (15) (Fig. 3B) where, for the processes based on SC and NY, the constant of anthocyanins extraction (k_A) was 4.028 and 3.290 d^{-1}, the constant of anthocyanins degradation (k_{dA}) was 0.019 and 0.015 d^{-1}, and the maximum anthocyanin concentration ($c_{Ant}^{\max}$) was 0.360 and 0.538 g L^{-1}, respectively. In this regard, although the extraction constant was less with NY, the degradation constant was higher with SC, which allowed obtaining higher anthocyanin concentrations with the native strain.

The degradation of anthocyanins with the fermentation has been reported in other works. Wang *et al*. (2015) obtained a maximum peak of anthocyanins concentration on the first day of fermentation in the production of wine with blackberry. Also, Sun *et al*. (2011) found a higher quantity of anthocyanins on the second day of fermentation with grapes, but then the amount decreased. In the present work, the fruit of *A. compressa* was fermented with the skin from day zero to day 10, so it is assumed that there was a continuous transference of anthocyanins from the epidermis to the fermentation medium. However, the rate of extraction in the first few days was greater than the degradation or conversion to other compounds. In this regard, it was accepted that the alcohol acted as a solvent in a solid-liquid extraction phenomenon. Also, the concentration of monomeric anthocyanins changes constantly during fermentation, where products of the yeast metabolism, such as pyruvate, acetaldehyde, and

vinylphenols, interact with anthocyanins to produce pyranoanthocyanins, anthocyanin oligomers, and polymeric pigments (Ruta and Farcasanu, 2019).

The color intensity (CI) was 0.465 with SC and 0.598 with NY at the fermentation beginning (Fig. 4A). Free or associated anthocyanins and the copigmentation effect with other phenolic compounds are important factors in the color of a wine. With maturation and aging, red tones shift towards red-orange, due to appearance of oligomeric and polymeric pigments (Ribéreau-Gayon *et al*., 2021). During the first days, the intense red color in red wines increases due to intra- and inter-molecular co-pigmentation reactions and self-association of anthocyanins, but later, co-pigmentation is interrupted by the alcohol production, giving rise to yellow tones (Casassa, 2017). Therefore, the higher CI value in NY systems was a consequence of the higher concentration of anthocyanins in comparison with those based on SC.

Likewise, during the 2-3 d of fermentation, the CI increased in both cases, as a consequence of the increase in anthocyanins from the suspended solid phase. Subsequently, the CI value remained approximately constant, in the range from 0.84 to 0.90 in the system with NY and from 0.58 to 0.70 with SC. In addition, young wines exhibit maximum absorption at 520 nm and a shift towards higher absorbances at 420 nm occurs with maturation, thus the tone is evaluated as the ratio of the latter and that of the former (Ribéreau-Gayon *et al*., 2021). In the present work, the initial tone value was 0.70 in systems with SC and 0.51 with NY. In both, the value increased with fermentation (Fig. 4B), consistently with the kinetics reported for a traditional wine (Ribéreau-Gayon *et al*., 2021). In fact, the percentage of yellow shades increased during fermentation in both treatments, while that corresponding to red shades tended to decrease (Fig. 4C and 4D).

The antioxidant activity (Aox) was 5,110.0 (±188.6) and 8,333.0 (±205.7) μmol L^{-1} with the FRAP assay at the beginning of fermentation with SC and NY, respectively (Fig. 5A). Meanwhile, the ABTS procedure yielded 8,253.0 (±151.7) μmol L^{-1} in both cases (Fig. 5B). The difference between methods is due to the first evaluates transference of hydrogen atoms, while the second evaluates transference of electrons (Shahidi and Zhong, 2015). The native yeast produced higher Aox than *S. cerevisiae* and this property increased rapidly between the beginning and the fourth day, although showed minor changes subsequently, reaching values of 10,406.0 (±119.0) and 12,211.0 (±396.6) μmol L^{-1} with SC and NY, respectively, according to FRAP and values of 16,832.4 (±343.0) and 18,614.2 (±386.4) μmol L^{-1}, respectively, according to ABTS. In fruits, different compounds interact to provide specific antioxidant properties. In grape, anthocyanins, flavan-3-ols, and flavonols are found mainly in the skin, seed and stalk, while non-flavonoids predominate in the pulp (Obreque-Slier *et al*., 2010). Also, yeast metabolism products like acetaldehyde and other phenolics, such as hydroxycinnamic acids, participate in the formation of pigments derived from anthocyanins during and after alcoholic fermentation (Lingua *et al*., 2016).

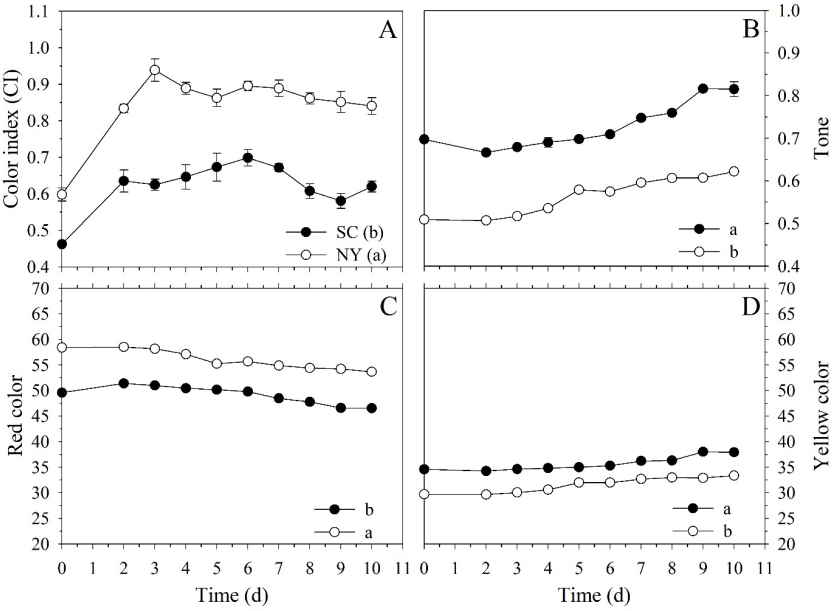

Fig. 4. Changes in color index (CI) (A), hue (B), percentage of red color (C), and percentage of yellow color (D) during the alcoholic fermentation of *Ardisia compressa* fruits using *S. cerevisiae* and native yeast. Equal letters in legends indicate non-significant difference (Tukey, 0.05).

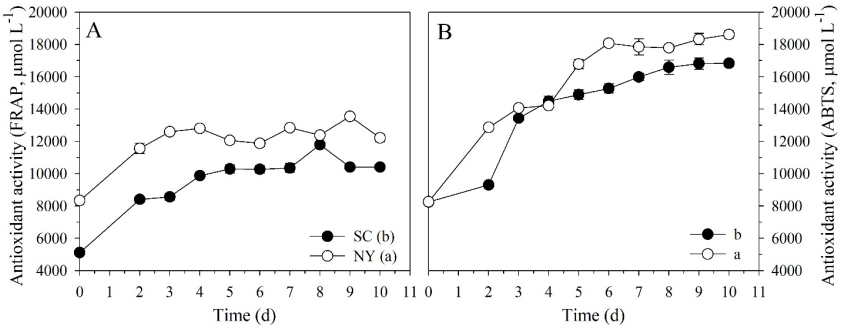

Fig. 5. Modification of the antioxidant capacity evaluated with the FRAP (A) and ABTS (B) assays during the alcoholic fermentation of *Ardisia compressa* fruits using *S. cerevisiae* and native yeast. Equal letters in legends indicate non-significant difference (Tukey, 0.05).

According to Sun *et al*. (2011), the behavior of the antioxidant activity in a vinification process is due to a higher content of anthocyanins and total phenols during the initial days, while in the advanced fermentation phase the antioxidant property is associated with a higher percentage of catechins, di and trimers of polymerized procyanidins, and polyphenols.

Conclusions

A fermented beverage was obtained from fruits of *Ardisia compressa*. The process was feasible both with a commercial strain of *Saccharomyces cerevisiae* (SC) and with native yeast (NY). The process with NY exhibited higher alcohol production and higher sugar consumption than with SC. The content of total soluble phenols was similar; however, the content of anthocyanins and antioxidant activity were higher with NY. Likewise, there was a greater change in anthocyanins with NY, which affected the color of the product and the antioxidant activity. The use of modeling allowed explaining the variation of yeast concentration, an increment of soluble phenols, and a depletion of anthocyanins. The native yeast was a better option to prepare a fermented beverage from *A. compressa* fruits.

Nomenclature

A_{420}, A_{520}, A_{620}, A_{700}	Absorbances at 420, 520, 620, and 700 nm, respectively.
A	Absorbance obtained with Equation (2).
Ant (mg L^{-1})	Anthocyanin concentration (milligrams of anthocyanins per liter of sample).
B (d^{-1})	Rate of alcohol produced (grams of alcohol produced per gram of active yeast per day).
B_{max} (d^{-1})	Maximum rate of alcohol produced (grams of ethanol produced per grams of active yeast per day).
c_{Ant} (g L^{-1})	Anthocyanin concentration (grams of anthocyanins per liter of fermentation medium).
c_{Ant}^{max} (g L^{-1})	Maximum anthocyanin concentration (grams of anthocyanins per liter of fermentation medium).
c_{Ay} (g L^{-1})	Active yeast concentration (grams of active yeast per liter of fermentation medium).
c_{Et} (g L^{-1})	Ethanol concentration (grams of alcohol per liter of medium fermentation).
CI	Color index (dimensionless).
c_S (g L^{-1})	Sugar concentration (grams of sugars per liter of medium fermentation).
c_{TSP} (g L^{-1})	Total soluble phenols concentration (grams of soluble phenols per liter of fermentation medium).
c_{TSP}^{max} (g L^{-1})	Maximum concentration of soluble phenols (grams of soluble phenols per liter of fermentation medium).
c_{Ty} (g L^{-1})	Total yeast concentration (grams of yeast per liter of fermentation medium).
ε (L moL^{-1} cm^{-1})	Molar extinction coefficient of cyanidin-3-O-glucoside.
k_1 (d^{-1})	Constant of extraction of soluble phenols (grams of soluble phenols extracted per grams of ethanol per day).
k_A (g L^{-1} d^{-1})	Constant of anthocyanins liberation (grams of anthocyanins extracted per liter of fermentation medium per day).
k_d (g L^{-1} d^{-1})	Rate of biomass inactivation (grams of inactivated yeast per liter of fermentation medium per day).

k_{dA} (d^{-1})	Constant of anthocyanins degradation (grams of anthocyanins degraded per gram of alcohol per day).
k'_d (d^{-1})	Sensitivity of the yeast to ethanol (grams of inactivated yeast per grams of ethanol per day).
k_s (g L^{-1})	Monod constant (grams of sugar per liter of fermentation medium).
M	Molecular weight of cyanidin-3-O-glucoside.
μ (d^{-1})	Specific rate of biomass production (grams of total yeast produced per gram of active yeast per day).
μ_{max} (d^{-1})	Maximum specific rate of yeast growth (grams of total yeast produced per gram of active yeast per day).
r^2	Determination coefficient.
Re	Red percentage (dimensionless).
T	Tone (dimensionless).
t (d)	Process elapsed time (d).
x_{exp}	Experimental data.
x_{mean}	Mean experimental data.
x_{mod}	Data estimated by the model.
Ye	Yellow percentage (dimensionless).
$Y_{Et/S}$	Ratio of alcohol production rate to sugar consumption rate (daily grams of alcohol produced per daily grams of sugar consumed).

Acknowledgment

Authors are grateful for the support received from Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT) of Mexico.

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