Research article

The effect of ion-exchange resin treatment on grape must composition and fermentation kinetics

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ABSTRACT

The aim of this study was to determine the influence of ion-exchange resin treatments of white grape must for the production of sparkling base wines, on the grape must chemical composition and fermentation kinetics. The experiment included an untreated grape must with a pH of 3.2, and resin-treated musts with pH 3.14, 3.07, and 3.01. Analysis of pH, total acidity, optical density (O.D.) at 420 nm, total phenolic content, tartaric acid, ammonium-nitrogen (AN) and cations (Ca2+, Mg2+, Cu2+, K+, and Na+) was performed. The fermentations were monitored by mass reduction (CO2 release g/L), and fermentation parameters obtained from a modified non-linear sigmoidal equation. The analyses of the musts showed differences in the concentration of metals, total acidity, tartaric acid, AN and O.D. 420 nm. The kinetic parameters of the fermentation showed significant changes between the control must and the ion-exchange treatments. This study showed that the treatment with cationic resins at the tested level significantly affects the chemical composition of the musts, interfering with the fermentation kinetics. In addition, reductions in the must pH index close to 0.1 unit can lead to positive results in the characteristics of the base must of sparkling wines.

1. Introduction

Grape must pH is considered an important indicator of wine quality. On basic wines to produce sparkling wines, low pH values affect the final product increasing freshness, vivacity, and brightness. Moreover, low pH interferes with redox reactions increasing shelf-life, stability, and the action of active SO2 (Ribéreau-Gayon et al., 2007; Moreno and Peinado, 2012). The relative concentration of organic acids is an important factor as it directly affects wine pH (Sowalsky and Noble, 1998). In addition to the traditional techniques of pH control on grape must and wines, such as the addition of organic acids, other techniques are gaining importance, such as acidification by electro dialysis, and cationic-exchange resins treatment, which have a lower cost for application and also aids in tartaric stabilization of wine (Mira et al., 2004; Lassanta et al., 2013; Ibeas et al., 2015; Ponce et al., 2018; Togores, 2018; Comuzzo and Battistutta, 2019).

According to the International Organization of Vine and Wine (OIV) in the International Oenological Codex 2018 (OIV, 2018), the ion exchange treatment of musts and wines is a technique of partial physical extraction of cations of grape musts or wines intended to increase the total acidity leading to pH decrease.

Cationic exchange resins for oenological use consist of synthetic polymerized materials formed by styrene and divinylbenzene. The resins have a three-dimensional (0.3–1.2 mm of diameter) and porous matrix that supports the ion-exchanging groups, consists of a synthetic resin that is obtained by condensation and polymerization (Mira et al., 2004, 2006).

Ion exchange resins are activated by treatment with a mineral acid, usually sulfuric acid, incorporating H+ ions. The activated resin exchanges their H+ ions with cations (e.g. K+, Ca2+, among others) present in grape must and wines. This ionic exchange (stoichiometric exchange) causes a pH decrease and total acidity increase, by the liberation of organic acids, like tartaric acid, from their corresponding salt (AEB-Group, 2013).

Cationic resins follow the laws of affinity by which cations with higher valence are preferably exchanged than cations of lower valence (Al3+ > Ca2+ > K+). Thus, bivalent cations, like magnesium and calcium,
of must and wines, are fixed in the cationic resin in preference than monovalent ions, like sodium and potassium. However, if two cations have the same valence, preference is for that of a higher atomic number (Ribéreau-Gayon et al., 2007).

Initially, the regulation of the European Parliament (n° 606/2009) had authorized the use of cationic resins for tartaric stabilization of wines, removing part of the cations responsible for insoluble salts. In 2012, the OIV approved its use in musts and wines for tartaric stabilization and an increase of total acidity (decrease in pH). In the sequence, the European Parliament (n° 144/2013) adopted the OIV International Oenological Codex (OIV, 2012a, b). In Brazil, the use of resins in musts and wines is regulated by the RDC n° 123/2016 National Health Surveillance Agency - ANVISA (Brazil, 2016).

In this context, the objective of this study was to analyze the effect of cationic resin treatment on the physicochemical and mineral composition of white musts (Chardonnay), as well as its influence on the kinetics of alcoholic fermentation.

2. Materials and methods

2.1. Materials

The ion exchange resins (pH-Stab) and the sulfuric acid activator (ACID +) used in this study were acquired from AEB Biochemistry Americana S.A. (São José dos Pinhais, Paraná, Brazil). The doses, application method, washing and regeneration (sulfuric acid activator) procedures of the resins followed the manufacturer's recommendations. Briefly, after each use the resin was washed with demineralized water twice the volume the resin used, after that, it was regenerated with the detergent ACID + (in 1.5x volume of the total solution – 17.5% of regeneration agent). After regeneration the resin was washed 5 times with demineralized water (monitored by pH) and one time with must to make sure that there is no detergent residue on the resin, except H⁺ ions.

2.2. Base must

The study demanded 75L of Chardonnay grape must. The must was obtained by pressing the grapes with a pneumatic press PPC 9 (Enoveneta S.P.A., Piazzola Sul Brenta PD, Italy), followed by enzymatic treatment performed with Zimopec PML (Perdomini-IOC S.P.A, San Martino Buon Albergo, VR, Italy), sulfitation (50 mg/L of SO₂) and fining with silica sol Xiles 40 (Perdomini-IOC S.p.A., San Martino Buon Albergo, VR, Italy) and gelatin Gecoll (Laffort, Bordeaux, France). After settling (Débourbage), the musts were separated in volumes of 5.5 L for the resin treatment.

2.3. Must treatment with ion exchange resin

The performing of the ionic exchange on a pilot scale, between the resins and the grape must require an experimental glass device developed in a cylindrical shape, with a volumetric capacity of 600 ml (350 g of resin) equipped with a Teflon valve, and a porous disc to retain the resins. The circulation of the must through the resin was performed manually until the entire must reached the desired pH levels. Afterward, the treated musts were separated for fermentation and chemical analyses.

The treatments consisted of control must with pH 3.2 which was called ST (standard treatment), and treated musts with a pH reduction of approximately 0.06 units, obtained by the passage through the ion exchange resin. Treated must have pH values around 3.14 termed TR1, pH 3.07 (TR2) and pH 3.01 (TR3). The resin treatments, fermentation, and the analysis were performed in triplicate.

2.4. Experimental fermentation and monitoring

The musts were inoculated with 20 g/L strain of Saccharomyces cerevisiae Zymaflore X5 from Laffort (Bordeaux, France). Yeast was previously rehydrated according to the manufacturer's recommendation: diluted in grape must (200 ppm) and incubated at 37 °C for 20 min. After 48 h from the beginning of the alcoholic fermentation (F.A.), 30 g/L of Nutristart, fermentation nutrient (Laffort, Bordeaux, France) and 15 g/L of bentonite Microcol Alpha (Laffort, Bordeaux, France) were added in order to maintain a safe fermentation and a limpid resulting wine. Fermentation was conducted at 20 °C. The fermentation process was monitored by the release of carbon dioxide (g/L), measured by mass loss with two measurements per day. Fermentations were stopped when mass loss did not variate in two consecutive days.

The data were analyzed according to a modified non-linear sigmoidal equation of Gompertz (López et al., 2004; Rinaldi et al., 2006) and expressed as; duration of the lag phase for CO₂ production (lag = h), total release of CO₂ (Vₘₐₓ = g/L), and maximum release of CO₂ (Vₘₐₓ = g/L per day). The data were subjected to variance analysis and comparison of means (Tukey test - P ≤ 0.05).

2.5. Chemical and physical analyses

The total soluble solids content (SST) of the grape must was determined by refractometry with a portable digital refractometer PAL-3 (Atago, Bellevue, Washington D.C., USA) and the result was expressed in °Brix (numerical scale of refractive index). The turbidity of the initial must before the ion exchange resin treatment was measured by a 2100P benchtop turbidimeter (HACH, Loveland, CO, USA), calibrated with standard solutions of 1.0, 10 and 100 NTU (Nephelometric Turbidity Unit) and the results were expressed in NTU (Ribéreau-Gayon et al., 2007).

The reducing sugars were quantified by the Fehling method (Pregnolatto and Pregnolatto, 1985; AOAC, 1995) which is based on the property of sugars reducing copper sulfate in hot and alkaline media. The values found were expressed in g/L of reducing sugars. The pH determination in the musts was performed with an MPA210 benchtop pH meter (Tecnol commercially Piracica, SP, Brazil (2013). Total acidity (TA) was measured by neutralization with 0.1 N sodium hydroxide solution and using phenolphthalein as an indicator (Brazil, 1986). Results were expressed in g/L tartaric acid.

The levels of tartaric acid (C₄H₇O₇) were verified through enzymatic kit and equipment WINEFLOW (Gibertini, Novate, MI, Italy) which measures the colorimetric intensity of the enzymatic reaction occurring between tartaric acid and vanadic acid to form a colored compound whose absorption at 492 nm is proportional to the concentration of tartaric acid in the sample, expressed as g/L.

The ammonium-nitrogen (AN) was measured by steam distillation and posterior titration with 0.01 N sulfuric acid according to the method described by Rizzon and Salvador (2010) and AOAC (1995). When a must sample is heated in an alkaline medium, ammonium hydroxide (volatile compound) is formed and can easily be determined by titration with an acidic substance in the presence of boric acid. The concentration was expressed in mg/L of ammonium-nitrogen (NH₄⁺).

The quantification of total phenolic content was estimated, based on the reducing capacity, by the colorimetric method of Folin-Ciocalteu in ultraviolet absorption equipment, spectrophotometer UV/VIS T92 (PG Instruments, Leicestershire, EM, England) (Rockenbach et al., 2008). For the quantification, a standard curve was constructed with five gallic acid concentrations in 70% (v/v) ethanol and the polyphenols concentration was evaluated by the linear line equation. The results were expressed in mg/L of gallic acid.

The UV/VIS T92 spectrophotometer (PG Instruments Leicestershire, EM, England) was used to evaluate the yellow color component of the musts. For this evaluation, the samples were poured directly into a 10 mm cuvette. The absorbance reading was measured at the optical density (O.D.) at 420 nm, which is the characteristic absorption wavelength of yellow substances (Ribéreau-Gayon et al., 2007).
For the analysis of the metals of the grape must, atomic absorption and flame emission spectrophotometry was used. The preparation of the samples to be quantified varied for each analyzed element. We used the wavelengths and slot width specific for each metal and made a standard curve for each element, according to the method proposed by Rizzon and Salvador (2010). The alkaline earth metals, calcium (Ca), magnesium (Mg) and the metallic ion copper (Cu) in the musts were determined by an atomic absorption spectrometer AAnalyst (PerkinElmer, Waltham, Massachusetts, USA). Potassium (K) and sodium (Na) determination were carried out by a flame photometer, model B462 (Micronal S.A., São Paulo, Brazil) (Rizzon and Salvador, 2010; Fonseca and Pulcineli, 2013). The results were expressed in mg/L.

The results were submitted to analysis of variance (ANOVA) followed by a comparison of means with a probability lower than 0.05 (Tukey’s test). Statistical analyses and graphs were generated using Graphpad Prism®, ASSISTAT and Microsoft Excel software.

3. Results and discussion

3.1. Physicochemical parameters and mineral composition

The Chardonnay juice used for the experiment, exhibited specific characteristics for the sparkling base wines (Togores, 2018), with total soluble solids (TSS) concentration of 17.6° Brix, 170 g/L of sugars, total acidity of 9.7 g/L expressed in tartaric acid, pH 3.2, and a turbidity of 35 NTUs (Nephelometric Turbidity Unit). Total soluble solids, sugars, and NTU did not show significant variation after resin treatment and were not include in the tables.

Must analyze showed an increase in total acidity in the resin-treated musts (Figure 1A). This increase was directly related to the reduction of must pH and associated with an increase in the amount of available tartaric acid (Figure 1B). An increase in total acidity and tartaric acid is expected since the cationic resin reduces the concentration of salts (e.g. potassium tartrate) exchanging the cations (K⁺, Na⁺, etc.) by H⁺ ions.

![Figure 1](image-url)

Figure 1. Total Acidity (A), tartaric acid (B), ammonium-nitrogen (C), total phenolic content (D) and O.D. 420 nm (E). Distinct letters are significantly different by the Tukey test (P < 0.05). AT = Total Acidity (expressed as tartaric acid); A.N. = Ammonium Nitrogen; C₆H₄O₆ = tartaric acid chemical formula; O.D. 420 nm = absorbance at 420 nm in spectrophotometer.
Although examples of must treatments with cationic resins are scarce, similar results were obtained in previous works using cationic resins on wines (Mourgues, 1993; Walker et al., 2003; Ponce et al., 2018). Moreover, wines obtained from resin-treated musts (Cisilotto et al., 2019) showed an increase in total acidity and tartaric acid concentration proportional to resin-treatments.

A significant difference was found in the ammonium-nitrogen (NH₄⁺) content of the control must and the largest treated must (TR3). The ST had 71 mg/L (AN), while TR3 showed only 59 mg/L, a 17% reduction (Figure 1C). Nitrogen in the form of ammonium is the most rapidly assimilable source of nitrogen for yeast, and its levels must exceed 50 mg/L for fermentation to proceed smoothly (Moreno and Peinado, 2012).

Figure 2. Determination of calcium, magnesium, copper, potassium and sodium cations. Distinct letters are significantly different by Tukey test (P ≤ 0.05). A = Ca (calcium); B = Mg (magnesium); C = Cu (copper); D = K (potassium); E = Na (sodium).
Positive results were shown in a work with an ion exchange resin to remove ammonia and ammonium in wastewater treatments (Ding and Sartaj, 2016; Imchuen et al., 2016). However, the effect on grape musts was not found in the literature.

The O.D. 420 nm (yellow color) evaluation (Figure 1E) showed a decrease in absorbance in resin-treated musts compared with the control sample. In the study by Tamasi et al. (2018) the authors obtained similar results with the use of cation exchange resin in samples of dessert wines. In the study by Cisilotto et al. (2019), lower susceptibility to browning overtime was reported in wines that had their must treated with ion exchange resins. In previous studies (Benítez et al., 2002) showed reduced susceptibility to browning in white wines submitted to resin treatments. Although the analysis of total phenolic content has no significant differences (Figure 1D), the O.D. 420 nm reduction can be explained by the decrease in the oxidation catalyst metals concentration as shown below in Figure 2.

The analysis of metal ions (Figure 2) allowed to evidence a reduction of all the ions evaluated in musts treated with the cationic resin. Moreover, the reduction of the ions was proportional to the resin treatment intensity determined by pH reduction. In a comparison between the control must and the strongest resin treatment (TR3), the highest decrease was observed in the calcium concentration (67%). The other divalent cations showed a reduction of 27% and 16%, for copper and magnesium, respectively. Among monovalent ions, both sodium and potassium showed similar reduction after resin treatment, 25.7 and 26%, respectively.

Although magnesium and copper are divalent cations, the results show a smaller reduction compared to monovalent sodium and potassium. This may be due to a lower concentration of these cations in the juice, decreasing the interaction with the resin. In regards to calcium, the affinity of the resin to these cations is relevant. Bearing a lower solubility than that of potassium, its stabilization is more difficult, where, upon removal, there is a lower possibility of calcium tartrate formation in the wine (Ribéreau-Gayon et al., 2007).

As reported by Creswell and Eschenbruch (1981) and Walker (2004), magnesium is very important in the process of industrial fermentation, being necessary to activate several glycolytic enzymes, influencing the multiplication and growth of yeasts. The magnesium reductions verified in the musts affected by the passage through the ion exchange resin may have contributed to the kinetic differences observed in the fermentations of the different treatments.

Copper content, in wines, has been widely studied for its interference in the fermentation kinetics of yeasts, as well as its role as a catalyst of wine oxidation (Azevedo et al., 2003; Danilewicz, 2003; Stehlik-Tomas et al., 2003). Benítez et al. (2002) analyzed 12 Chardonnay wines that had undertaken treatments with two types of ionic resin. The copper decrease ranged from 38.4% to 96.3%.

In the study of Ponce et al. (2018), carried out on laboratory and industrial scale, different metals content in wines under cationic resin treatment were quantified, including samples of the Chardonnay variety, it was also observed that, in almost all cases, occurred a decrease in the levels of Na⁺ with differences in pH similar to those of the present study. In the work of Tamasi et al. (2018), two types of resin were tested, and reductions of several cations were reported in sweet and dessert wines after treatment.

According to the results obtained in the grape musts analyses, the passage through the resin with the decrease in the pH index around 0.1 units (TR1 and TR2) was sufficient to significantly reduce the concentrations of potassium, sodium, calcium and color intensity, having a minor influence on the ammonium-nitrogen. The treatment TR3, with a higher decrease in pH, had a greater reduction in copper concentration although a significant decrease in the amount of AN was also observed.

### 3.2. Fermentation kinetics

The fermentation kinetics graph (Figure 3) shows the daily development of the fermentations, comparing the treatments. The obtained results showed differences in the fermentation time. The ST treatment finished fermentation in 10 days, while treatments TR1, TR2 and TR3 required 12, 13 and 14 days, respectively. At the end of the fermentations, we performed the analysis of reducing sugars, alcohol and volatile acid, and did not evidence significant differences, indicating that despite the kinetic differences all the treatments complete alcoholic fermentations within two weeks.

**Table 1** shows the values obtained through the modified Gompertz model for alcoholic fermentation parameters. The results (Table 1) showed that the greater the pH reduction, the shorter the lag phase time. This result may have been due to the decrease in some metals that inhibit the adaptation and growth of yeast such as copper (Figure 2. Cu), used as a fungicide in grape production (Azevedo et al., 2003; Danilewicz, 2003; Stehlik-Tomas et al., 2003).

For the total CO₂ released (Ymax), there is a tendency that the higher the pH reduction by resin treatment, the lower the CO₂ released during the whole fermentation (Table 1). In a previous study, Cisilotto et al. (2019) showed that wines obtained from cationic resin-treated must exhibit a lower concentration of acetates, who were perceived in the sensory analysis as lower fruity aromas. The relation between pH

![Fermentation kinetics](image)

**Figure 3.** Monitoring of alcoholic fermentation - monitoring of fermentation by CO₂ (g/L) detachment rate over the days of alcoholic fermentation.

|      | ST          | TR1        | TR2        | TR3        |
|------|-------------|------------|------------|------------|
| Lag  | 18.2 ± 0.53 a | 14.3 ± 0.63 b | 7.0 ± 0.99 c | 2.7 ± 0.91 d |
| Ymax | 69.8 ± 0.23 a | 69.1 ± 0.09 ab | 68.7 ± 0.11 b | 68.3 ± 0.07 b |
| Vmax | 18.36 ± 0.46 a | 16.39 ± 0.23 b | 14.33 ± 0.43 c | 12.61 ± 0.65 d |

Lag = lag phase (hours); Ymax = weight loss (CO₂ g/L); Vmax = maximum CO₂ release (g/L/day). Means values ± standard deviation followed by different letters within a line, are significantly different according to Tukey's test (P ≤ 0.05).
reduction, slow fermentation, and wine composition may be studied further.

The maximum rate of CO₂ released per day (Vmax), showed a reduction as the intensity of ion exchange treatments increased (Table 1). In addition to the increase in total acidity and the decrease in pH, the decrease in fermentation rate may be associated with the reduction of ammonium-nitrogen caused by passing through the resin (Figure 1. D). Decreased Mg concentration (Figure 2 B) may also have influenced the fermentation rate as a sum of factors, as it is an important element for yeast multiplication and metabolism (Creswell and Echenbruch, 1981).

4. Conclusions

The pH control through a pre-fermentation treatment with ion exchange resins is efficient, increasing the must acidity, decreasing the metals and the color. The reduction of ammonium-nitrogen suggests that in winery practice, this must correction analysis should always be performed after treatment with ion exchange resins. A metal reduction may be an important factor for better tannic stability from a must with lower potassium and calcium concentrations. The reduction in copper levels under resin treatment is interesting considering its catalytic role in oxidation, yeast inhibition, and heavy metal status. Significant differences occurred in the fermentation speed of up to four days to complete, which should be considered in the logistics of the companies during the grape harvest. According to the results obtained in the analysis of musts, we think that the moderate passes through the resin with decreases in the pH index around 0.06 and 0.13 units (TR1 and TR2) was more interesting because, despite not having decreased the copper concentrations, it was sufficient to reduce the amount of potassium, sodium, calcium and decrease the O.D. 420 nm, with little influence on ammonium-nitrogen.

Declarations

Author contribution statement

Bruno Cisilotto, Sergio Echeverrigaray: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Simone B. Rossato, Evandro Ficagna: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Luisa C. Wetzstein: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Angelo Gava, Gisele M. Gugel: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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