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# Antioxidant activity of sparkling wines produced by *Champenoise* and *Charmat* methods

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ABSTRACT

The objective of this study was to evaluate the antioxidant activity of 19 Brazilian sparkling wines produced by *Champenoise* and *Charmat* methods. All sparkling wines tested showed significant antioxidant activity, both *in vivo* and *in vitro* assays. In general, the *Charmat brut* possessed more antioxidant activity than *Charmat demi-sec* and *Champenoise* samples. In most of the sparkling wines studied, the majority compound found was gallic acid, although *trans*-resveratrol, (+)-catechin, (−)-epicathechin and procyanidins $B_1$, $B_2$, $B_3$ and $B_4$, were also identified. Significant differences were observed in the concentrations of these compounds, when considering the *assemblage* used and the production methods.

The wine industry around the world uses similar oenological technologies and the wines are divided into categories, for example, in relation to sugar concentration or elaboration methods. The findings of this study would help the wineries to determine the sugar contents and time to mature (*sur lie*) appropriate for sensorial characteristics desired by the winemakers and consumers. Furthermore, the data can offer an improvement in the biological properties of the sparkling wines.



## 1. Introduction

The process of making sparkling wines begins by obtaining the base wine from white grapes (*blanc de blancs*) or from white and red grapes (*blanc de noirs*). White base wines are obtained when fermentation takes place without contact between the must and the grape skins. The wines obtained will be red or *rosés*, depending on the time and intensity of this maceration (Hidalgo et al., 2004). The base wine is then submitted to a second fermentation, in order to produce carbon dioxide naturally by *Charmat* (in large containers) or *Champenoise* methods (in the bottle). Sparkling wines may be varietals (a single grape) or *assemblage*/*coupage* (two or more varieties and vintages) (Ribéreau-Gayón, Glories, Maujean, & Dubourdieu, 2003). The second fermentation can be followed by ageing of the wine with yeasts and the sparkling wines may be classified according to sugar content.

Sparkling wines are rich in phenolic compounds (Chamkha, Cathala, Cheynier, & Douillard, 2003; Ibern-Gómes et al., 2000; Pozo-Bayón, Hernández, Martín Álvares, & Polo, 2003), with known antioxidant activity (Cartron et al., 2003; Roig, Cascón, Arola, Bladé, & Salvadó, 2002; Satué-Garcia, Andrés-Lacueva, Lamuela-Raventós, & Frankel, 1999; Yilmaz & Toledo, 2004). The content of these compounds, however, depends on several factors, including variety of grape, fruit growth and ripening conditions, quality of the base wine, yeast used and *sur lie* (time needed to mature) (Cortell, Halbleib, Gallagher, Righetti, & Kennedy, 2005; Delgado, Martín, del Álamo, & González, 2004; Mazauric & Salmon, 2005, 2006).

Several studies have already been performed in order to evaluate the antioxidant activity of red and white wines (Cartron et al., 2003; De Beer, Joubert, Gelderblom, & Manley, 2003; Jamroz & Beltowski, 2001; Landrault et al., 2001); however, there are few data on the antioxidant capacity of sparkling wines (Cartron et al., 2003; Satué-Garcia et al., 1999). Furthermore, there are no reports on the influence of the different methods for making and/or sugar concentration on the biological activity of these wines.

Therefore, the purpose of this study was to determine the antioxidant capacity of Brazilian sparkling wines *in vitro* (scavenging capacity of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and *in vivo* (in eukaryotic cells of the *Saccharomyces cerevisiae* yeast). The influence of the different methods used in manufacture (*Charmat* or *Champenoise*), the concentration of sugar (*brut* or *demi-sec*) and the phenolic composition on the antioxidant activity of these wines was also evaluated.

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## 2. Material and methods

### 2.1. Samples

Nineteen sparkling wines were studied: 12 *Charmat* (seven *brut* and five *demi-sec*) and seven *Champenoise*, made by seven different wineries in the "Serra Gaúcha", the mountains situated in the south of Brazil. In each of these groups, the performance in relation to its respective base wine was evaluated too. The main characteristics of the sparkling wines used are shown in Table 1. These samples were made from 12 varieties: Pinot Noir (PN), Chardonnay (CH), Italian Riesling (IR), Semillon (SE), White Muscat (WMu), Merlot (ME), Cabernet Sauvignon (CS), Prosecco (PR), White Malvasia (WMa), Candia Malvasia (CMa), Canelli Muscat (CMu) and Alexandria Muscat (AM). The first fermentation (base wines) was performed at 15 °C for an average of 16 days. In the second fermentation, the mean temperature was 12 ± 2 °C with the foam formation time varying between 30 and 90 days. The ageing period varied from zero to 540 days (Table 1). Except for sparkling wines **9**, **10** and **11**, obtained from fermentations with *S. cerevisiae*, in all the others *S. bayanus* was used in both fermentations. In order to perform the assays, the sparkling wines were previously degassed, using a vacuum pump with a valve for air removal, coupled to a workbench agitator.

### 2.2. Chemical reagents

DPPH·, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, gallic acid and procyanidin $B_3$ were acquired from Sigma–Aldrich, St. Louis, MO. The procyanidins $B_1$, $B_2$ and $B_4$ were kindly provided by Dr. Regina Vanderlinde (Instituto Brasileiro do Vinho, Bento Gongalves, Brazil). The anthocyanins cyanidin-3-glycoside, delphinidin-3-glycoside, peonidin-3-glycoside and malvidin-3-glycoside were acquired from Extrasynthese, Genay, France. The other reagents were acquired from E. Merck, Damstadt, Germany.

### 2.3. Oenological analysis of sparkling wines

The alcohol content, total acidity, pressure, volatile acidity, pH, free and total $SO_2$, dry extract and reduced dry extract, concentration of sugar and ascorbic acid were determined using the methods described by Zoecklein, Fugelsang, Gump, and Nury (2000). All analyses were performed in duplicate.

### 2.4. Determination of polyphenols by UV spectrophotometry

Total polyphenols and total hydroxycinnamates were quantified by measuring the absorbances at 280 and 320 nm (UV-1700 spectrophotometer, Shimadzu, Kyoto, Japan), respectively. The results of total polyphenols were expressed as mg/l of catechin and those of hydroxycinnamates as mg/l of caffeic acid. The total flavonoids (*TF*) were calculated using the following formula, as described by Iland, Ewart, Sitters, Markides, and Bruer (2000):

$$TF = [(A_{280} - 4) - 0.66] \times (A_{320} - 1.4).$$

The results were expressed in mg/l of catechin. All the analyses were performed in duplicate.

### 2.5. Determination of polyphenols by HPLC

A 5 ml aliquot of each sample was filtered through a cellulose membrane with a 0.20 μm diameter just before the analysis of the major phenolic compounds by high performance liquid chromatography using a Hewlett–Packard (Palo Alto, CA) 1100 series

**Table 1**
Main characteristics of the sparkling wines (SW) studied.

| SW | *Assemblage* | Wineries | *Sur lie*[1] (days) | Ascorbic acid ± SD[2] (mg/l) | Sugar ± SD (g/l) | TP[3] (mg/l of C[6]) ± SD | *TF*[4] (mg/l of C) ± SD | THC[5] (mg/l of CA[7])* ± SD |
|---|---|---|---|---|---|---|---|---|
| *Charmat brut* | | | | | | | | |
| 1 | 40% PN, 60% CH | A* | 30 | 33.3 ± 0.03[a**] | 12.5 ± 0.23[a] | 354 ± 4.19[a] | 156 ± 2.94[a] | 39.8 ± 0.22[a] |
| 2 | 60% PN, 40% CH | B | 126 | 21.9 ± 0.01[b] | 5.48 ± 0.26[b] | 185 ± 7.13[b] | 52.3 ± 4.19[b] | 26.6 ± 0.61[b] |
| 3 | 50% IR, 30% SE, 20% WMu | C | No ageing | 28.9 ± 0.03[c] | 11.4 ± 0.08[c] | 164 ± 4.19[c] | 6.16 ± 1.68[c] | 31.5 ± 0.50[c] |
| 4 | 50% IR, 33% PN, 17% CH | B | 60 | 11.4 ± 0.04[d] | 9.33 ± 0.20[d] | 193 ± 1.68[b] | 23.8 ± 1.67[d] | 33.9 ± 0.55[d] |
| 5 | 30% IR, 70% CH | D | 60 | 21.8 ± 0.03[b] | 8.94 ± 0.17[d] | 520 ± 0.00[d] | 302 ± 1.67[e] | 43.7 ± 0.33[e] |
| 6 | 100% CH | E | 30 | 22.6 ± 0.06[b] | 10.5 ± 0.22[e] | 420 ± 3.35[e] | 220 ± 12.15[f] | 40.1 ± 3.05[a] |
| 7 | 40% IR, 10% ME, 50% CS | B | 193 | 32.6 ± 0.04[a] | 10.6 ± 0.29[e] | 1350 ± 3.35[f] | 780 ± 6.70[g] | 113 ± 0.66[f] |
| *Champenoise* | | | | | | | | |
| 8 | 40% PN, 60% CH | A | 180 | 11.9 ± 0.03[d] | 5.84 ± 0.14[b] | 362 ± 0.84[a] | 192 ± 1.67[h] | 34.1 ± 0.22[d] |
| 9 | 100% PR | F | 150 | 20.5 ± 0.02[e] | 7.28 ± 0.15[f] | 483 ± 5.86[g] | 234 ± 5.87[f] | 50.0 ± 0.05[g] |
| 10 | 100% CH | F | 540 | 16.7 ± 0.03[f] | 8.04 ± 0.19[d] | 620 ± 4.19[h] | 365 ± 4.19[i] | 51.3 ± 0.00[h] |
| 11 | 20% PN, 80% CH | F | 270 | 21.2 ± 0.01[b] | 7.24 ± 0.11[f] | 493 ± 2.93[g] | 242 ± 5.03[f] | 50.4 ± 0.39[g] |
| 12 | 10% CH, 60% ME, 30% PN | F | 365 | 79.4 ± 0.02[g] | 9.91 ± 0.07[d] | 2790 ± 41.90[i] | 1870 ± 22.63[j] | 185 ± 3.89[i] |
| 13 | 50% PN, 50% CH | F | 150 | 43.6 ± 0.04[h] | 6.59 ± 0.20[g] | 616 ± 37.71[h] | 301 ± 21.37[e] | 63.3 ± 3.33[j] |
| 14 | 20% PN, 80% CH | G | 365 | 46.5 ± 0.03[i] | 5.89 ± 0.19[b] | 375 ± 0.84[j] | 216 ± 0.42[f] | 31.8 ± 0.11[c] |
| *Charmat demi-sec* | | | | | | | | |
| 15 | 50% IR, 30% SE, 20% WMu | C | No ageing | 29.0 ± 0.03[c] | 37.5 ± 0.39[h] | 185 ± 0.84[b] | 15.4 ± 1.68[k] | 34.2 ± 0.16[d] |
| 16 | 30% IR, 70% CH | D | 60 | 19.1 ± 0.07[j] | 51.1 ± 0.14[i] | 509 ± 3.35[k] | 306 ± 9.22[e] | 42.8 ± 0.44[e] |
| 17 | 100% CH | E | 30 | 19.5 ± 0.04[j] | 54.4 ± 0.09[j] | 523 ± 3.35[d] | 297 ± 1.68[e] | 45.4 ± 0.33[k] |
| 18 | 56% WMa, 25% CMa, 10% CMu, 9% AM | B | 8 | 61.3 ± 0.03[k] | 36.2 ± 0.13[k] | 340 ± 0.84[l] | 146 ± 0.42[l] | 38.9 ± 0.05[a] |
| 19 | 74% IR, 14% PN, 12% CH | B | 30 | 15.7 ± 0.03[l] | 36.4 ± 0.04[k] | 217 ± 0.42[m] | 32.6 ± 0.42[m] | 36.9 ± 0.05[l] |

[1] Time needed to mature.
[2] Standard deviation.
[3] Total polyphenols (TP).
[4] Total flavonoids (*TF*).
[5] Total hydroxycinnamates (THC).
[6] Catechin (C).
[7] Caffeic acid (CA).
* Distinct letters corresponding to different wineries in the Serra Gaúcha/Rio Grande do Sul/Brazil.
** Data followed by different letters for each column differ significantly by Kruskal–Wallis *H* test ($p \leqslant 0.05$).

LC liquid gradient, with a Diode Array Detector (DAD). A Zorbax 300 SB C18 (12 mm × 4.6 mm × 5 μm) pre-column and a C18-ODS (150 mm × 4 mm × 5 μm) (Agilent Technologies, Santa Clara, CA) column were used. The specific phenols quantified were *trans*-resveratrol (Jeandet et al., 1995), anthocyanidins (OIV – Resolution OENO 22/2003), procyanidins $B_1$, $B_2$, $B_3$ and $B_4$, (+)-catechin, (−)-epicatechin and gallic acid (Lamuela-Raventós & Waterhouse, 1994).

### 2.6. Evaluation of antioxidant activity in vitro

The scavenging capacity of free radical DPPH· was measured by adding to the sparkling wines, pure or diluted in distilled water [0.1%; 1.0%; 10% and 50% (v/v)], a tris–HCl buffer solution (100 mM, pH 7.0) containing 250 μM of DPPH· dissolved in ethanol. In the control tube, sterilised distilled water was used in lieu of sparkling wines. The tubes were kept in the dark for 20 min and the absorbance was measured at 517 nm (Shimadzu UV-1700 spectrophotometer) (Yamaguchi, Takamura, Matoba, & Terão, 1998). The results were expressed in values of $IC_{50}$ (quantity of sparkling wine needed to reduce 50% of the free radical DPPH·), calculated by polynomial regression graphs (Mensor et al., 2001), using the mean of triplicates.

### 2.7. Evaluation of antioxidant activity in vivo

The assays *in vivo* were performed using cells of *S. cerevisiae* XV 185-14c yeast (MAT α ade 2-1, arg 4-17, his 1-7, lys 1-1, trp 1-1, trp 5-48, hom 3-10), kindly provided by Dr. R.C. Von Borstel (Department of Genetics, University of Alberta, Canada). Cell suspensions of $2 \times 10^6$ cells/ml obtained from the exponential growth phase were treated with hydrogen peroxide (75 mM), in the presence and absence of sparkling wines. The tubes were incubated for 1 h at 28 °C. Then the samples were diluted in a 0.9% (w/v) sodium chloride solution, seeded into a YPD culture medium (10 g/l of yeast extract, 20 g/l of peptone, 20 g/l of dextrose and 20 g/l of agar–agar) and incubated for 72 h at 28 °C. After incubation, the colonies were counted, and 100% survival was considered the total of colonies observed on the control plate (untreated cells) (Wilmsen, Spada, & Salvador, 2005).

### 2.8. Data analysis

The data were analysed using the following tests: Kruskal–Wallis *H*, Spearman Correlation and Principal Components Analysis (PCA), using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

## 3. Results and discussion

### 3.1. Oenological analysis and determination of polyphenols by UV spectrophotometry

The alcohol contents of the different sparkling wines analysed varied from 11.23% to 13.05% (v/v), and total acidity from 5.08 to 8.21 g/l of tartaric acid. The mean levels of pressure, volatile acidity and pH were 5.7 ± 0.2 atm, 0.588 ± 0.091 g/l of acetic acid and 3.29 ± 0.14, respectively. The mean concentration of free $SO_2$ was 20.0 ± 8.12 mg/l and total $SO_2$ was 122 ± 37.3 mg/l. The values were below the allowed level for volatile acidity, free $SO_2$ and total $SO_2$, indicating that the grapes were healthy and that good vinification practices were used (Boulton, Singleton, Bisson, & Kunkee, 1995). The analysis of the dry extract and reduced dry extract showed, respectively, values of 23.5 ± 3.57 and 19.1 ± 2.95 mg/l for the *brut* samples, and 58.3 ± 9.93 and 16.1 ± 2.69 mg/l for the *demi-sec* sparkling wines (data not shown). The sugar concentration varied from 5.48 to 12.5 g/l, for the *brut* samples, and from 36.2 to 54.4 g/l for the *demi-sec* sparkling wines (Table 1). These values are within the range established by Brazilian law (Brasil, 1990) for sparkling wines.

The ascorbic acid content of the sparkling wines studied varied from 11.4 to 79.4 mg/l (Table 1). Up to the present, there are few data in the literature concerning the content of this acid in sparkling wines. We know that its concentration is the result of the variety of grape, degree of maturity (Ribéreau-Gayón et al., 2003) and the amount of sunlight on the vine (Valpuesta & Botella, 2004), for example. Fig. 1a shows that sparkling wines had an increase in ascorbic acid concentration, in comparison to the base wine (samples **2**, **15** and **19**). Although the use of the ascorbic acid in wines is a well-known practice (Marks & Morris, 1993), we believe that this result is probably due to yeast metabolism (Hancock, Galpin, & Viola, 2000; Sauer, Branduardi, Valli, & Porro, 2004; Smir-

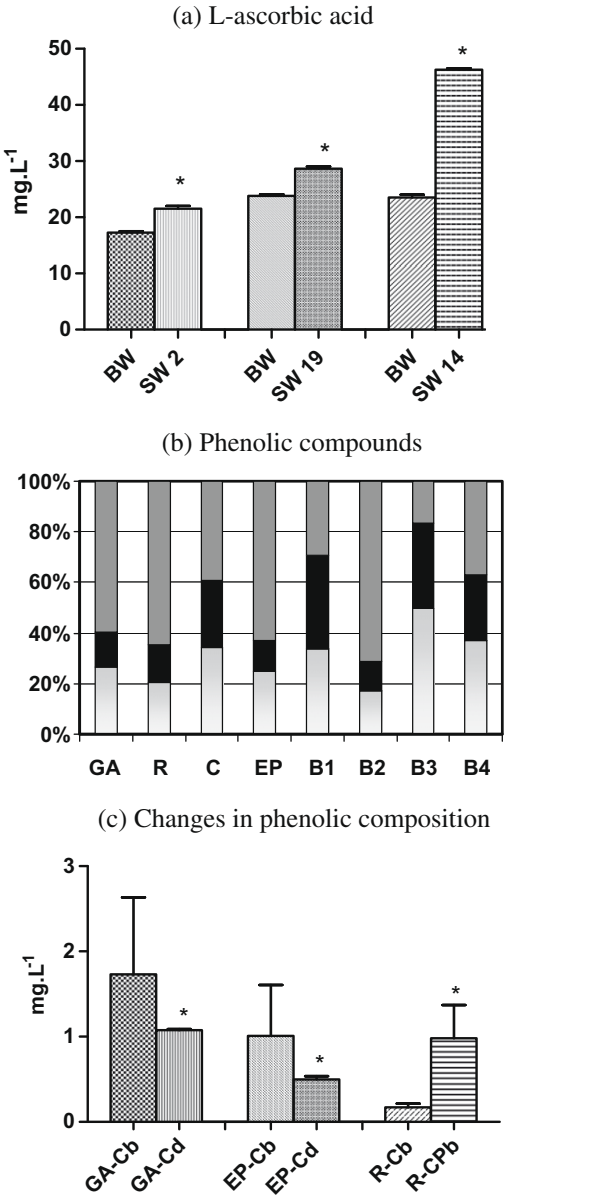


**Fig. 1.** Profiles of L-ascorbic acid (a) in base wine (BW) and respective sparkling wine (SW), polyphenols (b) by HPLC: □; *Charmat brut* (Cb), ▒; *Champenoise* (CPb), ■; *Charmat demi-sec* (Cd), gallic acid (GA), *trans*-resveratrol (R), catechin (C), epicatechin (EP), and procyanidins ($B_1$–$B_2$–$B_3$–$B_4$); and changes in phenolic composition (c) mediated by *sur lie* and sugar concentration. (a) L-Ascorbic acid, (b) phenolic compounds, and (c) changes in phenolic composition.

noff, Conklin, & Loewus, 2001), as there was no ascorbic acid addition to the sparkling wines assayed. Furthermore, in sample **14**, for which the *sur lie* period was 360 days (Table 1), the concentration of vitamin C practically doubled. New tests to evaluate the biosynthesis of ascorbic acid by oenological yeasts are currently being carried out as a result.

Higher values of total polyphenols, total flavonoids and total hydroxycinnamates were found, obviously, in red sparkling wines (samples **7** and **12**). As for the whites, major variations were observed, depending partially on the method by which the sparkling wine was made (Table 2). Sample **14** (*Champenoise*) showed a higher concentration of total polyphenols and total flavonoids compared to the base wine of origin. On the other hand, the concentration of these compounds diminished in *Charmat* samples (both *brut* and *demi-sec*) compared to their respective base wines (Fig. 1b). The mean reduction of total polyphenols and total flavonoids levels observed in *Charmat* sparkling wines was 24.58 ± 0.72% and 57.19 ± 4.22%, respectively. The concentration of total hydroxycinnamates diminished after the second fermentation, independent of the method by which they were made (Fig. 2), probably due to the action of yeasts, which can metabolise and/or adsorb up to 20% of the content of these compounds (Ribéreau-Gayón et al., 2003; Zoecklein et al., 2000).

### 3.2. Polyphenols analysis by HPLC

Fig. 1b shows the percentile differences on the phenolic profile of all sparkling wines. Some specific differences have been noted: sample **12** had the highest contents of all polyphenols analysed; larger amounts of *trans*-resveratrol, (+)-catechin and procyanidins $B_1$, $B_3$ and $B_4$ were observed in sample **7**; higher values of (−)-epicatechin were obtained in sample **1** and of procyanidin $B_2$ in sample **10**; in all sparkling wines analysed, the main phenolic component was gallic acid (data not shown).

**Table 2**
DPPH· mean values for the different sparkling wines analysed and mean survival values of the *Saccharomyces cerevisiae* yeast treated with hydrogen peroxide ($H_2O_2$) 75 mM in presence and absence of different sparkling wines.

| Sparkling wines | DPPH· | | Survival | |
|---|---|---|---|---|
| | $IC_{50}$* ± SD** | Rank# | ±SD (%) | Rank |
| *Charmat brut* | | | | |
| 1 | 11.80 ± 1.28[a] | 2 | 93.55 ± 0.25[a] | 1 |
| 2 | 13.03 ± 1.19[b] | 3 | 93.65 ± 4.75[a] | 1 |
| 3 | 1.83 ± 0.49[c] | 1 | 96.90 ± 3.10 [a] | 1 |
| 4 | 80.76 ± 2.75[d] | 8 | 100.00 ± 0.00[a] | 1 |
| 5 | 20.76 ± 5.12[e] | 4 | 78.70 ± 3.70[b] | 3 |
| 6 | 20.26 ± 0.32[e] | 4 | 96.75 ± 0.35[a] | 1 |
| 7 | 31.50 ± 0.42[f] | 5 | 62.43 ± 8.30[c] | 4 |
| *Champenoise* | | | | |
| 8 | 9.05 ± 1.04[a] | 2 | 84.22 ± 1.91[d] | 2 |
| 9 | 19.63 ± 0.63[e] | 4 | 56.80 ± 1.00[e] | 5 |
| 10 | 30.83 ± 2.01[f] | 5 | 53.58 ± 0.95[e] | 5 |
| 11 | 11.09 ± 1.81[a] | 2 | 34.80 ± 0.50[f] | 6 |
| 12 | 32.83 ± 0.13[f] | 5 | 85.50 ± 0.90[d] | 2 |
| 13 | 16.77 ± 0.54[b] | 3 | 65.41 ± 1.21[c] | 4 |
| 14 | 39.53 ± 1.53[g] | 6 | 85.05 ± 3.35[d] | 2 |
| *Charmat demi-sec* | | | | |
| 15 | 26.87 ± 0.58[e] | 4 | 65.45 ± 1.05[c] | 4 |
| 16 | 32.56 ± 0.43[f] | 5 | 64.85 ± 2.35[c] | 4 |
| 17 | 25.04 ± 0.13[e] | 4 | 79.20 ± 3.60[b] | 3 |
| 18 | 23.73 ± 0.57[e] | 4 | 100.00 ± 0.00[a] | 1 |
| 19 | 26.10 ± 1.93[e] | 4 | 95.30 ± 4.70[a] | 1 |
| Catechin (control) | 63.68 ± 1.42[h] | 7 | | |
| $H_2O_2$ (control) | | | 27.95 ± 0.25[g] | 7 |

* $IC_{50}$ (% of amount of samples necessary to scavenge 50% of DPPH·).
** Standard deviation.
# Rank in crescent order according to statistical significance (Kruskal–Wallis *H* test, $p \leqslant 0.05$) among the values of each parameter.

So far no studies have been performed on the phenolic composition of *Charmat* sparkling wines. As to the *Champenoise*, it was observed that the Brazilian sparkling wines possessed similar phenolic profiles to those reported for Spanish sparkling wines (Ibern-Gómes et al., 2000; Pozo-Bayón et al., 2003; Satué-Garcia et al., 1999), as well as in French Champagnes (Chamkha et al., 2003). To our knowledge this is the first report on the presence of procyanidins $B_1$, $B_2$ and $B_4$ in sparkling wines.

For the red and *rosé* sparkling wines the contents of four important anthocyanins found in red grapes and wine (Zoecklein et al., 2000) were also quantified, and as expected, sample **13** (*rosé*) possessed lower concentrations among the four compounds analysed compared to samples **7** and **12** (red). The main and most plentiful anthocyanin found in red varieties, malvidin monoglycoside (Ribéreau-Gayón et al., 2003), was the main compound in the three sparkling wine samples evaluated (data not shown).

Fig. 2 shows the phenolic profile of the samples assayed. The second fermentation of base wines increased the concentration of gallic acid in the *brut* sparkling wines, probably due to the hydrolysis of procyanidins that are esterified with this acid (Jordão, 2000; Stevens et al., 2002). Conversely, in *demi-sec* sparkling wines this type of reaction seems to be a disadvantage, suggesting that sugar concentration affects the phenolic composition. The same relationship was verified on (−)-epicatechin values. A higher concentration in *brut* (*Charmat* or *Champenoise*) sparkling wines than in their respective base wines were observed (Fig. 2), and no changes were observed in the *demi-sec* sparkling wines compared to the respective base wines.

The *trans*-resveratrol contents (Fig. 1b) were lower in the *Charmat* sparkling wines (both *brut* and *demi-sec*), compared to the base wines, probably due to the finishing stages, such as clarification and or filtration (Threlfall, Morris, & Mauromoustakos, 1999; Vrhovsek, Wendelin, & Eder, 1997). With the *Champenoise* method, however, there was a higher content of this compound than in its base wine. Both the malolactic fermentation (Pezet & Cuenat, 1996) and the action of yeasts with over expression of the β-glycosidase enzyme (Vrhovsek et al., 1997) may increase the concentration of *trans*-resveratrol in wines. Furthermore, sample **5** was obtained with a longer time *sur lie* compared to the other samples (Table 1). A positive correlation was observed between the concentration of *trans*-resveratrol and the *sur lie* time ($r = 0.456$; $p = 0.05$), suggesting an effect of contact time between the yeasts and the sparkling wine in the *trans*-resveratrol concentration. New assays to evaluate the β-glycosidase performance during the vinification are currently being carried out.

Comparing the base wines, it is observed that the (+)-catechin concentration changes in relation to the *sur lie* (Table 1). Samples **3**, **15** and **19**, elaborated with minimal *sur lie* times did not show differences. For a medium *sur lie* (sample **2**), the values of this phenolic compound increased. Conversely, for greater periods of *sur lie*, the (+)-catechin levels were lower (sample **14**). A negative correlation was found between the reduced dry extract content (which had a direct relationship with the yeast metabolism) and the concentration of (+)-catechin ($r = -0.619$; $p = 0.01$). Procyanidin $B_1$, $B_2$, $B_3$ and $B_4$ contents were lower after the second fermentation (data not shown). It is possible that, as seen in red wines, this reduction is due to condensation reactions, e.g., of procyanidins with proteins and polysaccharides, to the polymerisation reactions among the different procyanidins and to oxidative degradation phenomena (Lopez-Toledano, Mayen, Merida, & Medina, 2002; Ribéreau-Gayón et al., 2003). These interactions may be influenced by the different techniques adopted during the manufacturing/maturing process (Boulton et al., 1995; Flanzy, 2003; Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003), accounting, at least in part, for the differences found.

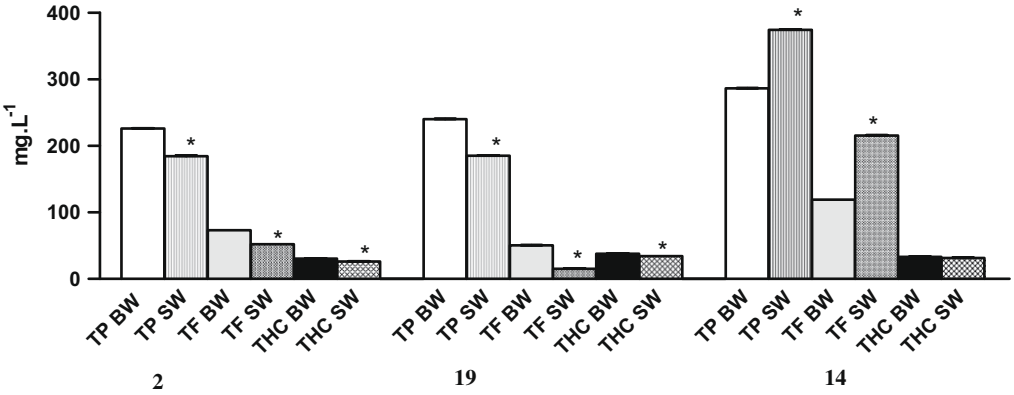


**Fig. 2.** Level of total polyphenols (TP), total flavonoids (*TF*) and total hydroxycinnamates (THC) in base wines (BW) (**2**, **19** and **14**) and in their corresponding sparkling wine (SW) *Charmat brut* (**2**), *Charmat demi-sec* (**19**) and *Champenoise* (**14**).

### 3.3. Evaluation of antioxidant activity in vitro

Table 3 shows that sample **3**, which was elaborated without *sur lie* (Table 1) possessed the highest degree of antioxidant activity ($IC_{50}$ = 1.83 ± 0.49%). Furthermore, sparkling wines **6** (*Charmat*) and **10** (*Champenoise*) were prepared exclusively with the Chardonnay variety, and the greatest capacity for scavenging of free radical DPPH· (Table 3) was observed in sample **6**, which was produced with a small *sur lie*, and possessed a higher ascorbic acid content (Table 1) and a higher concentration of major phenolic compounds analysed by HPLC than sample **10** (Table 2).

Sparkling wines **11** and **14** were prepared using the *Champenoise* method by different wineries. Sample **11** possessed a greater capacity to scavenge free radical DPPH· than sample **14** (Table 3). The latter showed lower (+)-catechin, (−)-epicatechin, gallic acid and procyanidin $B_1$, $B_2$ and $B_4$ contents (Table 2), indicating the influence of the vinification techniques (as for example the *sur lie* period) adopted by each winery on the phenolic composition and antioxidant activity of the sparkling wines. Interestingly, the similar *assemblage* (40% Pinot Noir and 60% Chardonnay) submitted to different methods of vinification in the same winery showed small differences in the antioxidant capacity. Sample **8** *sur lie* (Table 1; *Champenoise*), which presented higher antioxidant activity than sparkling wine **1** (*Charmat*) was only a little greater (Table 2), suggesting one more time the influence of this technique. Higher values of total flavonoids and procyanidins $B_2$ and $B_3$ (Table 2) were found in sparkling wine **8**. New assays about the influence of the *sur lie* time on the antioxidant activity and phenolic profile are being currently carried out. Beyond *sur lie*, the sugar concentration also influenced results.

**Table 3**
Variance explained by the first principal components (PC).

| PC | Eigenvalue | Explained variance (%) | Cumulative variance (%) |
|---|---|---|---|
| *Charmat brut* | | | |
| 1 – *TF*[a] | 11.83 | 51.45 | 51.45 |
| 2 – DE[b] | 4.70 | 20.44 | **71.89** |
| *Champenoise* | | | |
| 1 – *Sur lie* | 15.94 | 69.29 | 69.29 |
| 2 – TP[c] | 3.92 | 17.03 | **86.32** |
| *Charmat demi-sec* | | | |
| 1 – Sugar | 12.41 | 53.95 | 53.95 |
| 2 – Alcohol | 4.37 | 19.00 | **72.95** |

[a] Total flavonoids.
[b] Dry extract.
[c] Total polyphenols.

Samples of *Charmat brut* **3**, **5** and **6** showed a higher antioxidant capacity than their respective *demi-sec* peers (samples **15**, **16** and **17**) (Table 2). Higher values of (+)-catechin, procyanidin $B_2$ and gallic acid (Table 1) were found in these *brut* sparkling wines, compared to their *demi-sec* peers.

Apparently, antioxidant activity does not depend exclusively on the total polyphenols content. Samples **7** and **12** (red sparkling wines) with significant amounts of phenolic compounds (Tables 1 and 2) did not show the highest antioxidant activity. Studies have already demonstrated that the biological activity of resveratrol, specifically the inhibition of the tyrosinekinase protein p56, is diminished when glycosilation of the hydroxyl groups occurs (Soleas, Diamandis, & Goldberg, 1997). Therefore, it is possible that some polyphenols, when connected to carbohydrates (for instance the anthocyanidins of red wines) possess less antioxidant activity than their respective aglycones, which might account at least in part for the results observed. Among the red sparkling wines, the presence of a negative correlation between the antioxidant capacity *in vitro* and the concentrations of cyanidin-3-glycoside, peonidin-3-glycoside and malvidin-3-glycoside (all with a value of $r$ = 0.985) and delphinidin-3-glycoside ($r$ = 1), at a level of significance of $p$ = 0.01 corroborates this hypothesis. New assays examining the sugar influence on antioxidant activity are being currently carried out.

### 3.4. Evaluation of the antioxidant activity in vivo

In order to determine antioxidant activity *in vivo*, the highest non-cytotoxic concentration of sparkling wines was used, i.e., 10.0% (v/v) (data not shown). All the samples evaluated were able to protect the yeast cells against damage caused by hydrogen peroxide (Table 3).

Of the two red sparkling wines studied, sample **12**, which possessed the highest polyphenol and ascorbic acid contents of all the sparkling wines evaluated (Tables 1 and 2), showed higher antioxidant activity *in vivo* than sparkling wine **7** (Table 2).

Differences in the antioxidant activities of sparkling wines because of the *Charmat* and *Champenoise* methods used to make them are shown in this study. Similarly to what was observed *in vitro*, the *Charmat brut* sparkling wines possessed on average a higher antioxidant capacity (88.85 ± 1.79) than those prepared by the *Champenoise* method (60.77 ± 3.31), including the cases in which the sparkling wines were made using the same varieties/*assemblages* and/or by the same wineries (i.e., samples **1** and **8**, and **6** and **10**).

The greatest differences between the two methods of making sparkling wines are the *sur lie* time and the area of contact between

the sparkling wine, the yeasts, vinary containers and sugar concentration. It has already been demonstrated that the yeasts are able to adsorb different compounds present in the wines, including catechin and epicatechin (Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003). The samples of *Charmat brut* (**1** and **6**) prepared with a shorter *sur lie* time than their respective peers *Champenoise* (samples **8** and **10**) (Table 1), possessed higher (+)-catechin and (−)-epicatechin contents (Table 2). A negative correlation was observed between the *sur lie* period and antioxidant activity *in vivo* ($r = -0.519$; $p = 0.01$), i.e., the longer the *sur lie* period, the smaller was the antioxidant capacity of the sparkling wines. This correlation was even greater in the group of *Champenoise* sparkling wines ($r = -0.842$; $p = 0.05$). The statistical analysis of the principal components (PCA), for all sparkling wines, revealed that the first two principal components explain more than 70% of the total variance (Table 3). This analysis corroborated our data, since the *sur lie* variable appears as one of the factors of biggest influence on the antioxidant activity in the *Champenoise* (Fig. 3a), when compared with *Charmat* sparkling wines (Fig. 3b and c). Furthermore, the *Charmat demi-sec* sparking wines (samples **15**, **16** and **17**) possessed lower antioxidant activity (Table 2) than observed for their respective *Charmat brut* peers (samples **3**, **5** and **6**), similarly to what was observed *in vitro*. Negative correlations between the *in vivo* antioxidant capacity and the sugar contents ($r = -0.476$; $p = 0.01$) and dry extract ($r = -0.346$; $p = 0.05$) were found in these two groups of sparkling wines. This is the first time that differences are shown in the antioxidant potential of sparkling wines as a function of its sugar concentration. Orange juices with added sugar showed less antioxidant activity than juices without added sugar (Franke et al., 2004), corroborating the results found in our work. The PCA analysis showed that, for the group of *Charmat demi-sec* sparkling wines, the sugar concentration was one of the main variables that had influenced the antioxidant response of these wines (Fig. 3b).

The phenolic compounds have an acknowledged antioxidant capacity, and can scavenger free radicals, chelate metals, and diminish lipid peroxidation (Cartron et al., 2003; Jamroz & Beltowski, 2001; Roig et al., 2002; Yilmaz & Toledo, 2004). Polyphenol formation by the vine is influenced by natural factors, such as variety of grape, genetic susceptibility to diseases, climate and soil, besides viticultural management (rootstock, vigour, exposure to sunlight as a function of the conduction system, fertilisation, etc.; Cortell et al., 2005; Delgado et al., 2004; Ribéreau-Gayón et al., 2003). Oenology also plays an important role in determining the phenolic profile of the final product, using techniques such as industrial maturing, press yield, maceration time and temperature, yeast used, fermentation period, clarification, stabilisation, filtration, maturing, ageing, etc. (Mazauric & Salmon, 2005; Ribéreau-Gayón et al., 2003).

In this study, the phenolic compounds showed an important role in the antioxidant activity. Interestingly, the PCA analysis showed a stronger association between the phenolic compounds and the antioxidant activity for the *Champenoise* than *Charmat* sparkling wines (Fig. 3). Therefore, the role of phenolic compounds may be greater in the antioxidant activity of the sparkling wines made by the *Champenoise* method.

Summarising, the results presented in this study show that: (a) the *Charmat* and *Champenoise* sparkling wines, both *brut* and *demi-sec*, possess significant antioxidant activity, which is associated with the presence of phenolic compounds; (b) *Charmat brut* sparkling wines possess higher antioxidant activity than *demi-sec Charmat* and *Champenoise*; and (c) there are major differences in the concentrations of phenolic compounds in sparkling wines as a function of the type of grape/*assemblage* used and the method used for manufacture.

New hypotheses about sparkling wine antioxidant activity, in relation to phenolic composition, were discovered with our tests and they will be evaluated individually. However, data shown in this work suggest that it is possible to obtain a specific phenolic profile in sparkling wines by the oenological practices adopted, thus enabling the production of sparkling wines with greater antioxidant activity and thus with a higher added value. Furthermore,

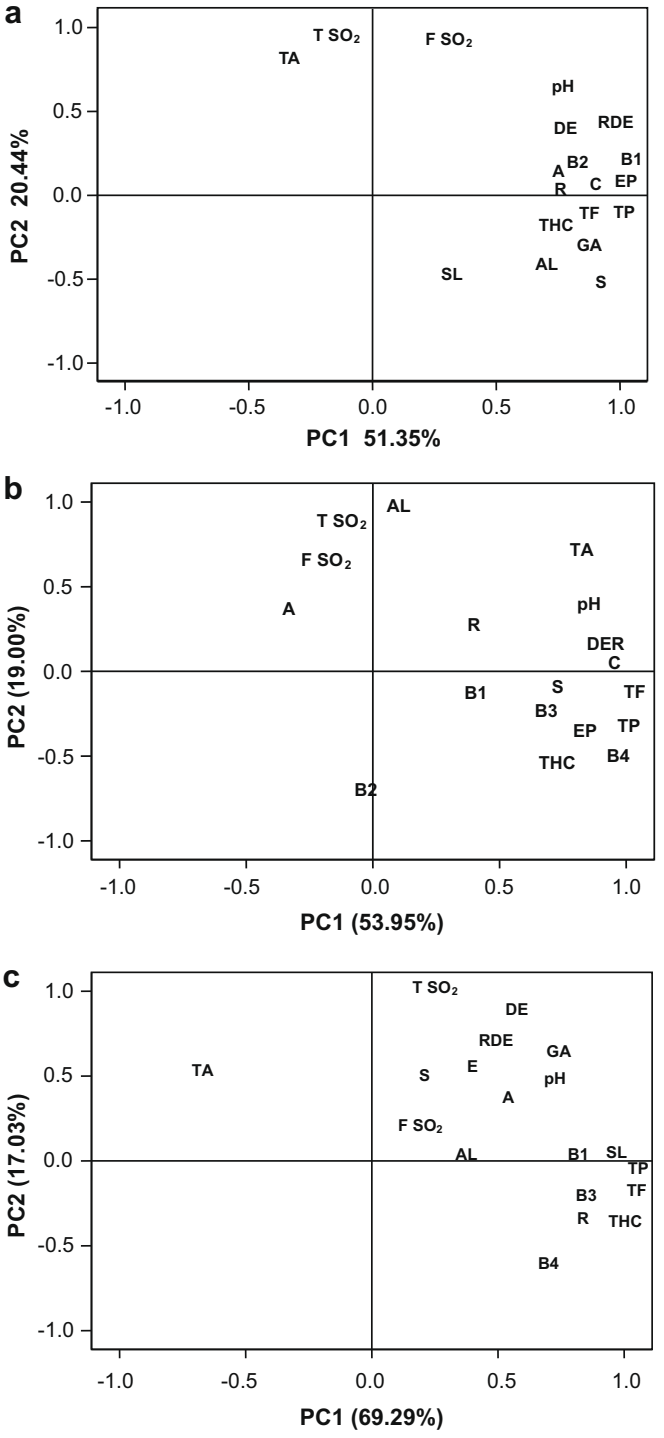


**Fig. 3.** Scores plots (PC1 vs. PC2) of the phenolic compounds and main characteristics of sparkling wines (a) *Champenoise*, (b) *Charmat demi-sec* and (c) *Charmat brut* (pH, *trans*-resveratrol (R), L-ascorbic acid (A), sugar concentration (S), catechin (C), procyanidins ($B_1$–$B_2$–$B_3$–$B_4$), gallic acid (GA), epicatechin (EP), total polyphenols (TP), total flavonoids (*TF*), total hydroxycinnamates (THC), dry extract (DE), reduced dry extract (RDE), total acidity (TA), *sur lie* (SL), alcohol (AL) and free and total $SO_2$ ($FSO_2$–$TSO_2$)).

the moderate/guided consumption of sparkling wines may be a positive choice in seeking a healthy life.

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