Short Communication

Antioxidant Activity and Phenolic and Mineral Content of Rose Grape Juice

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ABSTRACT There are many studies related to the antioxidant activity of grape products; however, they concern only purple
and white grape varieties. Up to now, there are no reports of studies on the Goethe rose grape variety, either on its an-
tioxidant activity or on its phenolic and mineral quantification. Thus, the purpose of this study was to evaluate in vitro
and in vivo antioxidant activity, as well as to quantify total phenolic compounds, ascorbic acid, and mineral content, in a Goethe
rose grape juice. The results obtained showed that the Goethe rose grape juice is a great polyphenol source, which contains
catechin, epicatechin, and procyanidins (B1, B2, B3, and B4). Of all metals analyzed, potassium, calcium, magnesium, and
iron showed the highest values. We found that this rose grape juice shows an important antioxidant activity in in vitro (2,2-
diphenyl-1-picrylhydrazyl radical scavenging activity) and in vivo (using the Saccharomyces cerevisiae yeast cells) assays.
The antioxidant activity could be explained by the significant phenolic content and ascorbic acid levels found in the juice.
The results showed that rose grape juice is an excellent antioxidant source, which could contribute to the prevention of many
diseases related to oxidative stress, such as atherosclerosis and Parkinson’s disease.

KEY WORDS: • antioxidant • ascorbic acid • grape juice • phenolic compounds

INTRODUCTION

It has already been reported that a moderate intake of grape products—wines or juices—have health pro-
tection effects. In this sense, some of these activities can be attributed to the phenolic compounds present in
grapes.1 Grape juices can be made from hundreds of differ-
ent grape varieties, but the Vitis labrusca species, which includes the Goethe variety, is the most commonly
used. This is a rose grape variety, also known as Roger’s, originally from the United States, but which can also be
found in several other countries, e.g., Brazil, China, Ger-
many, France, India, and Italy, according to the 2002 Eu-
ropean Vitis Database.2

As already reported in other studies, white and purple
grape juices are a source of antioxidants, especially of pheno-
lic compounds such as catechin and procyanidins, which have well-known antioxidant activity.3,4 Neuropro-
tective and hepatoprotective effects in Wistar rats were also attributed to grape juices.5,6 However, no study has
yet been conducted to quantify the chemical and pheno-
lic compounds of rose grape juice (RGJ) and their an-
tioxidant activity.

Thus, the purpose of this study was to evaluate the ca-
pacity of RGJ to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (in vitro assay) as well as its in vivo an-
tioxidant activity, by using Saccharomyces cerevisiae yeast cells exposed to hydrogen peroxide (H2O2). This study was
undertaken to extend the database on (+)-catechin, (−)-epi-
catechin, procyanidins, and vitamin C amount and mineral
content in RGJ.

MATERIALS AND METHODS

Samples

The RGJ, produced in 2005 from V. labrusca Goethe va-
riety grapes, was kindly donated by a local winery and was
kept in a controlled-temperature room. The main charac-
teristics of the juice are shown in Table 1.
TABLE 1. MAIN CHARACTERISTICS OF THE GOETHE RGJ

<table>
<thead>
<tr>
<th>Parameter analyzed</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total acidity (g/100 mL)</td>
<td>0.497 ± 0.02</td>
</tr>
<tr>
<td>Volatile acidity (g/100 mL)</td>
<td>0.018 ± 0.01</td>
</tr>
<tr>
<td>Ethyl alcohol (% vol/vol)</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>Relative density 20/20°C</td>
<td>1.0770 ± 0.03</td>
</tr>
<tr>
<td>pH</td>
<td>3.37 ± 0.01</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>12.51 ± 0.05</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Alimentary fiber (%)</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>86.48 ± 0.02</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.25 ± 0.00</td>
</tr>
<tr>
<td>Caloric value (kJ/100 mL)</td>
<td>214 ± 0.20</td>
</tr>
</tbody>
</table>

**Chemicals**

DPPH, trans-resveratrol, (+)-catechin, (-)-epicatechin, gallic acid, and procyanidins B1, B2, B3, and B4 were all obtained from Sigma-Aldrich (St. Louis, MO). The anthocyanin pigments cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside were obtained from Extrasynthese (Gennai, France). Methyl parathion was obtained from Bayer, and the acetylcholinesterase kit was bought from UFRJ (Rio de Janeiro, Brazil). All other chemicals were purchased from E. Merck (Darmstadt, Germany).

**Chemical analysis and nutritional evaluation of the grape juice**

Alcoholic grade, total acidity, volatile acidity, pH, total SO2, and ascorbic acid were determined using the methods described by Zoecklein et al.7 All analyses were performed in duplicate. The levels of carbohydrates, food fiber, saturated fats, proteins, humidity, and caloric value were determined according to the official methodologies of analysis of the Association of Official Analytical Chemists International.8

**Phenolic compound content**

Total phenol content was quantified using the Singleton and Rossi modification of the Folin-Ciocalteau colorimetric method.9 High-performance liquid chromatography analysis was used to quantitate individual phenolic compounds. Before high-performance liquid chromatography analysis, 5 mL of each sample was filtered through a cellulose membrane with a 0.20-mm diameter. The equipment used in the analysis consisted of a liquid gradient chromatographic system (LC-DAD Series 1100, Hewlett Packard, Palo Alto, CA) with a diode array detector system. A Zorbax 300 SB C18 (12 mm × 4.6 mm × 5 μm) precolumn and a C18-ODS (150 mm × 4 mm × 5 μm) column (Agilent Technologies, Palo Alto) were used.

In order to quantify procyanidins B1, B2, B3, and B4, (+)-catechin, (-)-epicatechin, and gallic acid we used a mobile phase with solvent A (50 mM ammonium hydroxide diphosphate, pH 2.6), solvent B (20% solvent A and 80% acetonitrile), and solvent C (0.2 M orthophosphoric acid, pH 1.5), in a constant flow of 0.5 mL/minute, in a controlled-temperature room at 40°C. The peak was detected at 204 nm, and the amount of sample injected was 5 μL. The elution conditions were standardized according to the procedure of Lamuela-Raventós and Waterhouse.10

**Particle-induced X-ray emission (PIXE)**

Metals quantified in grapes were analyzed by means of the PIXE method.11 In this assay, 400 mL of RGJ was dried and transformed in tablets. PIXE analysis was carried out at the 3-MV Tandetron accelerator facility at the Instituto de Física of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. All measurements were performed using a 2-MeV proton beam with an average current of 5 nA. The acquisition time for each sample was approximately 10–20 minutes. The beam spot at the target position was about 9 mm2. The filters containing grape juice, blank, and calibration targets were placed in a target holder, which accommodates up to 10 samples. Each sample was positioned in the proton beam by means of an electric-mechanical system. The characteristic X-rays induced by the proton beam were detected by a high-purity germanium detector (GLP series, EG&G ORTEC® Redwood City, CA), with an energy resolution of 180 eV at 5.9 keV. The detector was positioned at 45°C in relation to the beam axis. The electronics consisted of a Telenec 245 amplifier associated with a PCA3 multichannel analyzer (Oxford Instrument, Memphis, TN), running on a PC-compatible computer. GUPIX code12 was used for data analysis.

**Antioxidant activity**

The antioxidant activity of the juice was measured by in vitro (DPPH radical scavenging activity) and in vivo (S. cerevisiae yeast cells) assays.

Scavenging of DPPH was measured using a method modified from that of Yamaguchi et al.,13 in which grape juice solutions were added to obtain final concentrations of 0.1%, 1.0%, 10.0%, 50.0%, and 100.0% (vol/vol). The tubes were stored in the dark for 20 minutes, after which absorbance was measured at 517 nm. The results were expressed as the concentration of juice necessary to scavenge 50% of DPPH radical (IC50). The control used distilled water instead of antioxidant solutions. Catechin was used as the standard.

The determination of the in vivo antioxidant activity of the juice was performed using eukaryotic cells of S. cerevisiae XV 185-14c yeast (MATa, ade2-2, arg4-17, his1-7, lys1-1, trp5-48, hom3-10), kindly provided by Dr. R.C. Von Borstel, Department of Genetics, University of Alberta, Edmonton, AB, Canada, treated with H2O2 to induce oxidative stress.14–16 Suspensions containing 2 × 107 cells/mL (exponential phase), with or without H2O2 (75 mM) and different concentrations of the juice, were incubated for 1 hour.
The juice concentrations chosen for this assay were 10%, 25%, and 50% (vol/vol), the last one being the highest non-cytotoxic dose. After incubation, samples were diluted in 0.9% (wt/vol) saline solution, plated onto YPD (0.5% yeast extract, 2% bactopeptone, and 2% glucose), and incubated for 48 hours at 28°C. Colonies were then counted and compared to the control plates, which were considered to represent 100% of survival of yeast cells.

**Statistical analyses**

Except for catechin, epicatechin, and procyanidins, all assays were performed in triplicate. Data were analyzed by analysis of variance, and means were compared using Tukey’s test. All tests used the SPSS version 12.0 software package (SPSS, Chicago, IL).

**RESULTS**

The main findings about RGJ are shown in Table 1. Through these results we can observed that RGJ is an important nutritional source. Total phenolic compounds found in RGJ were 156.6 ± 5.15 mg of catechin/mL of juice. Specific phenolic compounds found and their contents were catechin (2.2 ppm), epicatechin (1.68 ppm), and procyanidins B1 (4.22 ppm), B2 (1.95 ppm), B3 (2.14 ppm), and B4 (1.71 ppm). In a comparison with White Niagara and Purple Bordo, it was seen that RGJ presented higher levels of ascorbic acid, carbohydrates, and fibers among these three kinds of juices (Table 2).

Fifteen metals were analyzed in RGJ. The main metals found were potassium (290.9 ppm), calcium (97.7 ppm), magnesium (96.5 ppm), and iron (65.8 ppm). Phosphorus, chloride, sulfur, copper, zinc, and manganese were found in lower levels (<10 ppm). The remaining five metals (sodium, aluminum, silicon, titanium, and nickel) were below the level of detection.

In the in vitro assay, RGJ showed an IC_{50} value of 14% (catechin standard, 8.03%). In the in vivo assay, noncytotoxic concentrations (10%, 25%, and 50% vol/vol) of RGJ were chosen for the test. RGJ was able to inhibit damages caused by the stressing agent (H_{2}O_{2}) in all concentrations. The 50% and 25% (vol/vol) concentrations showed the same antioxidant activity, both being better than the 10% (vol/vol) concentration (Fig. 1).

**DISCUSSION**

It is well known that grapes and grape juices are an important nutritional source. RGJ was shown to be an important nutritional source with significant vitamin C and polyphenol contents. RGJ presented higher values of ascorbic acid, carbohydrates, and alimentary fiber than Niagara white and Bordo purple commercial grape juices analyzed in another study by our group (Table 2). The difference could be attributed to several factors, such as grape cultivar, soil, climate, processing methods, etc.

Although there are other juices richer in ascorbic acid content than RGJ, e.g., orange (1,379 mg/L) and grapefruit (337 mg/L), RGJ juice showed a significant ascorbic acid content (48.4 mg/L) similar to other fruit juices such as peach (66 mg/L) and apple (57 mg/L). Vitamin C plays a protective role against reactive species generated during photosynthesis and the respiration processes in plants and also has antioxidant and antimutagenic effects.

RGJ was richer in total phenolic (156.6 ± 5.15 mg of catechin/mL) content than the Bordo (119 ± 5.33) and Niagara (39.95 ± 1.06) commercial grape juices (Table 2) analyzed by our group. Many factors could influence the phenolic content, for example, the grape juice production process and the ripeness level. According to the literature, the main compounds found in grape juices made from *Vitis vinifera* and *V. labrusca* are (–)-catechin, (+)-epicatechin, and the procyanidins dimers. RGJ showed higher catechin (2.2 ppm) values when compared with a *V. labrusca* purple grape.

![FIG. 1. Survival of *S. cerevisiae* strains untreated and treated with RGJ and/or the stressing agent H_{2}O_{2} (75 mM). Different letters correspond to values statistically different by analysis of variance and Tukey’s post-test: *P* < .05.](image)
juice (1.2 ppm) (Concord variety).2 This difference occurs because phenolic compounds are secondary metabolites produced and accumulated in plant tissues, and changes in phytopathogenesis, among other factors, may result in different concentrations of these compounds in plant organs.22

This is the first study showing the mineral composition of the V. labrusca grape juice. Minerals, such as iron, magnesium, sodium, and others, have important physiological functions in the human body.23 Although more studies are necessary, RGJ is capable of supplying minerals important to human health.

Except for one study showing in vitro antioxidant activity of white grape juice,24 all other studies about antioxidant activity of grape juices are related to juices produced with V. vinifera purple grapes.1,24,25 This is the first study that showed the in vitro and in vivo antioxidant activity of RGJ from V. labrusca species (Goethe variety). In vivo antioxidant activity was assessed using S. cerevisiae yeasts cells, which are a useful model to screen natural antioxidants.14–16

In this model, RGJ was able to protect yeast cells from damages induced by H2O2. This important biological activity could be attributed, at least, in part, to high levels of phenolic compounds and ascorbic acid found in RGJ. In fact, it has already been shown that phenolic compounds and ascorbic acid contents are related to antioxidant activity.22,26,27

Although the results show an important antioxidant activity of RGJ, in both in vitro and in vivo assays, more studies are needed. The data are very useful since RGJ could become an important component in the diet of adults and children. Our findings show that RGJ could be considered a potentially active compound to be used in conditions where reactive oxygen species are involved, thus protecting against important diseases, such as arteriosclerosis, Parkinson’s disease, cancer, and others.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES


