Polyphenols and Antioxidant Content in Grape Juice

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ABSTRACT

Changes of polyphenols (PP) content and antioxidant capacity were investigated in grape juice produced from the cultivars Isabel, BRS Violeta, BRS Rúbea, BRS Carmem and from the selections 1 and 2, in four harvest periods (Tb1, two weeks before; Tb2 one week before; Tn, normal harvest; Ta, one week after the normal harvest). Statistical analysis performed within Tn of each cultivar and selection revealed that the trolox equivalent antioxidant capacity (TEAC) presented highly significant differences for all cultivars ($P<0.01$), except between Isabel and Carmem ($P>0.05$). Significant ($P<0.05$) and very significant ($p<0.01$) differences were observed for total PP. No significant differences were observed between the lowest producers Isabel and Carmem, between the highest producers Violeta and selection 2 and between selection 1 and Violeta ($P>0.05$). A significant difference was found between Rúbea and selection 2 ($P<0.05$). The difference among all other means were highly significant ($P<0.01$). Some cultivars and selections exhibited a gradual and significant increase of TEAC throughout the period of harvest.

RESUMEN

La composición del jugo de uva es influenciada por la variedad de la uva y su madurez. Cambios en el contenido de polifenoles (PP) y capacidad antioxidante (TEAC) fueron investigadas en el jugo de uva producido de las variedades Isabel, BRS Violeta, BRS Rúbea, BRS Carmem y de las selecciones 1 and 2, en cuatro períodos distintos (Tb1, dos semanas antes; Tb2, una semana antes; Tn, período normal de cosecha; Ta, una semana después). El análisis estadístico de TEAC en Tn de cada variedad o selección reveló diferencias altamente significativas, excepto entre Isabel y Carmem. Diferencias significativas y altamente significativas fueron observadas en los PP totales. No se observaron diferencias significativas entre las variedades de baja producción Isabel y Carmem, entre las variedades de alta producción Violeta y la selección 2, y entre la selección 1 y la variedad Violeta. Diferencia significativa fue encontrada entre la variedad Rúbea y la selección 2. Las diferencias entre todas otras medias fueron altamente significativas. Algunas variedades y selecciones exhibieron un incremento gradual y significativo de TEAC por todo el período de cosecha.

INTRODUCTION

The human health is strongly linked to the composition of the food intake. The polyphenols and antioxidant are plant metabolites. The human health benefits attributed to the consumption of fruit and vegetables are related, at least in part, to their antioxidant activity. The polyphenols and antioxidant act on several fronts. They have the ability to inhibit low-density lipoprotein oxidation, macrophage foam cell formation and atherosclerosis (Aviram et al., 2002). A specific antioxidant compound called resveratrol suppresses cell transformation and induces programed cell death through activation of p53 activity (Huang et al., 1999). It is well known that p53 is a protein involved with the repair and survival of damaged cells, and also can eliminate severely damaged cells from the replicative pool to protect the organism. Antioxidants have some protective effect against cronical diseases such as coronary heart disease. Grapes treated with trans-resveratrol solution had positive behaviour concerning the conservation of berries during storage, doubled their normal shelf-life at room temperature and maintained their post-harvest quality within 10 days

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(Montero et al., 2003). Nevertheless, it is known that resveratrol is immediately degraded by tyrosinase if the activity of this enzyme is not inhibited (Gilly et al., 2001).

The concentration of these compounds is higher in red than in white wines. Differences in antioxidant activities among cultivars for red and white wines are also expressive (de Beer et al., 2003, 2005) as well as among cultivars for grape juice (da Silva et al., 2006, 2007). Although most antioxidant studies have focused on genetic inheritance, experimental evidences have demonstrated that external factors such as the presence of phytopathogen or antagonic microorganisms on grapevine leaves can also induce plant response, enhancing thè production of resveratrol (Bernard et al., 1998). The accumalation of resveratrol in plants is related to disease resistance (Hipskind and Paiva, 2000; Schoonbeek et al., 2001). The major interest is focused on the antioxidant capacity of wine but the concentration of these compounds can be higher in grape juice than in red wine (da Silva et al., 2006) or be much more effective than red wine in inhibiting atherosclerosis(Vinson et al., 2001).

The composition of juice from any grape species can never be accurately predicted because it is known that the juice composition varies from year to year. Changes can continually occur during the maturation and ripening processes in the field and also depends on the place, the soil, and the climatic conditions (Morris, 1989) The grape juice composition is influenced by grape cultivar and fruit maturity. The main purpose of this work was to investigate the changes of phenols content and the antioxidant capacity in grape juice produced from the cultivars Isabel, BRS Violeta, BRS Rúbea, BRS Carmem and from the selections 1 and 2, in four different harvest dates.

MATERIAL AND METHODS

Cultivar and Selections

The cultivars Isabel, BRS Violeta, BRS Rúbea, BRS Carmem and the selections 1, 2 were obtained by crossbreeding (Camargo et al., 2008). The plants were grown on pergola trellises. A total of four harvests date were performed. The grapes were harvested two weeks (Tb1) and one week (Tb2) before and one week after (Ta) the normal date of harvest (Tn).

Juice

Immediately after the harvest, the grapes were manually destemmed without crushing. The Welch process was used . In brief, the grapes (20Kg) were transferred to a heat juice extractor, which is composed of a vapor source compartment, a juice collector, and a grape reservoir.

Antioxidant analysis

The trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) of grape juice was measured by means of scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH -Sigma-D-9132) radical described elsewhere (Brand-Williams et al., 1995; Bondet et al., 1997; Molyneux, 2004). In brief, the DPPH working solution was prepared by adding 40 ml methanol to 10 ml of a 24 mg(100 ml)$^{-1}$ DPPH stock solution. The absorbance of this new solution was adjusted, when necessary, with methanol or DPPH staviramock solution to give 1.1 at 515 nm. The trolox stock solution was prepared by diluting 25 mg trolox q.s.p. 10 ml methanol. A standard curve of trolox with 20 different concentration of trolox was prepared by diluting the trolox stock solution to give 378.79 to 26.72 μM trolox. A 950 μL DPPH working solution was added to 50μL trolox standard curve or sample and the mixtures were shaken gently and allowed to stand for 30 minutes at 25 °C. The absorbance was measured at 515 nm using a spectrophotometer (Lambda Bio UV/Vis Spectrometer, Perkin-Elmer, USA), using a 1.0-cm path-length cuvette.

Phenol analysis

The total phenols concentration in grape juice was determined using the Folin-Ciocalteu reagent according to Singleton et al. (Singleton et al., 1999) In brief, besides the Folin-Ciocalteu reagent, a 200 gL^{-1} anhydrous sodium carbonate solution was used. A standard curve with 11 different concentrations of gallic acid was prepared from a 500 mg(100mL)$^{-1}$ stock solution. The reaction mixture consisted of the following concentrations and components: 1.58 mL H_2O, 20 μL standard solutions or samples, 100 μL Folin-Ciocalteu reagent, after 30 seconds, 300 μL of $NaCO_3$ was added. The mixture was allowed to stand for 2 hours at room temperature and then the absorbance was measured at 765 nm using a spectrophotometer (Lambda Bio UV/Vis Spectrometer, Perkin-Elmer, USA), using a 1.0-cm path-length cuvette.

Statistical analysis

The experimental design was entirely randomized with three repetions.The analysis of variance (one-way

Anova), the difference between means by Tukey test and regression analysis were performed using the R program (Version 2.6.2) (Venables et al., 2004). The difference were considered significant and highly significant at $P<0.05$ and $P<0.01$, respectively.

RESULTS AND DISCUSSION

Antioxidant and Harvest Date

Statistical analysis performed within Tn of each cultivar and selection revealed that the trolox equivalent antioxidant capacity (TEAC) presented highly significant differences for all cultivars ($P<0.01$), except between Isabel and Carmem ($P>0.05$) (Tab. 1).

With regard to cultivar Isabel and considering the four weeks of harvest, there was no progressive increase in the amount of TEAC. However, a week after the normal harvest time, the value of TEAC was 25% greater than in all other periods of harvest. Isabel is an important cultivar for juice in Brazil. The cultivar Carmem has behaved differently. A progressive increase in the level of antioxidant during the first three weeks was observed. The maximum production was obtained at the time of a normal harvest (5412.167 μM). When the harvest was extended for an additional week, a 5% reduction in the concentration of antioxidant was obtained.

Table 1: Trolox equivalent antioxidant capacity (TEAC) and Gallic acid equivalent (GAE) of the grape juice obtained from cultivars and selections at normal harvest date

Antioxidant and Total Phenol Analysis		
Cultivar and Selection	TEAC ((μM))	GAE (mgL^{-1})
BRS Violeta	37551.99 A [1]	6193.78 bcC[1]
Selection-1	33706.24 B	7219.06 cB
Selection-2	28465.22 C	5412.25 cC
BRS Rúbea	21916.03 D	3933.18 B
BRS Carmem	5412.17 aE	1416.5 aA
Isabel	5760.07 aE	1720.75 aA

[1] Means followed within the same column by different small and capital letters are significantly different at the 0.05 and 0.01 levels of probability, respectively, by Tukey's test.

BRS Rúbea and selection 2 showed greater stability in the concentration of antioxidant throughout the evaluation. However, it was observed, with the cultivar BRS Rúbea, an increase of 8.9% in the concentration obtained a week after the normal harvest period. BSR Violeta and selection 1 were those that had the greatest concentration of antioxidant. Throughout the evaluation (Tab. 2), BSR Violeta and selection 1 presented an exponential increase, reaching maximum mean values of 39679.52 μM and 35428.52 μM TEAC, respectively, in the last week of harvest. Their production rates were 0.0712 and 0.125 $\mu Mweek^{-1}$, respectively. The equations obtained that explain the increase in the concentration of antioxidant over the period of harvest of Violeta and selection 1 were:

Cultivar Violeta

$$TEAC = 29974.34e^{0.071246t} \qquad (1)$$
$$r^2 = 0.9899$$

Selection 1

$$TEAC = 22081.6e^{0.12549t} \qquad (2)$$
$$r^2 = 0.9612$$

The cultivars and selections behaved quite differently with regard to antioxidant production and evolution over time. Moriartry et al. (2001) observed that the resveratrol concentrations of two Californian table grape cultivars depended upon the time of harvest. The similar behaviour observed with the cultivar Violeta and the selection 1 could be explained by the genetic relationship. They have both one parent in common. This fact does not explain the similarity obtained because the cultivar Carmem has the same common parent and behaved like Isabel. Pressure, maceration time and grape variety are believed to cause difference between wines (Villano et al., 2005). The difference between grapes can be related not only to the concentration of antioxidant molecules but to the composition of these molecules. Villano et al. (2006) showed that when the

Table 2: Trolox equivalent antioxidant capacity (TEAC) of the grape juice obtained from cultivars and selections in four harvest dates. Tb1, two weeks before the normal harvest period; Tb2, one week before the normal harvest period; Tn, normal harvest period; Ta, one week after the normal harvest period

Antioxidant Analysis				
Cultivar and Selection	Tb1 (μM)	Tb2 (μM)	Tn (μM)	Ta (μM)
BRS Violeta	32273.7	34226.97	37551.99	39679.52
Selection-1	24736.17	28235.45	33706.24	35428.52
Selection-2	25278.72	24960.23	28465.22	28353.42
BRS Rúbea	20333.78	22134.56	21916.03	23874.35
BRS Carmem	5274.02	5376.68	5412.17	5088.34
Isabel	5995.82	6087.7	5760.07	7504.68

number of phenoxyl groups increases the antioxidant action also augments.

Polyphenols and Harvest Date

The variance analysis within Tn of each cultivar and selection revealed highly significant difference among at least two means (P<0.01). The Tukey's test showed no significant difference only between the cultivars Isabel and Carmem, between Violeta and selection 2 and between the selection 1 and Violeta (P >0.05). It there was significant diference between Rúbea and selection 2 (P<0.05). The difference among all other means were highly significant (P<0.01) (Tab. 1).

Although the selections and some cultivars are genetically related (half-sister), they behaved differently with regard not only to antioxidant and phenols production but also with regard to these metabolites evolution during the maturation process.The cultivar Rúbea as well as the selection 1, over the four weeks (Tab. 3), showed an exponential increase in the concentration of phenols with rates of 0.087 and 0.144628 $mgL^{-1}week^{-1}$, respectively. The equations which accounted for this increase were the following:

Cultivar Rúbea

$$y = 3038.122e^{0.087342t} \tag{3}$$

$$r^2 = 0.9908$$

Selection 1

$$y = 4664.155e^{0.144628t} \tag{4}$$

$$r^2 = 0.998$$

The cultivar Violeta and the selection 2 also showed a progressive increase in the production of total phenols.

Table 3: Galic acid equivalent (GAE) of the grape juice obtained from cultivars and selections in four harvest dates. Tb1, two weeks before the normal harvest period; Tb2, one week before the normal harvest period; Tn, normal harvest period; Ta, one week after the normal harvest period

Total Phenols				
Cultivar and Selection	Tb1 (mgL^{-1})	Tb2 (mgL^{-1})	Tn (mgL^{-1})	Ta (mgL^{-1})
BRS Violeta	5881.39	5780.98	6193.78	6939.44
Selection-1	5424.31	6160.53	7219.06	8332.2
Selection-2	4838.19	4953.32	5412.25	6075.86
BRS Rúbea	3349.85	3569.18	3933.18	4339.18
BRS Carmem	1174.38	1261.49	1416.5	1401.77
Isabel	1725.23	1723.95	1720.75	2368.31

This behaviour was described by the following equations:

Cultivar Violeta

$$y = 6359.73 - 698.88x + 211.51x^2 \tag{5}$$

$$r^2 = 0.998$$

Selection 2

$$y = 4962.51 - 268.40x + 137.12x^2 \tag{6}$$

$$r^2 = 0.999$$

The correlation between TEAC and GAE was high (r=0.97). Similar results were obtained by da Silva et al. (2006) in grape juice, by Silva et al. (2007) in 15 Amazonian plant species and in red and white wines (de Beer et al., 2003; Fernandez-Pachon et al., 2004). The analysis of variance was highly significant, which means that TEAC is linearly related to the GAE ($P<0.01$). The following equation shows that TEAC increases 5.6 μMmg^{-1} GAE. Different phenolic compounds have distinct antioxidant potentiality. Flavonoids, a group of phenolic compounds, are considered products of secondary metabolism of plants with antioxidant activity. It was also showed that the anthocyanins, a flavonoid compound, of pomegranate juice account for only 1,400 μM TEAC of a total of 20,500 μM TEAC (Gil et al., 2000). These results demonstrate that more coloured juice does not necessarily mean juice with more antioxidant capacity.

The correlation equation and the coefficient of correlation were the following::

$$TEAC = -2225.1123 + 5.6443GAE \tag{7}$$

$$r = 0.97185$$

CONCLUSION

Among the six grape juices, those obtained from the new cultivars BRS Violeta, and BRS Rúbea and from the selections 1 and 2, due to their high antioxidant properties, suggest to possess a remarkable potential to reduce the risks of cardiovascular and degenerative diseases and to minimize the oxidative stress.

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