Comparison of antioxidant capacity in urine by ORAC analysis after consumption of muscadine grape juice

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INTRODUCTION
Approximately 2.3% of oxygen consumed during cellular aerobic metabolism is converted into free radicals. Antioxidants and phenolic compounds work to remove the free radicals before damage can occur. Most fruits and vegetables contain high levels of antioxidants. Muscadine grapes, which are native to the southeastern United States, contain high levels of antioxidants. In this study, urine was analyzed before and after the consumption of muscadine grape juice to compare the antioxidant statuses. The Oxygen Radical Absorbance Capacity assay (ORAC) measures the antioxidant scavenging activity against peroxyl radicals which is induced by 2,2’-Azobis(2-amidinopropane) dihydrochloride (AAPH). ORAC was used to determine the entire antioxidant status of each sample but uric acid, which is naturally produced in the body, was found to overshadow the effects of the juice consumption. The uric acid and vitamin C concentration were determined for the control and experimental data using high pressure liquid chromatography.

METHODS

ORAC
Each urine sample was diluted by 100-fold before performing the ORAC analysis. Folin reagent was used as the fluorescent probe and Trolox was the standard. Loss of fluorescence over time is due to the peroxyl-radical formation by the breakdown of AAPH. The results were analyzed in Excel and statistical analyses were performed with IBM SPSS Statistics Software.

Uric Acid Analysis
High Pressure Liquid Chromatography (HPLC) was used to determine uric acid concentration. Daily aliquots for the control, day 1-10, were combined so a approximate uric acid content could be determined. The same protocol was used for experimental days. Vitamin C concentrations in the grape juice and each sample were also determined in this test.

Total Phenolics
This spectrophotometric test measures all the phenolic compounds in the sample. The Folin reagent used causes a color change based on the ability of each sample to be oxidized. A calibration curve of catechin was used to estimate the phenolic concentration of the muscadine grape juice. The wavelength used was 765 nm.

RESULTS

Antioxidant content of urine before and during Muscadine juice consumption

Figure 2. Statistical significance was determined for 2 participants when comparing the averages of the control and experimental data while 8 participants had no significant increase.

Uric acid content of urine before and during Muscadine juice consumption

Figure 3. Uric acid (µg/mL) in combined urine samples by subject and juice consumption status. Generally higher uric acid concentration can be seen in the control days. Significance was not determined.

DISCUSSION

• As a population, there was no significant change in the antioxidant capacity of urine before the consumption of grape juice compared to the antioxidant capacity of urine after grape juice consumption. This was determined by the ANOVA statistical analysis.
• The experimental t-test analysis showed that two participants had statistical increase in the antioxidant capacity after consuming the grape juice. This is shown by the asterisks above the bars in the ORAC graph.
• Uric Acid analysis was performed to determine if the uric acid in the samples was overshadowing the antioxidant effects from the grape juice. Urinary uric acid concentration was lower in nearly all subjects after the consumption of muscadine grape juice. It is unknown if the uric acid decrease is due to juice consumption but any changes in urinary ORAC due to juice consumption were obscured by concomitant changes in urinary uric acid.
• Although some participant’s initial urine sample did not contain Vitamin C, in those that did, an increase was seen after the consumption of the grape juice.
• The total phenolics analysis of the grape juice indicated that 115.4 µg/g is available for each sample consumed.

CONCLUSIONS

• Positive variance was found in two participants consistent with a positive correlation between antioxidant consumption and antioxidant absorption.
• Since uric acid was found to play a major role in total antioxidant capacity, future studies will account for this contribution.
• The availability of the analytical methods for antioxidant assays in urine and other biological samples have provided means for observing enhanced antioxidant concentration in urine.

BIBLIOGRAPHY


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