

# Source Variation in Antioxidant Capacity of Cranberries from Eight U.S. Cultivars

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## ABSTRACT

Antioxidants prevent the oxidation of low-density lipoproteins (LDL) that contributes to cardiovascular disease. While cranberries have been shown to contain antioxidants, cranberries from different sources may possess different antioxidant capacities. The purpose of this study was to determine if antioxidant capacity varies among select cranberry cultivars. Eight U.S. cranberry cultivars were assayed for antioxidant activity. The cranberries were homogenized in a blender to extract juice. Cranberry juice was then added to isolated LDL from five humans and oxidized with copper sulfate. Thiobarbituric acid reactive substances (TBARS) formation was used to determine the antioxidant capacity of each of the cranberry cultivar extracts following oxidation. Sample absorbances at 532 nm varied significantly among the tested cranberry juice extracts. These results may be beneficial to both producers and consumers of cranberry juice. Producers may market and provide a cranberry juice that offers the best health benefits, while consumers may be certain that they are purchasing cranberry juice that provides the best health benefits.

## INTRODUCTION

Studies have shown that antioxidants have positive effects on the prevention of cardiovascular disease (CVD) by inhibition of circulating plasma low-density lipoprotein (LDL) oxidation. Endogenous production of reactive oxygen species (ROS) is essential to the maintenance of homeostasis in biological systems (Kandaswami and Middleton, 1994). However, when ROS generation is in excess, deleterious consequences to biological systems ensue. If endogenous antioxidants do not occur at greater levels than prooxidants, the pathophysiological events leading to CVD begin.

When circulating LDL is oxidized, it participates in the formation of atherosclerotic plaques in blood vessel walls. ROS exert their action by abstraction of hydrogen from a carbon on a fatty acid or a fatty acid side chain of lipids, thereby forming lipid peroxides (Kandaswami and Middleton, 1994). Accumulation of lipid peroxides and cholesterol esters is observed in atherosclerotic plaques (Kandaswami and Middleton, 1994). The formation of atherosclerotic plaques is a major factor in the development of high blood pressure, stroke, and myocardial infarction.

Lower rates of CVD have been reported for years in European populations in which red wine consumption is high (Frankel et al., 1995). Phenolic chemicals (flavonoids) in the grapes of the wines have been implicated for these observed antioxidant effects (Fuhrman et al., 1995). More recent research has shown cranberries to be a potentially rich source of antioxidants and inhibitors of platelet aggregation *in vivo* in both human and animal models due to their content of polyphenolic flavonoids (Wilson et al., 1998 and Xia et al., 1998).

ROS production is inhibited by various flavonoids (Kandaswami and Middleton, 1994). Mangiapane et al (1992) demonstrated an 80% drop in oxidation of Cu<sup>2+</sup>-oxidized human LDL in the presence of the flavonoid (+)-catechin compared to oxidation in its absence. Additionally, evidence from the "Zutphen Study" demonstrated an inverse correlation between elderly subjects with high flavonoid intake and mortality from coronary heart disease (Xia et al., 1998).

Recent studies at the University of Wisconsin-La Crosse have shown that cranberry juices may have CVD preventative activity similar to purple/red grape juices. The study showed that when LDL oxidation occurs in the presence of cranberry juice, oxidation, as measured by the formation of thiobarbituric acid reactive substances (TBARS) and LDL electrophoretic mobility, is decreased (Wilson et al., 1998). In addition, research has shown that cranberry juice also vasodilates isolated arteries and transiently reduces blood pressure in anesthetized and conscious rats (Maher et al., 1999). This evidence suggests that the polyphenolic flavonoids obtained from the consumption of cranberry juice can be of benefit to cardiovascular health.

The antioxidant capacity that different cranberries possess may vary between different cranberry strains and cultivars. If a certain strain or cultivar of cranberries is found to embody a greater antioxidant capacity, this could provide greater CVD protection to the consumer. Likewise, cranberry juice producers could endorse their product as providing the greatest health benefits, which could enhance their sales. In a state such as Wisconsin, where cranberry cultivation and juice production is a prominent industry, improving the marketability of cranberry juice could be beneficial to the state's economy. The purpose of this study was to conclude if cranberries from different cultivars and strains do, in fact, possess significantly differing antioxidant capacities.

## **METHODS**

### **Cranberries**

Eight U.S. cranberry cultivars were obtained from a local cranberry company and shipped frozen. Ben Lear and Stevens cultivars were obtained from Nantucket, Massachusetts (MA); Manitowish Waters, Wisconsin (WI); and Warrens, WI. Crowley and Stevens cultivars were obtained from Brandon, Oregon (OR); Crowley was substituted for the Ben Lear cultivar which was not available from this site. The samples remained frozen until the day of juice production. All juices were prepared from berries thawed in a cold room for 24 hours at 8°C. The juice was extracted by homogenizing the berries from individual cultivars in a blender for 10 minutes. The homogenate was then centrifuged using a Sorvall RC2-B and a GSA rotor for 20 minutes at 16,270 x G and 6°C. Each sample was then titrated to pH 7.40 with 30% hydrochloric acid and 10% sodium hydroxide. The juice extracts were individually stored in 15 mL centrifuge tubes at -20°C until used.

### **Isolation of human LDL**

Human blood (28 mL) was collected from 4 male and 1 female volunteer(s) after an overnight fast into vacutainers containing liquid K<sub>3</sub>EDTA. Plasma was collected from the blood following centrifugation at 1,500 x G for 15 minutes. The lipoproteins were isolated by sequential ultracentrifugation using a Sorvall OTD55B ultracentrifuge and a T-1270 rotor. NaCl (2.2 mL) was placed on top of 4.4 mL of each plasma sample and then centrifuged at 230,000 x G for 12 hours. The VLDL fraction was removed from the top 2.2 mL and added to 2.2 mL of 1.182 g/mL NaBr. This mixture was then centrifuged for 18 hours at 230,000 x G and the top 2.2 mL was removed (LDL fraction).

### Antioxidant assay

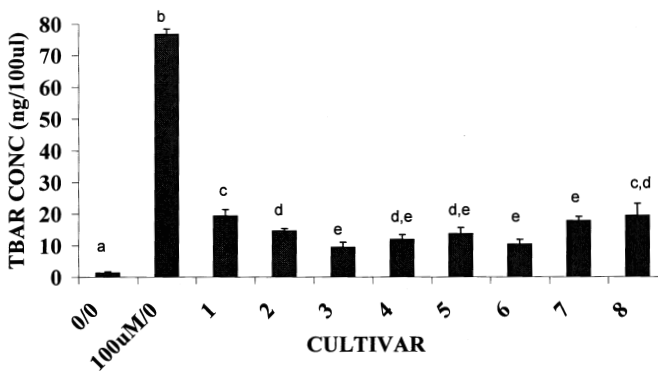
Equal concentrations (100 µg LDL/mL) of LDL were added to a 96 well plate along with cranberry juice extracts final dilutions of 1:5,000 and 1:15,000. Oxidation was induced by 10 µM CuSO<sub>4</sub> during a 95 minute incubation at 37°C. Control wells were prepared using PBS in place of cranberry juice with no CuSO<sub>4</sub> and using wells with PBS in the presence of CuSO<sub>4</sub> and no cranberry juice.

### Measurement of LDL oxidation-TBARS assay

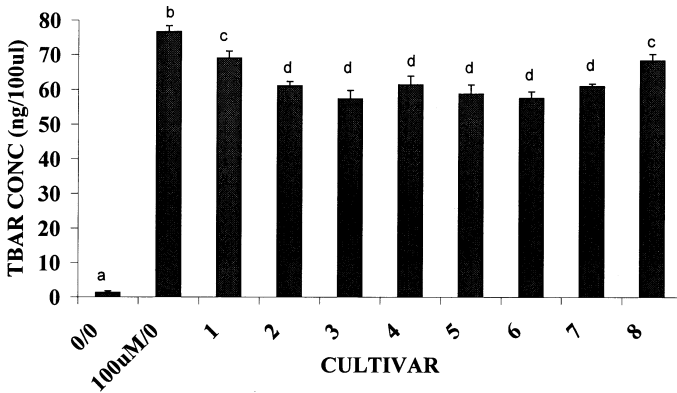
Trichloroacetic acid (400 µL) was added to as many 1 mL microfuge tubes as there were wells containing LDL. Following incubation, 150 µL of each LDL sample was added to its corresponding labeled microfuge tube. Warm thiobarbituric acid (400 µL) was immediately added to each microfuge tube and incubated, uncapped, at 95°C for 30 minutes on a heating block. The tubes were capped and cooled at room temperature for 15 minutes following incubation, and then centrifuged at 14,000 x G for 5 minutes. Each sample's supernatant (700 µL) was added to cuvettes and absorbances were read at 532 nm in a spectrophotometer. A standard curve was generated using tetramethoxypropane standards of 0.0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 ng/µL and measuring absorbance at 532 nm in a spectrophotometer.

## RESULTS

One way ANOVA revealed significant source variation and Tukey's post hoc analysis was used to distinguish differences among cultivars. The copper catalyzed oxidation of LDL was most effectively inhibited by Stevens cranberry cultivars at both 1:5,000 and 1:15,000 with mean TBARS concentrations of 12.98 ng/100 µL and 59.22 ng/100 µL, respectively (Figures 1, 2). At the 1:5,000 dilution, WI cultivars had a mean TBARS concentration of 15.24 ng/100 µL, while the non-WI cultivars had a mean TBARS concentration of 13.78 ng/100 µL (Figure 1). The WI cultivars had a greater inhibitory effect on oxidation at 1:15,000 dilution with a mean TBARS concentration of 61.41 ng/100 µL compared to 62.18 ng/100 µL for non-WI cultivars (Figure 2).



**Figure 1: TBARS IN CU<sup>++</sup> Oxidized LDL IN THE PRESENCE OF EIGHT CRANBERRY CULTIVAR EXTRACTS AT 1:5,000 DILUTION. #1=Crowley-Brandon, OR, #2=Stevens-Brandon, OR. #3=Stevens-Nantucket, MA, #4=Ben Lear-Nantucket, MA, #5=Ben Lear-Warrens, WI, #6=Stevens-Warrens, WI, #7=Stevens-Manitowosh, Waters, WI, #8=Ben Lear-Manitowosh Waters, WI. Means of groups that share a letter were not significantly different.**



**Figure 2: TBARS IN CU++ Oxidized LDL IN THE PRESENCE OF EIGHT CRANBERRY CULTIVAR EXTRACTS AT 1:15,000 DILUTION. #1=Crowley-Brandon, OR, #2=Stevens-Brandon, OR. #3=Stevens-Nantucket, MA, #4=Ben Lear-Nantucket, MA, #5=Ben Lear-Warrens, WI, #6=Stevens-Warrens, WI, #7=Stevens-Manitowosh, Waters, WI, #8=Ben Lear-Manitowosh Waters, WI. Means of groups that share a letter were not significantly different.**

## CONCLUSION

The TBARS assay in this study revealed that significant differences exist among the antioxidant capacity of different cranberry cultivars. These results further support the belief that cranberries do, in fact, possess antioxidant properties. Furthermore, it is evident from this study that certain cranberry cultivars may contain greater antioxidant capacity than others. Cranberry juice producers may be able to use these results to market and provide consumers with cranberry juice that is most effective against LDL oxidation and the prevention of CVD.

This study provides a basis for further research into the variance of antioxidant capacity among cranberry cultivars. Variance in vitro of cranberry cultivars was shown, but whether or not the same results would occur in vivo could be of great interest. Also, cranberry juice producers would be interested to know if the variance found in this study is enough to create a greater CVD protective effect that is of any health-promoting significance.

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