TOTAL ANTIOXIDANT CAPACITY OF FRESH, FROZEN, AND JUICED BLUEBERRIES

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INTRODUCTION

Fruits and vegetables are known in common culture for their disease-fighting and general health benefits (Joshipura et al. 2001, Ford and Mokdad 2001, Gandini et al. 2000). Antioxidants, found in abundance in fruits and vegetables, are often considered to be the source of many of these healthful properties (Anraku et al. 2009, Belch et al. 1989, Cantuti-Castelvetri et al. 1999, rice-Evans and Miller 1997, Seis 1997, Valko 2007). Although the amounts of antioxidants in many foods are known, the effect of freezing and baking on antioxidant levels of specific foods has not been well studied. Thus, the objective of this study is to compare the antioxidant capacity of commercially available blueberries in the fresh, frozen, and baked forms using a buffered DPPH assay and VCEAC. This study will show the dietary value of different types of blueberry foods, enabling the healthiest choices to be made by consumers.

Antioxidant is a broad label for any molecule that delays or inhibits oxidation of other molecules (Sies 1997). Free radicals are molecules that cause this oxidation, and usually contain single unpaired electrons, which make them extremely reactive. Free radicals will pull electrons from biomolecules, destroying them and leading to cellular damage (Valko et al. 2007). Components of cells that are often damaged by free radicals are DNA, lipids of cell membranes, and proteins (Sies 1997, Valko et al. 2007), which compromise a considerable amount of animal bodies. Antioxidants combat the effects of free radicals by acting as reducing agents, supplying the electrons that free radicals take from biological molecules, thereby sparing these molecules from damage.

Both antioxidants and free radicals belong in balance in the body. Free radicals are created in, and indeed required for, many normal biological processes. They are used by the body in many cellular signaling systems, as well as in the destruction of antigens (Valko et al. 2007). They are created in the electron transport chain at the end of the complex process of ATP synthesis, when oxygen is reduced to water in the mitochondrial membrane (Nelson and Cox 2008). Sometimes, this process creates free radicals such as the superoxide anion radical (O2ˉ), hydrogen peroxide (H2O2), and the hydroxyl radical (OH, the neutral form of hydroxide, OHˉ). Antioxidant systems are present in the body to counteract the potentially damaging effects of free radicals, however an imbalance in the favor of free radicals can occur, and oxidative stress is the result. Oxidative stress leads to cellular and DNA damage, which has been linked with degenerative symptoms of ageing (Valko et al. 2007), as well as many common diseases, including various types of cancers, cardiovascular disease and atherosclerosis (Belch et al. 1989), and neurodegenerative diseases (Cantuti-Castelvetri et al. 1999).

Sometimes, the body’s antioxidant systems may not be enough to counter oxidative stress. This is where dietary antioxidants may be important to supplement the body’s natural defenses. Absorption of dietary antioxidants into the body, as well as their beneficial effects on health, is well documented (Anraku et al. 2008, Hollman et al. 1996, Rice-Evans et al. 1997).

An assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a standard way of measuring antioxidant capacity (Sharma et al. 2009, Milardovec et al. 2005, Kim et al. 2002). DPPH is a violet-colored reactive species that is stabilized by the delocalization of its unpaired electron (Sharma and Bhat 2009). When DPPH reacts with an antioxidant, the DPPH loses its color, thus enabling the use of spectrophotometry to determine the free radical scavenging capacity of an antioxidant (Milardovic et al. 2006).

Vitamin C equivalent antioxidant capacity (VCEAC) method is the most common and preferable measurement of antioxidant capacity because it is easier to comprehend than other measurements (Kim et al. 2002). It uses vitamin C as a reference to which other antioxidants are compared. DPPH assay data are calculated in mg/100g, a unit that represents the milligrams of vitamin C that will donate electrons to the same number of free radicals as a given mass of a sample of food.

METHODS

 Blueberry solutions will be made for each type of blueberry treatment. (Number) units each of the same brands of fresh blueberries will be obtained from Ingles in Swannanoa, North Carolina. The blueberries will be divided into 3 groups, and (20) samples will be made from each: one will be made into a solution immediately, one will be frozen for later testing, and one will be baked at (blank) degrees for (time). The fresh, baked, and frozen samples will be mashed or defrosted and mashed. A mixture will be made consisting of 1 g of each sample and 1 mL methanol. Mixtures will be centrifuged for 5 minutes. The supernatants will be decanted, and stored in a refrigerator in foil-wrapped vials at 4°C. The fresh and baked samples will be tested immediately.

A 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay will be used to measure the VCEAC for the samples, according to the method proposed by Sharma and Bhat (2009). DPPH will be purchased from Sigma-Aldrich (USA). A 0.5 μM solution will be prepared by mixing 0.0106 g DPPH in 500 mL of reagent grade buffered methanol. The buffered methanol will be prepared with 40 mL of 0.1 M acetate buffer (pH 5.5) and 60 mL methanol. The DPPH solution will be stored in a dark bottle wrapped in foil at -18°C until use.

The free radical scavenging capacity will be analyzed with an Ocean Optics Ultra Violet/Visible Light Spectrophotometer set to 517 nm. Two mL of the DPPH solution will be placed in the cuvet. One hundred μL of each blueberry sample will be added. The absorbance will be recorded every 5 seconds for 1 minute, and every minute thereafter for 5 minutes. The replicates from each treatment will be averaged and the VCEAC and standard deviations will be calculated for each treatment. An ANOVA test will be used to determine if there is a difference in the mean antioxidant level between fresh, frozen, and juiced blueberries.

LITERATURE CITED

Anraku, M., T. Fujii, N. Furutani, D. Kadowaki, T. Maruyama, M. Otagiri, J.M. Gebicki, and H. Tomida. 2009. Antioxidant effects of a dietary supplement: Reduction of indices of oxidative stress in normal subjects by water-soluble chitosan. Food and Chemical Toxicology 47:104–109

Belch, J.J., M. Chopra, S. Hutchison, R. Lorimer, R.D. Sturrock, C.D. Forbes, and W.E.Smith. 1989. [Free radical pathology in chronic arterial disease](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T38-47NVN1F-6T&_user=8495685&_coverDate=12%2F31%2F1989&_alid=1079702723&_rdoc=1&_fmt=high&_orig=search&_cdi=4940&_sort=r&_docanchor=&view=c&_ct=125938&_acct=C000073608&_version=1&_urlVersion=0&_userid=8495685&md5=248c79e7b885cdea4ddf56cdf03a3299). Free Radical Biology and Medicine 6:375-378.

Cantuti-Castelvetri, I., B. Shukket-Hale, J.A.Joseph. 1999. Neurobehavioral aspects of antioxidants in aging. International Journal of Developmental Neuroscience 18:367-381.

Ford, E.S., and A.H. Mokdad. 2001. [Fruit and Vegetable Consumption and Diabetes Mellitus Incidence among U.S. Adults](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WPG-4576DG9-2B&_user=8495685&_coverDate=01%2F31%2F2001&_alid=1079675344&_rdoc=1&_fmt=high&_orig=search&_cdi=6990&_sort=r&_st=13&_docanchor=&_ct=7&_acct=C000073608&_version=1&_urlVersion=0&_userid=8495685&md5=1a929a34532151f2164eebfedf9b6881). Preventive Medicine 32:33-39.

Gandini, S., H. Merzenich, C. Robertson, and P. Boyle. 2000. [Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients](http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T68-3YWWYBW-D&_user=8495685&_coverDate=03%2F31%2F2000&_alid=1079689749&_rdoc=1&_fmt=high&_orig=search&_cdi=5024&_sort=r&_docanchor=&view=c&_ct=31375&_acct=C000073608&_version=1&_urlVersion=0&_userid=8495685&md5=c2cd873fee261f3033d07294a42d96cb). European Journal of Cancer 36: 636-646

Holloman, P.C., M.V. Gaag, M.J. Mengelers, J.M. Van Trijp, J. H. Vries, and M.B. Katan. 1996. Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Radical Biology & Medicine 21: 703-707.

Joshipura, K.J., A. Ascherio, J.E. Manson, M.J. Stampfer, and E.B. Rimm et al. 1999. Fruit and Vegetable Intake in Relation to Risk of Ischemic Stroke. Journal of the American Medical Association. 282:1233-1239

Kim, D., K.W. Lee, H.J. Lee, and C.Y. Lee. 2002. Vitamin C equivalent antioxidant capacity of phenolic phytochemicals. Journal of Agricultural and Food Chemistry 50:3713-3717.

Milardović, S., D. Ivekovic, and B.S. Grabarić. 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry 68:175-180.

Nelson, D.L., and M.M. Cox. 2008. Lehninger: principles of biochemistry. 5th ed. W.H. Freeman and Company, New York, NY, U.S.A. 720 p.

Rice-Evans, C., and N. Miller. 1997. Measurement of the antioxidant status of dietary constituents, low density lipoproteins and plasma. Prostaglandins, Leukotrienes and Essential Fatty Acids 57:499-505.

Sies, H. 1997. Oxidative stress: oxidants and antioxidants. Experimental Physiology 82:291-195.

Sharma, O., and T. Bhat. 2009. DPPH antioxidant activity revisited. Food Chemistry 113:1202-1205.

Valko, M., D. Liebfritz, J. Moncol, M. Cronin, M. Mazur, and J. Tesler. 2007. Free radicals and antioxidants in normal physiological functions and human disease. [The International Journal of Biochemistry & Cell Biology](http://www.sciencedirect.com/science/journal/13572725) 39:44-84.

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**PROPOSED BUDGET**

|  |  |  |  |
| --- | --- | --- | --- |
| ITEM | QUANTITY | SOURCE | UNIT PRICE |
| 2,2-DIPHENYL-1-PICRYHYDRAZYL | 1 g | SIGMA D-9132 | $45.30  |
| METHANOL | 1 L | SIGMA 179337 |  32.40 |
| ACETIC ACID | 100 mL | SIGMA 695092 |  26.10 |
| SODIUM ACETATE | 250 g | SIGMA S-2889 |  24.10 |
| BLUEBERRY JUICE CONCENTRATE – 8 oz. | 3 BOTTLES | INGLES GROCERY |  16.77 |
| BLUEBERRIES, FRESH | 3 CARTONS | INGLES GROCERY | ~ 15.00 |
| BLUEBERRIES, FROZEN | 3 BAGS | INGLES GROCERY | ~15.00 |
|  |  | **TOTAL** | **$174.67** |

THE ESTIMATED TOTAL COST OF THE PROJECT WILL BE $174.67