To Freeze or Not to Freeze: An Analysis of Antioxidants in Blueberries

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Introduction

The subject of much fanfare in wellness and nutrition-conscious circles, antioxidants serve a crucial role in the body. But what are antioxidants, really? Essentially, antioxidants are a family of molecules which counter oxidation of the body's structures by free radical ions, which are unstable molecules with positive charge. Free radicals are naturally occurring, resultant of anabolic biochemical pathways, but are also a consequence of exposure to such environmental factors as sunlight, air pollution, and cigarette smoke.[1] Antioxidants inhibit the damaging oxidation process, which is thought to be related to cardiovascular disease, diabetes, and Alzheimer's Disease, among others,[1] by donating electrons to free radicals, thereby stabilizing them.

It is the purpose of this research project to investigate and compare the antioxidant concentrations in blueberries stored at -17.8 °C and those stored at 1.7 °C, in order to determine if storage temperature is a factor in the preservation of antioxidant content. The tested blueberries were obtained (nonorganic) from a local grocery store. They were stored immediately at their respective temperatures and tested one week later.

Materials and Methods

Beer's Law states that concentration is directly proportional to absorbance. The absorbance, and therefore the concentration, all other variables being constant, of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) may be determined through visible light spectrophotometry. $ABTS^+$ is a free radical commonly used as a reagent in laboratory applications. Critically, it becomes clear (has zero absorbance) when reacted with an antioxidant. Therefore, the amount of antioxidants in blueberry extract may be determined by reacting the extract with $ABTS^+$ reagent of known concentration, and measuring the absorbance of the products.

The following procedure was used to obtain extract from both the frozen and refrigerated blueberries: Approximately five blueberries were crushed with a mortar and pestle until yielding a visually homogenous liquid. This liquid was then vortexed with 10 ml of acidic methanol for a total time of one minute. This solution was filtered through two paper filters into a vacuum flask. This extract was used to create a 1:10 dilution with acidic methanol. The 1:10 dilution was added to 12.0 ml $ABTS^+$ reagent in 0.5, 1.0, and 1.5 mL amounts. Acidic methanol was used as a matrix to keep volumes constant.

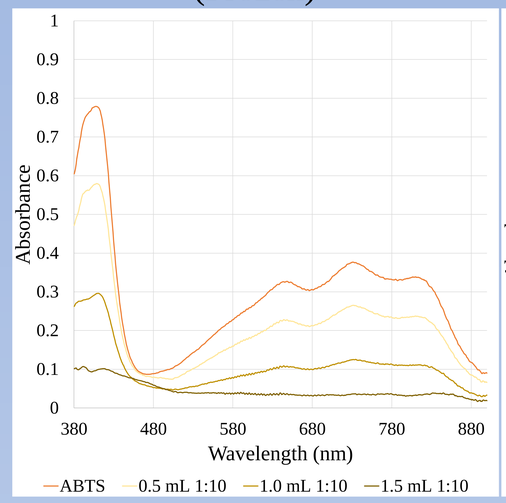
Visible light spectrophotometry was performed with the $ABTS^+$ reagent solutions, determining their absorbances. Using the principle described above, the concentration of $ABTS^+$ was determined as $c = \frac{A}{\varepsilon}$ where c is concentration, A is absorbance, and ε is the molar absorptivity coefficient of $ABTS^+$, which is $0.012867\ \frac{1}{\mu M \cdot cm}$.

Results

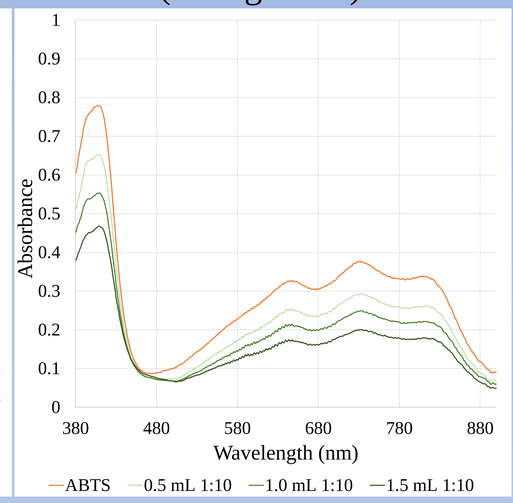
In each case (0.5, 1.0, and 1.5 mL of 1:10 dilution extract), the blueberries stored at -17.8 °C reacted to produce a lower absorbance value in the $ABTS^+$ reagent solution than did the blueberries stored at 1.7 °C. This indicates that the concentration of $ABTS^+$ was lower in the solutions with frozen blueberry extract. Because it is the function of antioxidants to react with $ABTS^+$, forming ABTS and reducing its absorbance to zero, it may be concluded that there were more antioxidants present in the frozen blueberries.

The graphs below represent the absorbance data obtained directly from the spectrophotometer for wavelengths of visible light.

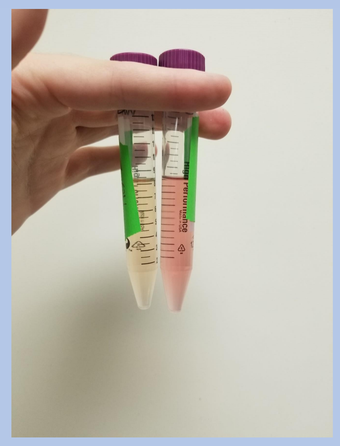
Absorbance vs. Wavelength (Frozen)



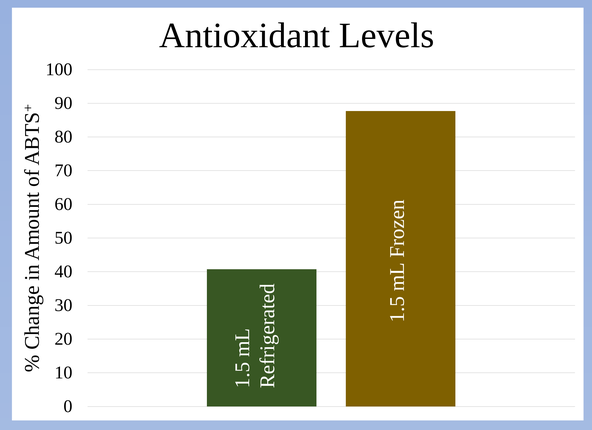
Absorbance vs. Wavelength (Refrigerated)



Visible light spectrophotometry is performed on an array of extracts and dilutions.



Refrigerated (left) and frozen (right) extracts demonstrate substantial difference in color upon visual inspection.



The above graph reflects an average value A for all wavelengths $c = \frac{A}{\varepsilon}$.

Discussions and Conclusion

The results of this experiment suggest that blueberries should be stored frozen in order to preserve antioxidant content. It is commonly known that the nutritional value of food deteriorates with time, and that freezing slows this process. Common sense does not reveal, however, the extent to which the antioxidant content of refrigerated blueberries decreases compared with frozen blueberries. It is important to quantify this loss so that consumers can make informed decisions about their nutrition.

It should be noted that the health benefits of antioxidants are a current area of research, and while the ingestion of antioxidant-rich foods has been correlated with superior health outcomes, it has not been demonstrated that elevated antioxidant intake is beneficial.[1]

Potential sources for error in this experiment include the extraction procedure and the spectrophotometry itself. Objective metrics were not used to ensure refrigerated and frozen blueberry samples were equally homogenized by mortar and pestle. Additionally, the absorbance of the extract dilutions was not accounted for and considered negligible.

Future research may be directed to determine the rate of decrease in antioxidant content as blueberries age. This might be accomplished by testing a sample initially, and comparing the results with identical samples tested later, at regular intervals. This might be done for both refrigerated and frozen blueberries, comparing the rates of decrease.

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References

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