Experiment 7: Determination of titratable acidity, total phenolic compounds, and total antioxidant activity of cherry juice.

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Abstract

A sample of cherry juice was analyzed for three relevant molecule types, acids, phenolic compounds, and antioxidants. There are many natural acids found in fruits, and they give a sour taste. The predominating acid in cherry juice is malic acid. The total acid was determined by titration with sodium hydroxide and phenolphthalein to be 0.146 equivalents per liter or 9.79 g/L malic acid. Phenolic compounds have a characteristic aromatic ring structure and often give fruits and vegetables their colour. They also are beneficial for our cells by acting as antioxidants. Total phenols in cherry juice was determined using a colourimetric assay and the Folin-Ciocalteau reagent. Standardized tannic acid solution was used for preparation of a standard curve and the concentration of phenols was 1.50 grams of tannic acid equivalent per liter. The total antioxidant activity of the cherry juice was also estimated using an assay for free radical scavenging activity. The assay uses the stable free radical molecule DPPH which undergoes a colour change when reduced, and the synthetic antioxidant BHA as a positive control. The total free radical scavenging activity of the juice was determined to be 3.288 mmol/mL of BHA equivalent. Interpretation of these phenol and antioxidant assays can be challenging since there are many unique species being quantified, and the reported values are best when compared to other samples analyzed in the same way.

Introduction

It is important to know what is in food, and developed countries often have entire government agencies responsible for regulating what can be sold as food. The field of biochemistry is often concerned with specific compounds and their interaction with cellular machinery, making the chemical composition of foods relevant. Demand of effective ways to detect certain compounds in food is partially the basis for food chemistry fields. Detecting and understanding potentially dangerous or unhealthy compounds in food is only part of the story, and studying beneficial compounds, or compounds that simply affect taste is also prevalent. In this experiment, a sample of cherry juice is analyzed for determination of three particularly relevant types of compounds: acids, phenols, and antioxidants.

Acidity

Acidity of foods is what determines their sourness[1], and fruits are among the most naturally acidic foods[4]. Natural acidity is often produced as the byproduct of normal cell processes, but it can also be produced by microorganisms, which can indicate spoilage.[1] Consumption of highly acidic foods can sometimes be harmful, especially with certain preexisting conditions,[5] meaning a determination of total acidity is often useful. The easiest way to determine acid content is by titrating a sample with a base like sodium hydroxide and using an indicator with an endpoint close to pH 7. The volume and concentration of base used to reach neutral pH is then used to calculate the equivalents of acid in the sample. The acid content can then be reported as molar concentration, mass of acid, or percent by weight or volume. These require knowing which acid predominates in the sample. Common naturally occurring acids are citric, acetic, tartaric, butyric, lactic, malic, succinic, oleic and more[1]. In this experiment, a sample of cherry juice was titrated with sodium hydroxide using phenolphthalein indicator to determine acid content. The predominant acid in cherry juice is malic acid, and contains only small amounts of citric, quinic and fumaric acid[4].

Phenolic compounds

Another relevant class of compounds are phenols, which in biochemical context, are a group of secondary plant metabolites with an alcohol group off an aromatic ring. Members of this class of compounds include flavonoids, lignans, and tannins[6,7]. Some of these classes are polyphenols, which are larger molecules made up of many smaller phenolic structures[7]. Many studies have correlated phenolic intake to lower risk of many diseases and fruits and vegetables are the best sources of these nutrients[6,7]. Much of their benefits come from their antioxidant activity, which delays oxidation of other important macromolecules in the cell[7]. Coloured flavonoids also help give fruits and vegetables bright colours like orange, red and blue. A common method for determination of total phenolic compounds in a sample is a colourimetric assay using Folin-Ciocalteau (FC) reagent. This reagent contains phosphomolybdic and phosphotungstic acids, which are large molecules that are initially yellow in colour, but form a complex called molybdenum blue when reduced[1,8]. A weak base, usually sodium carbonate, is used to raise the pH enough to deprotonate any phenolic compounds in the sample, allowing them to react with the FC reagent and form the molybdenum blue complex[1,8]. Spectrophotometric measurements can then be used to determine total phenols. In this experiment, total phenols in a cherry juice sample was determined using FC reagent and saturated sodium carbonate solution. Tannic acid, which is a polyphenol found in many plants, was used as a standard to construct a standard curve from absorbance measurements.

Antioxidant activity

One of the major threats that cell can face is oxidative stress, where a large number of reactive oxygen species (ROS) are produced during aerobic metabolism. ROS, and other reactive species can damage important macromolecules like proteins or DNA. The cell relies on antioxidants, which can be small organic molecules or large multi-subunit enzymes to neutralize the reactive species and prevent damage, especially to DNA[11]. Many natural antioxidants and antioxidant precursors are found in fruits and vegetables, including vitamin C (ascorbic acid), vitamin E, carotenoids, glutathione and many polyphenols[11]. A majority of ROS and other reactive species are free radicals, which have an unpaired electron in their structure[11]. Many antioxidants have 'free radical scavenging' activity and donate either a hydrogen atom or an electron to free radicals and neutralize them[10,11]. A common way to determine the total antioxidant activity of a sample is using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is a stable free radical molecule and is purple in colour. Upon reaction with a free radical scavenger antioxidant, DPPH is reduced and picks up a hydrogen, becoming DPPHH, which is yellowish in colour[1,9,10]. Absorbance measurement can therefore be used to determine the total radical scavenging activity of a solution. In this experiment, this DPPH spectrophotometric assay was used to determine total antioxidant activity in a cherry juice sample. Butylated hydroxyanisole (BHA) is a synthetic antioxidant that was used as a positive control, representing complete reduction of DPPH[1]. Establishment of a blank when taking spectrophotometric measurements in especially important in this assay since the colour change in DPPH results in an absorbance decrease (colour change is purple to yellow).

Methods

This experiment procedure was adapted from the Fall 2025 BIOL 4150 Laboratory Manual.[1]

To prepare a cherry juice sample for analysis, 25.0 mL was filtered using a 0.45 µm syringe filter into a 100.0 mL volumetric flask. The juice was then diluted to a total volume of 100.0 mL using deionized water. This juice sample was used for all subsequent analyses.

<u>Determination of acid content:</u>

The determination of acid content in the juice was completed by titration with 0.1 M sodium hydroxide. A 5.0 mL aliquot of the diluted juice was dispensed into a 250 mL Erlenmeyer with about 100 mL of deionized water and 300 µL of phenolphthalein indicator. Sodium hydroxide solution was added using a buret until the endpoint was reached and the volume was used to calculate the acid content.

<u>Determination of total phenolic compounds:</u>

Determination of phenols in the juice sample was completed spectrophotometrically using the Folin-Ciocalteau (FC) reagent and saturated sodium carbonate solution. In a test tube, 1.0 mL of diluted juice sample, 7.5 mL of deionized water and 0.5 mL of 1M FC reagent were added and mixed well. After three minutes, 1.0 mL of saturated sodium carbonate solution was added and the tube was incubated at room temperature for one hour. For preparation of a standard curve, 500 ppm tannic acid stock solution was used to prepare five standards of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm tannic acid. A blank was also prepared using deionized water instead of tannic acid stock. The appropriate volume of stock solution and water was added to five test tubes, along with 0.5 mL of 1M FC reagent. After mixing and three minute incubation, 1.0 mL of saturated sodium carbonate was added and the standards were incubated for one hour. The total volume in all tubes was 10.0 mL. After incubation, each standard was diluted to the target concentration of 0.5 – 2.5 ppm by adding 1.0 mL of standard to 9.0 mL of deionized water. Multiple dilutions of the incubated juice sample solution were also prepared to ensure the sample's absorbance fell within the linear range of the calibration curve. The dilutions prepared were a 1:10, 1:25, 1:50 and 1:100. The absorbance of each solution was then measured at 725 nm using a spectrophotometer.

Determination of total antioxidant activity:

The determination of total antioxidant activity in the juice sample was completed spectrophotometrically using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Three treatments were prepared, each in a test tube: the juice sample, a positive control, and a blank. The juice sample contained 0.5 mL of 0.5 mM DPPH in methanol, 1 mL of diluted juice sample and 2.0 mL of methanol. The positive control tube used 2 mL of 0.5 mM butylated hydroxy anisol (BHA) in methanol, and then 0.5 mL of DPPH solution and 1.0 mL of deionized water. The blank contained 0.5 mL of DPPH solution, 1.0 mL of deionized water and 2 mL of methanol. Each tube had a total volume of 3.5 mL. The tubes were then mixed well, wrapped in foil to prevent light exposure, and incubated at room temperature for 45 minutes. The absorbance at 515 nm of each solution was measured using a spectrophotometer and the antioxidant activity of the juice was determined by comparison of absorbance to the positive control. Notably, this assay will produce negative absorbance values relative to the blank since DPPH gets lighter in the presence of active antioxidants.

Results

Determination of acid content:

An estimation of total titratable acid in cherry juice was completed by titration with 0.1 M sodium hydroxide. The endpoint was reached with 1.83 mL of NaOH meaning the 5.0 mL of diluted juice contained 1.83×10^{-4} equivalents of acid. The original filtered juice sample was diluted fourfold, meaning the acid concentration in the undiluted juice is 0.146 mol equivalents per liter.

$$0.00183L\ NaOH \times \frac{0.1mol}{L} = 1.83 \times 10^{-4} mol$$

$$1.83 \times 10^{-4}\ mol \times \frac{1}{0.00500\ L} \times 4 = 0.146\ eq/L$$

Since the predominant acid in cherry juice is malic acid, the acid content can be expressed as mass of malic acid per volume[4]. Malic acid has a molar mass of 134.09[1], but two titratable protons, meaning the equivalent weight is 67.045 g/mol. The malic acid content of the cherry juice was 9.79 g/L.

$$0.146\frac{mol}{L} \times \frac{67.045\ g}{mol} = 9.79\ g/L$$

Determination of total phenolic compounds:

A determination of total phenols in cherry juice was completed using FC reagent, saturated sodium carbonate, and absorbance measurement at 725 nm. A standard curve was constructed using tannic acid stock solution and standards ranging from 0.5 to 2.5 ppm. The standard absorbances ranged from 0.032 to 0.162 (Table 1).

Table 1. Tannic acid concentration and measured absorbance of six standard solutions used for determination of total phenols.

Standard	**Volume of 500 ppm tannic acid stock (mL)**	**Dilution factor**	**Con'c Tannic Acid (ppm)**	**Avg. Absorbance @ 725 nm**
S0	0.0	10X	0.0	0.000
S1	0.1	10X	0.5	0.032
S2	0.2	10X	1.0	0.064
S3	0.3	10X	1.5	0.097
S4	0.4	10X	2.0	0.125
S5	0.5	10X	2.5	0.161

A standard curve was constructed (Figure 1) using the absorbance of these standards. The coefficient of determination (R^2) was 0.9993 and the equation of the trendline was $y = 0.0639x - 0.0003$. Dilutions were also prepared of the sample tube containing cherry juice and both the 1:50 and 1:25 dilutions fell within the standard curve's linear range. The average absorbances of these dilutions were 0.047 and 0.099 respectively.

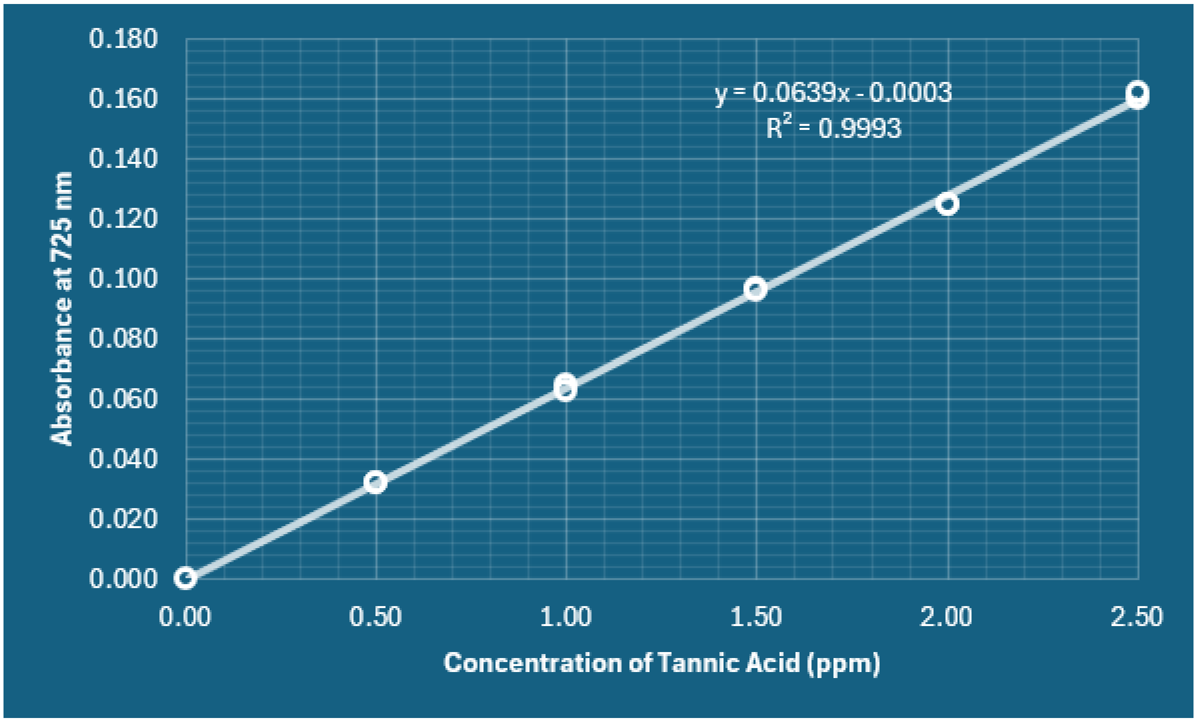

Figure 1. Calibration curve constructed from tannic acid standard solutions incubated with FC reagent and saturated sodium carbonate for the determination of total phenols.

Using the equation of the trendline and appropriate dilution factors, the phenol concentrations in the original sample tube was calculated to be 37.6 ppm. The concentration in the original undiluted cherry juice was then calculated to be 1.50 g/L ± 2.86% of tannic acid equivalent. Since tannic acid is a relatively large polyphenol, the molar concentration of total phenolics is likely higher than this relative value since many phenols have a molar mass lower than tannic acid.

$$\text{sample tube con'c} = \frac{(0.0478 - 0.0003)}{0.0639} \times \frac{10\ mL}{0.2\ mL} = 37.6\ ppm$$

$$\text{cherry juice con'c} = 37.6 \frac{mg}{L} \times \frac{10\ mL}{1\ mL} \times \frac{100\ mL}{25\ mL} = 1504\ mg/L$$

Determination of total antioxidant activity

A spectrophotometric assay using 0.5 mM DPPH was used to determine the total antioxidant activity of cherry juice. This assay measures the radical scavenging activity of a solution, where active antioxidants produce a colour change in the DPPH. Each tube contained 500 µL of 0.5 mM DPPH or 2.5×10^{-7}mols DPPH. A tube containing BHA was used as a positive control and represents complete radical scavenging of DPPH. The absorbance at 515 nm of each solution was taken in duplicate (Table 2). The positive control had an average absorbance of -0.9055 and the diluted juice sample had an average absorbance of -0.744.

Table 2. Absorbance measurements of three solutions each containing 2.5×10^{-7}mols DPPH in methanol.

Treatment	Absorbance @ 515 nm	Average absorbance @ 515 nm
Cherry juice	-0.744, -0.744	-0.744
Positive control (BHA)	-0.906, -0.905	-0.9055
Blank	0.000, 0.000	0.000

The DPPH radical scavenging activity of the juice sample can be given as a proportion of BHA activity, using the assumption that absorbance is linearly correlated to antioxidant activity:

$$\frac{-0.744}{-0.9055} = 82.2\%$$

The radical scavenging activity of antioxidants in the cherry juice was 82.2% of BHA activity, which is a relatively high antioxidant activity level. Since the positive control was known to contain 1.0 mmols/mL of BHA, the undiluted cherry juice was calculated to contain 3.288 mmols of BHA equivalent per mL.

Discussion

The analysis of a cherry juice sample was done to estimate the total acid, total phenolics and total antioxidants. For determination of total acid, a diluted sample was titrated with sodium hydroxide using phenolphthalein indicator. An important consideration in this method was diluting the red coloured juice enough to visualize the pink endpoint of phenolphthalein. The acid content was calculated to be 0.146 mol equivalents per liter or in terms of malic acid, 9.79 g/L. This value agrees within an order of magnitude to another cherry juice analysis[4] which reported 6.80 g/L malic acid. To improve the precision of this assay, multiple titrations should be done and an average volume of 0.1 M NaOH should be used for calculations. Increasing the volume of sample so that between 10-20 mL of titrant are required to reach the endpoint also increases precision since it adds a significant figure to the volume measurement.

An assay for determination of total phenolic compounds was also completed. This was a spectrophotometric assay using the FC reagent, which undergoes a colour change when reduced by phenols. A polyphenol, tannic acid, was used to construct a calibration curve which had good linearity. The equation of the trendline was used to calculate the concentration of phenolics in the original cherry juice to be 1.50 g/L of tannic acid equivalent. When determining the concentration of a molecule class, the individual species vary greatly in molecular weight, meaning that there is a lot of inherent error in this reported mass. Since tannic acid is actually a mixture of many similar polyphenols, its exact molar mass cannot be reliably used to calculate the amount of molar equivalents of phenols. This means the interpretation of the reported value, 1.50 g/L of tannic acid equivalent, can be challenging. Furthermore, the acid species in the FC reagent are susceptible to reduction by non-phenol reducing agents like ascorbic acid, and there are also some phenolic compounds that do not react with the FC reagent to produce the colour change[7,8]. These considerations show that this assay is not useful for high precision determinations of phenolic compounds, but is suitable for relative comparisons between multiple samples.

The total antioxidant activity of a cherry juice sample was measured using DPPH and spectrophotometric measurement. DPPH is a stable free radical that undergoes a colour change when reacted with a free radical scavenging molecule. BHA, a synthetic free radical scavenging (FRS) antioxidant, was used as a positive control and the cherry juice sample was compared to this control for a relative measure of FRS activity. The undiluted cherry juice contained 3.288 mmols of BHA equivalent FRS molecules. A point of consideration in this assay is the blank, which should contain DPPH but no antioxidants. Since the stable free radical form of DPPH appears purple, and loses colour to become yellowish when reduced to DPPHH, the blank will have a higher absorbance. If the spectrophotometer is simply blanked with this solution, the BHA containing positive control and sample solution will produce a negative absorbance, and the antioxidant activity of a sample is given by the proportion of sample absorbance of the positive control. The other option is to blank the spectrophotometer with the positive control, which will cause the negative control (blank solution) and the sample solution to produce positive absorbance values. The FRS activity of the sample is then given by the equation:

$$\frac{Abs_{neg.\ ctrl} - Abs_{sample}}{Abs_{neg.\ ctrl}} \times 100\%$$

In this experiment, the instrument was blanked with the negative control, and negative absorbance values were used to determine FRS activity. Notably, both calculation methods rely on the assumption of linearity between absorbance and FRS activity. Since only antioxidants which act as free radical scavengers react with DPPH to produce a colour change, this assay does not measure any antioxidants which act by another mechanism, though those are less common[10,11]. As with the assay for total phenols, the DPPH assay aims to quantify many individual molecules at once, making calculation of an exact molar or mass concentration difficult. Interpretation of the reported value, 3.288 mmols BHA equivalents, can also be challenging, and is best when directly compared to other samples. For increased precision, this assay would also benefit from multiple replicates, most easily done by preparing multiple tubes worth of each treatment.

Literature Cited

1. Ramroop-Singh, N. (2024). Biochemical Techniques I, Laboratory Manual. Thompson Rivers University Department of Biological Sciences.
2. LibreTexts. (2020, January 13). 7: Titration of Fruit Juices. Chemistry LibreTexts. https://chem.libretexts.org/
3. Damar, İ., & Ekşi, A. (2012). Antioxidant capacity and anthocyanin profile of sour cherry (Prunus cerasus L.) juice. Food Chemistry, 135(4), 2910–2914. https://doi.org/10.1016/j.foodchem.2012.07.032
4. Krueger, D. (2014). COMPOSITION OF SWEET CHERRY JUICE. https://www.kfl.com/kflimages/poster-sweetcherry.pdf
5. Mayo Clinic. (2008, February 10). When And How Often You Consume Acidic Foods Or Beverages Affects Dental Health. ScienceDaily. www.sciencedaily.com/releases/2008/02/080205204535.htm
6. Luna-Guevara, Ma. L., Luna-Guevara, J. J., Hernández-Carranza, P., Ruíz-Espinosa, H., & Ochoa-Velasco, C. E. (2018, January 1). Chapter 3 - Phenolic Compounds: A Good Choice Against Chronic Degenerative Diseases (Atta-ur-Rahman, Ed.). ScienceDirect; Elsevier. https://www.sciencedirect.com/science/article/abs/pii/B9780444641793000037
7. Lin, J.-Y., & Tang, C.-Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101(1), 140–147. https://doi.org/10.1016/j.foodchem.2006.01.014
8. Pérez, M., Domínguez-López, I., & Lamuela-Raventós, R. M. (2023). The Chemistry behind the Folin–Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. Journal of Agricultural and Food Chemistry, 71(46). https://doi.org/10.1021/acs.jafc.3c04022
9. Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C.-M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus Religiosa. Molecules, 27(4), 1326. https://doi.org/10.3390/molecules27041326
10. Stone, W. L., Pham, T., & Mohiuddin, S. S. (2020). Biochemistry, Antioxidants. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK541064/
11. Dj, B. (2000, February 1). What Is Oxidative Stress? Metabolism: Clinical and Experimental. https://pubmed.ncbi.nlm.nih.gov/10693912/