Investigating the Effects of Natural and Synthetic Antioxidants on Oxidising Apples

Group 1-34

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Abstract

Antioxidants have been commonly used as preservatives in beverages like fruit juices. However, most of the current antioxidants used are considerably toxic and pose a harm to human health, some even being carcinogenic. Thus, the aim of this study was to find suitable antioxidants which are conveniently found and effective in delaying oxidation as alternatives to commercially-used antioxidants. Varying masses of antioxidants were mixed with Mutsu Fuji apple juice at constant room temperature and tested for concentrations of potassium ions, glucose and chlorogenic acid. These 3 nutrients are easily oxidised, with chlorogenic acid itself sometimes acting as an antioxidant. The results obtained have distinctly shown the order of effectiveness of each antioxidant, but no trend could be established when different masses of antioxidants were used. Should the results have been more accurate, the most suitable antioxidants for replacing harmful antioxidants within beverages could be more accurately identified given more replicates in the study.

Introduction

Oxidation is a natural chemical process that destroys many nutrients and is also the same process that turns an apple's flesh brown when the plant tissues are exposed to oxygen in the surrounding air. The exposure to oxygen causes an enzyme in the flesh known as polyphenol oxidase (PPO) to be triggered, which will then start to oxidise the originally antioxidant polyphenols in the apple. The product of this reaction, o-quinones, subsequently reacts with amino acids to produce brown melanins (Petruzzello, M., n.d.). Vitamins A, C and E are particularly prone to oxidation. In addition, with longer exposure to air and light, the concentration of vitamins, as well as that of the polyphenols, in the flesh is lowered (Shingai. & Toshiya, 2011). Although storage under low temperatures delays oxidation, oxidation cannot be completely prevented (Holford, P., 2005).

Furthermore, many antioxidants like vitamin C are used as preservatives in beverages. As such, this project aims to find out what antioxidant, natural or synthetic, would be most effective at retaining the nutrients in the apples and preventing them from being destroyed.

Research has shown apples to possess a wide range of positive impacts on human health. In the Nurses' Health Study and the Health Professionals' Follow-Up Study involving over 100,000 men and women, fruit and vegetables were associated with a 21% reduced risk in lung cancer. Women consuming at least one serving per day of apples and pears had a reduced risk of lung cancer (Feskanich, D., 2000). Despite having been found to possess strong antioxidant activity (Boyer & Liu, 2004), apples still undergo oxidation. Their main protection against oxidation is their skin, as it contains many polyphenols with strong antioxidant activity. In the flesh, there are catechin, procyanidin, epicatechin, and phloridzin, but these are found in higher concentrations in the skin (Escarpa, & González, 1998). Apples with the skin had higher antioxidant activity than apples without them and were better able to inhibit cancer cell proliferation (Eberhardt & Liu, 2000). Apples have many immense positive health benefits but are vulnerable to losing them once oxidised.

Sodium chloride is known to be commonly used in households to wash fruit, although its true antioxidant effects may be unclear. An experiment investigating the antioxidant activity of sodium chloride based on the inhibitory effect of a sodium chloride solution on the oxidation of lipids and lipid-related compounds has indicated that sodium chloride has strong antioxidant activity on the aqueous oxidation of compounds tested (Miyashita, K., et al., 2003). Vitamin C is a powerful antioxidant, having the ability to donate a hydrogen atom and form a relatively stable ascorbyl-free radical and is suggested to decrease oxidative damage and lower the risk of certain chronic diseases (Pehlivan, 2017). Furthermore, lemon juice was also found to act as a powerful antioxidant because it contains citrates, flavonoids, vitamin E, vitamin C, and limonoids. The researchers also reported that vitamin C works as a strong antioxidant (Quita, S. M., 2016).

An increase in intake of potassium ions by humans is known to be able to reduce the risk of cardiovascular diseases, such as heart disease and stroke, by lowering blood pressure (Wax, E., 2018). Glucose is metabolised by the body so as to generate energy stored in the form of adenosine triphosphate (ATP) (Brennan, J., 2018). Chlorogenic acid has been found to have anxiety-reducing effects, coupled with antioxidant activity, as shown in a study on the effects of its extracts from certain prunes (Bouayed & Soulimani, 2007), but is also a good substrate, in other words supporting surface, for PPO oxidation (Wildermuth, S. R., 2016). As these 3 substances hold certain health benefits but are sometimes lost easily through oxidation, the concentration of these 3 substances in the apple juice were set as criteria to evaluate the effectiveness of antioxidants tested.

Finally, research regarding the effects of various natural or synthetic antioxidants on apples has not yet been conducted. This project thus aims to determine these effects and through it suggest less harmful alternatives to commercially-used antioxidants. Examples would be potassium sorbate, sodium metabisulphite and sodium benzoate. Although potassium sorbate has not shown any side effects in beverages (Hecht, M., 2017), sodium metabisulphite is known to be toxic by inhalation, irritate human skin and tissue and form a corrosive acid when mixed with water. A large concern over the use of sodium benzoate is its ability to convert to benzene, a known carcinogen. Benzene can form in soda and other drinks that contain both sodium benzoate and vitamin C (McCulloch, M., 2019).

Objectives

The objectives of this study are to find the effects of adding various antioxidants on the apples ability to retain the nutrients of apples and the extent to which different concentrations of the added antioxidants slows oxidation.

Hypotheses

The hypotheses of this study are that:

I. Ascorbic acid will be the most effective in percent retention of concentration of potassium ions, glucose and chlorogenic acid concentrations in apple juice tested, followed by freshly squeezed lemon juice, sodium chloride, sodium bicarbonate and deionised water

II. In higher concentrations, all antioxidants will show a higher rate of slowing oxidation.

Materials & Methods

a) Materials

Sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), ascorbic acid, also known as vitamin C, ($C_6H_8O_6$) and 40% potassium sodium tartrate solution ($KNaC_4H_4O_6$) were procured from GCE Laboratory Chemicals. Chlorogenic acid ($C_{16}H_{18}O_9$) standard curve under High-Performance Liquid Chromatography (HPLC) system and 1% 3, 5-dinitrosalicylic acid solution ($C_7H_4N_2O_7$) were obtained from Sigma-Aldrich, while potassium ion (K^+) standard curve under HPLC system was obtained from PerkinElmer. Mutsu Fuji apples (*Malus domestica "Mutsu"*) and Meyer lemons (*citrus limon "Meyer"*) were purchased from NTUC FairPrice Co. (Singapore).

b) Methods

(i) Preparation of set-ups

Apples were rinsed, cut and sliced thinly, then juiced to obtain apple juice. Lemon juice was obtained via cutting and squeezing the lemons. Antioxidants used were weighed using an electronic balance before being added into the set-ups. For each experimental set-up, 75 ml of apple juice was added into a glass bottle, followed by a specific amount of the respective antioxidant - 5.0 g, 7.5 g, 10.0 g, 12.5 g and 15.0 g, and finally 45 ml of deionized water. The negative-control set-up did not contain any antioxidant. The glass bottles were sealed and left in a room at a constant temperature of 28°C for 1 day. Afterwards, 2 5 ml samples from each bottle of juice were sent for testing of concentration of specific substances. Despite the initial plans, as a result of time constraints, experiments were only carried out for samples containing 5.0 g and 7.5 g of antioxidants.

(ii) Testing effectiveness of added antioxidants in retaining apple nutrients

It was initially planned for the high-performance liquid chromatography (HPLC) system to measure the concentration of glucose and cholorogenic acid while the atomic absorption spectrophotometer (AAS) was used to measure the concentration of potassium ions in the apple juice samples. There were time constraints and the HPLC system and AAS were unable to be used after the reopening of the facilities. As it was not possible to test for potassium ions and chlorogenic acid without using the HPLC system and AAS within the limits of our facilites, results for the concentrations of those compounds could only be retrieved later. The best replacement for the testing of glucose was to run a reducing sugars test using 3, 5-dinitrosalicylic acid ($C_7H_4N_2O_7$) (DNS). For this test, a known concentration of glucose in 1.5 ml of deionised water was added to a centrifuge tube, followed by 1.5 ml of 1% DNS solution. After heating in a boiling water bath for 5 minutes and cooling in an ice bath for 2 minutes, 0.5 ml of 40% potassium sodium tartrate solution was added to every centrifuge tube. A standard curve was plotted by using the (UV-VIS) spectrophotometer to measure the absorbance of the DNS at 575 nm and relate this value to its respective concentration of reducing sugars. For the juice samples, DNS tests were conducted in the same way, except for the replacement of 1.5 ml of glucose solution with 1.5 ml of juice. For samples heated that had too high a light absorbance, a dilution using water to the same volume of 1.5 ml was conducted. Then, using the standard curve obtained, after measuring the light absorbance of the DNS used in the unknown concentration of reducing sugars in the apple juices, the concentration of reducing sugars can be determined by checking against the curve and multiplying by the dilution factor if any.

Results and Discussion

a) **Chlorogenic acid**

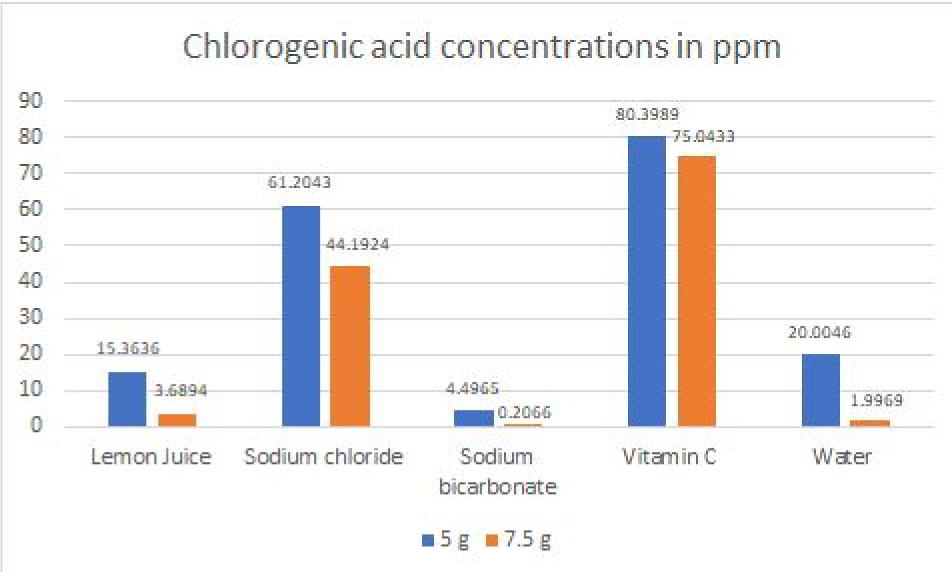

Fig 1.1: Concentration of chlorogenic acid in the control and experimental set-ups.

From Fig 1.1, the concentration of chlorogenic acid in the control set-up for 5.0 g was 20.0046 ppm compared to 15.3636, 61.2043, 4.4965 and 80.3989 ppm for the lemon juice, sodium chloride, sodium bicarbonate and ascorbic acid, respectively. The concentration of chlorogenic acid in the control set-up for 7.5 g was 1.9969 ppm compared to 3.6894, 44.1924, 0.2066 and 75.0433 ppm for the lemon juice, sodium chloride, sodium bicarbonate and ascorbic acid, respectively. These results indicated that juice samples with sodium chloride and ascorbic acid added to them had higher concentrations of chlorogenic acid than the control set-up, while that of lemon juice was lower, while sodium bicarbonate fared even worse than the control set-up. This deviated from the first hypothesis by a fair bit.

b) Potassium ions

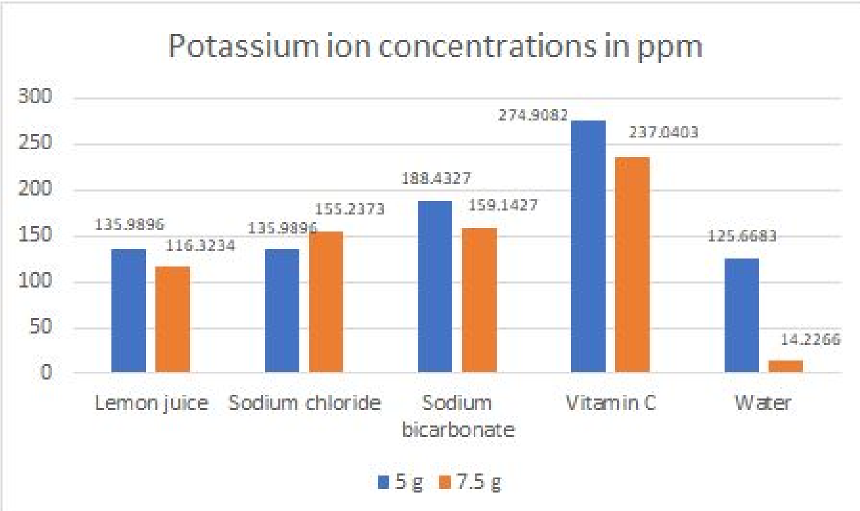

Fig 2.1: Concentration of potassium ions in the control and experimental set-ups.

From Fig 2.1, the concentration of potassium ions in the control set-up for 5.0 g was 125.6683 ppm compared to 135.9896, 135.9896, 188.4327 and 274.9082 ppm for the lemon juice, sodium chloride, sodium bicarbonate and ascorbic acid, respectively. The concentration of potassium ions in the control set-up for 7.5 g was 14.2266 ppm compared to 116.3234, 155.2373, 159.1427 and 237.0403 ppm for the lemon juice, sodium chloride, sodium bicarbonate and ascorbic acid, respectively. These results have shown that the juice sample with ascorbic acid added remained as the sample with the highest concentration of substance measured, followed by samples with lemon juice, sodium chloride and sodium bicarbonate added to them, each having around the same percent retention of potassium ions. These results were closer to the first hypothesis made, as compared to those in Fig 1.1, as the concentration of potassium ions in the control set-up has fallen below that of the experimental set-ups.

c) **DNS light absorbance and derived reducing sugars concentration**

DNS light absorbance at 575 nm:			
Antioxidants:	7.5 g (1st):	7.5 g (2nd):	7.5 g (average):
Lemon juice	1.106	1.000	1.053
NaCl	8.355	8.415	8.385
$NaHCO_3$	0.147	0.147	0.147
Ascorbic acid	0.038	0.044	0.041
Water (control)	0.044	0.033	0.0385

Fig 3.1: 1st, 2nd and average DNS light absorbance readings in the control and experimental set-ups.

Derived reducing sugars concentration (mg/100 ml):			
Antioxidants:	7.5 g (1st):	7.5 g (2nd):	7.5 g (average):
Lemon juice	185.644	167.988	178.816
NaCl	1412.97	1422.96	1417.965
$NaHCO_3$	9.581	9.581	9.581
Ascorbic acid	7.749	8.748	8.2485
Water (control)	8.748	6.916	7.832

Fig 3.2: Derived concentration of reducing sugars in the control and experimental set-ups.

From Fig 3.1 and 3.2, it can be observed that there is a rather different trend in the concentration of reducing sugars derived, as compared to that of potassium ions and chlorogenic acid. However, there were inevitable errors with the usage of such a method to measure the concentration of reducing sugars within juice samples. First, the original colour change of each juice sample could have affected the light absorbance reading and hence the derived concentration. Second, there were reducing sugars present in the lemon juice added, thus the derived reducing sugars concentration may inaccurately depict the antioxidant properties of the lemon juice tested. Excluding the juice sample with lemon juice added to it, the antioxidant which had the highest percent retention of reducing sugars was sodium chloride, followed by sodium bicarbonate, ascorbic acid and finally the control set-up. However, the large deviation between the percent retention of reducing sugars if sodium chloride and that of the other antioxidants, disregarding lemon juice, shows the results obtained for sodium chloride to be anomalous. It was observed that through heating the sample with added sodium chloride during the DNS test turned abnormally dark. A possible reason for this anomaly would be that the juice sample we used had been contaminated, resulting in a light absorbance which was much higher than the actual value, in turn affecting the derived reducing sugars concentration. During the conduction of the DNS test, many readings for 5 g antioxidant samples were anomalous. Due to the shortage of time, the DNS test was not conducted on those samples.

Conclusion and Recommendations for Future Work

From the concentrations of substances obtained, the effectiveness in percent retention of nutrients overall, in descending order were ascorbic acid, sodium chloride, sodium bicarbonate and lemon juice. All antioxidants showed a higher percent retention of nutrients as compared to the negative control set-up, but showed a lower percent retention of nutrients in higher concentrations. Thus, antioxidants used in this experiment were able to slow down oxidation but did not increase in effectiveness of retaining apples' nutrients in higher concentrations. The antioxidants investigated in this study can be used as alternatives to common antioxidants in beverages as mentioned before which are known to harm human health.

More experiments on different masses of antioxidants and on other antioxidants and different types of drinks could be conducted with more repetitions being done for reading concentrations of chlorogenic acid, potassium ions and glucose using more accurate instruments when available for usage, like the Atomic Absorption Spectrophotometer and High-Performance Liquid Chromatography system which was intended to be used.

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