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Article

The effect of wild orange essential oil on ascorbic acid decay in freshly squeezed orange juice

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SUMMARY

Ascorbic acid, also known as Vitamin C, is an essential nutrient for human beings as those are deficient develop scurvy. Ascorbic acid, by nature, is not stable. Once the orange is peeled it is exposed to atmospheric oxygen, oxidation, which deactivates ascorbic acid, occurs. Due to being high in limonene, wild orange essential oil (EO) has purifying and cleansing properties that can be a natural food preservative. The goal of this project was to see if the addition of wild orange EO to freshly squeezed orange juice would help to slow down the decay of ascorbic acid when exposed to various temperatures, allowing vital nutrients to be maintained and providing a natural alternative to the chemical additives in use in industry today. We hypothesized that the addition of wild orange EO to freshly squeezed orange juice would slow down the rate of oxidation when exposed to various temperatures, reducing ascorbic acid decay. An iodine redox titration was used to measure ascorbic acid across four different wild orange EO volumes and four different temperatures. Data was collected and used to compare experimental samples to controls. On average, wild orange EO slowed down ascorbic acid decay in freshly squeezed orange juice by 15% at the three highest temperatures tested.

INTRODUCTION

A deficiency of ascorbic acid in the body can lead to a disease called scurvy. Scurvy affects the blood vessels, skin, and the body's ability to heal (1). Oranges, a widely available citrus fruit, are natural sources of ascorbic acid (2). Oranges have the highest amount of ascorbic acid among all citrus fruits. A regular-sized orange contains approximately 70 milligrams of ascorbic acid (3). Wild orange essential oil (EO) is extracted from the rind of the orange by a method called cold pressing. During cold pressing, the fruit travels across needle-like cylinders that puncture the peel's surface, causing tiny EO sacs to burst open. Water is then sprayed on the fruit, and the mixture is collected into a tank. The mixture is filtered to remove the solids, and the clean oil/water is transferred to another container. The oil naturally rises to the top and is then separated from the water (4). Wild Orange EO is made up of 97% of a natural molecule that is present in the orange rind called limonene. Limonene is a hydrocarbon which means the molecule contains only hydrogen and carbon (5). Limonene's arrangement of atoms, specifically its monoterpene 10-carbon backbone structure, gives it its powerful purifying and cleansing properties, which could make it a good option for a natural food preservative (6).

By its intrinsic nature, ascorbic acid is not stable (7, 8). Once an orange is peeled or juiced and exposed to the environment, a chemical reaction called oxidation begins. Oxidation is when electrons are lost in a reaction with a molecule, atom, or ion (9). The oxidation reaction causes the ascorbic acid to decay and reduces the nutritional value of the juice (10, 11). Five factors contribute to oxidation in citrus juice: oxygen, metal ions, pH of juice, light, and temperature (12). Temperature is a potentially attractive influence for addressing ascorbic acid decay. In order for a chemical reaction to occur, the reacting particles must collide (13). Research shows that when the temperature increases, the particles move around and become unstable. The higher the temperature, the faster the particles will move and react (14).

In our experiment, ascorbic acid in orange juice is measured by a redox titration using iodine and a soluble starch indicator. During the titration, iodine, which is the titrant, reduces to iodide. As long as there is ascorbic acid present in the analyte sample solution, there is no color change. Once the ascorbic acid has been oxidized and no longer present, the excess iodine is free to react with the starch indicator causing a color change from pale yellow to bluish-black for the standard solution and from orange to grayish brown for the fresh orange juice samples (15). The amount of titrant needed to complete the titration for the orange juice samples is compared to the amount of titrant needed to complete the titration for the standard solution allowing for the ascorbic acid content of each orange to be calculated .

Slowing down the oxidation and natural decay of ascorbic acid is important, particularly in developing countries where storage conditions are not ideal, and disease is more widespread. Most of the orange juice on the market today is chemically engineered (16). Once oranges are picked, they are juiced, pasteurized to remove oxygen, and then stored in tanks for up to a year. Prior to packaging, manufacturers artificially alter the flavor by adding potential cancer-causing chemicals such as ethyl butyrate (17).

In this experiment, we investigated the potential for wild orange EO, in varying volumes, to reduce the amount of temperature-induced oxidation of ascorbic acid. Four different

temperature conditions were tested, and all samples were exposed to various temperatures at all times, to study the possible benefit of a natural preservative for freshly squeezed orange juice. On average, ascorbic acid decay in freshly squeezed orange juice was slowed down by wild orange EO by 15% at the three highest temperatures tested. Based on our understanding of oxidation protection, we believe that wild orange EO has the potential to maintain the vital nutrients to protect human beings from diseases such as scurvy, as well as be a natural alternative to the chemical additives being used today in the orange juice industry.

RESULTS

To explore if the addition of wild orange EO to freshly squeezed orange juice would slow down the rate of oxidation of ascorbic acid, navel oranges were purchased in bulk at the beginning of every trial. After juicing the oranges, the initial average ascorbic acid levels across all EO volumes were within 0.02 to 0.07 mg. There was a 0.35 to 1.26 mg variation in EO between temperature trials. This is due to the natural variation in navel orange ascorbic acid levels.

For 110° F, the largest difference was 72 hours. The samples with the higher volumes of EO (1.8 mL and 1.2 mL) were 7.52 mg and 7.13 mg, respectively, vs. 6.53 mg and 6.82 mg for the control (0.00 mL EO) and 0.6 mL EO. The ascorbic acid decayed 0.78 to 1.14 mg from the initial (freshly squeezed) through the 48-hour point. The mean absolute deviation for the 110° trials ranged from 0.01 mg to 0.08 mg (**Figure 1**). The mean absolute deviation of a data set is the average distance between each data point and the mean. It gives us an idea about the variability in a dataset.

For 120° F, there was a consistent difference between each of the EO volumes at the 24, 48, and 72-hour points. At the 96-hour point, the ascorbic acid levels across all EO volumes were reasonably similar. The mean absolute deviation for the 120° F trials ranged from 0.009 mg to 0.06 mg (**Figure 2**).

For 130° F, the ascorbic acid decay for the EO volumes of 0.6 mL, 1.2 mL, and 1.8 mL was quite similar. There was a consistent difference between the samples with EO added and the control (0.00 mL EO added). The greatest difference was at the 24 and 48-hour points. The mean absolute deviation for the 130° F trials ranged from 0.01 mg to 0.09 mg (**Figure 3**).

For 140° F, the ascorbic acid decay for the EO volumes of 0.0 mL and 0.6 mL were similar. In addition, the ascorbic acid decay for the EO volumes of 1.2 mL and 1.8 mL were similar. The EO volumes of 1.2 mL and 1.8 mL had a more significant effect on ascorbic acid decay. The greatest difference was at the 48-hour point. At the 96-hour point, the ascorbic acid levels across all EO volumes were fairly similar. The mean absolute deviation for the 140° F trials ranged from 0.01 mg to 0.08 mg. (**Figure 4**).

In general, the total ascorbic acid decay (the difference between the initial ascorbic acid level and the ascorbic acid level after 96 hours) increased as the temperature increased regardless of the amount of EO added, with 130° and 140° F temperatures having similar decay and wild orange EO affected ascorbic acid decay across all temperatures.

Based on a statistical two-way ANOVA analysis, we were able to conclude that our hypothesis was supported. Time has no significant effect on ascorbic acid concentration in orange juice. The F distribution of the ANOVA analysis with (5, 15) degrees of freedom had a F-ratio of 7.45 at a $p < 0.05$ level of significance and a critical value = 2.9. The F-ratio of 7.45 which is greater than the critical value of 2.9 indicates the result is significant at p<0.05 probability. There was a significant effect of time on ascorbic acid concentration in

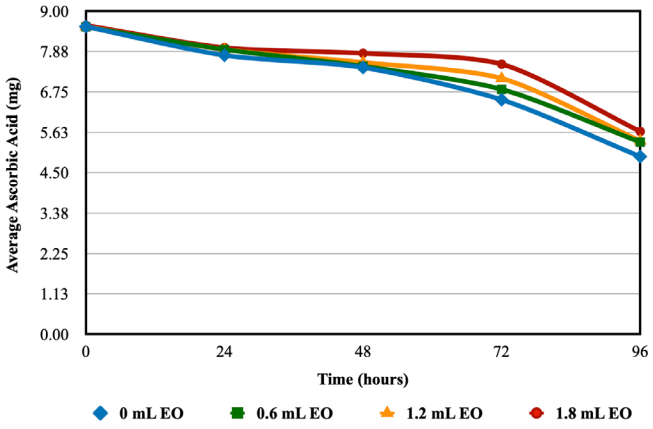

Figure 1. Ascorbic acid decay over time at 110 degrees Fahrenheit. N=3. Line graph showing average ascorbic acid in mg vs. time in hours for each wild orange essential oil (EO) tested at 110 degrees F. Different volumes of wild orange EO (0.0, 0.6, 1.2, 1.8 mL) were added to freshly squeezed orange juice in varying volumes and kept at a constant temperature of 110 degrees F over the course of 96 hours.

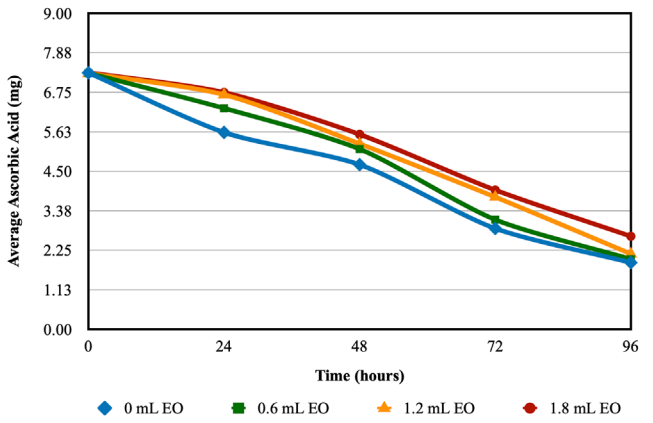

Figure 2. Ascorbic acid decay profile as a function of time - 120 degrees Fahrenheit. N=3. Line graph showing average ascorbic acid expressed in mg vs. time expressed in hours for each wild orange essential oil (EO) tested at 120 degrees F. Different volumes of wild orange EO (0.0, 0.6, 1.2, 1.8 mL) were added to freshly squeezed orange juice in varying volumes and kept at a constant temperature of 120 degrees F over the course of 96 hours.

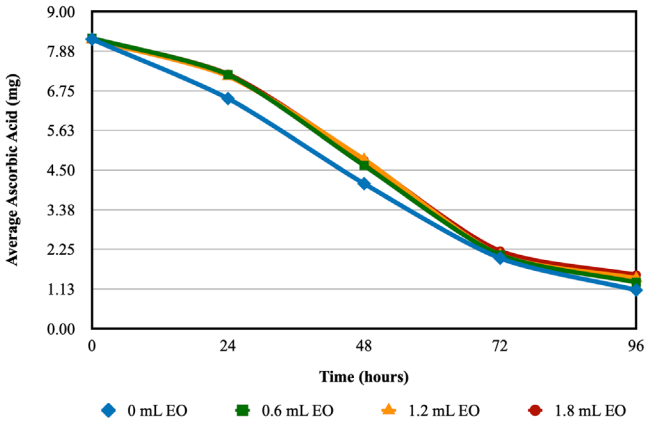

Figure 3. Ascorbic acid decay profile as a function of time - 130 degrees Fahrenheit. N=3. Line graph showing average ascorbic acid expressed in mg vs. time expressed in hours for each wild orange essential oil (EO) tested at 130 degrees F. Different volumes of wild orange EO (0.0, 0.6, 1.2, 1.8 mL) were added to freshly squeezed orange juice in varying volumes and kept at a constant temperature of 130 degrees F over the course of 96 hours.

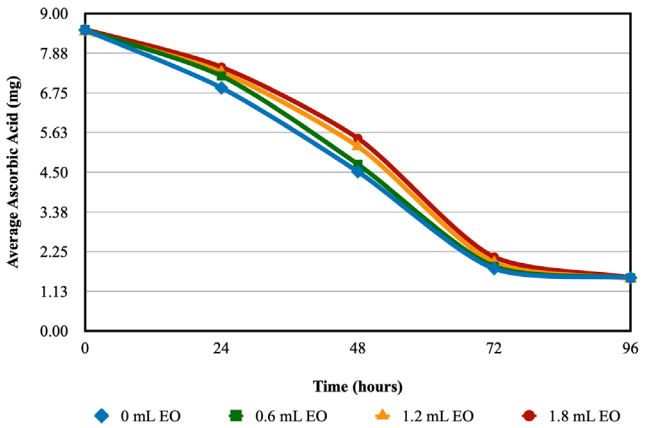

Figure 4. Ascorbic acid decay profile as a function of time - 140 degrees Fahrenheit. N=3. Line graph showing average ascorbic acid expressed in mg vs. time expressed in hours for each wild orange essential oil (EO) tested at 140 degrees F. Different volumes of wild orange EO (0.0, 0.6, 1.2, 1.8 mL) were added to freshly squeezed orange juice in varying volumes and kept at a constant temperature of 140 degrees F over the course of 96 hours.

orange juice. The F distribution of the ANOVA analysis with (3, 15) degrees of freedom had a F-ratio of 3.35 at a $p < 0.05$ level of significance and a critical value of 3.29. The F-ratio of 3.35 which is greater than the critical value of 3.29 indicates the result is significant at p<0.05 probability. Temperature had a significant effect on ascorbic acid concentration in orange juice. The F distribution of the ANOVA analysis with (15,15) degrees of freedom had a F-ratio of -0.67 at a $p < 0.05$ level of significance and a critical value of 2.25. The results from the statistical two-way ANOVA analysis strongly indicate that time and temperature interaction have a significant effect on ascorbic acid concentration in orange juice (**Figure 5**).

DISCUSSION

Ascorbic acid decay was measured across temperatures from 110-140° Fahrenheit. These temperatures were chosen so that the rate of ascorbic acid decay could be measured within the four-day testing period. Initially, room temperature and 40° Fahrenheit were tested and there was no significant evidence of change in ascorbic acid decay. This is essential to note because it shows that the EO added to the freshly squeezed juice did not increase initial ascorbic acid concentration. Interestingly, orange juice that was not tested at an elevated temperature spoiled before decay could even be measured.

The biggest limitation experienced was that samples could only be pulled every 24 hours, taking into consideration sleep and everyday school and work. Although we did not have access to advanced equipment while performing this experiment, in the future, using a thermocouple would provide a more accurate reading of temperature.

After completing the experiment, we hypothesized that when the EO is added to the orange juice, the hydrocarbon oil, which is made up of 97% limonene, creates a microemulsion barrier in the solution (13). The barrier could protect the ascorbic acid molecules from decomposition. There is a possibility that there is a correlation between temperature and ascorbic acid decay, as when the temperature increases, the ascorbic acid decay increases.

Wild orange EO slowed ascorbic acid decay across all temperatures. The 110° Fahrenheit trial temperature had the greatest difference of average ascorbic acid at 72 hours. The 120° Fahrenheit trial temperature had a consistently lower average ascorbic acid between each of the EO volumes at all sample points. The 130° Fahrenheit trial temperature had an ascorbic acid decay rate consistently lower amongst samples treated with higher levels of EO and the control

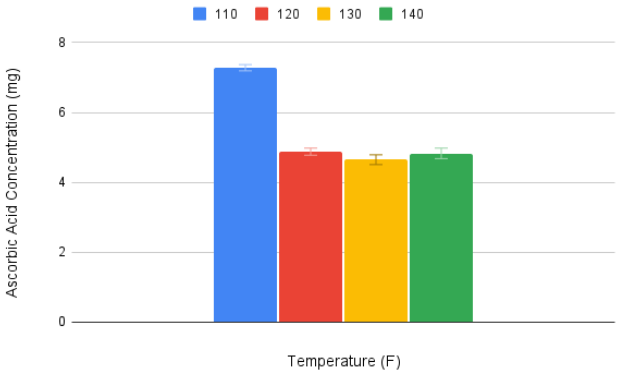

Figure 5. Ascorbic acid concentration as function of temperature. Bar graph showing ascorbic acid concentration expressed in mg and temperature expressed in Fahrenheit. Statistical two-way ANOVA analysis was completed and strongly indicates that time and temperature interaction have a significant effect on ascorbic acid concentration in orange juice, after 96 hours.

at all sample points. The 140° Fahrenheit trial temperature had a consistently lower ascorbic acid decay rate at the two higher volumes of EO added versus the control and lowest concentration of EO added. In general, the more EO added, the slower the ascorbic acid decay. Furthermore, a lower sample temperature slowed ascorbic acid decay regardless of the amount of EO added.

In the future, several more experiments could be completed to continue the exploration on the effect of EO on ascorbic acid decay. Different brands and higher volumes of wild orange EO could be used. Other citrus juices and essential oils such as lemon, lime, and grapefruit could also be used. Finally, higher temperatures could be tested to model the pasteurization process.

MATERIALS AND METHODS

Four different temperatures were tested (110° F, 120° F, 130° F, and 140° F). A water bath was prepared using a clear storage container and sous vide machine, set at the appropriate temperature for the trial. Four mason jars were labeled to indicate the trail number and control (no EO added), ten drops, 20 drops, and 30 drops, respectively. The samples for a trial were prepared by measuring 400 mL of freshly squeezed orange juice in each of the four mason jars and adding the appropriate amount of wild orange EO to the mason jars. The mason jars were weighed down to prevent them from floating and were placed in the covered water bath. The steps above were repeated for each temperature tested. A titration on each of the four samples was completed after 24, 48, 72, and 96 hours (**Figure 6**).

For each temperature tested, three trials were completed according to the procedure with four different wild orange EO volumes (0 mL, 0.6 mL, 1.2 mL, and 1.8 mL) per 400 mL of freshly squeezed orange juice. Wild orange EO was added in drops and the volumes calculated based on the total ml in the bottle (15 mL) and the number of drops in each bottle (250 drops) as determined by the manufacturer.

The wild orange EO concentration calculation follows:

Wild orange EO concentration = (15 ml / 250 drops) x number of drops added to the mason jar

A titration solution was made by combining 300 mL of a 1:10 titration solution with 30 mL of Lugol's Iodine Solution, and 270 ml of distilled water.

An ascorbic acid standard solution was made to compare the ascorbic acid of a known solution to the ascorbic acid of the orange juice samples. A mortar and pestle were used for crushing one 250 mg chewable Vitamin C tablet into powder form. The powder was transferred into a 250 mL beaker and was then dissolved in distilled water to bring the total volume to 250 mL. A standard titration was completed by measuring 20 mL of the ascorbic acid standard solution and pouring it into a 125 mL Erlenmeyer flask. Ten drops of 1% soluble starch solution were added using a plastic pipette. To titrate the ascorbic acid standard solution, the flask was swirled while adding the dilute iodine solution one drop at a time until the solution in the flask turned from a pale yellow to a blue/black color for more than 20 seconds. The initial and final iodine solution levels were measured at the bottom of the meniscus and recorded in milliliters. The titration oxidation amount for the standard solution was then calculated. For each standard solution, three titrations were completed to ensure the titration oxidation volumes were within 0.2 mL. The steps above were repeated at the beginning of each trial (**Figure 7**).

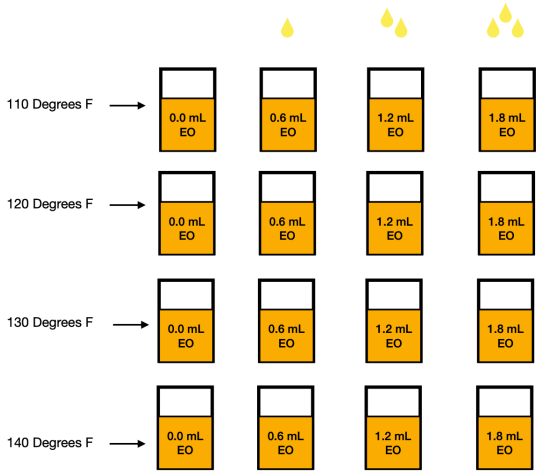

Figure 6. Employed experimental design in this study. Addition of four different volumes of wild orange essential oil to orange juice kept at four different temperatures.

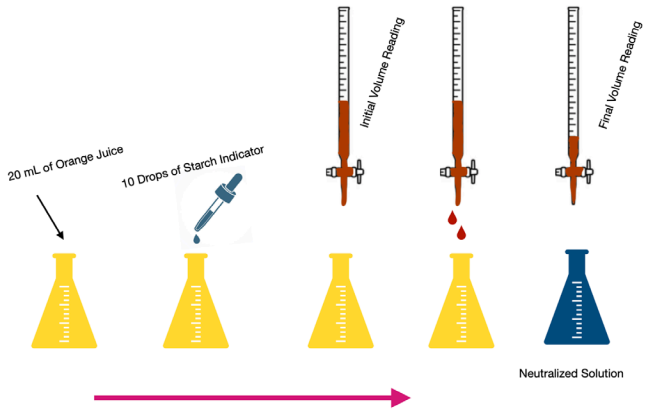

Figure 7. Titration method. The five steps on how to complete an iodine redox titration are illustrated in the figure.

The standard solution titration oxidation amount calculation follows:

Standard Solution Titration Oxidation Amount = (Final Iodine Solution at the bottom of meniscus) - (Initial Iodine Solution at the bottom of meniscus)

The average standard solution oxidation amount was calculated, recorded in milliliters, and used later to calculate each sample's ascorbic acid for that trial.

Navel oranges were juiced prior to each trial, and initial titrations of 20 mL each were completed. The initial and final iodine solution levels were measured at the bottom of the meniscus and were recorded in milliliters. The titration oxidation amount for each sample was then calculated. For each sample, three titrations were completed to ensure the titration oxidation volumes were within 0.2 mL.

To complete the sample titrations, 50 mL of orange juice sample was poured from the mason jar and was allowed to come to room temperature. Using a graduated cylinder, 20 mL of the 50 mL room temperature orange juice sample was measured and poured into a 125 mL Erlenmeyer flask. Ten drops of 1% soluble starch solution were added using a plastic pipette, and the dilute iodine volume measurement was recorded using the volume markings on the burette at the bottom of the meniscus. The freshly squeezed orange juice was titrated by swirling the flask and adding the dilute iodine solution one drop at a time until the solution in the flask turns from an orange juice color to a gray/brown color for more than 20 seconds while swirling.

The final dilute iodine volume measurement was recorded using the volume markings on the burette at the bottom of the meniscus.

The titration oxidation amount calculation follows:

Titration Oxidation Amount = (Final Iodine Solution at the bottom of meniscus) - (Initial Iodine Solution at the bottom of meniscus)

The average oxidation amount was then calculated and recorded in milliliters. The titration process was repeated two additional times to ensure the titration amount was within 0.2 mL.

The ascorbic acid was then calculated and recorded in milligrams. 20 mg was used in the calculation below for the "ascorbic acid of the standard" since the standard solution was 1 mg/mL, and 20 mL was used for all titrations.

The ascorbic acid calculation follows:

Ascorbic Acid = [(Average Titration Oxidation Amount) x (Ascorbic Acid of Standard)] / Average Titration Oxidation Amount of Standard

For each temperature tested, three trials were completed. The average ascorbic acid was calculated for each EO concentration at the five different time intervals (initial, after 24 hours, after 48 hours, after 72 hours, and after 96 hours).

After the ascorbic acid of each EO concentrations at the five different time intervals (initial, after 24 hours, after 48 hours, after 72 hours, and after 96 hours.) were calculated, the mean absolute deviation was calculated. This calculation was done to measure how far the data points were from the mean. In addition, a statistical two-way ANOVA analysis was completed to test the null hypothesis of time, temperature, and the interaction of time and temperature on the ascorbic acid concentration in orange juice.

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