TED ANKARA COLLEGE FOUNDATION PRIVATE HIGH SCHOOL

Investigating the effect of microfluidization and ultrasonication on the ascorbic acid concentration of orange juice

Extended Essay (Biology)

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Abstract

The aim of this study was to determine the effect of microfluidization and ultrasonication processing techniques used in the industrial mercantile on the ascorbic acid concentration of orange juice.

The research question was: "Is there a significant mean difference between the ascorbic acid concentrations in the fresh, microfluidized and ultrasonicated orange juices?" It is hypothesized that there should be a significant mean difference between the ascorbic acid concentration in the fresh, microfluidized and ultrasonicated orange juices since the microfluidization and ultrasonication dilute and shear the orange juice by removing and breaking down its particles which may reduce the ascorbic acid content. A common titration technique was used to calculate the ascorbic acid concentration in the orange juices. With the help of the reagents indophenol (blue colored indicator), metaphosphoric acid and ascorbic acid, standard values of ascorbic acid was collected to use in the formula which is used to determine the ascorbic acid concentration in the orange juice. By the use of indophenol in the titration, the color of the orange juice solution will turn into pink as the indophenol nullifies the ascorbic acid content in the orange juice solution. The volume of indophenol solution will be recorded in order to use in the formula which is to find the amount of ascorbic acid in the orange juice. More over, the blank trial and standardization data are recorded to be used in the same formula. The Anova results, P-value and graphical investigation accept the alternate hypothesis which suggests there should be a difference between average ascorbic acid concentrations of processed and unprocessed juices. The average concentration of ascorbic acid in the fresh orange juice was 4.82 g/lt, the concentration of ascorbic acid in the microfluidized orange juice was 4.10 g/lt and the concentration of ascorbic acid in the ultrasonicated orange juice was 3.00 g/lt.

As a result, the experiment results and the Anova test showed that there was a significant mean difference between the concentrations of ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices.

334 words

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Introduction/Background

Proper nutrition is a very important part of a healthy life. Unfortunately, our changing life style has increased our tendency to consume rather than produce. As a natural consequence of this, food industry developed technologies that increase the amount and shelf life of foods to meet the demands of consumer society. These technological modifications however, brought about a decrease in nutritional value of foods, namely, the concentrations of carbohydrates, lipids, proteins, and especially vitamins.

Vitamins are vital in human life, as they are essential to protect the body from oxidative damage and play important role in body functions, in repair and growth.[1] There are 13 vitamins; Vitamin A, B complex, C, D, E and K.[2] Vitamin B complex and C are water soluble vitamins that can't be stored, so foods containing these vitamins have to be taken daily.

Vitamin C, as known as ascorbic acid, is necessary in human body for the functioning of the cardiovascular system, immune system, and collagen synthesis and it has antioxidant activity. Severe deficiency of vitamin C causes scurvy. Despite being rare, scurvy includes potentially severe consequences and can cause sudden death. Vitamin C has many additi onal uses but not all of them are proved to success. For example, researches on asthma, cancer and diabetes remains inconclusive, and no benefits have been found for the prevention of cataracts or heart disease.[3] It is necessary to consume vitamin C including nutrients in order to prevent these kinds of disorders. There are various food sources containing Vitamin C like: red and green hot chili peppers, guavas, bell peppers, fresh herbs, broccoli, cauliflower, Brussels sprouts, kiwi fruits, papayas, tangerines and strawberries.[4]Some of these food sources can be presented in the form of processed food like fruit juices.

Industrial fruit juices are especially preferred by countries that cannot provide fresh sources of fruits and vegetables. Many people prefer consuming fruit juices due to their long shelf life and hygiene. However, although these commercially available products offer easy access, they can't be said to have nutritional quality comparable to fresh fruits. In the industrial mercantile the vitamin C containing fruit juices are subjected to several processes before they will be in the shops. Vitamin C is highly vulnerable and easily degraded due to the handling methods such as freezing, soaking, streaming, boiling, baking[5]

[1] http://www.livestrong.com/article/271425-why-are-vitamins-important-to-your-body/
[2] http://www.fauxpress.com/kimball/med/essentialv.htm
[3] http://www.mayoclinic.com/health/vitamin-c/NS_patient-vitaminc
[4] http://www.healthaliciousness.com/articles/vitamin-C.php
[5] http://www.whfoods.com/genpage.php?tname=nutrient&dbid=109

and increased storage time[6]. Streaming, a process necessary to get rid of contaminants like bacteria; result in about 25% loss in vitamin C content. Cooking can cause a loss of 50%. Furthermore, when the foods are canned and reheated, about 66% of the vitamin C is lost. In addition, after the peeling of the foods such as tangerines, pectin enzyme in them gets activated resulting in the formation of a precipitate. This will affect the homogenic property of the fruit juice thus the vitamin C concentration.

Two of the well-known food processing tools are necessary to increase the physical quality of the product in order to attract more customers, which are microfluidization and ultrasonic treatment. Microfluidization, which is the homogenization of certain fluids, is necessary in industrial fruit juices for making them more appealing for the customers and more hygienic. This process mostly separates large particles from the juice. As the fluid goes through the microfluidizer the particles in it are separated and the juice is homogenized.[7] Microfluidization process is the combination of a series of processes including exposure of the liquid to extremely high velocity, high frequency vibrations, high shear and high pressure in a very short time (Liu et al., 2009). High pressure causes the juice to flow rapidly in the microfluidizer's channels, which will divide into two smaller channels in order to shear the juice into smaller particles. Ascorbic acid is affected from most of the processes and the occurrence of loss of ascorbic acid depends on the treatment conditions (Vieira et al., 2000).

Ultrasonic treatment is another common process used in the preparation of industrial juices. Like microfluidization, it also includes high pressure cycles in order to shear the juice into smaller particles while separating the larger ones. The only major difference is that ultrasonic treatment raises the temperature of the juice extremely (5000 K). These high pressure and high temperature treatments cause the juice to break down to the smallest particles.[8]

I have always had a deep interest in nutritional health. All this information made me wonder about the effect of these processes on the vitamin C content of foods. Personally, I preferred consuming fresh juices rather than the industrial juices based on their potentially higher vitamin concentration.

To test this potential we can look at the literature review[9] which revealed that there is a simple way to measure the concentration of ascorbic acid in orange juice which is titration. The titration can be done with an indophenol solution prepared by combining dichlorophenolindophenol and sodium

[6] http://www.ultimatecitrus.com/vitaminc.html
[7] http://www.qualitydigest.com/inside/fda-compliance-article/high-shear-fluid-processing
[8] http://www.hielscher.com/ultrasonics/extraction_01.htm
[9] http://cygm.meb.gov.tr/modulerprogramlar/kursprogramlari/gida/moduller/GidalardaAskorbikAsitTayini.pdf

carbonate. After adding enough indophenol solution into the juice sample, the juice sample's color will turn to pink and stay pink for at least 20 seconds to indicate that the experiment is finished. The initial volume of the juice sample is subtracted from the final volume in order to determine the vitamin C concentration in the juice sample.[10]

This study compares ascorbic acid concentration of the industrial orange juices against the fresh orange juice. I think this topic is worth studying since it focuses on an issue that can affect our nutritional health. Knowing the effects of food processing techniques on vitamin concentration may help us in choosing what we should consume. Therefore, the research question of this study is "What are the effects of microfluidization and ultrasonication on the concentration of ascorbic acid in orange juice?"

Hypothesis

The microfluidization and ultrasonication techniques are commonly used in industrial processes in order to render the juices to the customers in an appealing way. The difference between the orange juice samples were the techniques whether the samples were processed or not. The first groups of orange juice samples were the control samples which weren't processed. The second group samples were processed by microfluidization which is the process that separates the big particles from the orange juice and homogenizes it. The expectation is that, the particles which separated during the microfluidization process can contain significant concentrations of vitamin C; the calculated concentration of vitamin C in the processed samples can drop down compared to the unprocessed samples. The third group samples were processed by ultrasonication. The ultrasonication is very similar to microfluidization except the high temperature rates. Relating to this hypothesis, the vitamin C concentration should be lower in the processed samples than the unprocessed ones based on the rate of (X) gram of vitamin C in (Y) liter of orange juice. Moreover, as the microfluidization process reduces the volume of the orange juice the reduction of the volume may be related to the vitamin C concentration in the orange juice. In addition, as the ultrasonication process includes high temperature treatment other than high pressure and shearing, we can assume that it will have a bigger impact than microfluidization in reducing the concentration of vitamin C in the orange juice. The ascorbic acid concentration of ultrasonicated orange juice will be lower than microfluidized orange juice which has lover ascorbic acid concentration than fresh orange juice. The hypothesis of this study is that, the concentration of ascorbic acid in the ultrasonicated orange juice should be the lowest, microfluidized should be the second lowest

[10] http://chemistry.about.com/od/demonstrationsexperiments/ss/vitctitration_3.htm

and the fresh should be highest. Given the information from the literature investigation, it is shown that the hypothesis is supported and the ascorbic acid concentration should be lowest in the ultrasonicated orange juice and highest in the fresh orange juice. (Yuce, 2011)

Method Development

This study investigates the effect of two food processing techniques on ascorbic acid concentration of orange juice. The first group is fresh orange juice which won't be processed, the second group is microfluidized and the third group is ultrasonicated. It is assumed that these processes will affect the concentration of ascorbic acid found in the orange juice. In order to calculate the most accurate results of ascorbic acid content in the orange juice, the fresh oranges are squeezed just before the titration experiment.

Titration is a way of analyzing which will allow us to indicate the endpoint of a reaction and the wanted quantity of reactant.[11] There are different reagents that can be used in ascorbic acid titration such as iodine[12] and iodide[13]. I chose to use indophenol reagent because it's easy to prepare and easier to use in titration because of its liquidity and color. The titration technique is used because it is appeared to be the simplest technique to determine the ascorbic acid content of a juice.[14] I've chosen to perform the titration process on the juices to determine the concentration of ascorbic acid content. The reason why I've chosen to perform titration is because it's easy and fast to perform, while the results are accurate and precise too. There are various reagents that will be used in the experiment in order to determine the ascorbic acid concentration in the orange juice which are indophenol, dichloroindophenol, metaphosphoric acid and pure ascorbic acid. There will also be two control experiments in order to make a comparison. One of these control experiments is named standardization. Purpose of the standardization is to determine the indophenol consumption to titrate pure ascorbic acid in order to make a comparison with the fresh orange juice samples. The value of indophenol consumption will give us the standardization constant which is V_1. This constant will be used in the formula that will determine the ascorbic acid concentration in the orange juice. Similar to the standardization, another control experiment is a blank trial which has a purpose of nullifying the reducing agents of dichloroindophenol other than ascorbic acid. The indophenol consumption to titrate

[11] http://www.dartmouth.edu/~chemlab/techniques/titration.html
[12] http://chemistry.about.com/od/demonstrationsexperiments/ss/vitctitration.htm
[13] http://www.outreach.canterbury.ac.nz/chemistry/vitamin_C_iodate.shtml
[14] http://cygm.meb.gov.tr/modulerprogramlar/kursprogramlari/gida/moduller/GidalardaAskorbikAsitTayini.pdf

the blank trial will also be recorded in order to be used in the formula. These control experiments' titration results and the orange juices' titration results will be used in this formula to determine the exact concentration of ascorbic acid in the orange juice. The concentration of ascorbic acid in the juice will be determined using the following:

Ascorbic acid concentration in the orange juice (g/lt) =

$$\frac{V_2 \times 2}{(V_1 - V_0) \times m}$$

Where;

V_0 = The volume of indophenol solution used to titrate the blank trial (mL)

V_1 = The volume of indophenol solution used to titrate the standardization trial (mL)

V_2 = The volume of indophenol solution used to titrate the orange juice solution (mL)

m = The volume of orange juice in the orange juice solution (mL)

As indicated in the formula there is several different control trials that will be needed in order to determine the volume of ascorbic acid in the juice. To perform the titration on the control trials some reagents must be prepared. These are methaphosphoric acid solution, ascorbic acid solution, indophenol solution and orange juice solution. The preparations of these solutions will be explained in the procedure section. The methaphosphoric acid solution, ascorbic acid solution and indophenol solution are prepared before the orange juice solution because these three solutions that can be stored and won't lose their quality for a few days. However, the orange juice is unstable because as soon as we start to squeeze the fresh oranges the ascorbic acid concentration in the orange will decrease over time. Finally, in order to determine the m constant; the m value is the orange juice volume in the orange juice solution. Orange juice solution is prepared by mixing methaphosphoric acid with orange juice. It is the final step of the titration process and its indophenol consumption will also be recorded to use in the formula. Furthermore, magnetic stirrer was used to mix the contents of the reagents because the reagents must be homogenized. If the magnetic stirrer wasn't used the reagents won't be homogenized and therefore the experiment will ve affected because of the concentration difference. Filtration paper was used to dilute to orange juice solution in order to determine the m constant easier. Distilled water is used in all the reagents because it's free of unnecessary minerals and other substances that could affect the experiment data. At last, the temperature was important because as the ultrasonication process heats the orange juice to extremely high temperatures we have to cool down the ulotrasonicated orange juice in order to collect the data properly. The experiment was performed in the METU Food Engineering labs.

Materials Used in the Experiment

- 2 x 50 mL graduated cylinder
- 3 x 500 mL graduated cylinder
- 2 x 250 mL graduated cylinder
- Magnetic stirrer
- 100 mL graduated cylinder
- Filtration paper
- 500 mL Methaphosphoric acid solution
- 200 mL Indophenol solution
- 100 mL Ascorbic acid solution
- 50 mL titration tube
- Electronic scale
- 1 L distilled water
- 500 mL orange juice solution

Method

Preparation of the reagents:

Methaphosphoric acid solution (500 mL):

- Take 15 g HPO_3 powder and mix it with 40 mL acetic acid.
- Take this solution and pour into 200 mL distilled water.
- Stir this solution with the magnetic stirrer until it is homogenized.
- Finally, complete this solution to 500 mL by adding distilled water.

Ascorbic acid solution (100 mL):

- Add 10 mL methaphosphoric acid solution to 100 mg pure ascorbic acid and dissolve the ascorbic acid.
- Then add 90 mL distilled water to complete the solution to 100 mL.

Indophenol solution (200 mL):

- Take 50 mg 2.6 dichlorophenolindophenol powder and dissolve it by mixing it with 50 mL distilled water.
- Add 42 mg sodium bicarbonate into the solution and dissolve it.
- Complete it to 200 mL by adding distilled water. The color of this solution should be blue.

Orange juice solution:

- Take 50 mL orange juice and 50 mL methaphosphoric acid solution and mix them.
- The 100 mL mixture is the orange juice solution. (Prepare this solution just before the titration of the orange juice solution.)

Control trials:

Standardization:

- Put 5 mL methaphosphoric acid solution into 50 mL graduated cylinder.
- Add 2 mL ascorbic acid solution.
- Titrate the solution using the indophenol solution.
- The color of the solution should turn to pink and stays that way for at least 15 seconds in order to determine that the experiment is finished.
- Measure the used volume of the indophenol solution from the titration tube (mL) and record it. (V_1)
- Take three standardization trials in order to get the most accurate results.

Blank trial:

- Add 5 mL methaphosphoric acid solution into distilled water.
- Determine the volume of the distilled that you're going to use in the blank trial from standardization. (The volume of distilled water that you are going to use must be equal to the indophenol solution volume used to titrate the standardization trial. (mL)) (V_0)
- Take three blank trials in order to get the most accurate results.

Orange juice solution trial:

- Filter the orange juice solution using the filtration paper
- Take 5 mL from the filtrated solution.
- Titrate this 5 mL filtrated solution using the indophenol solution.
- As soon as you get the pink color for at least 15 seconds record the used volume of the indophenol solution. (mL) (V_2)

Results

	Trials	Volume of methaphosphoric acid solution added (mL) ± 0.1	Volume of ascorbic acid added (mL) ± 0.1	Volume of distilled water added (mL) ± 0.1	Volume of indophenol solution consumed to titrate the solution (mL) ± 0.1
Standardization	1	5.0	2.0	250.0	8.2
	2	5.0	2.0	250.0	8.2
	3	5.0	2.0	250.0	8.3
Blank	1	5.0	-	8.2	0.8
	2	5.0	-	8.2	0.8
	3	5.0	-	8.3	0.9

Table 1: The volume of methaphosphoric acid solution, ascorbic acid solution and distilled water added to prepare standardization and blank trials and the volume of the indophenol solution consumed to titrate the standardization trial (V_1) and the blank trial (V_0).

Processed used on the orange juices	Trials	Volume of the orange juice in orange juice solution (mL) ±0.1	Volume of indophenol solution consumed to titrate the solution (mL) ± 0.1
None (fresh)	1	2.5	45.5
	2	2.5	43.6
	3	2.5	44.7
	4	2.5	45.1
	5	2.5	44.2
Microfluidized	1	2.5	37.9
	2	2.5	37.7
	3	2.5	38.5
	4	2.5	37.3
	5	2.5	38.1
Ultrasonicated	1	2.5	27.9
	2	2.5	27.1
	3	2.5	28.2
	4	2.5	27.9
	5	2.5	27.5

Table 2: The volume of the orange juice solution (m) and the volume of indophenol solution consumed to titrate the fresh orange juice solution, microfluidized orange juice solution and ultrasonicated orange juice solution (V_2).

Data Analysis

The following calculations will apply for "None (fresh)" orange juice, trial 1.

The formula of determining the ascorbic acid concentration (g/lt) in the orange juice was:

$$\frac{V_2 \times 2}{(V_1 - V_0) \times m}$$

Where;

V_0 = The volume of indophenol solution used to titrate the blank trial (mL)

V_1 = The volume of indophenol solution used to titrate the standardization trial (mL)

V_2 = The volume of indophenol solution used to titrate the orange juice solution (mL)

m = The volume of orange juice in the orange juice solution (mL)

If we put the appropriate data from the table into this formula our formula will be:

$$\frac{45.5 \times 2}{(8.2 - 0.8) \times 2.5}$$

And the result will be:

4.92 g/lt ascorbic acid

If we apply this formula to all of our trials the new table is:

Type of the process	None (fresh)	Microfluidized	Ultrasonicated
Ascorbic acid content in the orange juice (g/lt)	4.92	4.10	3.02
	4.71	4.08	2.93
	4.83	4.16	3.05
	4.88	4.03	3.02
	4.78	4.11	2.97

Table 3: The concentration of ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices (g/lt).

Average, standard error, standard deviation and confidence interval for this data will be constructed by descriptive statistics via Microsoft Word Excel 2010:

Type of the process	None (fresh)	Microfluidized	Ultrasonicated
Count	5	5	5
Average concentration of ascorbic acid (g/lt)	4.82	4.10	3.00
Standard Error	0.04	0.02	0.02
Standard Deviation	0.08	0.05	0.05
Confidence Interval 95%	0.10	0.06	0.06

Table 4: Average, standard error, standard deviation and confidence interval data for the ascorbic acid concentration (g/lt) in the fresh, microfluidized and ultrasonicated orange juices.

Hypothesis Testing

The p-value will be calculated by the Anova: Single Factor test by testing all the data of ascorbic acid concentration (g/lt) in the fresh, microfluidized and ultrasonicated orange juices via Excel:

Anova: Single Factor						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
None (fresh)	5	24.12	4.82	0.00683		
Microfluidized	5	20.48	4.10	0.00223		
Ultrasonicated	5	14.99	3.00	0.00227		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	8.44977	2	4.22489	1118.68138	2.30532E-14	3.88529
Within Groups	0.04532	12	0.00378			
Total	8.49509	14				

Table 5: Anova: Single Factor, which includes the p-value, test for the data of ascorbic acid concentration (g/lt) in the fresh, microfluidized and ultrasonicated orange juices.

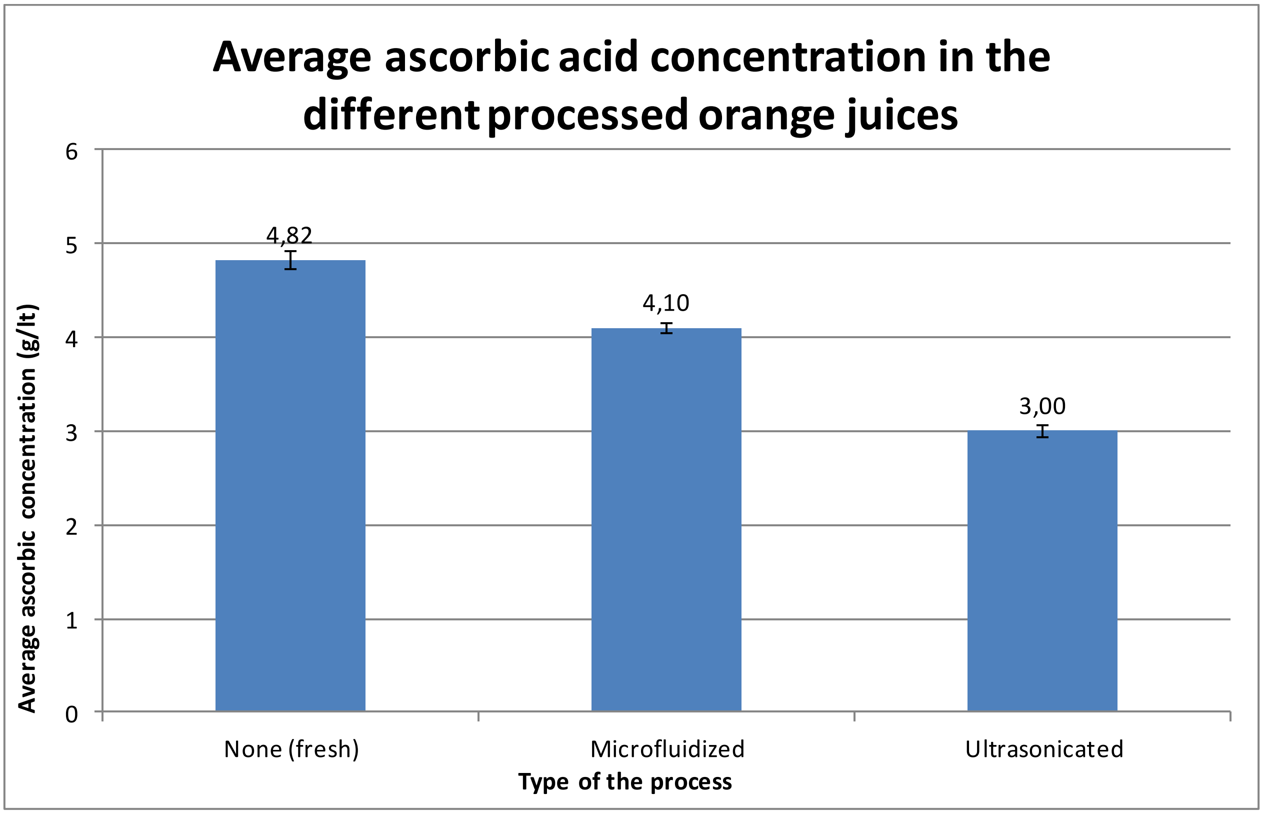
Null Hypothesis: The concentration of ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices are the same.

Alternate Hypothesis: The concentration of ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices are different.

P-Value: 2.30532E-14<0.05

According to the P-Value the null hypothesis should be rejected and the alternate hypothesis should be accepted, which means that there is a significant mean difference between the ascorbic acid concentrations of fresh, microfluidized and ultrasonicated orange juices.

Graphical Presentation



Graphic 1: Average ascorbic acid concentration (g/lt) in the fresh, microfluidized and ultrasonicated orange juices.

Evaluation

The aim of this study was to determine if there is a significant mean difference between the concentrations of ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices by using which is a common titration technique used, indophenol titration, to measure the ascorbic acid concentration. The research question of the study was "What are the effects of microfluidization and ultrasonication on the concentration of ascorbic acid in orange juice?" We can conclude the research question by looking at the data of the study which indicated the processed orange juices lost their ascorbic acid content. It was hypothesized that processed orange juices, which are microfluidized and ultrasonicated, should have less concentration of ascorbic acid compared to the fresh orange juice. The reason to that hypothesis was because the processed orange juices lost some of their content such as particulates due to the dilution and high temperature. This dilution and material loss in the orange juices could be related with the ascorbic acid concentration. Indophenol titration was used to determine the ascorbic acid concentration in the orange juice. At the end of the experiment ascorbic acid contents were found to be 4.82 (g/lt) for fresh, 4.10 (g/lt) for microfluidized and 3.00 (g/lt) for ultrasonicated orange juice.

My null hypothesis was that the ascorbic acid concentration in the fresh, microfluidized and ultrasonicated should be the same. However, the P-Value, which was 2.30532E-14 and significantly smaller than 0.05 showed that the null hypothesis must be rejected. That is, the difference between average ascorbic acid concentrations of fresh, microfluidized and ultrasonicated orange juice are statistically significant.

Experiment evidence supported the alternate hypothesis according to the acquired data and the P-Value. The reason of the loss of the ascorbic acid concentration in the microfluidized orange juice may be the dilution process. As indicated, the microfluidization process dilutes the fresh orange juice in order to make it compatible for industrial mercantile however it ends up with reducing its content such as the ascorbic acid concentration. In addition, if we look at the graphical presentation, we can easily see that the fresh orange juice has the highest ascorbic acid concentration, the microfludized orange juice has the second highest and the ultrasonicated orange juice has the lowest concentration. The reason of the loss of the ascorbic acid concentration in the ultrasonicated orange juice is similar to the microfluidization process although with an addition: high temperature. In addition to the dilution process, the high temperature infliction on the orange juice breaks down the particles in the juice to their smallest content which could decrease the ascorbic acid concentration in the juice. There are several error sources and limitations of this study. One of the most crucial variables in this experiment was the time interval. As the fresh orange juice sample stored even in a low temperature condition, as time passes the pectin enzyme in the juice activates which makes the juice precipitate with the result of the ascorbic acid concentration reduction. Even swilling the orange juice sample wouldn't be enough because if the orange juice is precipitated it means that the pectin enzyme was already activated and will affect the concentration of the ascorbic acid content. After swilling the orange juice sample may seem physically homogenized, although if we leave the sample without swilling for couple minutes, this time the orange juice sample will precipitate rapidly. In order to neglect this effect I performed the titration as soon as I squeezed the oranges. To get to the temperature factor, it is explained earlier that ascorbic content was affected negatively in high temperature conditions.[15] The time was also important on the reagents. In the instruction of the preparation of the reagents, it is indicated that all of the reagents can only stay

[15] http://www.dietitian.com/vitaminc.html

tender for maximum three days. After three days the reagents will begin to precipitate just like the orange juice sample and because of this the experiment data will differ. If the experiment could not be completed in one or two days, both the orange juice samples and the reagents must be prepared again. As I completed the experiment less than two days the time variable wasn't important for me. In order to improve the storage time, the orange juice samples and reagents were stored in a low temperature refrigerator, which will slow down the precipitation process. Moreover, after two days as the reagents start to lose their content, it would be necessary to prepare them again. However, this second preparation could be different from the first group of reagents ratio wise. Meaning, in the second group of reagents the ratios of the contents of reagents could be different because of the human error. This error could lead this study to different results. To get to the orange juice samples, different breeding styles of oranges will be resulted with different concentrations of ascorbic acid content. So it's important to use the same types of oranges in the experiment. To improve the concept with the oranges, which was bought from a regular grocery store, should be investigated if they were harvested at the same time and from the same place. However, the oranges can be stored longer without any loss of the ascorbic acid content if they aren't peeled off. To add more processing techniques to obtain industrial juices is pasteurization which is the heating process of a certain liquid in order to prevent contamination. This technique is usually used on milk products, however still effective on orange juice.[16] The results of my study were limited with orange juice and the processing techniques were microfluidization and ultrasonication only. For further investigations related to this study, additional processing techniques could be investigated rather than microfluidization and ultrasonication. Moreover, the ascorbic acid content of the different nutrients could be investigated rather than orange juice.

Conclusion

My research question was ""What are the effects of microfluidization and ultrasonication on the concentration of ascorbic acid in orange juice?" was answered with the help of this study and the hypothesis of the study was "There should be a significant mean difference of the ascorbic acid concentrations of fresh, microfluidized and ultrasonicated orange juices". As the experiment concluded and the results were investigated, it is cleared that there is a significant mean difference of the ascorbic acid concentration between the fresh, microfluidized and ultrasonicated orange juices.

[16] http://www.foodsci.uoguelph.ca/dairyedu/pasteurization.html

In the experiment used in this study, the ascorbic acid concentration in the orange juices was calculated with a pair of formulas which its data were collected with the titration using such as indophenol, metaphosphoric acid, ascorbic acid reagents. After the calculation of the concentration of the ascorbic acid in the fresh, microfluidized and ultrasonicated orange juices the alternate hypothesis, which was "There is a significant mean difference of the concentrations of the ascorbic acid between the fresh, microfluidized and ultrasonicated orange juices.", got accepted, where the fresh orange juice had the highest concentration, microfluidized was lower than the fresh but higher than the ultrasonicated and the ultrasonicated had the lowest.

The microfluidization and ultrasonication, which are industrial processes, are used to improve the storage time of the orange juices and they are used to attract the highest concentration of customers because these processes homogenize the orange juice and remove the big particles, which creates a healthier and fresher physical visual. However, this research shows that the microfluidization and ultrasonication processes will reduce the ascorbic acid content of the orange juice but ascorbic acid is crucial in the human life because it is used in several metabolic and immunity reactions. With the help of this research the customers can understand the differences between the processed orange j uices and fresh orange juice.

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