TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Effect of mixing lemon juice with water at different temperatures (4˚C, 24 ˚C, 37 ˚C, 50 ˚C and 100 ˚C) on the ascorbic acid content of lemon juice

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BIOLOGY EXTENDED ESSAY

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TABLE OF CONTENTS

I. INTRODUCTION/ BACKGROUND INFORMATION

Vitamins are the organic substances that we need to consume each day since human body is unable to produce them all by itself. The most known vitamins are A, C, D, K ,E and B. Many fruits and vegetables and animals like fish contain their own particular vitamin which we need to provide. However, especially when we think about the weather winter, the one that we often try to consume more is vitamin C, also known as ascorbic acid. Generally, citrus fruit contain vitamin C. Specifically, lemon, orange, grapefruit and mandarin orange are the ones which are commonly grown in Mediterranean region of Turkey and take place on the shelves of markets during winters.

Vitamin C acts as an antioxidant. As we can understand from the word "antioxidant", its chemistry prevents body cells or other cells of any kind of food products getting oxidized by removing oxygen from its surroundings. Getting oxidized is harmful for the proper functioning of body cells and being well-preserved structure of the cells considering the quality of a food product. If we focus on antioxidancy of vitamin C on body cells and it is appreciated contribution on the fight against the carcinogens, chemicals which cause cancer by forming oxide free radicals which are quite harmful to DNA so to the whole cell, vitamin C remains highly valuable. In addition, antiscorbutic nature of vitamin C, being reformative or resistant to scurvy disease cannot be ignored. This is because "the vitamin's wound healing and growth promoting properties are due to its participation in the synthesis of fibrous connective tissue in collagen, the most abundant protein of the Animal Kingdom."[1] Not just wound healing property but the other property which is strengthening the body's immune system in defensing it against the infections which may occur after any surgery or any kind of encounter with germs , is a good reason to emphasize again how useful and important the vitamin C for our body.

[1] http://pubs.rsc.org/ doi:10.1039/9781847552303-00074

It is known that L-ascorbic acid can be easily affected by the environmental factors. Its stability is influenced by the temperature, the kind of light(UV, visible light) it is exposed to, the material (copper, stain, plastic) used in the container which is used to keep the source during transportation or any processing, seasonal variations which the crops grow, the time of exposure to air. One of the most important factor is temperature. This research is about the effect of temperature on the stability of vitamin C which is found in lemon juice. As mentioned before, the common sources of vitamin C, specifically in Turkey, are lemon, orange, grapefruit and mandarin besides other vegetables and ready tablets sold in pharmacy. People often choose to drink their tea with some lemon juice or drink hot water with lemon juice droplets especially when they catch flue or get sick during winter season. In Turkey, it is believed that hot water or any hot herb tea with lemon juice cures the flue due to vitamin C content. The aim of using hot water is mainly about to soften the throat and get warm and obviously prepare tea. However, adding lemon juice to the tea is to get benefit from the vitamin C's healing property and its contributions to the immune system by boosting the recovery state. However, it is a good question to answer whether consuming lemon juice with hot water is better or not, instead of taking it with cold or warm water. Therefore, my research question is: How does the ascorbic acid(vitamin C) content of lemon juice change when equal amounts of lemon juice and water at 4˚C, 24˚C, 37˚C,50˚C and 100˚C mixed indicated by 760nm spectrophotometry. By this way, it will be possible to gain knowledge about something which is a habit for Turkish people during winter time.

[2]

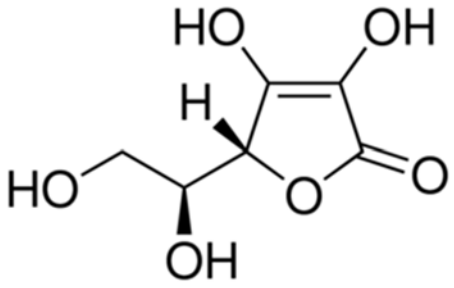


Figure 1, L-ascorbic acid or Vitamin C

[2] http://www.mpbio.com/product.php?pid=02194586&country=215

II. HYPOTHESIS

It is known that ascorbic acid found in fruits and vegetables is one of the most prone vitamin to degrade. The enzyme L-ascorbic acid oxidase(AAO) is the enzyme that oxidizes the ascorbic acid causing it to degrade its content. Knowing that any kind of enzyme's activity is related with temperature, different temperatures affect the enzyme's function so affects vitamin C content of a substance. Based on previous studies done, it was found out that as temperature gets close to 70˚C, AOO slows down, maintaining retention of vitamin C. Above 70˚C, the enzyme is destructed however vitamin C is also degenerated.[3] In light of this information, it was hypothesized that lemon juice mixed with water at different temperatures will contain different amounts of ascorbic acid(vitamin C). In addition, my null hypothesis is that the temperature would not affect the L-ascorbic acid content and there would not be a relation between chosen temperatures. Temperature is an effector of ascorbic acid and different temperatures affect the L-ascorbic acid content differently. By this way it will be possible to explore the suitable temperature of water to add lemon juice before consuming. I made inference based on previous data gathered on this subject that 100˚C will be the temperature that the ascorbic acid content of the lemon juice will be least, while 50 ˚C will be the most.

[3] http://www.ncbi.nlm.nih.gov/pubmed/20546391
http://www.dietitian.com/vitaminc.html#.Uq9Hx_RdUVV

III. METHOD DEVELOPMENT AND PLANNING

For the topic of my extended essay, I have raised the question which is "How does the ascorbic acid content of lemon juice change when equal amounts of lemon juice and water at different temperatures mixed?". I was curious about this due to my habit of drinking tea with lemon juice and the thought of vitamin C's effectiveness when I consume it in high temperatures. For my investigation, firstly, I should design the method. The first problem that I faced was finding an effective method for measuring ascorbic acid content of a solution. Then, there are some certain d variables which should be controlled throughout the study for all trials.

There are many analytical methods to determine ascorbic acid content of a solution such as iodine titration, liquid chromatography and spectrophotometry. In this experiment, I decided to use UV Spectrophotometric method to calculate the absorbance of the mixture of lemon juice and water which exposed to 5 different temperatures for 60 minutes.[4] The primary reason for why I have chosen this method (UV spectrophotometer) is that it is rapid in analyzing, have high accuracy and precision compared to titration and chromatography. "The spectrophotometer measures the amount of light absorbed by a solution." Therefore, it is possible to "quantitatively measure the absorbance which leads to determination of concentration of the absorbing molecule" which is ascorbic acid molecules of lemon juice.[5] In this experiment, the spectrophotometer will be used at 760 nm which is the suitable wavelength for the transmittance of ascorbic acid, red radiation. By using this data of transmittance, the absorbance of the vitamin C existing in the tube is automatically calculated

[4] http://www.idosi.org/wasj/wasj9(7)/13.pdf - World applied sciences journal 9(7): 800-803, 2010
[5] http://depts.noctrl.edu/biology/resource/handbook/spec.pdf

by the machine. As the value monitored increases, this means the concentration of the ascorbic acid increases, too.

Lemons chosen will be Interdonato lemons endemic in Turkey. They will be bought from the bazaar, from the same stand, confirming that they are the crops of the same field. The lemon samples will belong to the same field because it is known that time of harvest, the climate that the crop grew is effective on the concentration of ascorbic acid. The lemons will be cut and squeezed, they will be added in the same amounts to all temperatures after homogenized in the centrifuge.

Time of exposure (to oxygen or heat) is an important term for the stability of ascorbic acid content. In order to determine the time of exposure to heat in the incubator, some preparatory trials are done. Since preparatory trials showed that 5 minutes and 10 minutes do not create significant or any changes in concentration of ascorbic acid, 60 minutes is tried and ,at last, it is found suitable to wait for 60 minutes for the temperature to affect the ascorbic acid.

The temperatures are purposely chosen. +4 ˚C is the fixed temperature value for the storage of any gaseous drink so in order to see how adequate it is for the retention of ascorbic acid in the drink, it is an appropriate value. 24 ˚C which is in the range of accepted average room temperature, 37 ˚C is the average body temperature[6], 50 ˚C is the common temperature that we consume tea or coffee and 100 ˚C is the near temperature that water boils and then used for infusion of the tea. For one hour the samples belonging to a chosen temperature will be incubated and then applied to spectrophotometry.

[6] http://lamar.colostate.edu/~hillger/temps.html

Oxalic acid, phosphoric acid, ammonium molybdate and sulfuric acid are the chemicals which will be used to form the color of lemon juice and water solution in order to make it suitable for the measurement at UV spectrophotometry.[7] When these chemicals will be added to lemon juice- water mixture, a blue color formation will occur, and the tone of the blue color will change according to the amount of remaining ascorbic acid content. The frequency of the mixtures of all temperatures will indicate the temperature's effect on the content. These chemicals will be highly pure in order to minimize any error made.

As the samples does not include tea extract, the extent of this working is only limited to observing the effect of water at 5 different temperatures. It is highly possible that, the existence of tea in the mixture would change the degradation level of L-ascorbic acid. Although, the extract is not included in the experiment, still it is possible to observe what is aimed by this study, temperature's effect on vitamin C content found in lemon juice and water mixture which forms some part of act of consuming tea in daily life. If tea would be included in the experiment, it would be very time consuming and increase the risk of error as controlled extracts has to be prepared, heated,etc.

The balloon joji tubes (25 ml) are needed to be filled to the top, based on World Applied Journals reference before applied to spectrophotometry. While doing that, there are specific values for amount and molarities of color forming reagents and amount of water solution. 16 ml of distilled water with 3ml of lemon juice will be used other than chemicals. As water has electrostatic attraction and it is a solvent of vitamin C, it is effective on the content so the amount of water used has to be controlled.

[7] http://www.idosi.org/wasj/wasj9(7)/13.pdf - World applied sciences journal 9(7): 800-803, 2010

Finally, my research question is formed: “How does the ascorbic acid content of lemon juice change when equal amounts of lemon juice and water at 4˚C, 24˚C, 37˚C, 50˚C and 100˚C mixed indicated by 760nm spectrophotometry?”

Materials used in the experiment:

- 37.5 mL 0.5 M Oxalic acid
- 7.5 mL Phosphoric acid
- 15 mL Sulfuric acid 5% (v/v)
- 30 mL Ammonium Molybdate 5% (w/v)
- 135 ml Lemon juice (or 15 lemon)
- 1 L distilled water
- 15 × balloon joji/beakers
- Graduated cylinder (10 mL)
- 3×Pipet
- Incubator (Nüva)
- Centrifuge
- A Weighing
- Heater plate
- Chronometer
- A Spoon
- A knife

IV. METHOD

Gloves and glasses must be worn during the procedure at all times to prevent any harm that can come from chemicals used for formation of color.

Procedure;

1. Cut one lemon and squeeze it separating the seeds, centrifuge it in order to get away from the particulates for 10 minutes at 4000 rpm. The milliliter that you squeeze and centrifuge has got to be enough for 3 trials, 3 ml of juice for each. Therefore, there should be 9 ml at least of lemon juice.
2. For 3 trials of one of the temperature groups, add 3 ml lemon juice to the three balloon joji tubes, then, to all, add 10 ml distilled water which is heated or cooled(by heater plate) to the temperature that you choose to analyze first(one of 4, 24, 37, 50, 100˚C) by using pipets. Close the openings of tubes.
3. Place the three joji tubes carrying 3ml lemon juice and 10 ml of distilled water in the incubator. Wait for 1 hour (60 min) for heat to affect the ascorbic acid. (The temperature is the one that is chosen at the second step.)
4. At the end of the incubation add the color forming chemicals (in total 6 ml chemical for each tube)(listed in Appendix no.1) .
5. After addition of these chemicals, add 6 ml distilled water to each to fill the tubes to the 25 mL level. Again close the openings of tubes.
6. After waiting for another 2 hours of incubation at 25˚C for the formation of color, analyze the samples at 760 nm spectrophotometer. Record them.
7. Repeat the steps from the beginning for other 4 different temperatures. (use only one lemon for each trial.)
8. Each temperature tested independently for 3 times.

V. RESULTS

Table 1, raw data table, shows the concentration of ascorbic acid found in samples of applications of 5 different temperatures in 3 parallels.

Parameters	**Chemical Analysis Results: Amount of Ascorbic Acid (10^{-3}ml)(± 0.1)**		
Temperature ˚C (±0.01)	**Trial 1**	**Trial 2**	**Trial 3**
Freshly Squeezed Lemon Juice at 4˚C	74.8	78.9	76.3
Freshly Squeezed Lemon Juice at 24 ˚C	76.7	76.8	77.5
Freshly Squeezed Lemon Juice at 37 ˚C	75.2	78.1	78.1
Freshly Squeezed Lemon Juice at 50 ˚C	78.1	79.6	79.7
Freshly Squeezed Lemon Juice at 100 ˚C	63.4	62.6	65.7

VI. DATA ANALYSIS

The formulas[8] used to do anaylsis is given below;

Mean Value;

$$\bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

,

N is the number of trials (3 trials for each temperature in this experiment)

x_i is the mg of detected ascorbic acid in the lemon juice-water mixture

Standard Deviation;

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}, \quad \text{where} \quad \mu = \frac{1}{N}\sum_{i=1}^{N} x_i.$$

N is the number of trials (3 trials for each temperature in this experiment)

x_i is the mg of detected ascorbic acid in the lemon juice-water mixture

μ is the mean value for the corresponding temperature group

Standard Error of the Mean;

$$\mathrm{SD}_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

N is the number of trials (3 trials for each temperature in this experiment)

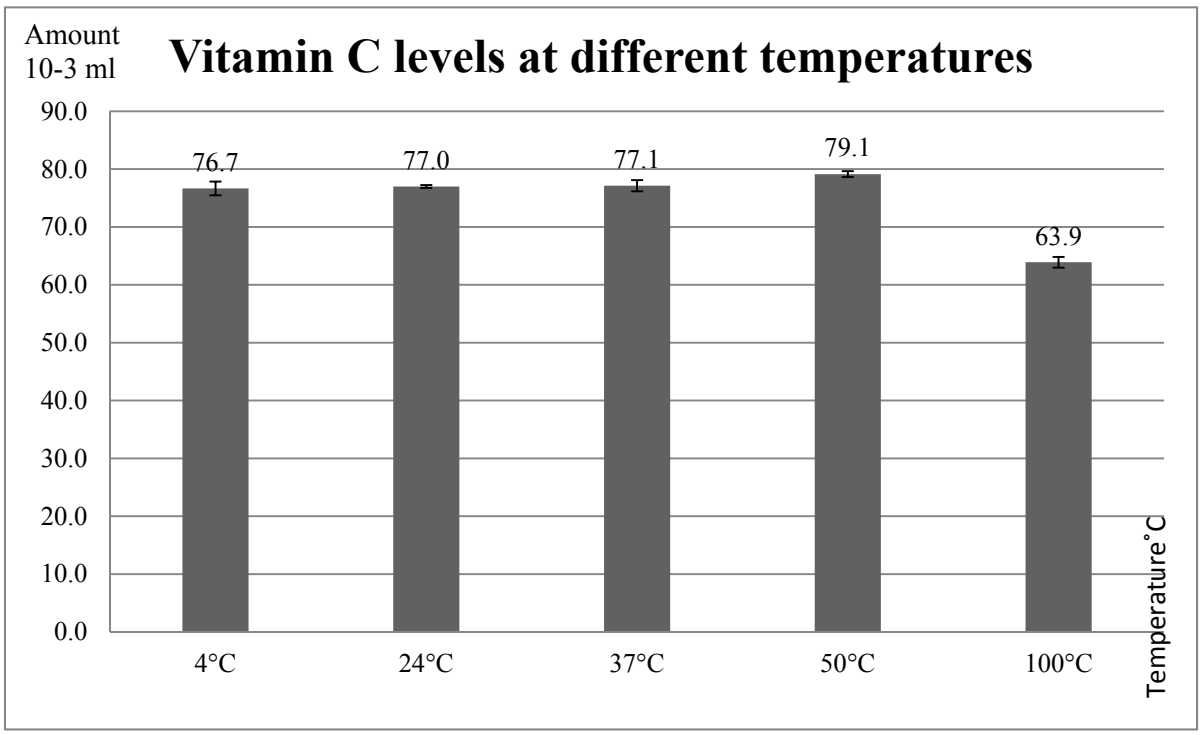
σ is the standard deviation of the corresponding temperature group

[8] http://en.wikipedia.org/wiki/standarderror/sd/mean

Table 2, the values of trials of all groups and their mean, standard deviation and standard errors.

Temperature(°C) (±0,01)	Trial 1	Trial 2	Trial 3	Mean	Standart Deviation	Standard Error
4,00	74,8	78,9	76,3	76,7	2,1	1,2
24,00	76,7	76,8	77,5	77,0	0,4	0,3
37,00	75,2	78,1	78,1	77,1	1,7	1,0
50,00	78,1	79,6	79,7	79,1	0,9	0,5
100,00	63,4	62,6	65,7	63,9	1,6	0,9

Graph 1, mean vitamin C levels of 5 temperature groups with error bars.



Groups	*Count*	*Sum*	*Average*	*Variance*
4°C	3	230	76,6666667	4,30333333
24°C	3	231	77	0,19
37°C	3	231,4	77,1333333	2,80333333
50°C	3	237,4	79,1333333	0,80333333
100°C	3	191,7	63,9	2,59

Table 3, Single factor Analysis of Varience (ANOVA) statistical calculation.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	454,0533	4	113,513333	53,0932336	1,062E-06	3,47805
Within Groups	21,38	10	2,138			
Total	475,4333	14				

Table 4, by using ANOVA statistical calculations and p-value of groups.

VII. EVALUATION

The purpose of this study was to answer the question "How does the ascorbic acid content of lemon juice change when equal amounts of lemon juice and water at 4˚C, 24˚C, 37˚C, 50˚C and 100˚C mixed indicated by 760nm spectrophotometry?". The experiment was a mimic, although not the exact same, of daily consummation of any kind of tea. It was hypothesized that the effects of each temperature would be different in means of remaining usable level of L-ascorbic acid in the mixture. The temperature which is considered to be optimum for L-ascorbic acid is thought to be 50˚C within the chosen temperatures since it is referenced and concluded from other studies which were mentioned in hypothesis section; it was expected to see significant degradation in the mixture above 70˚C, and below since the L-ascorbic oxidase enzyme breaks down with the increasing temperature so slowing down the degradation process of L-ascorbic acid.

After performing the experiment by using the instrument spectrophotometry at 760nm, the mg per 100 g of mixture of L-ascorbic acid content is recorded and needed calculations such as standard deviation, mean values and p-value of the groups and the graph are prepared by using Microsoft excel(Anova). Ascorbic acid amount remaining in the mixtures was seen the most in the trials of 50˚C, followed by 37˚C, 24˚C, 4˚C and 100˚C respectively. The 10^{-3}ml per 100g of mixture ranged between 74.8 to 78.9 10^{-3}ml with the mean value of 76.7 10^{-3}ml for 4˚C; 76.7 to 77.5 10^{-3}ml with the mean value of 77.0 10^{-3}ml for 24˚C; 75.2 to 78.1 10^{-3}ml with the mean value of 77.1 10^{-3}ml for 37˚C; 78.1 to 79.7 10^{-3}ml with the mean value of 79.1 10^{-3}ml for 50˚C; and 62.6 to 65.7 10^{-3}ml with the mean value of 63.9 10^{-3}ml for 100˚C.

My null hypothesis was that the temperature would not affect the L-ascorbic acid content and there would not be a relation between chosen temperatures. However, it is calculated by Anova that the p-value between groups was very close to zero and smaller than 0.05, it is 1.06E-06 as shown on Table 4 disproving the null hypothesis. Therefore, it is approved for the experiment that the temperature is effective on the ascorbic acid level of lemon juice and water mixture. My hypothesis is proved by the analysis, and the p-value. It is appropriate to say that temperature is an effector variable of L-ascorbic acid content and different temperatures have different influences on it.

The standard deviation value of 4˚C is found to be significantly higher than other temperatures with 2.1 and standard deviation of 24˚C is found to be significantly lower with the value of (0.4). This occurrence is likely related with the easiness of the control of temperature but also seems like random variation when other temperatures deviations considered. (37˚C, 50˚C, 100˚C: 1.7, 0.9, 1.6 respectively)

While performing the experiment, there was only one unexpected occurrence that have affected the course of the experiment. At first, it was planned to apply a temperature to the centrifuged lemon juice and water mixture for 5 minutes in the incubator. When the data is read from the spectrophotometry, it is realized that there was almost no difference between the vitamin contents of each temperature groups. Afterwards, 10 minutes is applied to the samples, this was also not enough effective on the vitamin content to be able to compare the temperatures. At last, 60 minutes was chosen. By this way, it is also discovered that the time the vitamin C is exposed to a temperature is also important. This could be used for future experiments while understanding the vitamin C and its effectors.

Besides this unexpected occurrence, there was not any prominent circumstance. However, it is realized that there are some error sources which could have affected the results of the experiment, although, it is thought to be not that much influential, they are given with suggestions for subsequent experiments:

1) Time of exposure to oxygen. As oxygen is an element that causes degradation of vitamin C by giving oxidation reaction, it has to be controlled throughout the experiment. However, as the lemons are cut and exposed to air while squeezing their juice, while adding chemicals in to the beaker where lemon juice and water mixture is put and while the incubation (although beakers are closed with a tap, still some air remains in them), it was not possible to control it. For every trial, there is no certainty of time of exposure to air, some trials took longer, some took shorter. In order to overcome this effect, the time of exposure to air has to be controlled in future experiments by using chronometer and being exact at each step.
2) Usage of metal spoon to get rid of seeds and extract the juice. It is known that metals that have physical interactions with the vitamin C can start reactions, causing it to degrade. Tin is a well-known example of metal that should be avoided to contain substances with vitamin C content. Also, it is known that instead of using knife to cut fruits and vegetables, ripping them by hand seems like a better choice in order to conserve vitamin C. In spite of these information, at the first step of the method, while cutting the lemon, getting rid of the seeds of the lemon and squeezing it , metal spoon and knife are used. This act has a role in degrading the content of the vitamin C as well as the temperature. In order to prevent this

uncontrolled variable using plastic knives and spoons can be quite effective for future experiments.

3) Only using water in this experiment does not mimic exactly the consuming act of lemon juice with any kind of tea. It is not known exactly whether black tea or any other herbal tea gives a reaction with L-ascorbic acid, causing it to degrade its content. If one chosen type of tea would have been used it could give a better understanding of vitamin C and its best way of usage in daily life. Therefore, this creates another newer and detailed topic for future experiments of vitamin C in tea with the independent variable as temperature.
4) There are different types of lemons in the market. Different lemons' ascorbic acid contents can be compared in future experiments. In addition, the freshly picked lemon's vitamin C content can be compared to stale lemon's. Since I hadn't the chance to obtain freshly picked, I bought lemons from the bazaar. It is important to note that, although it is confirmed that the lemons used in the experiment belong to the same field, the conditions that the lemons were under while transporting are probably different, thus the used lemons had different vitamin C contents, creating error. For instance, maybe some of them were at the upper parts of the truck, being exposed to sun more, so degrading its content more.
5) Although in this experiment for all trials the same amount of distilled water is used, different amounts of water can affect the ascorbic acid content due to its electrostatic nature. As vitamin C is a water-soluble vitamin there is a high chance of being affected by the water molecules. In future experiments, independent variable can be the amount of water, the source of vitamin C exposed.
6) Although there is no error source thought to be due to the time of exposure to heat, there is a suggestion to make about it. As well as time of exposure to air matters,

the time the vitamin is exposed to heat also matters. Therefore, this can be another experiment's independent variable.

VIII. CONCLUSION

My research question, "How is the temperature's effect on retention of L-ascorbic acid when UV spectrophotometry is applied to five different temperatures(4 ˚C, 24 ˚C, 37 ˚C, 50 ˚C, 100 ˚C) of water and lemon juice mixture?" is answered. It is seen that the level of L-ascorbic acid varies with the temperature and the greatest conservation was seen for 50 ˚C followed by 37 ˚C, 24 ˚C, 4 ˚C and 100 ˚C. Although, there are some errors and weaknesses in the experiment, I count this experiment and thesis successful.

The reason behind why I have chosen this topic is that I was always curious about how to consume a vegetable in its most beneficial way. As I use lemon juice in great deal of my life (from salads to lemonades, to teas), I considered about the most suitable temperature for retention of L-ascorbic acid. There are many studies about vitamin C and the factors affecting L-ascorbic acid content of citrus fruit and some vegetables. I haven't encountered to a one which specifically handles lemon and its interaction with the temperature. However, there is indeed information about temperature's effect on vitamin C. It was seen in another research that above 70˚C vitamin C becomes deformed. Therefore, for this experiment it was expected to see that 100 ˚C would be the most destructive or the only one within other temperatures. The results come up in favor of this assumption.

"Ascorbic acid is one of the important water soluble vitamins. It is essential for collagen, carnitine and neurotransmitters biosynthesis."[9] Unlike, most of the animals and plants, humans cannot produce their own vitamin C. Therefore, it is needed to be taken from outside, by plants or tablets. "Many health benefits have been attributed to ascorbic acid such as antioxidant, antiatherogenic, anti-carcinogenic, immunomodulator and prevents cold etc."

[9] http://www.nipne.ro/rjp/2008_53_1-2/0343_0352.pdf

That's why it should be taken in a way that makes the consumer benefit the most. Knowing the best temperature to use up vitamin C is a good start for it. This kind of studies carry weight for public to gain awareness in right consumption of vitamins. Noting that vitamin C is an antioxidant, and a helper against cancer, it is indeed essential to know the best way to benefit from it.

IX. APPENDIX

Figure 2, the step of measuring the amount of lemon juice squeezed.

Figure 3, the balloon joji tubes of the 5 temperature groups, filled with lemon juice, water and color forming chemicals.

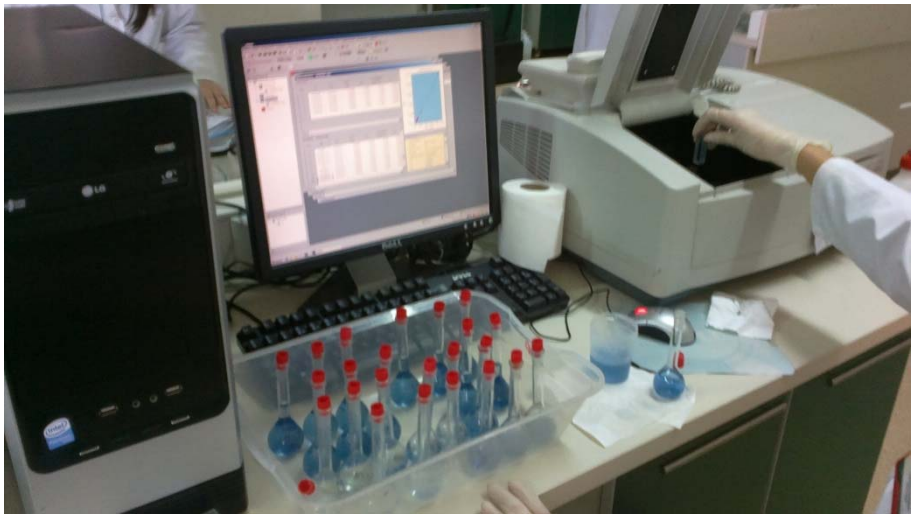


Figure 4, the detection of ascorbic acid content by using UV spectrophotometry .

No.1 Color forming chemicals used;

1) 2.5 mL 0.05 M Oxalic Acid
2) 0.5 mL Phosphoric Acid
3) 1 mL Sulfuric Acid (5% v/v)
4) 2 mL Ammonium Molybdate (5% m/v)