# INFLUENCE OF VARIOUS THERMAL TREATMENTS OVER VITAMIN C CONCENTRATION IN LEMONS

**Andrei I. Simion*, Lăcrămioara Rusu, Ioana Ştefănescu, Lucian Gavrilă**

*University of Bacău, Faculty of Engineering, Department of Chemistry and Engineering of Food Products and Environmental Protection, Calea Mărăşeşti 157, 600115 Bacău, Romania*

*Corresponding author: asimion@ub.ro

Received: 14/11/2008
Accepted after revision: 16/12/2008

**Abstract:** The chemical instability of vitamin C acid is due to the fact that it is a strong reducing agent and can be deactivated by a wide range of oxidizing agents. In food industry the use of various thermal processes has, in the most cases, a negative impact over the preservation of vitamin C content alongside raw materials conversion into the final products. A comparative study about the variations of vitamin C concentrations in lemons is presented in this paper, variations being produced by the uses of low, medium and respectively high temperature treatments.

**Keywords:** *vitamin C, food processing, citrus fruits, lemons.*

## INTRODUCTION

Taking into account the fact that scientific literature offers too little or disparate information about what is happening in time with vitamin C concentration in lemons in various thermal treatment conditions, this work is motivated in mapping with new data the behavior of vitamin C in this conditions. Performing on lemons is due to high vitamin C content (50 mg/100 g product) and for their wide spread uses in human alimentation.

Long before the discovery of vitamin C (ascorbic acid) in 1932, nutrition experts recognized that something in citrus fruits could prevent scurvy, a disease that killed as many as 2 million sailors between 1500 and 1800.

Vitamin C is a white crystalline compound highly soluble in water. It is not a carboxylic acid but a lactone, and owes its acidic properties and ease of oxidation to the presence of an enediol grouping [1]. The vitamin plays a role in controlling infections and the body's response to stress. It is also found to be a powerful antioxidant that can neutralize harmful free radicals; helps make collagen, a tissue needed for healthy bones, teeth, gums and blood vessels [2 - 4]. As early as the 1700's, vitamin C was referred to as the "anti-scorbutic factor", since it helped prevent the disease called scurvy. This disease was first discovered in British sailors, whose sea voyages left them far from natural surroundings for long periods of time. Their body stores of vitamin C fell below 300 mg, and their gums and skin lost the protective effects of vitamin C.

Vitamin C is reported to lower cancer risk, regenerate vitamin E supplies, improve iron absorption [5] and high-dose of it protects the eye against cataracts [6]. Vitamin C is said to have important interactions with other vitamins. For example, excessive intake of vitamin A is less toxic to the body when vitamin C is readily available [7].

***Table 1.*** *The amount of vitamin C in some vegetables and fruits*

| | **Plant source** | **Amount (mg/100 g)** | | **Plant source** | **Amount (mg/100 g)** |
|---|---|---|---|---|---|
| 1 | Kakadu plum | 3100 | 21 | Mandarin orange | 30 |
| 2 | Camu Camu | 2800 | 22 | Cabbage, raw green | 30 |
| 3 | Rose hip | 2000 | 23 | Mango | 28 |
| 4 | Baobab | 400 | 24 | Potato | 20 |
| 5 | Blackcurrant | 200 | 25 | Melon, honeydew | 20 |
| 6 | Red pepper | 190 | 26 | Cranberry | 13 |
| 7 | Guava | 100 | 27 | Tomato | 10 |
| 8 | Kiwifruit | 90 | 28 | Apricot | 10 |
| 9 | Broccoli | 90 | 29 | Blueberry | 10 |
| 10 | Redcurrant | 80 | 30 | Pineapple | 10 |
| 11 | Brussels sprouts | 80 | 31 | Grape | 10 |
| 12 | Strawberry | 60 | 32 | Plum | 10 |
| 13 | Orange | 50 | 33 | Watermelon | 10 |
| 14 | Lemon | 50 | 34 | Banana | 9 |
| 15 | Melon, cantaloupe | 40 | 35 | Carrot | 9 |
| 16 | Garlic | 31 | 36 | Peach | 7 |
| 17 | Grapefruit | 30 | 37 | Cherry | 7 |
| 18 | Lime | 30 | 38 | Apple | 6 |
| 19 | Spinach | 30 | 39 | Pear | 4 |
| 20 | Tangerine | 30 | 40 | Raisin | 2 |

Various reports show that fruits are excellent sources of vitamin C. Citrus fruits, tomatoes, strawberries, bell peppers and broccoli are good examples of food fruits rich in vitamin C (table 1) [4]. The reports of the vitamin C content of natural food-fruits

include the citrus fruits. A serving size ¾ cup of orange juice from frozen concentrate is reported to contain 75 mg of vitamin C; one medium size orange, 60 mg; half a white grapefruit, 40 mg; and one medium size tangerine, 25 mg of vitamin C [2]. Limejuice is reported to contain 29 mg of vitamin C per 100 g juice.
Though the literature is replete with the different types of methods for the analysis of such diversified products, efforts continue in the search of better methods. Such attempts to quantify ascorbic acid in these samples have resulted in a large number of methods: titrimetry, voltammetry, fluorometry, potentiometry, kinetic-based chemiluminescence (CL), flow injection analyses and chromatography [8, 9].
Nutritionists generally regard any ‘serving’ of food that provides 10 % to 25 % of the daily vitamin C need in a relatively low calorie package as a ‘good’ source. The 1989 recommended daily allowance (RDA) for an adult is 60 mg per day (this is based on the amount of vitamin C needed to prevent clinical scurvy and provide body stores sufficient to prevent scurvy for around 30 days plus “a margin of safety”). At April 1999 it was being officially recommended, based on new information that the RDA ought to be changed to 120 mg per day [7].
In the United States, the current (2000) recommended daily allowance (RDA) or Adequate Intake (AI) for vitamin C is 90 mg per day for men and 75 mg per day for women. The given AI value for men is for males 19 years and older, while that for women is for females 19 years and older. It is recommended that smokers consume 35 more milligrams of vitamin C per day. Pregnant and lactating women have AI values as high as 85 and 120 mg respectively for 19 years or older [10]. In the April 10, 2000 press release, National Academy of Sciences (NAS) set a Tolerable Upper Intake Level (UL) for vitamin C at 2,000 mg (2 g) for adults 19 years or older [10].
Recent surveys of the U.S. Department of Agriculture (USDA) show that the average intake of vitamin C by American adults was over the AI for vitamin C. Women tended to consume less than men of the same age. Taking too much vitamin C is reported to cause side effects such as nausea and diarrhea [4].

## MATERIALS AND METHODS

For a better mimic of thermal conditions in food processing the experimental program was created to cover a wide range of temperature and time intervals (table 2). The lemons fruits were cut into round slices and deposit at different temperature conditions and time intervals in closed glass vessels. Due to ascorbic acid instability at high temperature (dry oven conditions) the experimental time was narrowed at 5 hours, important for this part being the influence of temperature increment over intensification of vitamin C destruction. “Room conditions” is referring to an average temperature of 23 °C and normal conditions of humidity for temperate climate. This experimental part was limited at 6 day in order to exclude advance degradation in chemical composition and structure of lemon slices, which could influence in negative the test results. Lemons should be stored between 7 to 12 °C depending on the maturity-ripeness stage at harvest, season of harvest, storage time and production area. They can be stored for up to 6 months under the right conditions. The optimum room humidity (RH) is 85 to 95 %. Because lemons are chilling sensitive, they should not be stored for prolonged periods below 10 °C, although 3 to 4 weeks storage at 3 to 5 °C, which is typical for some receivers, is usually tolerated without harm.

***Table 2.*** *The experimental conditions, temperature and experimental time*

| Equipment | Temperature (°C) | Time interval |
|---|---|---|
| - | Room conditions (≈23 °C) | 6 days |
| Dry oven | 30 | 5 hours |
| | 40 | 5 hours |
| | 50 | 5 hours |
| | 60 | 5 hours |
| | 70 | 5 hours |
| | 80 | 5 hours |
| Freezer | 12 | 12 days |
| | 6 | 12 days |
| | 0 | 12 days |
| | -6 (whit slow defrosting) | 12 days |
| | -12 (whit slow and rapid defrosting) | 12 days |
| Lyophilized product | | 6 weeks |

Because moderate-low temperatures are recommended for storage of lemons fruits, the time in this experimental part was extended at 12 days and because lemons are chilling sensitive, for frozen products was choose two different approaches, whit slow or fast defrosting of lemon slices to observe a difference in loses of vitamin C if that exist.

A commercial lemon lyophilized product was also tested for variation in time of vitamin C concentration in 6 weeks of storage.

For determination of amount of vitamin C in the juice of lemon slices was use the standard iodometric determination method.

A standard iodine solution was prepared by dissolving 0.2 g of $KIO_3$ and 1.6 g of KI in some distilled water in a 500 mL volumetric flask. The KI was well in excess to keep the $I_2$ generated in solution as $I_3^-$. The solution was acidified by adding 1 mL of concentrated sulfuric acid. The mixture was swirled and the volume of solution raised to 500 mL with distilled water. The flask was stoppered and shaken to ensure homogeneity of content. Thus, the concentration of iodine solution was 5.6076 x $10^{-3}$ M. The iodine solution was kept in a closed cupboard [11].

After a predetermined time interval (after 1 hour for experiments created in dry oven and 1 day for experiments created in freezers and in "room conditions") the slices were squeezed to discharge their juice into a pre-washed beaker and the seeds were carefully picked out from the juice. A 15 mL aliquot of each juice was titrated against the standard 5.6076 x $10^{-3}$ M iodine solution to a light blue end point using freshly prepared starch as indicator.

## RESULTS AND DISCUSSIONS

The vitamin C amount in lemon slices juice placed at predetermined time intervals in different temperature conditions are presented in table 3.

***Table 3.*** *Variation in time of vitamin C concentration due to experimental temperature conditions*

| Temperature | Room conditions (≈23 °C) | | Dry oven 30 °C | |
|---|---|---|---|---|
| | **Time interval [days]** | **Amount [mg/100 g]** | **Time interval [hours]** | **Amount [mg/100 g]** |
| | 1 | 47.19 | 1 | 47.23 |
| | 2 | 46.29 | 2 | 46.49 |
| | 3 | 45.71 | 3 | 45.15 |
| | 4 | 43.26 | 4 | 43.67 |
| | 5 | 42.07 | 5 | 42.00 |
| | 6 | 40.11 | Initial amount 47.90 mg/100 g | |
| **Temperature** | **Dry oven 40 °C** | | **Dry oven 50 °C** | |
| | **Time interval [hours]** | **Amount [mg/100 g]** | **Time interval [hours]** | **Amount [mg/100 g]** |
| | 1 | 44.99 | 1 | 42.49 |
| | 2 | 43.34 | 2 | 41.18 |
| | 3 | 39.22 | 3 | 37.84 |
| | 4 | 36.94 | 4 | 34.37 |
| | 5 | 35.07 | 5 | 32.45 |
| | Initial amount 45.72 mg/100 g | | Initial amount 44.10 mg/100 g | |
| **Temperature** | **Dry oven 60 °C** | | **Dry oven 70 °C** | |
| | **Time interval [hours]** | **Amount [mg/100 g]** | **Time interval [hours]** | **Amount [mg/100 g]** |
| | 1 | 40.87 | 1 | 39.26 |
| | 2 | 37.44 | 2 | 34.58 |
| | 3 | 35.16 | 3 | 30.54 |
| | 4 | 31.58 | 4 | 26.58 |
| | 5 | 29.07 | 5 | 22.46 |
| | Initial amount 41.83 mg/100 g | | Initial amount 41.11 mg/100 g | |
| **Temperature** | **Dry oven 80 °C** | | **Freezer 12 °C** | |
| | **Time interval [hours]** | **Amount [mg/100 g]** | **Time interval [days]** | **Amount [mg/100 g]** |
| | 1 | 34.26 | 2 | 46.31 |
| | 2 | 30.62 | 4 | 44.50 |
| | 3 | 27.15 | 6 | 43.97 |
| | 4 | 19.14 | 8 | 40.41 |
| | 5 | 07.40 | 10 | 39.20 |
| | | | 12 | 37.67 |
| | Initial amount 39.81 mg/100 g | | Initial amount 47.12 mg/100 g | |
| **Temperature** | **Freezer 6 °C** | | **Freezer 0 °C** | |
| | **Time interval [days]** | **Amount [mg/100 g]** | **Time interval [days]** | **Amount [mg/100 g]** |
| | 2 | 45.03 | 2 | 46.89 |
| | 4 | 43.78 | 4 | 46.32 |
| | 6 | 39.41 | 6 | 44.97 |
| | 8 | 35.90 | 8 | 43.09 |
| | 10 | 34.09 | 10 | 42.00 |
| | 12 | 33.34 | 12 | 40.80 |
| | Initial amount 46.34 mg/100 g | | Initial amount 47.15 mg/100 g | |

| Temperature | Freezer -6 °C (slow defrosting) | | Freezer -12 °C (slow defrosting) | |
|---|---|---|---|---|
| | Time interval [days] | Amount [mg/100 g] | Time interval [days] | Amount [mg/100 g] |
| | 2 | 43.20 | 2 | 36.90 |
| | 4 | 40.35 | 4 | 32.43 |
| | 6 | 36.57 | 6 | 31.15 |
| | 8 | 33.01 | 8 | 29.59 |
| | 10 | 30.88 | 10 | 27.90 |
| | 12 | 28.82 | 12 | 24.88 |
| | Initial amount 43.92 mg/100 g | | Initial amount 38.71 mg/100 g | |
| Temperature | Frezeer -12 °C (fast defrosting) | | Lyophilized product | |
| | Time interval [days] | Amount [mg/100 g] | Time interval [days]* | Amount [mg/100 g] |
| | 2 | 47.32 | 1 | 41.12 |
| | 4 | 46.46 | 2 | 41.03 |
| | 6 | 45.26 | 4 | 40.70 |
| | 8 | 44.12 | 6 | 40.40 |
| | 10 | 43.26 | 8 | 40.20 |
| | 12 | 41.92 | 10 | 40.20 |
| | Initial amount 47.40 mg/100 g | | 12 | 39.89 |

*presentend only for 12 days from 6 weeks

The vitamin C losses determined by the experimental conditions are presented in tables 4 – 6.

***Table 4.*** *The drop of vitamin C concentration in "room conditions" and at various temperature conditions in dry oven*

| | Temperature [°C] | Initial concentration [mg/100 g] | Final concentration [mg/100 g] | Losses [mg/100 g] | Losses [%] |
|---|---|---|---|---|---|
| 1 | ≈23 | 47.19 | 40.11 | 07.08 | 15.03 |
| 2 | 30 | 47.23 | 42.00 | 05.23 | 11.03 |
| 3 | 40 | 44.99 | 35.07 | 09.92 | 20.51 |
| 4 | 50 | 42.49 | 32.45 | 10.04 | 23.62 |
| 5 | 60 | 40.87 | 29.07 | 11.80 | 28.80 |
| 6 | 70 | 39.26 | 22.46 | 16.80 | 42.79 |
| 7 | 80 | 34.26 | 07.40 | 26.86 | 78.42 |

In table 4 is clearly presented the fact that the drop in vitamin C concentration for a period of 6 days (the time was choose for avoiding an accentuated degradation of lemon slices) in "room conditions" (approximately 23 °C), are around 15 %. Heat treatments in the dry oven applied to lemon slices modified the vitamin C concentrations more drastically, the augmentation of heat temperature having like results a rapid drop in concentrations: from a minimum of 11.03 % for 30 °C (the lowest temperature used in oven) to a maximum of 78.42 % for 80 °C (the highest temperature used). The same information it could be observed in the figure 1, the differences between start concentrations are explained by the fact that for this experiment were use lemons with different initial vitamin C concentration (the concentration of vitamin C in lemons is dependent on the maturity-ripeness stage at harvest, season of harvest, storage time and production area). Due to the fact that in 5 hour was lost for temperatures of 30 and 40 °C approximately the same amount of vitamin at 23 °C, in normal conditions, for a extended period of 6 days, suggest the negative impact of heat treatments over the chemical stability of ascorbic acid compound.

***Table 5.*** *The drop of vitamin C concentration in freezer conditions*

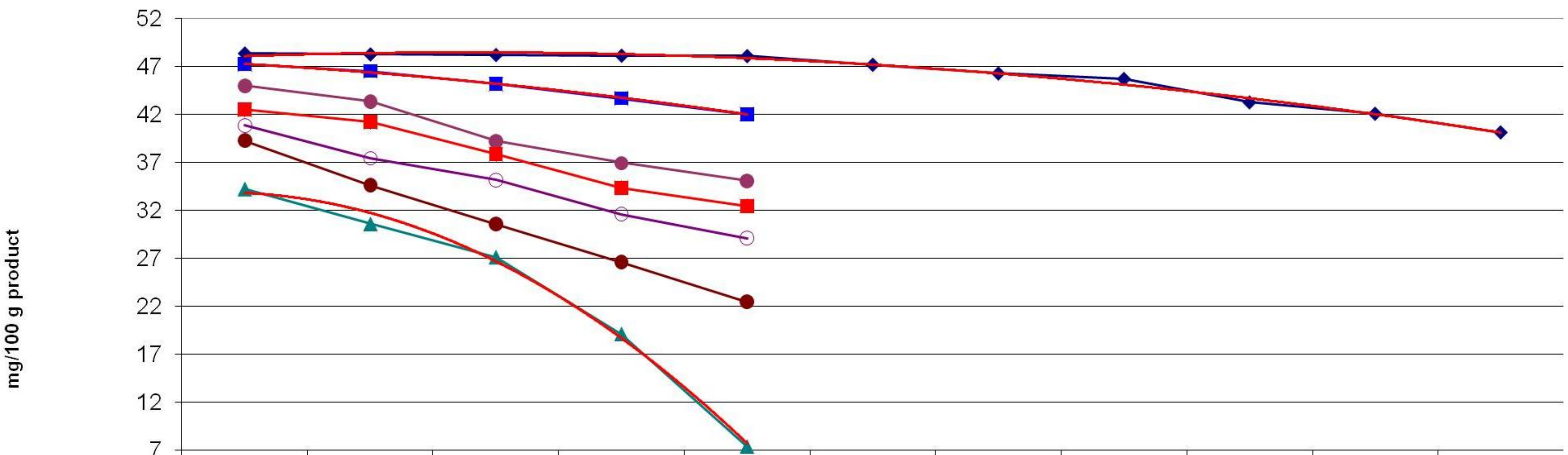
| | Temperature [$^0$C] | Initial concentration [mg/100 g] | Final concentration [mg/100 g] | Losses [mg/100 g] | Losses [%] |
|---|---|---|---|---|---|
| 1 | -12 rapid defrosting | 47.32 | 41.92 | 05.40 | 11.40 |
| 2 | -12 slow defrosting | 36.90 | 24.88 | 12.02 | 32.57 |
| 3 | - 6 slow defrosting | 43.20 | 28.82 | 14.38 | 33.28 |
| 4 | 0 | 46.89 | 40.80 | 06.09 | 12.98 |
| 5 | 6 | 45.03 | 33.34 | 11.66 | 25.89 |
| 6 | 12 | 46.31 | 37.67 | 08.64 | 18.65 |

In table 5 is presented loses in concentrations of vitamin C for low temperature storage and treatments. Temperatures of 12 °C and 6 °C create, relatively, the same drop in concentration, these temperatures being used for normal storage of lemons (7 to 12 °C) for a recommended 6 months.
At 0 °C and -12 °C (fast defrosting) are recorded the minimum loses in vitamin, 12.98% and 11.4%, for a period of 12 days, that suggest a better conservation on vitamin. In figure 1, is presented the fact that in 6 days the amount drop in vitamin C was half in compare with storage at 23 °C, in the end the after 12 days the final loses being fewer than 15% (see figure 4).

***Table 6.*** *The drop of vitamin C concentration in different temperature conditions*

| | Temperature [°C] | Time [hours] | Initial concentration [mg/100 g] | Final concentration [mg/100 g] | Losses [mg/100 g] |
|---|---|---|---|---|---|
| 1 | -12 | 288 | 47.32 | 41.92 | 05.40 |
| 2 | 20 | 144 | 47.19 | 40.11 | 07.08 |
| 3 | 30 | 5 | 47.23 | 42.00 | 05.23 |
| 4 | 80 | 5 | 34.26 | 07.40 | 26.86 |

An interesting fact was observed when was compare two experiments at -12 °C where was changed the modality of defrosting. A significant loss in vitamin C was recorded for slow defrosting product due to an accentuated oxidation of ascorbic acid in air and juice dilution, with water from fruit exterior ice, when this was collected. In compare between fast defrosting were loses was 11.4% and slow defrosting 32.57%, the drop in concentration was approximate three time more accentuated (figure 2).
For commercial lyophilized product, the drop in vitamin C was 18.6% in 12 days, but is irrelevant at this time because in technical literature the loses of vitamin C in lyophilization process are around 10 to 15%, in our case 11%, and in this light the real concentration loses in storage time was only 7.6% value that drop only at 7.9% in 6 weeks (figure 5).




| Hours | 1 | 2 | 3 | 4 | 5 | 24 | 48 | 72 | 96 | 120 | 144 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 degrees C | 47,23 | 46,49 | 45,15 | 43,67 | 42 | | | | | | |
| 40 degrees C | 44,99 | 43,34 | 39,22 | 36,94 | 35,07 | | | | | | |
| 50 degrees C | 42,49 | 41,18 | 37,84 | 34,37 | 32,45 | | | | | | |
| 60 degrees C | 40,87 | 37,44 | 35,16 | 31,58 | 29,07 | | | | | | |
| 70 degrees C | 39,26 | 34,58 | 30,54 | 26,58 | 22,46 | | | | | | |
| 80 degrees C | 34,26 | 30,62 | 27,15 | 19,14 | 7,40 | | | | | | |
| 23 degrees C | 48,38 | 48,28 | 48,22 | 48,15 | 48,1 | 47,19 | 46,29 | 45,71 | 43,26 | 42,07 | 40,11 |

***Figure 1.** Variation of vitamin C concentration for 5 hours in dry oven in compare with "room conditions" in 6 days (144 hours)*

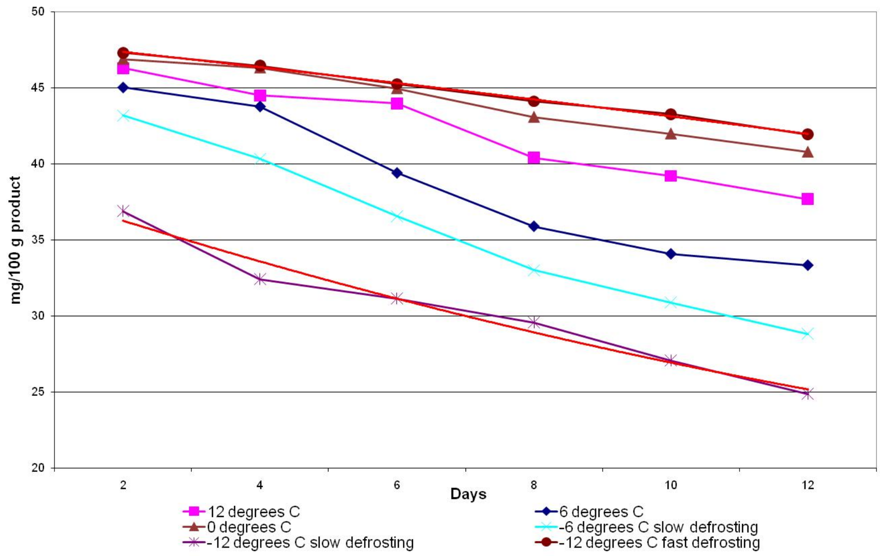



***Figure 2.*** *Variation of vitamin C concentration in freezer conditions for 12 days*

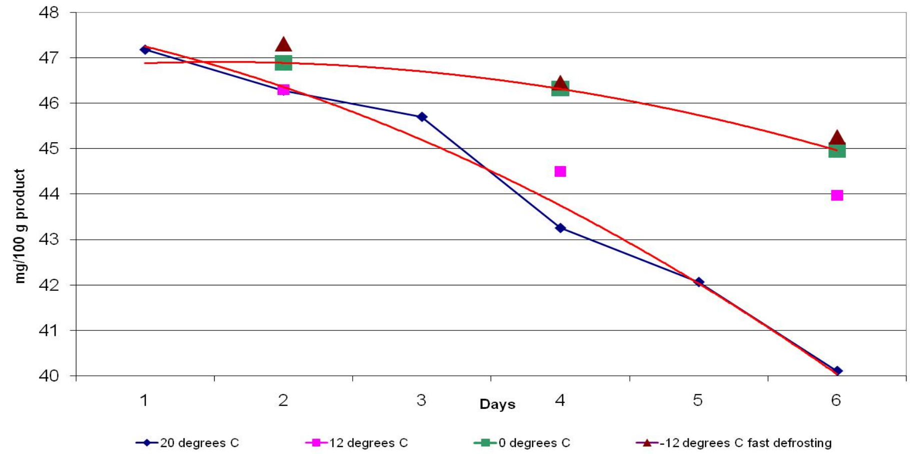


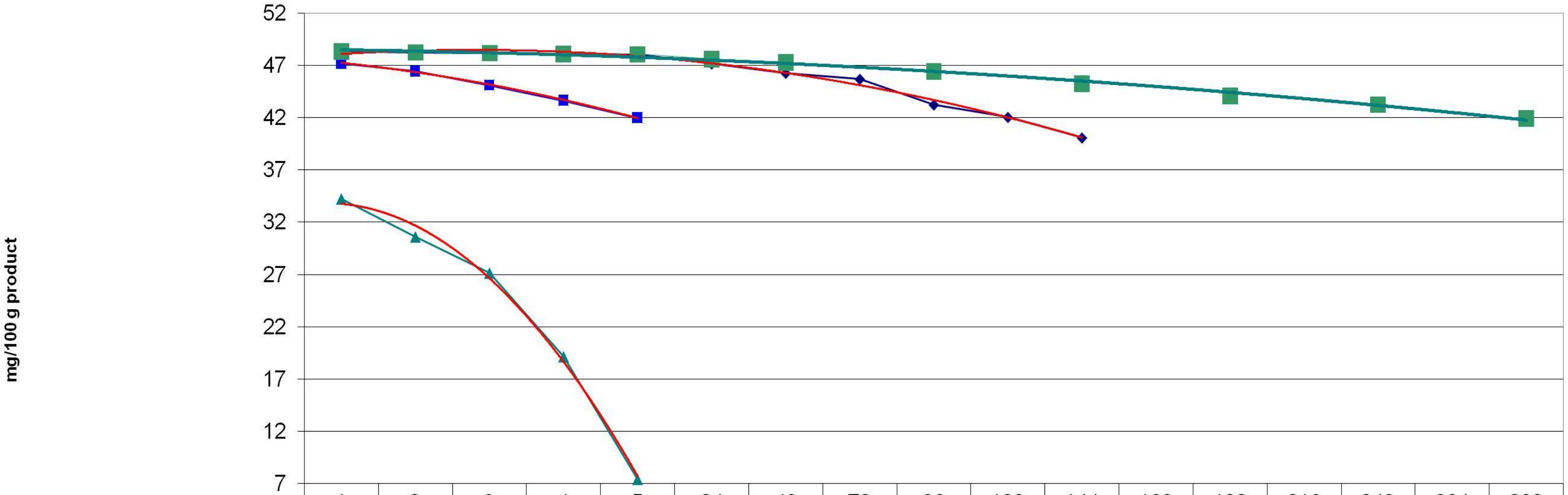

***Figure 3.*** *Variation of vitamin C concentration in freezer conditions in compare with "room conditions" in 6 days*

## CONCLUSIONS

According whit theoretical and experimental researches is clear that the amounts of vitamin C in lemon are influenced by the nature, intensity and time exposure at heat treatments and low temperature treatments or storage.

The conclusions of this experiment were:

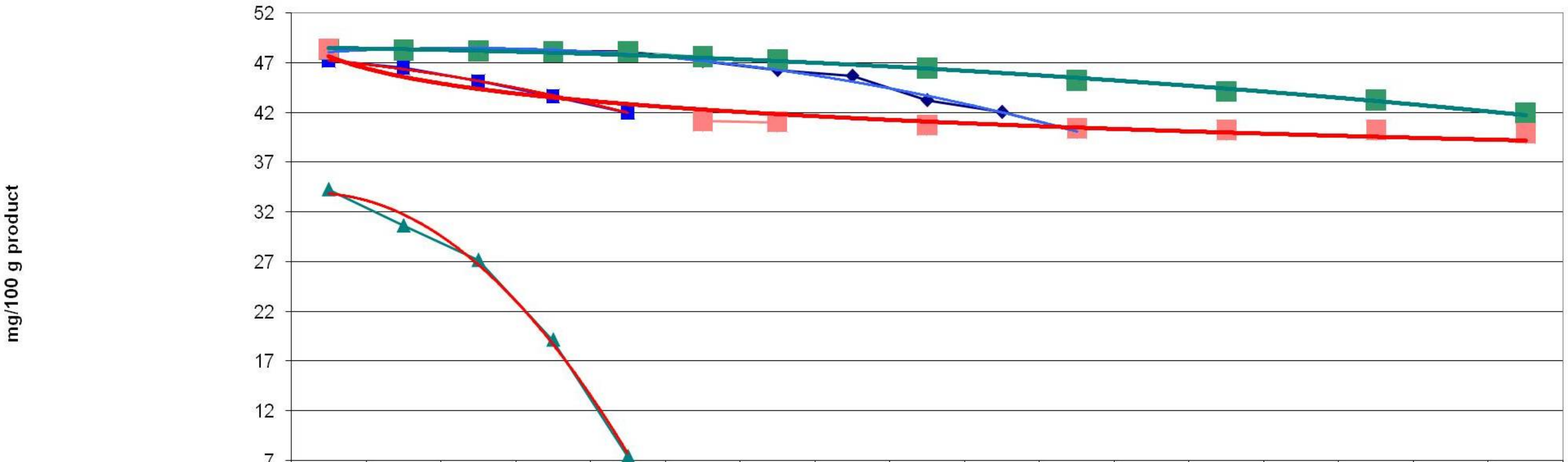
- Even that the contents of vitamin C suffers a small drop at 23 °C (room conditions), the exposure at open air, light and room humidity determined a significant degradation in flavor and exterior aspect of lemon;
- Moderate heat treatments (30, 40, 50 °C) increase the loses in vitamin C by 20 times in compare with 23 °C;




| Hours | 1 | 2 | 3 | 4 | 5 | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 | 216 | 240 | 264 | 288 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -30 degrees C | 47,23 | 46,49 | 45,15 | 43,67 | 42 | | | | | | | | | | | | |
| -80 degrees C | 34,26 | 30,62 | 27,15 | 19,14 | 7,40 | | | | | | | | | | | | |
| -23 degrees C | 48,38 | 48,28 | 48,22 | 48,15 | 48,1 | 47,19 | 46,29 | 45,71 | 43,26 | 42,07 | 40,11 | | | | | | |
| -12 degrees C fast defrosting | 48,38 | 48,28 | 48,22 | 48,15 | 48,1 | 47,63 | 47,32 | | 46,46 | | 45,26 | | 44,12 | | 43,26 | | 41,92 |

Hours

***Figure 4.*** *Variation of Vitamin C concentration in dry oven condition in 5 hour in compare with "room conditions" in 6 days (144 hours) and freezer conditions for 12 days (288 hours)*




| | 1 | 2 | 3 | 4 | 5 | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 | 216 | 240 | 264 | 288 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 degrees C | 47,23 | 46,49 | 45,15 | 43,67 | 42 | | | | | | | | | | | | |
| 80 degrees C | 34,26 | 30,62 | 27,15 | 19,14 | 7,40 | | | | | | | | | | | | |
| 23 degrees C | 48,38 | 48,28 | 48,22 | 48,15 | 48,1 | 47,19 | 46,29 | 45,71 | 43,26 | 42,07 | 40,11 | | | | | | |
| -12 degrees C fast defrosting | 48,38 | 48,28 | 48,22 | 48,15 | 48,1 | 47,63 | 47,32 | | 46,46 | | 45,26 | | 44,12 | | 43,26 | | 41,92 |
| lyophilized product | 48,38 | | | | | 41,12 | 41,03 | | 40,7 | | 40,4 | | 40,2 | | 40,2 | | 39,89 |

**Figure 5.** *Variation of Vitamin C concentration in dry oven condition in 5 hour in compare with "room conditions" in 6 days (144 hours), freezer conditions for 12 days (288 hours) and lyophilized product*

- High temperature treatments indicate an intensification of degradation over 100 times in compare with 23 °C times and up to 250 times more aggressive than 0 °C or more;
- The storage temperatures of 12 and 6 °C indicate an increase in lifespan of vitamin C in time of storage over 2 times in compare with 23 °C but without degradation in flavor and exterior aspect of lemon;
- The 0 °C is the most indicated storage temperature by the experiment and by technical literature because the lemon frozen temperature (-2.47 °C) is excluded and so the chemical degradations at this temperature grant a high time preservation of vitamin C in lemon and eliminate their chilling sensitivity.
- The temperatures of -12 and -6 °C are indicated for short time storage because they offer a better protection of vitamin C in lemons (even then storage at 0 °C) but they could generate in time chemical degradations of fruits and the energy consumption for this is high;
- Is necessary to avoid slow defrosting of fruits due to an accentuated oxidation of ascorbic acid in air and juice dilution, with water from fruit exterior ice, in case of fruits squeezing or cutting process ;
- For commercial lyophilized product, the drop in vitamin C was only 7.6% in 12 days and 7.9% in 6 weeks values that make this product the richest in vitamin C after a long storage period. The lyophilized product is easily rehydrated, recovering his form, volume, flavor, color and sensorial characteristics.

## REFERENCES

1. Roberts, J.D., Caserio, M.C.: *Basic Principles of Organic Chemistry*, W.A. Benjamin, Inc., California, **1977**;
2. Ohio State University, Department of Human Nutrition and OSU Extension 2004, *Vitamin C (Ascorbic Acid)*, HYG-5552-05;
3. Carr, A.C., Frei, B.: Toward a new recommended dietary allowance for Vitamin C based on antioxidant and health effects in humans, *Am. J. Clin. Nutr.*, **1999**, **69**, 1086-1107;
4. Hwang, M.Y.: How much vitamin C do you need?, *The Journal of the American Medical Association*, **1999**, **281**(15), 1640;
5. Mateljan, G.: *The world's healthiest foods: Vitamin C*. www.whfoods.org., **2007**;
6. Sardi, B.: *Is high-dose vitamin C risky?*, **2000**;
7. Levine, M., Rumsey, S.C., Daruwala, R., Park, J.B., Wang, Y.: Criteria and recommendations for vitamin C intake, *The Journal of the American Medical Association*, **1999**, **281**(15), 1415-1423;
8. Bates, C.J.: Vitamins: Fat and Water Soluble: Analysis*, Encyclopedia of Analytical Chemistry*, **1999**, **1**;
9. Matei, N., Birghila, S.: Determination of Vitamin C from fruit juices and pharmaceuticals, *Ann. Univ. Ovidius,* **2001**, **12**, 47-49;
10. http://en.wikipedia.org/wiki/Vitamin_C;
11. Izuagie, A.A., Izuagie, F.O.: Iodimetric Determination of Ascorbic Acid (Vitamin C) in Citrus Fruits, *Research Journal of Agriculture and Biological Sciences*, **2007**, **3**(5), 367-369.