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INFLUENCE OF TEMPERATURE AND TIME OF STORAGE ON AMOUNT OF VITAMIN C IN STRAWBERRIES

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Abstract

Nutritional quality of strawberries comes mostly due to the large amount of containing vitamin C. There is a need of proper strawberry storage to preserve the high amount of vitamin C.

Vitamin C was determined by the Tillmans method in strawberries stored at: room temperature, temperature of 4 ^{0}C (cooling) and at temperature of -18 ^{0}C (freezing). The storage was up to 11 days. Results were processed with *t*- test and after statistical processing we calculated the Pearson's correlation coefficient.

In all three storage conditions there is a reduction of vitamin C amount. The amount of vitamin C in fresh strawberries is 60.85 mg/100 g and after 5 days it is only 7.57 mg/100 g for the strawberries stored at room temperature, 43.30 mg/100 g for the strawberries stored under cooling conditions and 44.54 mg/100 g for the frozen strawberries. After 11 days, there is almost no vitamin C (0.55 mg/100 g) in the strawberries stored at room temperature, the amount of vitamin C in the frozen strawberries was 28.21 mg/100 g, and in the cooling strawberries there was the highest amount of vitamin C that achieved 37.92 mg/100 g. For strawberries stored at room temperature and for freezing strawberries there is the highest value for Pearson's correlation coefficient and r = 0.93871402. This coefficient it is lower for cooled strawberries and freezing strawberries, r = 0.887226935.

Vitamin C reduces differently in strawberries stored in different conditions. Until the fifth day, the tendency of vitamin C reduction is the smallest for the frozen strawberries, and after the fifth day it is the smallest for the cooling strawberries.

***Key words**: Strawberries, Vitamin C, Storage, Cooling, Freezing.*

1. Introduction

Vitamin C (ascorbic acid, AA) is water-soluble, unstable vitamin, which is a powerful antioxidant essential for the human body (Anitra and Balz, [1]). The main sources of vitamin C are: citrus fruits, strawberries, peppers, tomatoes, spinach and etc.

Strawberries are healthy, dietary and nutritionally rich products because of well a balanced composition. Large amounts of vitamin C, which they contain, determines their nutritional quality. It is recommended that they should be eaten fresh, to use the most as their nutritional components (Giampieri *et al*., [2]). Usually strawberries can't be eaten fresh as they are processed or stored.

The storage conditions are very important for the amount of vitamin C in them. If the fruit surface is damaged, that causes major losses of ascorbic acid. Fruits which have a low pH (citrus fruits) have smaller losses of ascorbic acid and fruit with a soft consistency, such as strawberries are more sensitive to external influences. By reducing of the temperature, we reduced possibility of losing vitamin C in fruits. Evaporation of strawberries is higher at the strawberries stored at a higher temperature, and therefore the losses of water soluble vitamin C are higher (Nunes *et al.,* [3]).

Also high temperature adversely affects the content of vitamin C, because it is unstable at the high temperature (Davey *et al*., [4]). To minimize the losses of vitamin C in strawberries, it is recommended that they are storage by cooling or freezing and not at room temperature. Depending on storage temperatures, strawberries anti-oxidative ability is changes. Therefore, the strawberries which are kept at a temperature between 10 ^{0}C and 5 ^{0}C have higher antioxidant capacity, and higher concentration of phenols and anthocyanins than those strawberries kept at a temperature of 0 ^{0}C.

Regarding different storage temperatures, the concentration of total phenols and flavonoids is almost the same (Zhao [5]). But the strawberries concentration of ascorbic acid is reduced, because it oxidizes to dehydroascorbic acid, which still has vitamins properties. Storage at the long period, dehydroascorbic acid oxidizes to dicetogulonic acid which has no vitamins properties (Pavlovska and Tanevska, Bode *et al*., [6 - 7]).

The temperature and time of storage affect many parameters of strawberries. This paper following the amount of vitamin C in strawberries during different storage conditions (room stored temperature, cooling and freezing) for a period of 11 days.

2. Materials and Methods

Garden strawberries from the region of Demir Hisar, Republic of Macedonia, are analyzed. Strawberries are harvested in May, 2014. The samples are divided into three parts and stored in various conditions. The first sample is strawberries left at room temperature (20 - 25 ^{0}C), another at a cool temperature of 4 ^{0}C and the third samples frozen at a temperature of -18 ^{0}C.

Determination of Vitamin C is performed in a homogenized strawberries with 2,6-dichlorofenolindofenol using Tilman´s method according to the AOAC [8]. The strawberries were pulverized in Ultraturex homogenizer (Ika Labortechnik T25).

Numerical statistical analyses of data are made with applying "Pearson's correlation coefficient" and the Student t-test [9 - 10]. Since the results are obtained under different storage conditions, a correlation coefficient between two sets of data results is calculated. "Pearson's correlation coefficient", or simply "the correlation coefficient" measures the relative strength of the *linear* relationship between two variables, or two sets of data obtained from different storage conditions. The correlation coefficientis obtained by dividing the covariance of the two variables by the product of their standard deviations.

Student's *t*-test deals with the problems associated with inference based on "small" samples. Two sets of data can be used to determine if the averages of your two samples are significantly different.

3. Results and Discussion

3.1 Determining the amount of vitamin C

Garden strawberries are analyzed at room and frozen temperatures for 1, 3, 4, 5, 7 and 11 days.

Over time the stored strawberries are succumbed to water reduction, therefore they lose their shine and succulence (juiciness) and their surfaces become wrinkled. Also they get darker in color as a result of anthocyanins synthesis. These changes occurred faster with room stored strawberries compared to samples stored cool.

Room stored strawberries became moldy after the fourth day. The frozen stored sample did not show the same signs. Frozen samples when melting were characterized by a diluted structure from disintegration due to their structure.

In the Figure 1 is shown the amount of vitamin C in room stored strawberries. Amount of Vitamin C is 60.85 mg/100 g in the fresh strawberries. The reduction of vitamin C is continuous, 8 - 9 mg per day, or 14 - 15% each day. In terms of the third day, declining vitamin C is very small after the fourth day and very big after the fifth day. After a week of storage at room temperature, the vitamin C in strawberries is almost decomposed and less than 2%, and after eleven days less than 1% relative to the initial amount of vitamin C in fresh strawberries.

The amount of vitamin C in strawberries stored under refrigeration is shown in Figure 2. The reduction in the content of vitamin C in strawberries stored cooling is leniently significant compared with the decrease of vitamin C in strawberries stored at room temperature. The biggest jump in reduction of vitamin C has after the first day is storage then it is smaller. After 11 days of storage the amount of vitamin C in strawberries is 37.92 mg/100 g, or it is reduced to less than 40% relative to the amount of vitamin C in fresh strawberries.

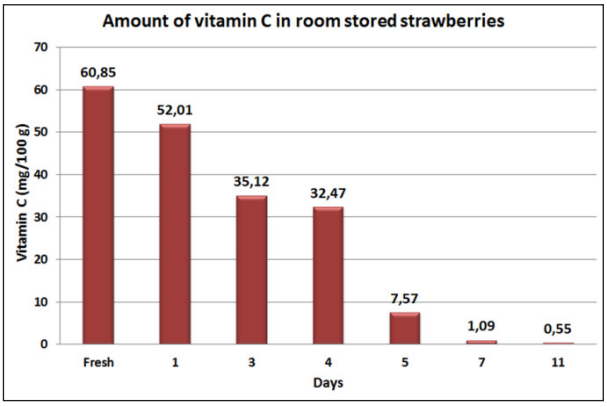

Figure 1. Amount of vitamin C in room stored strawberries

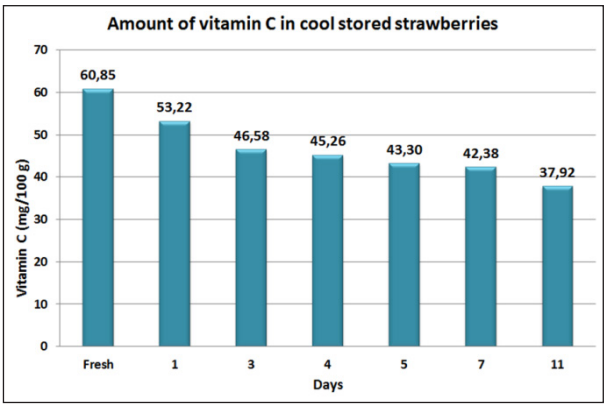

Figure 2. Amount of vitamin C in cool stored strawberries

Frozen strawberries or stored strawberries at the lowest temperature of -18 ^{0}C have a different trend in the reduction of vitamin C (Figure 3). The amount of vitamin C is quite well preserved in frozen strawberries in the first three stored days. The amount of vitamin C in strawberries has been reduced to only 1 mg/100 g after the first day of storage, and 6 mg/100 g after the third day. After one week of freezing, the amount of vitamin C in strawberries has reduced to less than 50%. Strawberries contain 28.21 mg/100 g of vitamin C after 11 days of storing.

The comparison of the amount of vitamin C at three different condition-stored strawberries is presented in Figure 4 with the trend in reduction of vitamin C. The highest amount of vitamin C is present in the frozen stored strawberries in the first five days, but after the seventh and after the eleventh day, highest amount of vitamin C is presentin the cool stored strawberries. Room temperature stored strawberries have the lowest quantity of vitamin C in all the days of storage.

3.2 Determining the correlation coefficient and Student's *t*-test

The correlation coefficient measures the strength and the direction of a linear relationship between two variables or two sets of data. It is calculated that the correlation coefficient between the data of strawberries stored at room temperature and cooling stored strawberries, was $r = 0.925769141$.The correlation coefficient between the data of strawberries stored at room temperature and frozen stored strawberriesis $r = 0.93871402$. And $r = 0.887226935$ is the correlation coefficient between the data of strawberries cooling stored and frozen stored strawberries. From the correlation coefficient result we can see that all groups are in strong linear correlations.

But very importantly, is the content level of vitamin C statistically significant different in room stored strawberries than cooled stored strawberries? Alternatively, is the content level of vitamin C statistically significantly different in room stored strawberries than frozen stored strawberries?

In Table 1 the statistical parameters are presented which have the following meaning:

$\bar{x}$ - The average value (mean) of the amount of vitamin C in mg/100g;

sd - The standard deviation measures the amount of variation or dispersion from the average. From the statistical results we can see how much each measurement deviates from the mean. It means in room stored strawberries we have a higher variation in the amount of vitamin C. For example, on the first day the amount of vitamin C is 60.85 mg/100g, on last day it is 0.55 mg/100g, the average is 27.094 mg/100g, so we can see a high dispersion of 19.52 from the average. In

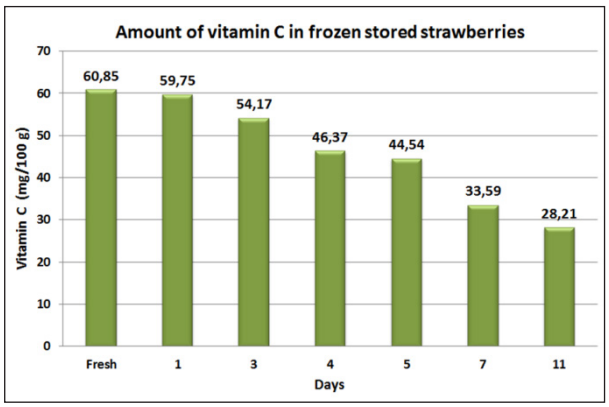

Figure 3. Amount of vitamin C in frozen stored strawberries

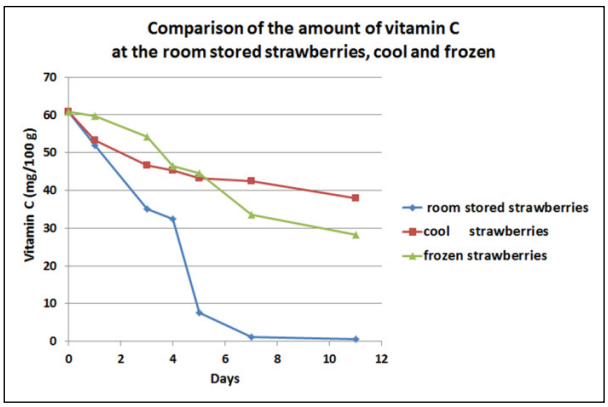

Figure 4. Comparison of the amount of vitamin C at the room stored strawberries, cool and frozen

Table 1. Representation of the parameters from Student's *t*-test

Statistical parameters	Room stored strawberries	Cooling storedstrawberries	Frozen stored strawberries
$\bar{x}$	27,094	47,074	46,784
sd	19.52	4,65	10,91
cv	72	9,8	23,3
t-test		- 3,39428	-4,97877
p-value (probability) (statistical significantly, $p < 0.05$)		0,00968	0,00209

cooled (refrigerated) and frozen stored strawberries we do not have so much variation in the amount of vitamin C during the stored process. Cooled stored strawberries have an sd of 4.65, and frozen stored strawberries have an sd of 10.91.

cv -The **variance** measures how far a set of numbers is spread out; a small variance indicates that the data points tend to be very close to the mean and hence to each other, while a high variance indicates that the data points are very spread out around the mean and from each other. The size of this difference in comparison to the variance (i.e. the range over which expression values fall) will tell us whether this expression difference is significant or not. Therefore, if the difference is large but the variance is also large, then the difference may not be significant. On the other hand, a small difference coupled with a very small variance could be significant.

We used the **t-test** for two groups to formalise this calculation. The tests return a p-value that takes into account the mean difference and the variance and also the sample size.

The **p-value** (probability) is a measure of how likely you are to get this spot data if no real difference exists. Therefore, a small p-value indicates that there is a small chance of getting this data if no real difference exists and therefore you decide that the difference in the group expression data is significant. A p-value is said to be significant if it is less than the level of significance, which is commonly 5% ($p < 0.05$).

From the results of both t-tests there are statistical significantly differences between the two groups of samples:

- Room stored strawberries and cooled stored strawberries, $t = -3.39428$; $p = 0.00968 < 0.05$
- Room stored strawberries and frozen stored strawberries, $t = -4.97877$; $p = 0{,}00209 < 0.05$.

In the first t-test (room stored and cool stored), levels of vitamin C are reduced in both cases but there are statistical significant differences in reducing the level of vitamin C in room stored then level of vitamin C in cooling stored strawberries. Cooled stored strawberries keep a statistically significant higher amount of vitamin C than room stored strawberries.

In the second t-test (room stored and frozen stored), the levels of vitamin C are reduced in both cases but there are statistically significant differences in reducing the level of vitamin C in room stored rather than the level of vitamin C in frozen stored strawberries. Frozen stored strawberries are kept statistically with a significantly higher amount of C vitamin than room stored strawberries.

From the statistical calculations and analysis above, we prove that the best situation is that it is necessary for strawberries to be kept for a longer period. The best way is for strawberries to be stored under refrigeration, or if it is necessary for strawberries to be kept for the longest period. Then we also get very good results for the level of the amount of vitamin C when strawberries are stored frozen.

4. Conclusions

- It is determined the amount of vitamin C in strawberries which are stored at three different conditions for a period of 11 days. Major losses of vitamin C occur in the strawberries stored at room temperature. After four days storage at room temperature, the amount of vitamin C is almost halved, and after 7 and 11 days, almost no vitamin C. Strawberries stored by freezing the highest values for vitamin C in the first 5 days of storage, and more storage for 7 and 11 days, it is best for strawberries to be stored under refrigeration.

- The correlation coefficient is stronger for strawberries stored at room temperature and freezing ($r = 0.93871402$), but the correlation coefficient is still strong between strawberries stored at cooling and freezing temperature ($r = 0.887226935$).

- From the results we can make the conclusion that the samples are in a strong correlation, which means a strong linear dependence between the two sets of data. In our case a very strong linear dependence of data of the amount of vitamin C in room stored (as a first variable) and the data of the amount of vitamin C in cooled stored strawberries (as a second variable). This means that in both cases, the amount of vitamin C is reduced by the same linear dependence. The same conclusion applies for room stored (as a first variable) and frozen stored strawberries (as a second variable), where by the level of content of vitamin C is reduced also.

- But most importantly is that with the Student t-test we statistically significant proved the differences between the samples. This means with numerical and statistical analyses we confirmed our experiment. We have a highest variation about the mean in room stored strawberries and also variation relative to the mean in the case of frozen and cooled stored strawberries. Following the t-test we calculated that the statistically significant differences are so much lower that 0.05 in both cases, in the first case: room and cool stored strawberries, and in the other case, room and frozen stored strawberries.

- With a strong correlation coefficient, and when the difference is statistically significant, we can state that it is likely that the difference is not caused by this random fluctuation. Therefore we confirm it is best for strawberries be stored under refrigeration.

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