RESEARCH REGARDING THE INFLUENCE OF STORAGE CONDITION ON BROCCOLI VITAMIN C CONTENT

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Abstract

The commercial potential of Broccoli (*Brassica oleracea*) is very high for the several ways: the vegetable can be utilized and also for its chemical composition, nutritional value and pleasant taste. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling procedures (*Palma A. et al., 2015*). The aim of this study is to highlight some metabolic changes, namely ascorbic acid in broccoli flower buds during short term refrigerated storage of broccoli heads under commonly applied conditions. Materials and Methods: It was studied one set of samples, having total weight of 200 – 250 g (8 – 10 florets), who were kept in plastic perforated trays both in open ambient storage conditions (19 ± 1^{0}C and 55 ± 2% Ur) and in laboratory refrigerated storage conditions (4±1^{0}C □i 50±2%Ur). The another set was packaged with commercial polypropylene film with 10 pin holes stored in the same conditions. The samples was evaluated in T_0 moments, after 1 day, 3 and 7 days of storage. The ascorbic acid was determinated by using the 2,6 – diclorphenol-indophenol method. The initial content in ascorbic acid of fresh broccoli floret's was 75,9 mg/ 100g which decreased during storage under ambient conditions from laboratory and the samples stored under 4^{0}C showed significant changes in ascorbic acid content by the end of storage (7 days). The ascorbic acid decreased rapidly in the florets unpacked, kept in open ambient plastic trays compared with the samples kept in refrigerated conditions.

Key words: ascorbic acid, broccoli, refrigerate, storage condition

Broccoli vegetable species belongs to the *Brasicaceae* family which is a large plant group that includes about 3000 species in 350 genere grouped, including different edible plant important from economically and agronomically point of view. According to *Gomez – Campo and Prakash (1999*), the principal genus *Brassica* is the most important in the *Brasiaceae* family, and includes vegetables and forage form such as *Brassica oleracea L., Brassica napus L., Brassica rapa L*. The same author stated that the *acephala* (kale, collard greens), *botrytis* (cauliflower, Romanesco broccoli, broccoflower), *capitata* (white cabbage), *capitata rubra* (red cabbage), *gemifera* (Brussels sprouts), *gongylodes* (kohlrabi), *italic* (broccoli), *sabauda* (Savoy cabbage) and *viridis* (collards, tree kale) are the most important cultivars of *Brassica oleareceae* in western- hemisphere countries.

The interest in *Brassica* plants exceeds the information on proteins, lipids, carbohydrates, vitamins, amino acids and minerals since other compounds exist that can explain their protective mechanisms in human health. Due to their agricultural importance, *Brassica* plants have been the subject of much scientific interest, particularly the *Brassica oleracea* species which have assumed an important role in human nutrition, as they assumed an important role in human nutrition, as they are the predominant dietary source of glucosinolates (*Cartea and Velasco, 2008*) but also have high contents of phenolics and other antioxidant compounds (*Ferreres et al., 2007*).

The literature show that the major antioxidant compounds, and thus the major protective dietary antioxidants, are vitamins C (*Murariu O., 2014*) and E, carotenoids, and polyphenols, especially flavonoids. It is well accepted now that these antioxidant compounds scavenge radicals and contribute both to the first and second defense lines against oxidative stress. As a result, they protect cells against oxidative demage, and may therefore prevent chronic diseases such as cancer, cardiovascular disease, and diabetes. *Broccoli*, like other vegetables, contains a considerable amount of vitamin C (113 mg/100 g) (*Davey et. al., 2000*). Several authors such us *Bernhardt and Schlich (2006)* and *Korus and Lisiewska (2007)* presented studies with the abundance of those compounds in *Brasiaceae* family, concluding that the cruciferous vegetables are a relatively good source of abundant antioxidants, highlighting that broccoli inflorescences, followed by Brussel sprouts and kale are amongst the Brassica vegetables with the highest content of vitamin C, β- carotene, lutein and DL-α-tocopherol (*Alfredo Aires, 2015*).

MATERIALS AND METHODS

The samples of *broccoli* was purchased from retail, where they were kept on refrigerated condition at 2 – 4 ^{0}C. It was studied one set of samples, having total weight of 200 – 250 g (8 – 10 florets), who were kept in plastic perforated trays both in open ambient storage conditions (19 ± 1^{0}C and 55 ± 2% Ur) and in laboratory refrigerated storage conditions (4±1^{0}C i 50±2%Ur). The another set was packaged usis commercial polypropylene film with 10 pin holes stored in the same conditions. The samples was evaluated in T_0 moments, after 1 day, 3 and 7 days of storage. The changes of ascorbic acid was determinated by using the 2,6 – *diclorphenol-indophenol* dye method. The principle of the method was the extraction of the ascorbic acid in the test sample with 2% oxalic acid solution and titrating with 2.6 - *diclorfenolindofenol* until a light pink color. *Broccoli* floret samples of 10 g were ground with 50 ml of 2 % oxalic acid, volume with distilled water in 100 ml beaker and filtered through Whatman no. 4. In an Erlenmeyer flask (50 cm^3 volume) are placed 5 cm^3 of acid extract of the sample and titrate quickly with indophenol dye solution stirring continuously, until the appearance of pink color that persists for 10 seconds.

Likewise is prepared the blank, in which the sample is replaced with the 5 cm^3 of extraction solution.

For reductones determination it proceed as: in a 50 cm^3 Erlenmeye flask it introduce the same amount of extract acid of the sample (5 cm^3), to which it's added 1 cm^3 of copper sulfate solution. The mixture is ground and heated in a boiling water bath for 10 minutes. After cooling, the sample is titrated with indophenols dye solution.

The ascorbic acid content, express as mg/100 g as fresh- weight basis is calculated using Eq. (1):

$$\text{Vit. C} = = \frac{[V0 \quad (V1 + V2) x V3 x C}{V4 x m} \text{ x } 100 \text{ [mg/100 g]},$$

where: V_0 – the volume of indophenols dye solution used in sample titration (cm^3); V_1 – the volume of indophenols dye solution used to blank titrate (cm^3); V_2 - the volume of indophenols dye solution used in reductones titration (cm^3); V_3 – the total volume of acid extract of sample (cm^3); V_4 – the volume of acid extract sample taken for analyses (cm^3); C – the amount of ascorbic acid corresponding to 1 cm^3 indophenol dye solution (0.088); m – mass of the sample taken into analasys [g].

RESULTS AND DISSCUTIONS

Vitamin C exist in several forms in plant. The dehidroascorbic oxidized forms (DHA) and monodehidroascorbat (MDHA) are obtained by non enzymatic oxidation and L-ascorbate is oxidized by the ascorbate oxidase (AO) or ascorbate peroxidase (APX) (*Smirnoff, 2000b*). These forms can be degraded or reduced by two reductase (monodehidroascorbat reductase - MDHAR and dehydroascorbate reductase – DHAR) to form ascorbic again. This recycling of ascorbate reductase is the basis of the antioxidant role because it allows regeneration of the active form (reduced form) of ascorbic acid after use to reduce the reactive oxygen species *(Ishikawa et al., 2006*) and to mantain a vitamin C redox state in plant (reduced form and shape ratio of the total).

Vitamin C degradation pathways in plants are not fully elucidated, although several studies have shown the ascorbic acid catabolism in oxalate acid, tartric or threonic acid.

The rol of vitamin C in broccoli constitution is represented by the participation in the growth and development precesses associating division and cell expansion (*Smirnoff, 2000b)*, enzyme cofactor (*Ariggoni and De Tullio, 2002*), antioxidant, against pathogens and in the photosynthesis process (*Massot, 2011*). Some nutrients from broccoli such us the antioxidant, vitamins, carotenoids, tocopherols and ascorbic acid appear to play a double role in metabolism. These are required for normal growth and development and they appear to provide antioxidant protection against chronic diseases, including chronic heart disease, arthritis and cancer (*Krinsky et al., 2000*).

The daily requirement of vitamin C for a healthy adult body is 0 – 90 mg/day, for women during pregnancy 100 mg/day, for breastfeeding women 130 mg/day and for children 1,5 – 2 mg/kg body/ day depending on age.

The dynamic of vitamin C content during broccoli storage shows a decrease tendency. This vegetable reaches the retail market at least 1 – 2 days after harvest. Most of the time fresh looking, green color florets are preferred for consumption. This crop is generally sold in the retail market either without any packaging or sometimes as a packed form in polyethylene bags of 250 – 500 g. Most of the time, after purchase consumers of this vegetable is purchased at a high price due to its higher phytochemical properties, its degradation during storage in such conditions is not known.

Nath et al. (2011) studied the changes in ascorbic acid content of broccoli during refrigerated storage and they reported that the initial ascorbic acid content of fresh broccoli florets was 130 mg/100 g which decreased linearly during storage under different treatments.

The ascorbic acid content decreased rapidly in the florets kept in non packaged plastic trays in ambient compared to the florets kept in ambient on poliethylene micro-perfored packets and plastic tray refrigerated samples.

By 7 days of storage, ascorbic acid content decreased from 75,9 mg/ 100 g to 42,72 mg/ 100 g, a 43,71 % reduction in the non packeged ambient plastic trays samples, while the samples stored in open trays in refrigerated condintion showed a lower decline in vitamin C content after 7 days, namely from 75,9 mg/100 g to 64,18 mg/ 100 g, with 15,44 % reduction. The losses of umidity that occur during storage can explain the intensive losses of vitamin C content. It occurred also due to the effect of atmosphere change inside the package which promoted vitamin C retention as a function of the CO_2 increment as the O_2 reduction (*Barth and Zhuang, 1996*). *Lee and Kader, 2000* mentioned that in generally, high CO_2 concentrations cause vitamin C degradation.

Table 1

Influence of packaging and temperature on ascorbic acid content (mean ± S.E. in mg/ 100 g) of *Broccoli* flower bods short term storage

Acid ascorbic [mg/100g]	T_0	Days of storage				
		1 day		3 days		7 days
		19^0C	$2 – 4^0C$	19^0C	$2 – 4^0C$	$2 – 4^0C$
Non-packaged	75,9 ± 0,4	70,3 ± 0,3	67,32 ± 0,3	42,72 ± 0,4	70,06 ± 0,4	64,18 ± 0,2
Packaged		71,98 ± 0,4	66,42 ± 0,24	62,5 ± 0,42	67,14 ± 0,36	56,36 ± 0,4

Table 1

Statistical differences of ascorbic acid content of *Broccoli* flower bods due by packaging (Non- packaging vs. packaging) during storage

Storage condition	Days of storage	
	1 day	3 days
19^0C	*	***
$2 – 4^0C$	*	***

ANOVA Test: i.s. – insignificance differences(p < 0,05); * - semnificative differences (p > 0,05); ** - distinct semnificative differences (p < 0,001); *** = very significant differences (p > 0,001).

Table 3

Statistical differences of ascorbic acid content of *Broccoli* flower bods due by storage temperature (20^0C vs. $2 – 4^0C$)

Packaging condition	Days of storage	
	1 day	3 days
Non-packaged	***	***
Packaged	***	***

ANOVA Test: i.s. – insignificance differences(p < 0,05); * - semnificative differences (p > 0,05); ** - distinct semnificative differences (p < 0,001); *** = very significant differences (p > 0,001).

During storage, the samples kept in ambient temperature in open trays showed lower levels (42,75 mg/100 g) of ascorbic acid content compared to samples packaged in polyethylene, kept in refrigerated condition (67,14 mg/ 100 g) highlighting significant differences (p> 0,001) in statistical points of view(tab. 2).

At the end of storage (after 7 days) the samples kept in refrigeration condition in non packaged plastic trays shows higher values (64,18 mg/ 100 g) than the packaged samples kept in the same condition (56,36 mg/ 100 g) (tab. 1). This results are in agreement with those previously results reported in the literature by *Ray et al. (2008), Nath et al. (2011; 2015*) by similar studies conducted for broccoli.

Thus, it's releaved significant differences (p> 0,05) in vitamin C content of broccoli packaged in polyethylene samples compared with un packaged samples, in both cases (stored in ambient and refrigerated temperature) after 1 day and very significant differences (p> 0,001) in both cases after 3 days of storage (*tab. 3*).

Mapson (1970) mentioned that during storage, oxidizing enzymes like ascorbic acid oxidase, peroxidase, catalase and polyphenol oxidase might help in reducing the ascorbic acid of fruits and vegetable.

For the broccoli florets packaged and un packaged samples kept in refrigerated condition it's releaved a slight increase on vitamin C content between the first and third day of storage respectively from 67,32 to 70,06 for unpackaged samples and from 66,42 at 67,14 for packaged samples, which occurs because of vegetable stress. After *Rozek et al., 1994* the harvest, manipulation, cutting, and cooling (1^0C) can promote stress conditions, so the plant increases ascorbic acid synthesis as a protection mechanism.

CONCLUSION

The stabilization of chemical compounds with an important role in the daily needs ensuring of human nutrition should be provided through suitable methods for obtaining and storage.

In the current study is highlighted the favorable role of packaging on broccoli florets with polyethylene film when they are stored in ambiental temperature conditions. It also highlights the impact of storage temperature of the vitamin C content in broccoli florets which was superior on refrigerated samples.

The contribution of broccoli florets vegetables to health improvement can be related to their phytochemical composition. Therefore, it is fundamental to know how to act, either at domestic or industrial levels, in order to preserve this beneficial compounds. The different stages of the production chain such us storage, must be optimized in order to minimize the losses of beneficial nutraceutical and bioactive compounds, such as acid L- ascorbic.

The current researches releaved that the highest stability of vitamin C content from broccoli is ensured by keeping this vegetables in unpackaged trays in refrigerated conditions.

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