Romanian Biotechnological Letters *Vol. 21, No. 5, 2016*

ORIGINAL PAPER

Changes in the nutrients content of some green vegetables during storage and thermal processing

Received for publication, October 10th, 2015
Accepted, November 14th, 2015

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Abstract

Changes in vegetables nutrients composition occur during postharvest period resulting in qualitative losses so that fresh vegetables have a short shelf life before they become unsafe or undesirable for consumption. In order to minimize the losses of valuable nutrients appropriate storage methods should be used. Nowadays, chilling and freezing seems to be common preservation technologies applied depending on the desired shelf life. On the other hand, most vegetables are commonly cooked before being consumed. Samples of fresh, chilled, frozen and boiled broccoli, spinach, green beans and peas were analysed in order to assess the potential benefits of the use of frozen or cooked vegetables in comparison to the fresh ones. The analyses were performed using proper biochemical methods: reversed-phase HPLC for vitamin C determination and spectrophotometric method for carotenoids content. Losses of analysed nutrients were determined during prolonged storage by refrigeration and after thermal processing but total carotenoids have been proven to be more stable compared to vitamin C.

Key words: *vitamin C,* carotenoids, spinach, broccoli, beans, peas, refrigeration, storage, boiling

1. Introduction

In the recent years the consumer's interest are increasingly focused toward foods containing compounds with antioxidant activity that are supposed to provide health benefit. *In vitro*, antioxidant activity was associated with reduced DNA deterioration, decreased lipid peroxidation or inhibited malignant transformation. For this reason the intake of antioxidants was related with lower incidence of certain types of cancer and degenerative diseases. Vegetables are a major source of macronutrients such as fibre and micronutrients like minerals and vitamins. An important role in the activity of the body's nonenzymatic antioxidant defence system is assigned to essential micronutrient compounds such as ascorbate (vitamin C), vitamin E, carotenoids, polyphenols, selenium (SIES 1995, [1]). Empirical evidence suggests that a considerable fraction of all cancer incidences can be prevented by a higher intake of fruit and vegetables resulting in improved public health (KRIS-ETHERTON & al. 2002, [2]; GUNDGAARD & al. 2003 [3]). Leafy vegetables such as spinach (*Spinacia oleracea*) are known being a rich source of natural antioxidants including polyphenols, chlorophyll, vitamin C as well as carotenoids (ISMAIL & al. 2003 [4]; LIGOR & al. 2013 [5]; IGWEMMAR & al. 2013 [6]). Also broccoli (*Brassica oleracea* var. *italica*) is a vegetable with high antioxidant activity containing high levels of vitamin C, carotenoids and phenolic compounds, particularly flavonoids and has been described as a vegetable with high nutritional value (LIN & al. 2005 [7]; YUAN & al. 2009 [8]). Generally green vegetables including peas (*Pisum sativum*) and green beans (*Phaseolus vulgaris*) had a high carotenoids and polyphenols content, so they can be considered as a potential source of antioxidants (ORUNA-CONCHA & al. 1998 [9]; EDELENBOS & al.

2001 [10]; CHAURASIA & al. 2012 [11]). Changes in nutrients composition of vegetables occur postharvest due to its high water content (70-90%) and increased respiration rate resulting in qualitative losses such as loss in edibility, nutritional value and consumer acceptability (KADER 2005 [12]). The rate of biological and biochemical degradation depends on both postharvest handling and on storage conditions. Fresh vegetables have a short shelf life before they become unsafe or undesirable for consumption. Although freshly gathered vegetables contain great amount of vitamin C, it begins to degrade immediately after harvest. Green peas for example registered up to 51% loss of vitamin C content during the first 24–48 hours after harvesting (PHILIPS & al. 2010 [13]); in the case of broccoli 56% loss was registered after 7 days of storage at 20°C (HOWARD & al. 1999 [14]). The perishable nature of these seasonal products imposed the need to find appropriate storage methods to minimize the losses of valuable nutrients and to make them available for longer period. Nowadays, chilling is used for short-term preservation of vegetables, as for a longer period of storage, freezing seems to be one of the most common preservation technologies applied (HOWARD & al. 1999 [14]; TOSUN & al. 2008 [15]; BARRETT & al. 2012 [16]). Retention of nutrients in harvested vegetables is highly depending on the vegetables species and cultivar, on maturity stage at the harvesting time and on post-harvest processing conditions. Recent research noted that considering the time spent between the harvest and consumption of a fresh produce, the frozen equivalent may be nutritionally similar to the fresh one due to oxidative degradation of the biochemical compounds during handling and storage of the fresh vegetables (GEBCZYNSKI & al. 2007 [17]). Analysis of fresh, chilled and frozen vegetables available in retail markets would facilitate the estimation of the nutritional content of fruits and vegetables available to the consumer. However, vitamins are among the nutrients sensitive to heat, light and oxygen, making its susceptible to loss during vegetables storage ant thermal processing (BARRETT & al. 2012 [16]). On the other hand, most vegetables are commonly cooked before being consumed. Generally, vegetables are prepared at home depending on local convenience, on culinary tradition and on taste preference, rather than taking account on retention of nutrient and health-promoting compounds (MASRIZAL & al. [18]). Processing treatments during cooking affects water-soluble nutrients such as vitamin C, making it susceptible to loss due its sensitivity to heat, light and oxygen. Also fat-soluble nutrients such as carotenoids may be released from their cellular matrices by thermal, freezing or other preservation treatments. Considering these, the researches performed in this study aimed to evaluate the impact of storage by refrigeration under domestic conditions on vitamin C and carotenoid content of some green vegetables and also to examine the changes in the amounts of selected antioxidants produced by varied thermal treatment (boiling). The analyses were performed using proper biochemical methods: reversed-phase HPLC for vitamin C determination and spectrophotometric method for carotenoids. Samples of fresh, chilling, frozen and boiled broccoli, spinach, green beans and peas were analysed in order to achieve information allowing to assess the potential benefits of the use of frozen or cooked vegetables in comparison to the fresh ones.

2. Materials and methods

Samples

Four fresh and frozen green vegetables were purchased from the local supermarket which included: spinach (*Spinacia oleracea*), broccoli (*Brassica oleracea* var *italica*), green beans (*Phaseolus vulgaris)* and peas (*Pisum sativum).* The fresh vegetables were harvested on the same day they were purchased and the frozen ones had similar storage period according to the label. The analyses were performed on fresh vegetables harvested at full maturity stage and during its storage at chilling temperature at 4-6°C for seven days period. The temperature from the refrigerator was checked every 12 hours using a thermo-hygrometer with external calibrated sensor. Frozen and boiled vegetables were analysed too in order to evaluate the influence of the thermal processing on the vitamins content. For a correct comparison, all the thermal treatments were performed in the same conditions with the boiling period considered since the vegetables started to boil. Fresh produce was processed on the same day as it was acquired. The determinations were performed in triplicate. The extractions were conducted according to the protocol used for each determination.

Methods

The total carotenoids content was performed using a spectrophotometric method. 5 g of sample were grinded and extracted with acetone repeatedly until a colourless residue was obtained. The extracts were collected and filtered, than the volume was filled up to 25 ml with acetone. The absorbance at 452 nm was measured with a UV/Visible ThermoSpectronic Helios spectrophotometer. Total carotenoids content was estimated by mg/100 g FW. *Ascorbic acid* was extracted from a homogenized sample using ortho phosphoric acid solution. After appropriated dilution, the extract was filtered by 0.22 µm PVDF syringe filter. Quantification of vitamin C was performed by reversed-phase high performance liquid chromatography method. Chromatographic experiments were conducted using a WATERS ALLIANCE system equipped with auto sampler and UV detector. SUPELCOGEL 4.6mm x 25 cm column was used for vitamin C separation in isocratic mode, with 0.17 ml/min flow. Data acquisition and processing were performed with Empower 2.0 specialized soft. Ascorbic acid content was calculated and reported as mg/100g FW.

3. Results and discussions

3.1. Changes in vitamin C and total carotenoids content during storage at 4-6°C chilling temperature

The analyses of fresh vegetables (figure 1) revealed high amounts of vitamin C in broccoli (34.45 mg/100 g) and spinach (30.37 mg/100 g), while green beans registered the lowest content in vitamin C (15.2 mg/100g).

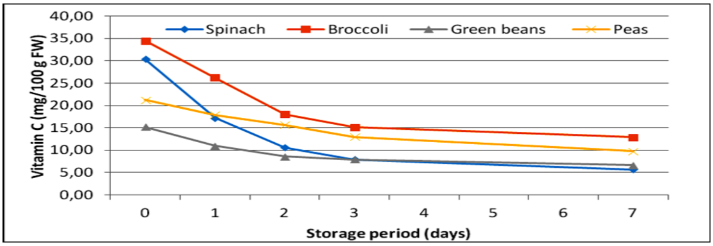

Figure 1. Dynamics of vitamin C content during storage at 4-6°C chilling temperature

Vitamin C is a less stable nutrient due to its high sensitivity to oxidation under the influence of heat and light therefore it degrades rapidly after harvest and during storage. The vitamin C content of the selected vegetables subsequently decreased during storage period, with the lowest values recorded after seven days (figure 1). However, the level of degradation depended on the vegetable species: fragile and perishable vegetables, expressing great sensitivity to manipulation and processing as spinach and broccoli were more affected regarding the vitamin C content. At the end of experiment spinach registered the highest loss of vitamin C (81.23 %) among the analysed vegetables. A significant concentration decrease was also observed for broccoli during refrigerated storage: final concentration, registered after 7 days, was 12.87 mg/100 g FW, therefore a loss of 62.66 % vitamin C content occurred. Green beans and peas registered a lower degradation of vitamin C content than those observed in the other analysed vegetables: 56.05 %, respectively 54 %. Probably their more fibrous texture and lower water content have a positive influence on preserving ascorbic acid content. Broccoli and spinach have a higher respiration rate correlated with more intense metabolic processes, including ascorbic acid oxidation. These results are supported by researches performed by other authors. According to the scientific literature retention of vitamin C can vary tremendously in all products depending on cultivar and processing conditions (HOWARD & al. 1999 [14]). Vitamin C losses in vegetables stored at 4-6°C for 7 days range from 15 % for green peas to 77 % for green beans (BARRETT & al. 2012 [16]). Total carotenoids are known as antioxidants present in high quantities in vegetables. Analyses performed in this study on the fresh vegetables indicated spinach as the richest in carotenoids (8.35 mg/100 g FW), while green beans was found to have the lowest amount of carotenoids among the studied vegetables (1.02 mg/100 gFW). Previous studies of β-carotene show no definite trend of nutrient retention within vegetables during prolonged chilled storage: after 14–16 days a 10% increase in the β-carotene content of carrots and a 10% loss in green beans were registered (HOWARD & al. 1999 [14]).

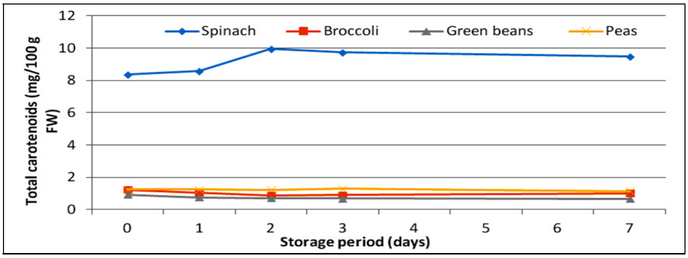

Figure 2. Dynamics of total carotenoids content during storage at 4-6°C chilling temperature

The researches performed indicated a marginal increase in carotenoids concentrations measured in spinach: from 8.35 to 9.47 mg/100g after 7 days of chilled storage (figure 2). These results do not show that chilled temperature could cause the synthesis of more carotenoids but it is possible to detect greater amounts of these compounds due to the leaves dehydration caused by respiration and transpiration processes. In order to limit the water loss, the vegetables were stored in sealed plastic bags. On the contrary, the other studied vegetables registered a decrease of the carotenoids content during the refrigeration period. Small values of the carotenoids losses were noticed for broccoli (8.9%) and peas (7.89%), but green beans recorded the highest loss in the carotenoids content (25.5%). However, analysing the obtained data it appears that carotenoids have been proven to be more stable than vitamin C during chilled storage of the studied samples.

3.2. Effect of thermal processing on the vitamins content

3.2.1. Boiling of fresh vegetables

Green vegetables can be eaten raw or cooked in different ways (boiled, steamed, fried) but the most common use worldwide is probable boiled vegetables. Advantage of this procedure is that it eliminates potential pathogens and poisonous or irritating compounds are neutralized at the same time (AGBEMAFLE & al. 2012 [19]). Also, it is known that the accessibility of the many valuable nutrients is enhanced when vegetables are cooked. However, cooking induces significant changes in physical and chemical properties of vegetables.

Table 1. Content in vitamin C (mg/100 g F.W.) in processed vegetables

Vegetable	Fresh	Boiled for 5 min	Boiled for 10 min	Boiled for 20 min
Spinach	35.37	20.69	14.76	11.40
Broccoli	34.45	22.18	16.18	13.60
Green beans	15.20	10.34	8.45	7.14
Peas	21.20	14.63	10.76	8.76

Cooking is often responsible for the greatest loss of vitamin C as it is very susceptible to chemical and enzymatic oxidation during the processing of vegetable. In order to assess the impact of thermal treatment on the nutritive value, the vitamin C and carotenoids content were analysed in the selected vegetables after different boiling time (5, 10 and 20 minutes since the vegetables started to boil). As expected, vitamin C decreased in all the analysed boiled samples (table 1). Increased boiling period resulted in significant reduction of the vitamin C concentration caused mainly by leaching into cooking water and thermal breakdown. Scientific literature reports that there is a significant negative correlation between the vitamin C concentration and the boiling period of different vegetables (OBOH [20]; AGBEMAFLE & al. 2012 [19]; IGWEMMAR & al. 2013 [6]).

At the end of boiling time, spinach registered a decrease of 23.97 mg/100 g in the vitamin C content, while in green beans it decreased with only 8.06 mg/100g (figure 3). The most severe reduction was observed in the first 5 minutes at all the analysed vegetables, and then as the boiling time increased the reduction became slower (figure 3). It is possible that the maximum amount of vitamin C has been destroyed by heating or leached into the water at the beginning of boiling so that only a small amount remained in the vegetal tissues during the further boiling duration. Also it can be observed that degradation of vitamin C was major in case of leafy vegetables as spinach (67.76% loss) at the end of boiling time in comparison to the other analysed green vegetables. It appears that greater loss of vitamin C in case of leafy vegetables is due to the structural aspects of storage location of ascorbic acid in plant tissues; leafy vegetables are juicier and contain more ascorbic acid as compared to root or stem vegetables (WAHEED-UZ-ZAMAN & al. 2013[21]). On the contrary, peas and green beans were least affected by thermal treatment regarding the amount of vitamin C which recorded smaller losses (58.67% and 53.02% respectively).

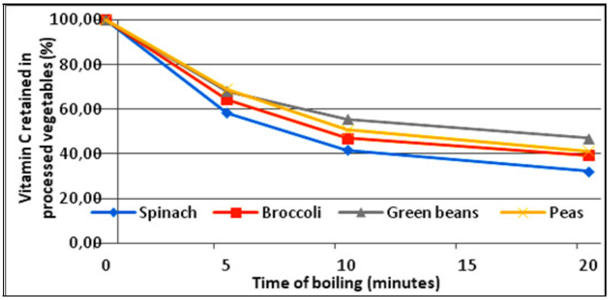

Figure 3. Effect of boiling time on vitamin C content

Regarding the changes occurred in total carotenoids content in the cooked vegetables compared to fresh produce there are discrepancies in the results reported by different authors. Some studies have found that thermal processing increases the carotenoid content determined in vegetables, possibly due of a greater extractability of carotenoids from vegetable matrix (GRANADO & al. 1992 [22]; KHACHIK & al. 1992 [23]). Similar results are noticed in recent papers: GLISZCZYŃSKA-ŚWIGŁO & al. 2006 [24] reported that boiling and steaming of broccoli resulted in an increase in carotenoids as compared with fresh broccoli; MIGLIO & al. 2008 [25] reported an increase in total carotenoids and ß-carotene content of boiled carrots compared to raw carrots, while other authors concluded that all the cooking method caused significant increase in total carotenoids (DE LA CRUZ-GARCIA & al. 1997 [26]; BEMBEM & al. 2014 [27]). Also, the scientific literature suggests that increased extractability attributed to heat treatment may be associated with a better bioavailability of carotenoids from cooked or processed vegetables compared to those from raw produce (HEDREN & al. 2002 [28]; DUTTA & al. 2005 [29]). This, however, contradicts the report of DIETZ & al. 1988 [30] that boiling for 30 minutes result in 53% and 40% decreasing of carotenoids in lettuce and carrots, respectively. ANJUM & al. 2008 [31] reported a significant reduction in ß-carotene content of selected Indian vegetables, while AGBEMAFLE & al. 2012 [19] noticed that the concentration of ß-carotene in the vegetables decreased continuously with increase in boiling time. The analyses performed on the selected vegetables indicated different dynamics of total carotenoids content under thermal processing depending on species (table 2). Spinach registered slight increase of carotenoids content (of 1.5 mg/100 g) at the end of boiling time. However, this increase in carotenoids content during thermal processing is not likely to be true but rather is a consequence of the analytical process. Significant reduction was observed at broccoli and green bean (around 1.5 times), while peas was less affected regarding the carotenoids content.

Table 2. Content in total carotenoids (mg/100 g F.W.) in processed vegetables

Vegetable	Fresh	Boiled for 5 min	Boiled for 10 min	Boiled for 20 min
Spinach	8.35	8.95	9.58	9.87
Broccoli	1.23	0.98	0.78	0.66
Green beans	1.02	0.72	0.68	0.64
Peas	1.14	1.03	0.96	0.92

The boiling time is also important, as it can be observed that the concentration of carotenoids in the vegetables varied continuously during thermal processing. Almost half of total loss in carotenoids content was recorded in the first 5 minutes of boiling, and then a slight reduction occurred. After 20 minutes of boiling peas retained the highest amount of carotenoids (80.70%), while in broccoli only 53.66% from the initial amount of carotenoids was preserved (figure 4).

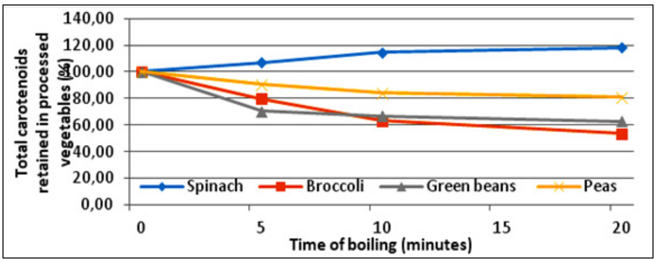

Figure 4. Effect of boiling time on carotenoids content

The losses in carotenoids content during boiling were probably caused by their heat sensitivity. HACKETT & al. 2004 [32] noticed that the loss in ß-carotene occurred because trans isomer changed to cis isomer, biologically inactive, as a result of the thermal processing. Comparing the obtained results a difference can be observed regarding the effect of heating on the analysed parameters: vitamin C was more affected than the carotenoids given that the losses recorded after 20 minutes of boiling varied between 53.02%-67.76% for vitamin C and only 19.29%-46.34% for carotenoids. This difference can be attributed to the fact that beside heat sensitivity, also the high solubility in water, result in high losses of vitamin C.

3.2.2. Boiling of frozen vegetables

Frozen vegetables are preferred by most consumers due to their prolonged shelf-life which enables an available consumption throughout the year. Certainly the frozen vegetables need to be processed before consumption, so the amount of vitamin C and total carotenoids were analysed in frozen vegetables and after boiling them to be eaten. The obtained results showed good levels of vitamin C (figure 5) and carotenoids (figure 6) in frozen vegetables making them appropiate for consumption. The concentrations determined in frozen produce generally resembled those recorded in the corresponding fresh produce. Frozen spinach registered the highest amounts for both vitamin C (32.18 mg/100 g) and carotenoids (6.21 mg/100 g). Also frozen broccoli was found to be a good source of vitamin C (30.78 mg/100 g), while lower values were registered for peas and green beans. A comparative assessment of vitamin C loss after boiling indicated that frozen spinach was found to be the most susceptible to vitamin C degradation (67.24% loss), broccoli and green beans demonstrated a better retention (losses of 58.99% and 53.32% respectively), whereas peas showed a significant lower loss (only 35.83%).

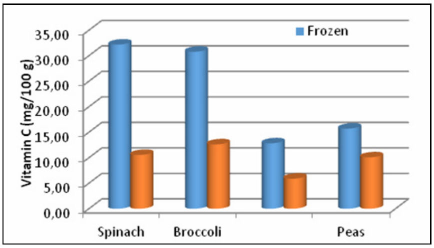

Figure 5. Vitamin C content in frozen vegetables

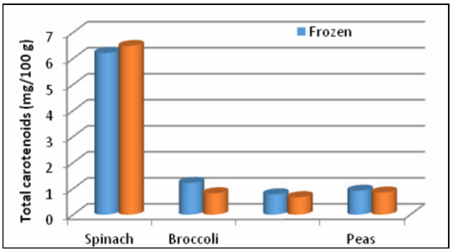

Figure 6. Total carotenoids content in frozen vegetables

The analysed amounts of carotenoids were found to have comparable values in frozen broccoli (1.22 mg/100 g), green beans (0.78 mg/100 g) and peas (0.92 mg/100 g). As expected, carotenoids contained in frozen vegetables were less affected by boiling compared to vitamin C. Frozen broccoli has been proven to be more sensitive to thermal processing regarding the carotenoids content which registered 32.78% loss. The lowest loss of carotenoids was calculated for peas (7.6%) as in the case of vitamin C. On the contrary, frozen as well as fresh spinach recorded after boiling a slight increase (4.34%) of carotenoids, which may be released better from their cellular matrices by thermal treatment.

4. Conclusions

The selected vegetables are good sources of vitamin C and total carotenoids in fresh status. Losses of nutrients occurred during prolonged storage at chilling temperature. Decrease of the vitamin C content was observed, with the lowest values recorded after seven days.

However, the level of vitamin C degradation depended on the vegetable species; sensitive and perishable vegetables as spinach and broccoli were more affected because their higher respiration rate that cause oxidative breakdown. Different behaviour of total carotenoids in selected vegetable was observed after seven days of chilled storage: they increased in spinach, while the others selected vegetables registered a decrease of the carotenoids content. Total carotenoids have been proven to be more stable during chilled storage compared to vitamin C. Frozen vegetables showed good levels of vitamin Cthat recomand them as an alternative to fresh ones. Thermal processing of both fresh and frozen vegetables severely affects vitamin C contents, especially in case of leafy vegetables. Broccoli and green beans demonstrated a better retention, whereas peas showed a significant lower loss of vitamin C after boiling. The stability of carotenoids differs in different vegetables, even when the same processing are used, but they were less affected by boiling compared to vitamin C. Frozen as well as fresh spinach recorded after boiling a slight increase of carotenoids, which may be released better from their cellular matrices by thermal treatment. Broccoli has been proven to be more sensitive to boiling regarding the carotenoids content as the lowest loss of carotenoids was calculated for peas. This study suggests that fresh vegetables are more appropriate for consumption because as long as fresh products undergo minimal storage and are handled at proper temperatures, they are superior to processed products in terms of nutrients content. Inevitable nutrient losses occurred during long-term storage, with longer processing time and higher processing temperatures which affect the optimal nutritional benefits of vegetables unless suitable preservation and preparation methods are used.

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