International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 21-25

Received: 18-04-2020
Accepted: 20-05-2020

Effect of heating and refrigeration durations on ascorbic acid, β-carotene and riboflavin contents of *Solanum tuberosum* and selected vegetables

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DOI: https://doi.org/10.22271/chemi.2020.v8.i4a.10267

Abstract

Aim: It has long been established that the quality and nutritional composition of vegetables vary according to the conditions under which they are stored or prepared. For this reason, the current research was designed to probe the effect of cooking as well as refrigeration time on the β-carotene, riboflavin and ascorbic acid contents of cabbage, lettuce, pumpkin, water leaf, onions, garden egg and Irish potatoes.

Methodology: The vegetable leaves and potato tuber were cooked in boiling water for 40 minutes to determine their β-carotene and riboflavin content, and 45 minutes for ascorbic acid content. Furthermore, portions of the samples were refrigerated for a period of 3 days and 7 days, and their β-carotene and ascorbic acid content assessed. Determinations were done using standard AOAC methods and methods reported by previous researchers.

Results: The amount of β-carotene in untreated samples ranged between 1,200 mg/100g in potato to 17,310 mg/100g in water leaf and between 504.00 mg/100g to 10,729 mg/100g in the same samples respectively after boiling for 40 minutes. The riboflavin content ranged between 3.80 mg/100g to 130.00 mg/100g in pumpkin and onions respectively. After boiling for 40 minutes, this dropped to within a range of 2.00 mg/100g to 74 mg/100g in both samples respectively. The ascorbic acid content was generally below that of β-carotene but higher than riboflavin and ranged between 504 mg/100g to 10,729 mg/100g and 39 mg/100g to 180 mg/100g before and after boiling for 45 minutes respectively. The β-carotene in cabbage was least affected by heat treatment with a percentage loss of 21.30% whereas ascorbic acid in onions was most affected at 82.21%. Refrigeration also showed marked impact on the vitamin content with the β-carotene and ascorbic acid content completely lost in lettuce after refrigeration for 7 days, whereas it showed no impact on the β-carotene content of cabbage after 3 days. The ascorbic acid content of garden egg was reduced by 80.01% upon refrigeration for 7 days while the β-carotene and ascorbic acid contents of lettuce were completely lost after the same duration of time. Generally, the contents of the vitamins decreased with increasing refrigeration time.

Keywords: Heating, refrigeration, β-carotene, riboflavin, ascorbic acid, vegetables

1. Introduction

The reliance on vegetables for nutritional requirements has increased tremendously as a result of their benefits for superior human health and socio-economic value. Vegetables furnish the body with vitamins, minerals, definite hormone originators alongside protein and energy (Waheed-UZ-Zaman *et al.* 2013) [37]. They are produced in larger amounts than most other crops and are known to provide a greater part of vitamins A and C in diets (Bosland & Votava, 2000) [9]. The intake of vegetables has become a major part of diet for people in sub-Saharan Africa as it is the main source of vitamins and minerals to the rural populace. Vitamin- A deficiency is one of the major key nutritional problems in developing nations. However, this assertion is not limited to vitamin A as many other vitamins may still be lacking in diets of people from these countries.

The consistent consumption of fruits and vegetables has been reported to defend the human body from deteriorative ailments (Iqbal *et al.* 2004) [22]. This protective function of vegetables is attributed to their inherent presence of antioxidants which prevent the chemical damage caused by reactive oxygen species (free radicals) that emanate from anthropogenic activities such as; vehicular emissions, organic solvents, pesticide residue and tobacco smoke that usually end up in the human body (Iqbal *el al.* 2004) [22]. Ascorbic acid occurs as a water soluble antioxidant in fruits and vegetables and has been found to be useful in abating tissue damage and in the prevention of sperm agglutination (Ogunlesi *et al.* 2009) [28]. β-carotene on the other hand is also obtained in many vegetables and fruits such as carrots, spinach, apricots and sweet potatoes (Girneno *et al.* 2001) [16]. It acts as a biological antioxidant (Khalil & Saleemullah, 2004) [25] playing a crucial role in human health by enhancing humeral and cell-mediated immune responses (Briton, 1995) [10]. Similarly, riboflavin (vitamin B2) is also an antioxidant which helps in energy- producing electron transport chain and the metabolism of fat molecules into chemically useful energy (George Mateljan Foundation, 2015) [35].

The presence of these vitamins in vegetables underscores their great importance in the diet of humans. However, food processing and storage has inevitably tampered with the novelty of these vitamins in various food groups. Although other vegetables like carrots, cabbage and lettuce are eaten raw, most others are consumed as boiled vegetable leaves (Agbemafle *et al.* 2012) [1]. In other instances, they are refrigerated or dried for the purpose of preservation to increase their shelf life. Hence, their vitamin content are likely to be destroyed when they are subjected to adverse handling and storage conditions as a consequence of higher temperatures, low humidity physical damage and chilling injury. These conditions induce reasonable changes in chemical composition, thus influencing the concentration and bioavailability of bioactive components in vegetables (Martin & Ruberte, 1998) [27]. The study investigates the effect of boiling and refrigeration on the ascorbic acid, β-carotene and riboflavin content of seven vegetables commonly consumed in the study area with the aim of providing vital dietary information for consumers.

2. Methodology

2.1 Sample collection and preparation

Freshly harvested potatoes were bought from tomato market, Feringada, Jos-Nigeria. Also, freshly harvested pumpkin leaves, water leaves, onions, garden egg, lettuce, and cabbage were bought from Wurukum market, Makurdi-Nigeria. The samples were transported to the laboratory and carefully washed without cutting, bruising or breaking. The washed samples were weighed in several portions of 10 g. Three portions each of the weighed samples were kept fresh, and four portions of each was kept in the refrigerator for 3 days and 7 days respectively. Each portion of the sample was boiled for 40 minutes. 0, 15, 30, 45 minutes for vitamin C content and 40 minutes for riboflavin and β-carotene content.

2.2. Extraction of ascorbic acid, β-carotene and riboflavin in samples

Ascorbic acid was extracted according to the method described by Bemuza & Azih (2005) [8] with slight modifications. A 10 g was homogenized with an extraction solution of acetone containing 0.5% oxalic acid. The mixture was centrifuged using a bench top centrifuge at 100 rpm for 15 minutes at room temperature. The supernatant was then filtered through a filter paper to obtain a clear extract.

The method of Tee *et al.* (1996) [34] was adopted for the extraction of β-carotene from the samples. Ethanolic potassium hydroxide was added to 10 g of the sample and left to stand for 3 minutes. The mixture was saponified by heating under reflux for about 30 minutes and later cooled to room temperature. The mixture was subsequently transferred into a separatory funnel to which 50 mL n-hexane was added. The funnel was inverted and then shaken vigorously for a few seconds and the layers were allowed to separate. The upper layer was pipette out and the aqueous layer re-extracted twice, each time with 50 mL n-hexane. The collections of the upper layers was pooled and washed with distilled water until it was free of alkali. The extract was further dried with anhydrous sodium sulphate to remove any water residue and the hexane residue was removed by evaporating in a warm water bath at 45 °C (AOAC, 1990; Ismail & Fun, 2003) [3, 23].

Riboflavin was extracted according to AOAC (1990) [3] method. 10 g sample was weighed, ground and transferred into a centrifuge tube and 17.5 mL of 0.1 M solution of tetraoxosulphate (VI) acid was added to it. The mixture was shaken vigorously for 1 minute and placed in boiling water for 30 minutes with occasional shaking at 10 minutes interval. The mixture was subsequently cooled in an ice bath before the addition of 2.5 mL amylase. After gentle mixing, the mixture was incubated at 55 °C for an hour in a shaking water bath. It was cooled and diluted to 25 mL with deionized water. The resulting mixture was centrifuged at 2500 rpm for 15 minutes at room temperature using a bench centrifuge. The supernatant was then filtered to obtain a clear extract (Tatsumi *et al.* 2002) [33].

2.3 Quantification of vitamins from the extracts

1 mL of the clear extract obtained was measured and diluted to 10 mL with distilled water and was titrated to a pink endpoint using 2, 6- DPIP indicator. The ascorbic acid content of the sample was estimated using the relationship;

$$\text{Milligram of ascorbic acid} = \frac{\text{mg of ascorbic acid}}{\text{ml of dye used for titration}} \times \frac{\text{Dilution of actor}}{\text{weight of sample}} \ldots (1)$$

β-carotene was quantified by addition of 1 mL chloroform to the hexane extract followed by the addition of 1 ml of antimony trichloride. The solution was then placed in the sample cell of a colorimeter and the absorbance measured 440 nm. β-carotene content was estimated using the Beer-Lambert law (Tee *et al.* 1996; Chaturvedi & Nagav, 2001) [34, 12].

Riboflavin was determined according to the method described by Gerald (2000) [15]. 1 mL glacial acetic acid was added to the clear extract followed by 0.5 mL of 3% $KMnO_4$ and was allowed to stand for 2 minutes. Furthermore, 0.5 mL of 3% H_2O_2 was added and the mixture was shaken vigorously. The absorbance reading was then taken at 440 nm using a colorimeter (WPA Colorwave CO7500), and the riboflavin content of the vegetable groups estimated using the Beer-Lambert law.

3. Results and Discussion

3.1 Amount of β-carotene, riboflavin and ascorbic acid in the fresh samples of studied vegetable groups

The results of the vitamin content of the fresh vegetable groups are presented in Tables 1 to 3. The amount of β-carotene in the vegetable groups was in the range of 1200 µg/100g in Irish potatoes to 17,310 µg/100g in water leaves. These amounts are far below the values of 45,490 µg/100g and 98,880 µg/100g reported by Park (1987) [31] for carrot and spinach but β-carotene content of fresh lettuce (14730 µg/100g) in the present study is in agreement with his finding of 10,600 µg/100g. Anjum *et al.* (2008) [2] had earlier reported β-carotene content ranging from 80 to 14,000 µg/100g which is in a fair agreement with the present study. Riboflavin in the vegetable groups was found present in the range of 3.80 µg/100g in pumpkins to 32.00 µg/100g in Irish potatoes.

These values are below similar research on riboflavin levels in leafy vegetables. Ismail & lain, 2003 [23] had earlier reported 0.26 and 0.22 mg/100g of riboflavin in organically and conventionally grown green vegetables. In a similar study by Caldwell & Enoch (2010) [11] on leafy vegetables, higher values in riboflavin content (0.1 - 0.3 mg/100g and 0.4 - 1.2 mg/100g) were reported for cultivated and non-cultivated leaves. The amount of ascorbic acid reported in the present study was in the range of 208 mg/100g to 347 mg/100g. The highest amount of this vitamin was found present in garden egg plant while the least occurred in Irish potatoes and onions. These range of ascorbic acid content are not in agreement with lower values reported for vegetable groups in similar studies. Watada (1987) [38] reported ascorbic acid content in the range of 16 to 77 mg/100g; 15.3 mg/100g and 124.8 mg/100g ascorbic acid have been reported by Ismail & Fun (2003) [23] for lettuce and Chinese mustard. Similarly, Alcran & Rehman reported ascorbic acid content of 8.5. 20.0, 24.3 and 30.0 mg/100g in fresh lettuce, potato, onions and cabbage while 41.0 mg/100g have been reported for fresh pumpkin (Bello & Fowoyo, 2014) [7]. Many factors which may influence the disparity in vitamin content of grown vegetables are; genetics, environment and post-harvest practices (Salunkhe & Desai, 1988) [32]. The differences could also be attributed to the analytical procedures employed in various studies.

3.2 Effect of heating duration on β-carotene, riboflavin and ascorbic acid

The effect of heating duration on the vitamin content of the studied vegetables is presented in Table 1. The percentage loss in β-carotene after 40 minutes of cooking was in the range of 21.3% in cabbage to 58.0% in Irish potatoes. The loss in riboflavin content of these vegetable groups was in the range of 21.43% in garden egg to 61.73% in water leaf. Furthermore, there was a greater loss of ascorbic acid content of the vegetables after 45 minutes of boiling in the range of 48.13% in garden egg to 82.21% in onions. The percentage loss of these vitamins was in the order; riboflavin< β-carotene<ascorbic acid in cabbage, lettuce and pumpkins while for onions, Irish potatoes and garden egg, the percentage loss of vitamins on boiling was in the order; riboflavin< β-carotene<ascorbic acid. However, water leaf had a different trend in the vitamin loss on boiling thus; p-carotene<ascorbic acid<riboflavin.

Studies indicate that boiling of food products has been implicated in the leaching of water-soluble vitamins into the water medium (Barrera, 2014) [6]. The water solubility of the vitamins analysed is in the order ascorbic acid>riboflavin>β-carotene- a precursor of vitamin A. Loss of vitamins can be induced by a number of factors such as cooking time, temperature and cooking method although some vitamins are heat-stable, others are heat labile (Tu. 1999) [36]. The heat labile nature of ascorbic acid might account for its predominant loss during boiling aside its solubility in water. However, β-carotene is relatively stable to heat while riboflavin is not susceptible to losses on heat but exposure to light in solution (Tu, 1999) [36]. Hachcti *et al.* (2002) had reported that the loss of β-carotene during processing is attributed to the changes that occur in its geometric isomers with the trans-form changing to the cis-form that is not biologically active.

Several studies have earlier reported varying percentage losses in vitamin during different cooking methods. Percentage loss in β-carotene in levels as high as 47-53%, 30% and 34.9% in lettuce, cabbage and artichoke which is in agreement with the findings in this research (21-58 5%) have been reported (Jane *et al* 1998; Anjum *et al*. 2008 [24, 2] and El Sohaimy *et al* 2013) [14]. After dehydration, the losses in β-carotene content of fluted pumpkins where reported in the ranges of 37.6 to 48.8%, 40.5 to 51.3% and 68.8-72.0% for steam-blanched, water-blanched and unblanched leaves respectively (Badifu *et al*. 1995). However, these findings disagree with the report of Granado *et al.* (1992) [18]; Han and Xu (2014) [20] where there was rather an increase in β-carotene content of different vegetable groups after boiling. This is however justified by the earlier submission of factors that could lead to these variations in vitamin content of vegetables. Osinboyejo *et al.* (2003) observed 100% loss in riboflavin, folic acid and thiamine including a 99% loss in ascorbic acid content of turnip greens during boiling water blanching. Varying degrees of percentage loss of ascorbic acid upon boiling have been documented which are in agreement with the present study. Ascorbic acid losses in boiled vegetables in the ranges of 42 to 85% and 33% have been reported (Agbemafle et al 2012; Yuan *et al.* 2009) [1, 39]. Bello and Fowoyo (2014) [7] reported a progressive loss of ascorbic acid of dark leafy vegetables and citrus on prolonged heating and an increase in heating temperature. A similar trend for the loss of ascorbic acid content of vegetables upon boiling was observed for vegetable groups in Pakistan (Waheed-Uz-Zaman *et al*. 2013) [37].

Table 1: β-Carotene (vitamin A), Riboflavin (vitamin B) in µg/l00g and Ascorbic acid (Vitamin C) in mg/100g content of fresh and boiled samples of vegetable groups

Sample	Vitamin con. in fresh samples			Vitamin conc. in samples at varied heating time			Loss (%)		
	A	B	C	A (40 mins.)	B (40 mins)	C (45 mins)	A	B	C
Cabbage	2,770.00	30.00	278.00	21,80.00	19.00	69.00	21.30	36.67	75.18
Lettuce	14,730.00	7.60	342.00	8,661.00	3.20	69.00	41.20	57.89	79.82
Pumpkin	17,310.00	3.80	342.00	7,314.00	2.00	139.00	41.02	47.37	59.36
Water leaf	12,400.00	8.10	331.00	10,729.00	3.10	107.00	38.02	61.73	49.55
Onions	2,370.00	130.00	208.00	1,185.00	74.00	39.00	50.21	43.08	82.21
Potato	1,200.00	32.00	208.00	504.00	23.00	69.00	58.00	28.13	66.83
Garden egg	4,694.00	28.00	347.00	2, 494.00	22.00	180.00	46.02	21.43	48.13

3.3. Effect of refrigeration on the β-carotene and ascorbic acid content of samples

This effect is shown in Table 2 and 3 below. After three days of refrigeration, there was no loss in β-carotene content of cabbage and potatoes as well as ascorbic acid content of onions. However, for the other vegetables, the percentage loss in their β-carotene content ranged from 2.5% (in garden eggs) to 36.70% (in lettuce). The percentage loss of ascorbic acid was in the range of 13.7 to 79.83% with cabbage and lettuce accounting for the least and greatest losses in each case. Furthermore, after the freezing time was increased to 1 week, there was a corresponding increase in the depletion of vitamins in the studied vegetable samples. There was a 100% loss of both β-carotene and ascorbic acid in lettuce, cabbage, pumpkins, onions, potatoes and garden egg recorded a <5% loss in their β-carotene content after one week of refrigeration. However, water leaf lost its β-carotene content above 20%. On the other hand, the loss in ascorbic acid content of the vegetable was greater in comparison with β-carotene. The loss was >60% in Irish potato, water leaves, pumpkins and garden egg while cabbage and onions recorded losses above 20%.

Table 2: β-Carotene (µg/100g) and Ascorbic Acid (mg/100g) content for fresh and refrigerated samples of vegetable groups (3 days of refrigeration time)

Sample	Concentration of vitamins in fresh samples		Concentration of vitamins in samples after refrigeration for 3 days		Loss of vitamins (%)	
	A	C	A	C	A	C
Cabbage	2,770.00	278.00	2,770.00	240.00	0.00	13.70
Lettuce	14,730.00	342.00	9,324.00	69.00	36.70	79.82
Pumpkin	17,310.00	342.00	16,773.00	208.00	3.10	39.18
Water leaf	12,400.00	331.00	11,258.00	139.00	9.21	58.01
Onions	2,370.00	208.00	2,304.00	208.00	2.78	0.00
Potato	1,200.00	208.00	1,200.00	139.00	0.00	33.17
Garden egg	4,620.00	347.00	4,504.00	208.00	2.51	40.08

Table 3: β-Carotene (µg/100g) and Ascorbic Acid (µg/100g) content for fresh and frozen samples of vegetable groups (7 days refrigeration time)

Sample	Concentration of vitamins in fresh samples		Concentration of vitamins in samples after refrigeration for 7 days		Loss of vitamins (%)	
	A	C	A	C	A	C
Cabbage	2,770.00	278.00	2,706.00	208.00	2.31	25.18
Lettuce	14,730.00	342.00	0.00	0.00	100.00	100.00
Pumpkin	17,310.00	342.00	16,547.00	69.00	4.41	79.82
Water leaf	12,400.00	331.00	9.536.00	69.00	23.10	79.13
Onions	2,370.00	208.00	2,249.00	139.00	5.26	33.17
Potato	1,200.00	208.00	1,170.00	69.00	2.50	66.83
Garden egg	4,620.00	347.00	4,449.00	69.00	3.70	10.01

The results are consistent with the values of 5.02 to 25% losses of carotenoids reported for leafy vegetables (Oulia *et al.* 2015) and the β-carotene range of 2.0 to 25.7% reported for refrigerated fluted pumpkin (Badifu *et al.* 1995). The decline in the β-carotene content of the vegetables may be as a result of degradation of pigments during refrigeration (Gareth, 1998). Earlier studies reported that loss of β-carotene during storage could emanate from oxidative and non-oxidative changes on exposure to light and oxygen (Arunal *et al* 1999; Dutta *et al.* 2005) [13]. The ease with which ascorbic acid oxidizes and enzymatic degradation as well as environmental stresses encountered before harvest could be responsible for its gradual decrease during refrigeration (Howard et al 1999; Oulia *et al.* 2015) [21]. Values of ascorbic acid losses upon refrigeration to the tune of 70% have been reported after one week of storage time (Howard *et al.* 1999) [21] which is in agreement with values in the present study. However, the ranges 3.56 to 25% and 11.43 to 44.68% stated by Oulia *et al.* (2015) for percentage losses in ascorbic acid after a refrigeration time of 5 and 15 days are lower than some values reported in the present study. The difference in values could be attributed to the differences in assay methods, storage temperatures and duration of storage. Granado *et al.* (1997) [18] have clearly stated that HPLC methods are more efficient than old spectroscopic methods in food analysis.

4. Conclusion

The results of the analysis confirms the general notion that fruits and vegetables are best served fresh or with minimal treatment. All of the vitamins were at their highest concentrations in fresh samples even though they tend to be influenced by similar treatment of heat and refrigeration to different degrees. Ascorbic acid in onions was lost the most on application of heat compared to the other vegetables, and amount of ascorbic acid lost was least in garden eggs. On the flip side, of all the vitamins, β-carotene appears to be the most stable to heat given its percentage loss on heating. Refrigeration also results in the loss of the vitamins, although to a lesser degree compared to heating, and the longer the duration of refrigeration, the greater the amount of vitamins lost.

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