EFFECT OF DIFFERENT PROCESSING CONDITIONS ON THE CAROTENE CONTENT OF DIFFERENT VEGETABLES

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Abstract
Benefits of beta (β)-carotene are well known, most important being the pro vitamin A activity but experimental evidence are lacking regarding the effect of processing on β-carotene. Processing is critical in determining the bioavailability of β-carotene from foods and there is a perception that β-carotene is destroyed by the heat process involved in cooking of vegetables. This study is intended to determine the stability of β-carotene when selected vegetables are subjected to various processing techniques. Carrot and spinach were chosen for this study since they are the two most important sources. β- Carotene was quantified from vegetables by Reversed phased High Performance Liquid Chromatography (HPLC) System. The result indicated the control sample reading of carrot and spinach were 16833µg/100g and 19383µg/100g, the β-carotene level increased substantially in all the processing conditions. Tray drying proved to be the best processing technique with highest percentage gain. Among the samples, Spinach was found to be the richest source of β-carotene.

Keywords: beta (β)-carotene, vitamin A, Reversed phased High Performance Liquid Chromatography (HPLC), processing, Tray drying

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INTRODUCTION

Carotenoids are pigments that can be found in higher plants, algae, fungus, bacteria, and animals like birds and crustaceans. Because plants and microorganisms can only synthesize carotenoids, their presence in animals is due to ingestion via food and accumulation in specific tissues, such as flamingo feathers, egg yolk, and invertebrate exoskeletons. They are found in subcellular organelles in plants i.e. chloroplasts and chromoplasts. Carotenoids are deposited in crystalline form (e.g., in carrot roots and tomato) or as oily droplets (e.g., in mango and paprika) in chloroplasts and serve as accessory pigments in photosynthesis, photoprotective pigments, and membrane stabilizers, whereas in chromoplasts, they are deposited in crystalline form (e.g., in carrot roots and tomato) or as oily droplets (e.g., in mango and paprika). (*Andreas Schieber* and Reinhold Carle*,2005)

Carotenoids are an essential source of colors and vitamins, and researchers are interested in finding efficient ways to produce them. Both plants and microorganisms are utilized as raw resources in the technological process. However, chemically synthesized carotenoids created in the 1950s were the primary source of carotenoid colors for a long time.

Carotenoids are derived from the leaves, flowers, fruits, seeds, roots, and tubers of plants. They are present in vegetables like carrots, pumpkins, spinach, tomatoes, and fruits like watermelon and raspberries. Carotenoids are significant for their metabolism as provitamin A, with **α** and β-carotene being the most abundant in plant tissues and having the highest activity (beta carotene has a biopotency of 100 percent while **α**-carotene has a biopotency of 53 percent). Human plasma contains oxygenated carotenoids (xanthophylls), which lack vitamin A activity in general, but their exact function, if any, is not defined.

β-Carotene is a carotenoid compound found in abundance in the human diet and, as a result, in all human tissues, including blood. It is also frequently utilized in medicine due to its potent bioactivity. Among the many roles of β-carotene in the human body, the most significant is provitamin A supply, affecting embryonic development, proper growth, and vision. β-

Carotene is used as an orange-red color in many products in the food industry, including non-thermally treated non-alcoholic beverages with a tropical fruit taste, edible fats, cheese, pastry, and ice cream. It acts as a gene inhibitor, as well as having anticancer and antioxidant effects. Orange carrots are the most common source of β-carotene. (*Food Quality and Safety*, Volume 2, Issue 2, May 2018)

Mamatha et al. (2010) have shown that when vegetables are subjected to various heat treatments before consuming, factors such as heat, light, chemical treatments, and oxygen exposure may have detrimental effect on several bioactive constituents. Thermal processing affected trans–cis-isomerization of b-carotene in carrot juice produced on a pilot plant and b-carotene-containing preparations. While pasteurization and sterilization at 121 °C. only resulted in minor isomerization, sterilization at 130 °C. and blanching resulted in higher quantities of cis-isomers. (*Marx, M*.2003).

The results of the present study were in accordance with the above studies and statements, as it was seen that β-carotene level increased substantially in all the processing conditions. Heating the food concentrates it and increases β-carotene but heating and overcooking for very long time, say over 30 minutes or an hour, decreases beta carotene. A growing body of literature exists on the benefits of β-carotene but experimental evidences are lacking on the effect of processing on β-carotene. Thus, the objective of this study is to determine the gain or loss of beta carotene when subjected to different processing conditions such as, blanching, open pan boiling, microwave cooking, pressure cooking and tray drying using high performance liquid chromatography (HPLC) system.

MATERIAL AND METHODS

β-Carotene was quantified from vegetables by Reversed phased HPLC System (LC- 10 A, Shimadzu, Kyoto, Japan) by the method of Khalil and Varananis (1996). HPLC is a rapid, efficient and sensitive technique for β-carotene analysis. HPLC is distinguished from traditional liquid chromatography because operational pressures are significantly higher, up to 350 bar. For extraction, Tetrahydrofuran (THF) was used in place of acetone for a highly efficient separation and analysis.

Sample collection

β-carotene determination was carried out in two common vegetables, carrot and spinach, grown in agro ecological conditions of Delhi. These vegetables were purchased from nearby Reliance Fresh store. One kg of each carrot and spinach were purchased, and from that 12 samples of 100gm were prepared (6 of carrot and 6 of spinach). The pre analyzed samples were washed with running tap water and rinsed with deionized water and kept in inert condition, at -4°C temperature (Khalil and Varananis, 1996). The carrot samples were grated and Spinach samples were sorted and cut into small pieces and then the samples were mixed well.

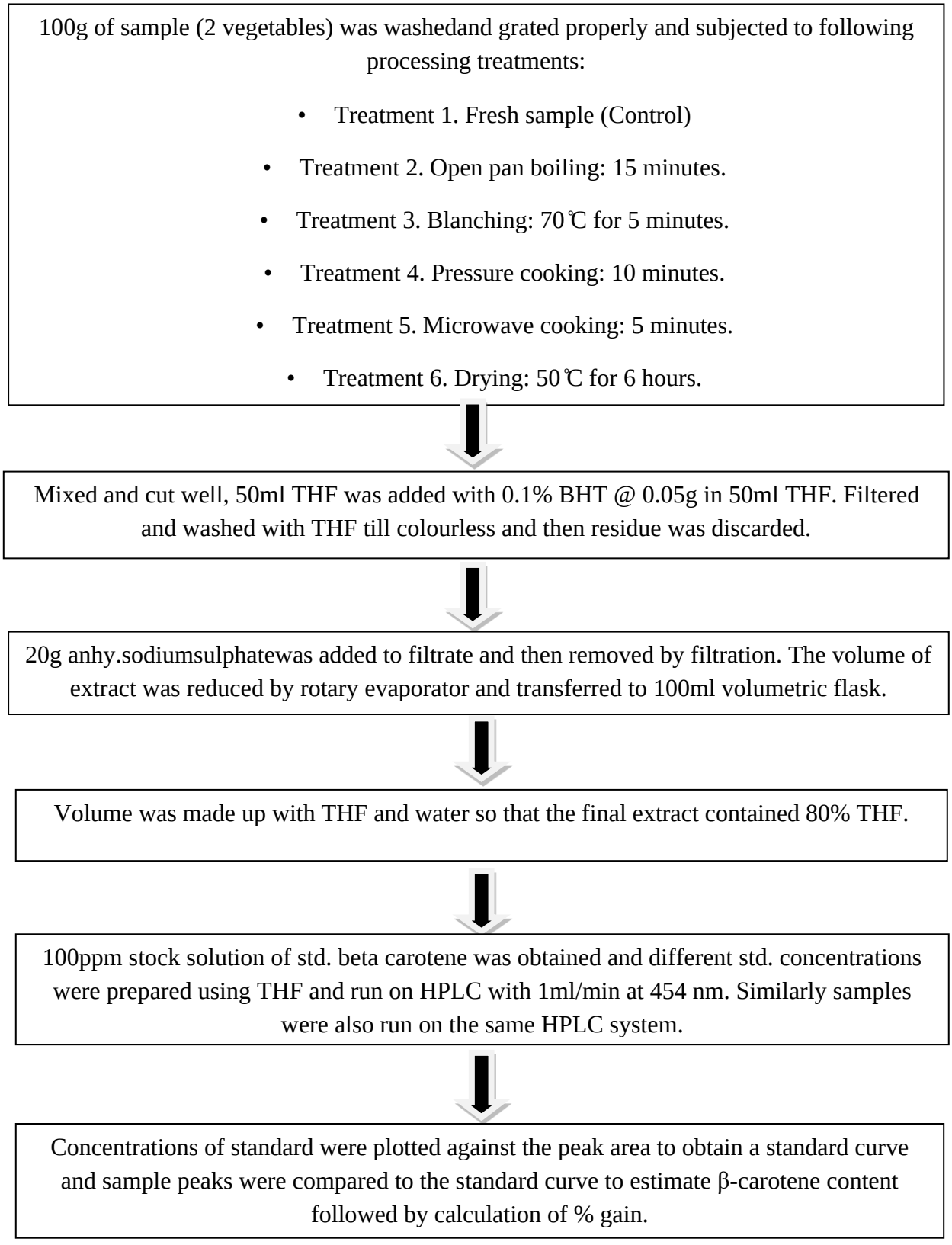
Extraction of sample

Carotene was extracted from vegetables by reversed phased HPLC system by the method of Khalil and Varananis, 1996. To determine the effect of processing, for both the vegetables, one sample of control was taken and five samples (10g) of each were subjected to five processing conditions:

A-blanching (70° C/5 minutes)
B-open pan boiling (15 minutes)
C-microwave cooking (5 minutes)
D-pressure cooking (10 minutes)
E-tray drying (60° C/5 hours).

Sample from each processing treatment was grinded by the use of mixer to convert it into pureed extract. This extract was then homogenized in 50 mL of THF and then 0.1% Butyrated Hydroxyl Toluene (BHT) was added as an anti oxidant at the rate of 0.05 g into 50 ml of THF. The resulting extract was filtered through Buchnar's funnel. The residue was washed twice with THF till it became colourless. About 20 g anhydrous sodium sulphate (a hygroscopic material) was added, and then removed through filtration. Volume of extract was reduced on rotary evaporator. The sample was further washed with 10 mL of THF

and refiltered. Filtration was repeated thrice to confirm purity. The extract was transferred to 100 mL volumetric flask and the volume was made up to the mark with THF and water, so that the final extract contained 80% of THF.



Standard preparation of β-Carotene solution
A 100 ppm stock solution of standard β-carotene was taken. From the 100 ppm stock solution, different concentrations of 10, 5, 3, 2 and 1 ppm standard β-carotene were prepared with volume made up by THF. The standard solutions were run on HPLC system by isocratic elution. The concentrations of the standards were plotted against the peak area to obtain a standard curve.

HPLC Analysis of β-Carotene
HPLC system was equipped with photodiode array (PDA) detector (Serial Presence Detect- M 20A, Shimadzu). β-carotene were separated on a C-18 column (25 cm– 4.6 mm i.d., 5-µm particle size) with 1 mL/min. flow rate. Two mobile phases were prepared. For the first one, acetonitrile (ACN) and water were taken in the ratio of 9:1 and the second was composed of ethylacetate. The pressure of the column was kept at 1800-2000 psi. All the carotenoids were monitored at 450 nm with PDA detector (Shimadzu, Japan). β-carotene was quantified from the peak area using the standard curve. The peak identity and λ_{max} of β-carotene was further confirmed by retention time and characteristic spectra. The beta-carotene peaks were integrated by the computer software to quantify the content of beta-carotene. The sample peaks were compared to the standards for estimation of the beta-carotene concentration.

Calculation
After obtaining the estimated concentration, β-carotene was estimated in µg/100g.

Concentration in µg/100g =

$$\frac{\text{Estd conc.} \times \text{vol.made up} \times 1000}{\text{Weight of sample}}$$

And then percentage gain was calculated using the formula:

Percentage gain (%) =

$$\frac{(\text{Sample Value} - \text{Control Value}) \times 100}{\text{Control Value}}$$

RESULTS AND DISCUSSION

The present study was conducted to determine the stability of β- carotene when vegetables, carrot and spinach, were subjected to different processing conditions. For both vegetables, one sample of control was taken and five samples (10g) of each were subjected to five processing conditions: A-blanching (70° C/5 minutes), B-open pan boiling (15 minutes), C-microwave cooking (5 minutes), D-pressure cooking (10 minutes) and E-tray drying (60° C/5 hours). β-Carotene was quantified from vegetables by Reversed phased HPLC System with flow rate of 1ml/min at 454 nm followed by calculation of percentage gain/loss.

Table 1. Final concentration and percentage gain for control and processed samples of carrot.

S.NO.	Sample	Estd Conc.	Vol. Make Up (ml)	Weight (G)	Final Conc. (µg/100g)	Percentage gain (%)
1.	Carrot Control	1.863	50	5.5338	16832.92	
2.	Carrot Blanched	3.309	50	5.1461	32150.56	91%
3.	Carrot Open Pan Boiled	1.712	50	5.0072	17095.38	1.5%
4.	Carrot Microwave Cooked	1.759	50	5.0110	17551.39	4%
5.	Carrot Pressure Cooked	2.663	50	5.2421	25400.13	51%
6.	Carrot Tray Dryed	19.441	50	5.0598	192112.34	1041%

Table 2. Final concentration and percentage gain for control and processed sample of spinach.

S.NO.	*SAMPLE*	*ESTD CONC.*	*VOL. MAKE UP (ml)*	*WEIGHT (g)*	*FINAL CONC. (µg/100g)*	*PERCENTAGE GAIN (%)*
1	*SPINACH CONTROL*	*2.317*	*50*	*5.9769*	*19383.96*	
2.	*SPINACH BLANCHED*	*3.152*	*50*	*5.3606*	*29399.69*	*51%*
3.	*SPINACH OPEN PAN BOILED*	*3.768*	*50*	*5.2807*	*35677.09*	*84%*
4.	*SPINACH MICROWAVE COOKED*	*4.037*	*50*	*5.1843*	*38934.86*	*100%*
5.	*SPINACH PRESSURE COOKED*	*3.098*	*50*	*5.1738*	*29939.31*	*54%*
6.	*SPINACH TRAY DRIED*	*23.704*	*50*	*5.1447*	*285955.56*	*1375%*

The carotenoid content of the control sample were carrot and spinach were 16833µg/100g and 19383µg/100g, respectively. The β-carotene level increased substantially in all the processing conditions. In carrots, percentage gain for A, B, C, D, E came out to be 91%, 1.5%, 4%, 51% and 1041%, respectively. In spinach, percentage gain was 52%, 84%, 100%, 55% and 1375%, respectively.

The results for the five processing conditions have been summed up in Table-1 and 2 and Figure 1 and 2, respectively.

CARROT

The β-carotene content of control sample of carrot was 16832.82 µg/100g. The β-carotene of carrot was in fair agreement with the result reported by Ahmad et al. (2010) in which beta carotene was 14000 µg/100g. The β-carotene content increased significantly in all the five processing conditions. In tray drying, the percentage gain was maximum1375 %. This was due to absolute removal of water and concentration of the sample thus the powder form being the richest β-carotene concentrate. In blanching, the content increased to 91%. Blanching being a mild heat treatment proved to the best cooking method followed by pressure cooking, 51%. High temperature treatments such as Open pan boiling and Microwave cooking had relatively very low increase of 1.5% and 4%, respectively. This was because carrot does not have moisture content as high as spinach so high temperature after certain time leads to losses in β- carotene. Ahmad et al. (2010) have reported that boiling for one hour of carrots resulted in approximately 50% loss. In this study, boiling (15 minutes) and microwave cooking (5 minutes) were both done for short intervals thus no decrease was seen but the increase was quite small. Hence, we can say high temperatures after a short while leads to losses thus cooking should to be optimized to prevent losses. Another study suggest (*Nauman*,2007) that carrot that contain maximum amount of beta carotene (11210 µg/100g). It appeared that their sample of carrot may have higher moisture content as compared to those analyzed in the present study and the variation in tomato content of β-carotene may be due to the use of immature samples, because content of beta carotene drops by 77% during the ripening process *(Rigo et al*. 1999).

SPINACH

The control sample reading of Spinach was 19382.96 µg/100g. This was different than the content reported by Ahmad et al. (2010), 9000 µg/100g. This was maybe due to variation in variety, area, season, soil, environmental conditions etc. In spinach also, all the five processing conditions showed substantial increase with tray drying showing the maximum gain of 1375%. In Spinach, in a slight contrast

to carrot, treatments that required very high temperatures such as microwave cooking and open pan boiling had a greater increase of 100% and 84%, respectively as compared to blanching, 52% and pressure cooking 55%. The reason for this contrast was maybe that spinach has a very high moisture content of about 95% so since the moisture content was high the concentration was greater in the case of spinach at very high temperatures and thus the high percentage gains. From another data (*Nauman*,2007) it was evident that dark green vegetables contained more β-carotene as compared to other vegetables e.g. spinach contained 9940 µg/100g, followed by mint, kulfa, lettuce and lady finger that were all dark green in appearance. Agte et al. (2000), who analyzed 24 green vegetables for different micro nutrients contents including beta carotene. They reported that beta carotene content in green vegetables ranges from 80-9204 µg/100gmThe above results were supported by Bioavailability of Carotene is Lower in raw than in Processed Carrots and Spinach in Women (Cheryl L).

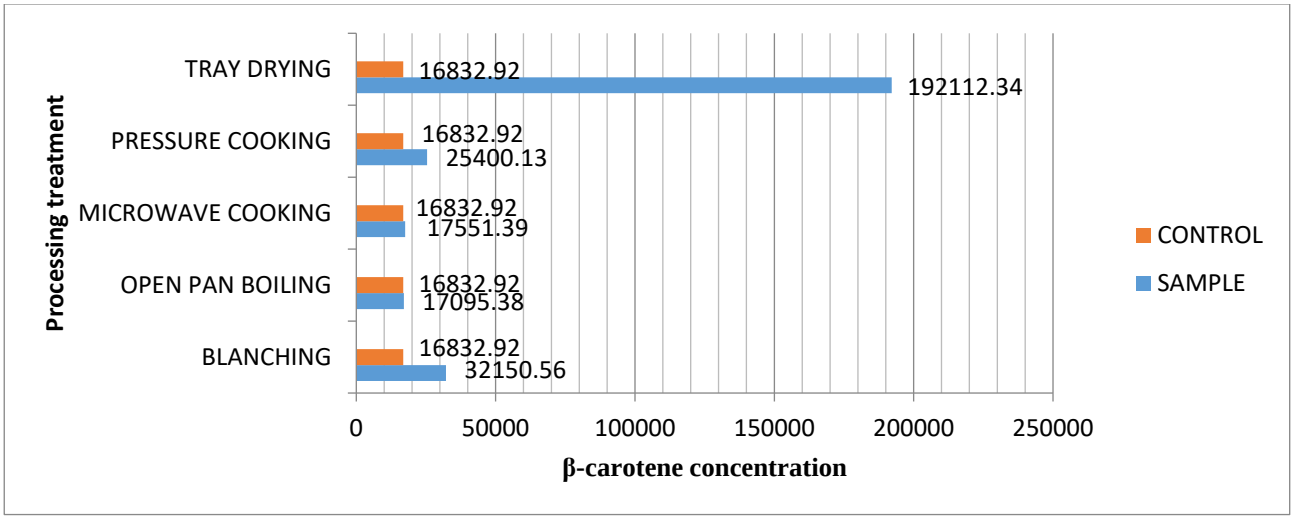


Figure 1: Effect of different processing conditions on the β-carotene content in carrot

(The above figure depicts the concentration (µg/100g) obtained when carrot was subjected to different processing conditions in comparison to the control sample)

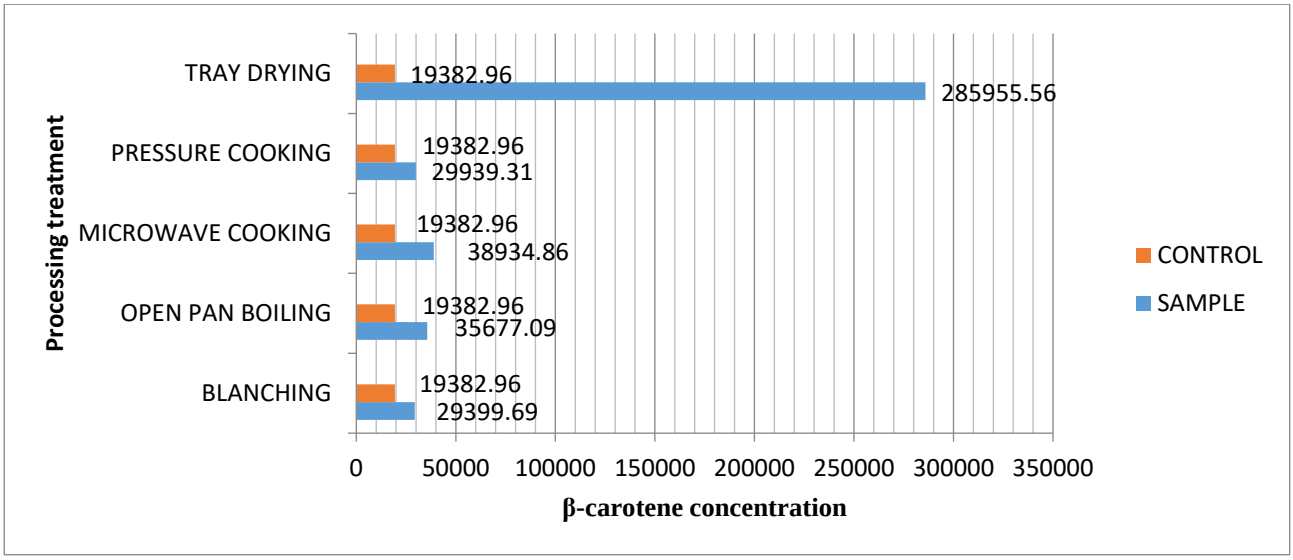


Figure 2: Effect of different processing conditions on the β-carotene content in Spinach

(The above figure depicts the concentration (µg/100g) obtained when spinach was subjected to different processing conditions in comparison to the control sample)

Heat treatment of vegetables promotes conversion of the trans isomer of β-carotene to cis forms, which is biologically inactive. As a point of practical application, the thermal processing of spinach and carrot did not negate the enhanced bioavailability of β-carotene, relative to raw vegetables. In fact, it was seen, carotene loss was minimal with moderate cooking, and in many cases, β- carotene become more bio available after cooking, probably because heat processing liberates them from cell matrices. Another reason for the retention of carotenoids may be the inactivation or reduction in the activity of peroxidase and lipoxygenase that are involved in carotenoid destruction. Thus, we can say, heating foods that contain beta-carotene doesn't destroy the beta-carotene. In fact, it makes it more available by breaking down the walls of the plant cells that contain beta-carotene. This is why, high percentage gains were noted in all the five processing conditions.

CONCLUSION

There is a perception that β-carotene is destroyed by the heat process involved in cooking of vegetables. In fact, carotenoid loss is minimal with moderate cooking, and in many cases, β-carotene become more bio available after cooking, probably because heat processing liberates them from cell matrices. Another reason for the retention of carotenoids may be the inactivation or reduction in the activity of peroxidase and lipoxygenase that are involved in carotenoid destruction. As a point of practical application, the thermal processing of spinach and carrot did not negate the enhanced bioavailability of β-carotene, relative to raw vegetables Thus, we can say, heating foods that contain beta-carotene doesn't destroy the beta-carotene. In fact, it makes it more available by breaking down the walls of the plant cells that contain beta-carotene.

The salient findings and conclusions emerging from this study are as follows:

Tray drying emerged as the best processing condition with a huge percentage gain of 1375% in spinach and 1041% in carrot. Blanching and pressure cooking proved to better cooking conditions in carrot while very high temperature treatments like open pan boiling and microwave cooking proved to be better in spinach owing it to it's very high moisture content. In carrot, the percentage gain for blanching and pressure cooking was 91% and 51%, respectively but for microwave cooking and open pan boiling was 4% and 1.5%. In contrast, spinach readings for blanching and pressure cooking were 51% and 54%, respectively but for microwave cooking and open pan boiling were very high, 100% and 84%. It was noted that beta carotene increased to high amounts for temperatures for short time. If subjected to continuous high temperature treatment for long intervals, losses may occur up to 50%. The widely held belief that fresh produce is nutritionally superior to commercially processed produce is not valid with regard to β- carotene because the bioavailability of β- carotene was enhanced as a result of food processing.

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