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PROCESSING EFFECT OF DIVERSE COOKING METHODS ON RETENTION OF -CAROTENE ALL TRANS AND CIS ISOMERS IN GREEN LEAFY VEGETABLES-INDIA

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The present study focuses on carotenoids all Trans and Cis isomers attendance in different Green Leafy Vegetables (GLV) and their retention using domestic processing methods. Carotenoids and their geometric isomers play an important role for the conversion of vitamin A protecting cells from night blindness, oxidation and cellular damages. GLV are pigment-rich and nutritionally significant functional food sources with unique phytochemical constituents that include carotenoids and their isomers which are precursors for vitamin A. Cooking processes and other factors such as temperature, light and alteration in moisture content generally promote either isomerization (Trans to Cis form) or oxidative degradation of carotenoids to epoxides. Studies pertaining to the effect of cooking methods on dietary carotenoids isomers and their retention percent using processing methods are insufficient, particularly in an Indian competition. The objective of the study was to determine the carotenoids all Trans and Cis isomers using different culinary practices and compute the retention percentages in GLV such as amaranth (Amaranthus gangeticus), spinach (Spinacia oleracea), fenugreek leaves (Trigonella foenum-graecum) and curry leaves (Murraya koenigii), when subjected to domestic cooking methods of microwave cooking, sautéing, pressure cooking, steaming and deep frying in oil, for a time duration of 8 and 12 minutes either with lid closed or open. The determination of carotenoids isomers and retention percentage were quantified by ultra high performance liquid chromatography (UHPLC) using vydac column (RP-C-18) with 100% methanol for first 5 minutes and methanol: chloroform (96:4) for the subsequent run as gradient mobile phase. The maximum retention percentage of β-carotene all Tran's isomers were observed with sautéing, micro wave cooking and steaming methods. The minimum retention was found in cooking method without lid in all the GLVs. (Spinach: 80.77% and 66.43%, Amaranth: 77.57% and 67.41%, Fenugreek leave: 82.60% and 68.81% and Curry leave: 66.74% and 58.23% respectively). The percentage of retention in the contents of carotenes all Tran's isomers in GLVs in correlation to various cooking methods are discussed which would be valuable for food researchers, nutritionists, health practitioners and dietitians to develop and promote the nutritionally balanced diets towards minimize vitamin A deficiency.

Keywords: Green leafy vegetables, Vitamin A, Carotenoids, All tran's isomers, Processing methods

INTRODUCTION

Micronutrient malnutrition (MNM) is widespread in the developing nations, but even more so in the regions of Asian and African continents of the world. It can affect all age groups, but young children and women of reproductive age tend to be among those most at risk of developing

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micronutrient deficiencies. They not only cause specific diseases, but they act as exacerbating factors in infectious and chronic diseases, greatly impacting morbidity, mortality, and quality of life (Anjali Verma, 2015). Worldwide the three most common forms of MNM are iron, vitamin A and iodine deficiency. It is estimated that over two billion people are anemic, just under 2 billion have inadequate iodine nutrition and 254 million preschool-aged children are vitamin A deficient (1-3 year). It has been anticipated that micronutrient deficiencies account for about 7.3% of the global burden of disease with vitamin A and iron deficiency ranking among the 15 leading causes of the global disease burden (World Health Report, 2002). According to National Nutrition Monitoring Bureau, National Institute of Nutrition (NNMB, NIN), 0.6% preschool children have bitot spot and 0.2% has night blindness (NNMB, 2006). Among the micronutrient malnutrition, vitamin A deficiency (defined by a plasma retinol concentration < 0.70 μmol/L) is a public health issue in numerous countries and affects an estimated 190 million preschool children and 19.1 million pregnant women around the world (WHO, 2002). Though India ranks second in the production of GLV next to China, immobile the night blindness and bitot spots crisis has been long-lasting in the nation.

Studies have shown that GLV, fruits and further vegetables have been cited as a latent source of micronutrients, predominantly carotenoids that can be absorbed and converted to vitamin A in the human body (MDIS, 1995; and WHO, 2009). Epidemiological studies designate that improved intake of GLV and vegetables are connected with decreased risk towards certain cancers, cardiovascular disease, cataract, macular degeneration, and other age-related ailments (Thurnham, 2007). In addition to serving as a critical source of micronutrients, leafy vegetables are also rich in many carotenoids including β-carotene (West *et al.*, 1998; and Stahl and Sies, 2005). Further 700 carotenoids have been notorious in nature. The largest part studied comprise lutein, zeaxanthin, lycopene, β-carotene, α-carotene, and β-cryptoxanthin (Sommerburg *et al.*, 1998). In addition the well-known provitamin A activity of some carotenoids (Mozaffarieh *et al.*, 2003), they also function as antioxidants and enhancers of the immune response, and as such are associated with lowering the risk of developing degenerative diseases (Block *et al.*, 1992; and Calvo, 2005).

Naturally, majority of the carotenoids occur as *trans*-isomer in plants. However, *cis*-isomers may amplify due to the isomerization of the *trans*-isomer of carotenoids during food processing (Schieber and Carle, 2005). Many studies have concerned in the analysis of dietary carotenoids and their potential isomers (Zepka and Mercadante, 2009; Mertz *et al.*, 2010; and Shi *et al.*, 2010), with much attention given to the geometric isomerization of carotenoids (Liu and Asato, 1985; Britton, 1998; Vasquez-Caicedo *et al.*, 2005; Aman and Schieber, 2005; Niedzwiedzki *et al.*, 2009; and Qiu *et al.*, 2009). The investigation of carotenoid contents in fresh, frozen and canned foods has been carried out (Rickman, 2007). However, a recent review on contents of carotenoids and their isomers from diverse GLV, fruits and vegetables has not been properly documented in Indian foods. Studies revealed that carotenoids isomers are also playing important role interns of vitamin A conversion in human. It indicates β-carotene is broken down by β-carotene dioxygenase in the mucosa of small intestine into two retinyl molecules, which is later reduced to vitamin A (retinol) (During, 2001). Naturally β-carotene is habitually found as all-*trans* isomers and lesser as *cis*-isomers with the relative abundances in the following order: all-*trans* > 9-*cis* > 13-*cis* > 15-*cis* (Guo, 2008). All-*trans*-β-carotene is very unstable and can be easily isomerizes into *cis*-isomers, when exposed to heat and light in processing methods. The retention of these molecules after processing using different cooking methods has not been documented in previous studies.

The other end in lower income countries about 80% of vitamin A must be obtained from a variety of carotenoids in plant foods (Fawzi *et al.*, 1993; and Giovannucci *et al.*, 1995). Recent studies publicized that Provitamin A carotenoids bioconversion in the body is evaluated to be 12 μg β-carotene: 1 μg vitamin A (retinol activity equivalent) with much lower values obtained when consumed with oil (Kohlmeier and Hastings, 1995). Improving vegetable processing and preparation that allow optimal retention of vitamin A activity (Mayne, 1996; and Santos *et al.*, 1996) is important to facilitate consumers and processors to choose the processing and storage conditions that favor retention of carotenoids isomers for maximum health benefits in order to overcome Vitamin A under nutrition. In Indian culinary practices, commonly GLV are cooked at home as per convenience and taste predilection, rather than on nutrient importance. Researchers have reported that 5 to 78% of β carotene degraded when vegetables were prepared using diverse cooking methods (Rahman, 1990; Speek, 1998; and Vimala *et al.*, 2011). Substantial quantities of carotenoids needed by individuals may be lost during household cooking of GLV and vegetables (Masrizal, 1997).

Apart from reduced provitamin A activities, *Trans-cis* isomerization also affects bioavailability and antioxidant ability of carotenoids. However, it must be distinguished that carotenoid geometry is only one factor that determines their bioavailability and that the food matrix, intracellular location, physicochemical characteristics, interaction between carotenoids, presence of dietary fat, and processing in particular mechanical disruption and heat treatment possibly will enhance carotenoid bioavailability (Castenmiller and West, 1998; Van het Hof *et al.*, 2000; and Yeum and Russell, 2002). Thus the information on the potential losses of carotenoids from GLV and vegetables during traditional cooking methods is of foremost importance (19 Gayathri *et al.*, 2004). Several reports have documented the losses of β carotene after boiling, frying, bleaching, and pressure cooking (Yadav and Sehgal, 1995; and Vimala *et al.*, 2011). However most of the reported data are on raw foods, although many are ingested after cooking. The best resources of obtaining data on cooked or processed foods are in the course of laboratory analysis. It is essential to determine the percentage of labile nutrients and other health promoting food components that is retained or lost during cooking practices and ascertain the factors that enhance or reduce degradation (De Sa and Rodriguez-Amaya, 2004). Hence this will afford guidelines for a healthy diet preparation that did not deplete nutrient content of vegetables (Leskova *et al.*, 2006). Indirectly this also contributes in recommending healthy cooking methods to the general public. Several factors affect the alteration and withholding of all Trans carotenoids to cis carotenoids from foods (Castenmiller and West, 1998). Among these, the matrix in which the carotenoids are embedded plays a crucial role.

GLV such as spinach, amaranth, curry leaves and fenugreek leaves are rich sources of carotenoids including â-carotene and are grown and frenzied in India. Recently there has been an mounting attention in assessing the conversions of all Tran’s isomers to cis isomers and their retention using domestic processing methods from GLV and fruits because of the multi-dimensional health benefits associated with consumption (Schieber and Carle, 2005). Information on the conversion of β carotene Trans and cis isomers to vitamin A from GLV is still inadequate in Indian scenario. Thus, due to the positive health effects of GLV in managing vitamin A deficiency, the present research outcome emphasizes on understanding to quantify the all Trans and cis β carotene isomers from GLV and to ensure the effect of diverse cooking practices on their retention.

MATERIALS AND METHODS

Materials

Four varieties of green leafy vegetables were selected based on popular consumption by Indians. The GLV were amaranth (*Amaranthus gangeticus*), spinach (*Spinacia oleracea*), fenugreek leaves (*Trigonella foenum-graecum*) and curry leaves (*Murraya koenigii*). Samples were purchased from local whole sale markets of twin cities of Hyderabad and Secunderabad and brought to the laboratory.

Sample Preparation

Upon arrival at the laboratory, the fresh and healthy samples were immediately washed under tap water and extreme water drained off. The samples were pat dried with towel. Edible portions were mixed well after unpalatable parts and stems were removed. One fraction of this illustration was retained raw while the other portions were cooked using household methods. The cooked samples were homogenized using stainless steel mixer grinder and transferred in to an air-tight container and stored at -20 °C, till carotenoids isomers analysis. All procedures were carried out carefully without much exposure to light, oxygen and atmospheric pressure (Ismail and Fun, 2003). Precautions (samples in ice, darkened room) were taken as per the procedure to prevent the nutrients loss while performing analytical procedures (Masrizal *et al.*, 1997). All the chemicals and reagents used were of analytical grades.

Cooking Methods

The common house hold processing methods adopted in the present study were cooking without lid and with lid, microwave cooking, sautéing, steam cooking and deep frying.

Cooking Procedure

The cooking time to cook all the samples was carefully noted. 500 gm of each sample was taken and cooked with 100 ml of water. All the GLV were cooked for 8-12 minutes. Each procedure was conducted three times in a randomized way, for all the six cooking methods. The cooked samples were frozen with liquid N_2 and kept in polyethylene bags at -20 °C.

True Retention of Carotenoids and Nutrient Losses

The True Retention (TR) is defined as the ratio of the content of carotenoids retained in the cooked portion to the content of carotenoids in the raw portion, and is therefore related to the loss of nutrients. This is adjusted for the weight loss or gain during processing in the calculations (Murphy *et al.*, 1975). The true retention was calculated using the following formula.

% TR = ((Nutrient content per g of cooked food × g of food after cooking)/(Nutrient content per g of raw food × g of food before cooking)) × 100

Extraction of Carotenoids

All Trans and cis isomers of carotenoids were determined according to the method of Nelis *et al.* (1983). Five grams of each GLV sample was taken in a mortar and ground with pestle using cold acetone. Extraction was repeated until the extract gives no color. The extracts were pooled and filtered. The filtrate was transferred to a separating funnel to which 10-15 ml of petroleum ether was added and mixed. The pigments got transferred into the petroleum ether phase, when diluted with acetone and water containing 5% sodium sulphate. Petroleum ether extracts were pooled and volume was made up to 25 ml with 3% acetone in petroleum ether. Analysis of all Trans and Cis isomers of β-carotene in GLV by RSLC:Raw and cooked GLV were homogenized separately in a commercial food blender, along with 1% BHT, for 3 min. The carotenoids were separated by a reverse-phase High Performance Liquid Chromatography (HPLC). The UHPLC consisted of a computer data system, an auto sampler maintaining samples at 10 °C, a column heater at 25 °C, and a programmable Diode Array Detector (DAD) using vydac column (RP-C18, 5 µm, 4.6 mm x 250 mm) with 100% methanol for first 5 minutes and methanol: chloroform (96:4) for the subsequent run as gradient mobile phase. The injection volume of the extract was 2 µl with 45 min run time. Quantification and quality control were performed by external standard calibration using peak areas. (Nelis *et al.*, 1983; Marx *et al.*, 2000; and Delia *et al.*, 2004). Quantification of β-carotene isomers was done by plotting the peak area of authentic standard versus concentration. Five concentrations of all-trans-β-carotene (Sigma Co. Chemical, USA) were used to prepare the standard curve. Duplicate analyses were performed and the mean value was ascertained.

Quality Control of the Analytical Method

Method validation was performed with regard to accuracy, linearity and precision. The recovery and reproducibility was confirmed using Standard Reference Material (SRM) No: 2385 NIST purchased from USA. The reported value for β-carotene was 1.92±0.29 (mg/kg) and the obtained average value is 2.21±0.18 (mg/kg).

Statistical Analysis

Descriptive statistics like Mean and SD values were calculated for all the variables. Mean value when compared across the methods by one way ANOVA with post hoc comparison (Bonferroni correction). Level of significance was 0.05. SPSS 19.0 was used for all statistical analysis.

RESULTS

The chromatographic separation of carotenoid stereoisomer's separated from GLV samples and of β-apo-8-carotenal used as an internal standard was shown in Figure 1. Carotenoid isomers were identified by their retention and their spectral data. Individual carotenoid peaks were monitored at their spectral maximum: all-trans β-carotene (455 nm), 9-cis-β-carotene (445 nm), 13-cis-β-carotene (472 nm), 15-cis-β-carotene (470 nm) and β-apo-8-carotenal (462 nm). Quantification was carried out both by external standards and by standard addition of β-apo-8-carotenal. Purity of the standards was checked before use. All standards displayed their characteristic absorption spectra and were eluted as individual peaks. The concentrations of the standard solutions were determined. The standard curves were linear and covered the concentration ranges of all samples.

The present study highlights various processing methods of GLV cooking and the retention of all Trans and cis isomers of β carotenes content. Different GLV such as spinach, amaranth, fenugreek leaves and curry leaves were subjected to both conventional and modern cooking methods of boiling (with or without lid), steaming, microwave, sautéing and deep frying to observe the retention percentage of isomers restricted in them. The weight basis yield factors for different GLVs showed maximum values of 105.4, 108.6, 108.4 and 103.0% with observe to cooking by steaming and least yield of 45, 58, 58 and 47 % were renowned in cooked by deep frying in GLVs shown in Table 1 respectively.

In Spinach, the retention percentages of â carotene all Tran's isomers were found maximum in uncooked yield at

Figure 1: Typical Chromatogram of All Trans and Cis Isomers of -Carotene Using UHPLC

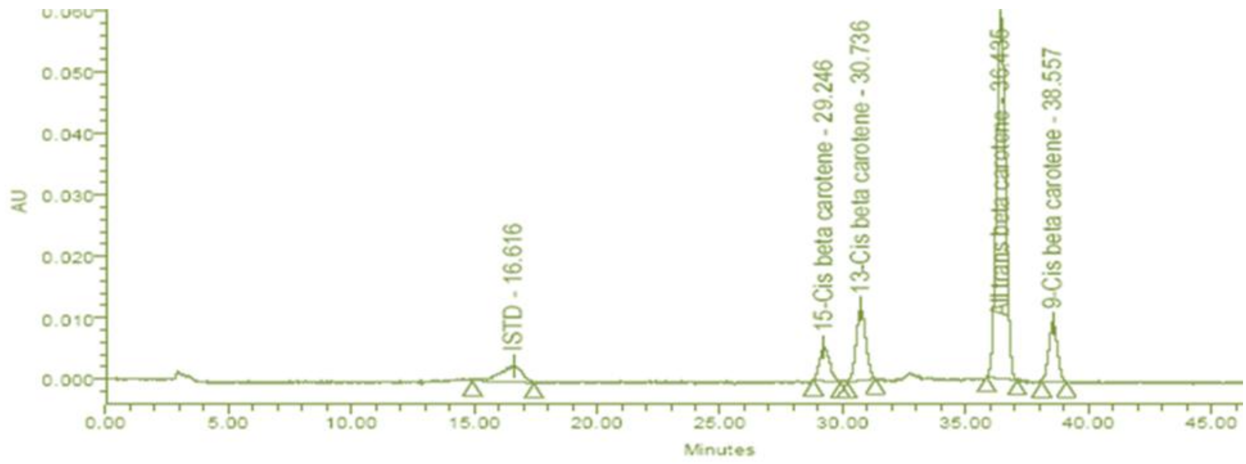

Table 1: Average Cooked Yield Percentage (%) of GLV for Different Cooking Methods

S. No	Green Leafy Vegetables	Boiling without Lid	Boiling with Lid	Steaming	Microwave Cooking	Sautéing	Deep Frying
1	Spinach	85.6	83.6	105.4	79.5	60.7	45
2	Amaranth	83.2	79.8	108.6	81.8	71.6	58
3	Fenugreek Leaves	92.5	81.4	108.4	78.8	66.4	58
4	Curry Leaves	80.1	82	103	75	74.3	47

Table 2: -Carotene All Trans and Cis Isomers from GLV Using Domestic Cooking Methods

Sample Name	Analytes	Uncooked	Cooking without Lid	Cooking with Lid	Steaming	Microwave	Sautéing	Deep Frying
Spinach leaves	All trans	3414.6±21^{a}	2268.6±15^{b}	2378.6±13^{c}	2591±15^{d}	2668±08de	2758.3±13^{e}	2655±09^{d}
	9-cis		376±0.7^{b}	392.3±1.1^{bc}	242.6±0.8^{cf}	495.7±1.3^{d}	547±1.2^{e}	453.6±0.8^{f}
	13-cis		232±0.9^{b}	250±0.7^{bc}	255±0.5^{bc}	276±0.7^{c}	329±0.4^{d}	278±0.3^{ce}
	15-cis		0±0[1]	0±0[1]	0±0[1]	0±0[1]	0±0[1]	0±0[1]

Note: Values in the same row and sub table not sharing the same subscript are significantly different at p<0.05 in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances. [1] This category is not used in comparisons because there are no other valid categories to compare.

the rate of 3414.6 µg/g followed by sautéing, microwave, deep frying, steaming, cooking with lid and without lid (2758, 2668, 2655, 2591, 2378 and 2268 µg/g) respectively. Approaching to the 9 cis isomers the maximum to minimum concentration level were found in sautéing and steaming process (547 to 242 µg/g) followed by microwave cooking, deep frying and cooking with and without lid (495, 453, 392 and 376 µg/g). The higher concentrations of 13 cis isomers were observed in sautéing followed by deep frying, microwave, steaming, cooking with lid and without lid (329, 278, 276, 255, 250 and 232 µg/g) respectively. 15 cis isomers were not detected in spinach. A significant difference in â carotene all Tran's isomers retention was observed among the fresh sample and different cooking methods. The cooking methods of steaming, microwave, sautéing and deep frying were comparable in all Tran's isomers. It was not observed in 9 cis β carotene isomers retention. In 13 cis isomers the cooking with and without lid, steaming methods were comparable, also microwave cooking and deep frying were comparable and differ with other processing methods. The results were shown in Table 2.

Amaranth is one of the most frequently consumed and leading GLV in Indian cooking. The retention of all Trans and cis isomers of β carotene using diverse food preparation methods was determined. The maximum retention percentage of all Trans isomers (8213 µg/g) was observed in uncooked yield, followed by microwave (6372 µg/g), steaming (6244 µg/g), sautéing (5918 µg/g), deep frying (5835 µg/g) and cooking with and without lid (5780, 5537 µg/g) processes respectively. With respect to 9 cis isomers of β-carotene, among various cooking methods, maximum retention (606 µg/g) was observed in microwave cooking followed by (591, 572 µg/g) in cooking with lid and without lid (531 µg/g) in steaming process (465 µg/g) in sautéing and (420 µg/g) in deep frying methods were observed. The elevated levels of 13 cis isomers were found in sautéing followed by cooking without lid and with lid, steaming and micro wave cooking methods (46, 43, 37, 354, and 27 µg/g) respectively. 15 cis isomers were also detected at minuscule quantities and the maximum to minimum levels were from (15 to 9 µg/g) of the sample respectively. A significant difference in retention of β carotene all Tran's isomers were observed among the fresh sample and different processing methods used in the present study. The processing methods of cooking with and without lid, steaming, microwave were comparable with respect of all Trans and cis isomers and distinction with sautéing and deep frying methods. In 13 cis isomers the cooking processes without lid and with lid were comparable also microwave cooking and deep frying methods were comparable, but differ with other methods. 15 cis isomers retention was also detected in amaranth and shown in Table 3

Fenugreek leaves are considered as traditional medicine and is one of the GLV in Indian cooking. The maximum retention of all Trans isomers (8942 µg/g) was found in uncooked yield similar to the other GLV, followed by the processing methods steaming (7386 µg/g), microwave (7100 µg/g), sautéing (6906 µg/g), deep frying (6833) and cooking with and without lid (6673, 6153) respectively. With admiration to â-carotene 9 Cis isomers, the maximum retention (951 µg/g) was observed in sautéing followed by (892, 850, 810, 790 and 780 µg/g) cooking methods deep frying, microwave, steaming and cooking with and without lid respectively. The superior concentrations of 13 cis isomers were found in sautéing followed by deep frying, micro wave, steaming and cooking without lid and with lid process (308, 249, 247, 247, 228 and 185 µg/g) respectively. 15 cis β carotenes isomers were also detected at tiny quantities while processing using different house hold methods. A significant difference in retention of β carotene all Tran's isomers were observed among the fresh sample and processed samples in different processing methods. It was observed that the cooking methods without lid, with lid, steaming, microwave cooking was comparable and differ with other methods. The results were shown in Table 4.

Curry leaves are used in many dishes in India, Sri Lanka, and neighboring countries. Often used in curries, the leaves are generally called by the name curry leaves. The maximum retention of all Tran's isomers (7139 µg/g) was observed in uncooked yield and it is comparable with other GLV. Followed by the processing, among the methods steaming (4765 µg/g) shown highest retention and sautéing (4684 µg/g) microwave (4562 µg/g), cooking with and without lid (4352, 4266 µg/g) and deep frying (4157 µg/g) in that order. With

Table 3: -Carotene All Trans and Cis Isomers from GLV Using Domestic Cooking Methods

Sample Name	Analytes	Uncooked	Cooking without Lid	Cooking with Lid	Steaming	Microwave	Sautéing	Deep Frying
Amaranth leaves	All trans	8213 ± 21^{a}	5537 ± 17^{b}	5780 ± 24^{bd}	6244 ± 23^{c}	6372 ± 13^{c}	5918 ± 21^{d}	5835 ± 25^{de}
	9-cis		572 ± 0.8^{b}	591 ± 0.2^{b}	531.6 ± 0.5^{bc}	606 ± 1.1^{b}	465.3 ± 1.3^{cd}	420 ± 0.7^{d}
	13-cis		43 ± 0.6^{be}	37 ± 0.2^{b}	35 ± 0.3^{c}	27 ± 0.2^{d}	46 ± 0.4^{e}	21 ± 0.1^{d}
	15-cis		15 ± 0^{1}	11 ± 0^{1}	9 ± 0^{1}	10 ± 0^{1}	0 ± 0^{1}	0 ± 0^{1}

Note: Values in the same row and sub table not sharing the same subscript are significantly different at $p<0.05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.[1] This category is not used in comparisons because there are no other valid categories to compare.

Table 4: -Carotene All Trans and Cis Isomers from GLV Using Domestic Cooking Methods

Sample Name	Analytes	Uncooked	Cooking without Lid	Cooking with Lid	Steaming	Microwave	Sautéing	Deep Frying
Fenugreek leaves	All trans	8942±17^{a}	6153±16^{b}	6673.3±15^{c}	7386.6±14^{d}	7100±12^{e}	6906±15^{ce}	6833.3±21^{c}
	9-cis		780±1.2^{b}	790±0.5^{b}	810.6±0.7^{bc}	850.3±0.5^{ce}	951±0.5^{d}	892.6±0.3^{e}
	13-cis		228±0.7^{bd}	185±1.1^{b}	247±1.6^{bc}	247±0.9^{bc}	308±0.8^{c}	249±0.6^{cd}
	15-cis		21±0.3^{b}	24±0.3^{b}	34±0.2^{c}	39±0.2^{c}	39±0.2^{c}	24±0.3^{b}

Note: Values in the same row and sub table not sharing the same subscript are significantly different at $p<0.05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.

Table 5: -Carotene All Trans and Cis Isomers from GLV Using Domestic Cooking Methods

Sample Name	Analytes	Uncooked	Cooking without Lid	Cooking with Lid	Steaming	Microwave	Sautéing	Deep Frying
Curry leaves	All trans	7139±23^{a}	4266±22^{be}	4352±24^{b}	4765.6±34^{c}	4562.6±14^{d}	4684.6±25^{cd}	4157.3±23^{e}
	9-cis		551±1.1^{a}	584.6±0.8^{ab}	622.6±1.5^{bc}	671.7±1.3^{c}	679.7±2.1^{cd}	643.7±0.7^{ce}
	13-cis		363±0.4^{a}	355±1.1^{ad}	334±0.9^{acd}	288±1.1^{b}	318±0.4^{bce}	329±0.6^{de}
	15-cis		46±0.5^{ab}	42±0.4^{ab}	51±0.3^{a}	47±0.5^{ab}	37±0.3^{b}	44±0.2^{ab}

Note: Values in the same row and sub table not sharing the same subscript are significantly different at $p<0.05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances.

Table 6: Retention Percentage of (%) -Carotene All Trans Isomers from GLV Using Processing Methods

Cooking Methods Adopted	Name of the Samples and Retention Percentage (%) of All Trans Isomers			
	Spinach	Amaranth	Curry Leaves	Fenugreek Leaves
Uncooked	100	100	100	100
Cooking without lid	66.43	67.41	59.75	68.81
Cooking with lid	69.65	70.37	60.96	74.62
Steaming	75.88	76.02	66.74	82.6
Microwave cooking	78.13	77.57	63.9	79.4
Sautéing	80.77	72.05	65.61	77.23
Deep frying	77.75	71.04	58.23	76.41

admiration to β-carotene 9 Cis isomers, the maximum retention (679 µg/g) was observed in sautéing followed by (671, 643, 622, 584and 551 µg/g) were establish in the cooking methods microwave, deep frying, steaming and cooking with and without lid process. The elevated levels of 13 cis isomers were found in cooking without lid followed by with lid, steaming, deep frying, micro wave, and cooking without lid and with lid process. Cooking without lid and with lid, steaming, sautéing and micro wave cooking methods (363, 355, 334, 329, 318 and 288 µg/g) respectively. 15 cis isomers were shown maximum comparable levels in all the processing methods shown in Table 5.

Retention percentage of β-carotenes all Tran's isomers in GLV after diverse cooking processes were tabulated in Table 6. It was observed that among the various processing methods that have been studied, a maximum retention of β-carotenes all Tran's isomers of 82.60%, 80.77%, 77.57% and 66.74% was found in Fenugreek leaves-Steaming, Spinach-sautéing, Amaranth-Microwave and curry leaves-Steaming process respectively. However the minimum retention percentage was also found with respect to 59.75% in curry leaves, 66.43% in spinach, 67.41% in amaranth and 68.81% in fenugreek leaves using cooking without lid process. The method of cooking without covering lid was observed to yield least percentage of β-carotene all Tran's isomers in all the studied GLV.

DISCUSSION

Weight Yield of Vegetables

Weight yield of the processed green leafy vegetables are given in Table 7. The yield values of each GLV, varied significantly depending on the cooking method employed, and the type of GLV. Steamed GLV had the highest mean yield values, due to absorption of water while steaming, while deep fried and sautéed GLV had the lowest mean yield values due to loss of water through evaporation. All the GLVs studied showed a gain of weight in the steaming process while reduction of weight was observed for the deep frying and sautéing processes. This could be attributed to the nature of leaf matrix of each GLV.

The effects of six different cooking methods (cooking without lid, cooking with lid, steaming, microwaving, sautéing and deep frying) on the contents of β-carotene all Trans isomers retention in GLV were investigated in the present study. The similar cooking time was maintained to all the methods.

Processing Methods Effect

Different types commonly used domestic cooking methods include cooking with and without lid, steaming, microwave, sautéing and deep frying are applied in the present study. It is hypothetically believed that processing causes rapid diminution in carotenoid isomers content. Many scientists also proved it by their research. Since experimental evidence of this was lacking in India it was of interest to determine the effect of processing on *cis-Trans* isomerization of β-carotene retention in GLV. But most of the similar work was done in foreign countries. Several studies shown that isomerization energy is involved in repositioning of the single or double bond of one form of carotenoid into another (47 Kuki *et al.*, 1991; and ESA, 2009). Besides processing of GLV and fruit could result in significant *cis-Trans* isomerization of β-carotene which was shown by the formation of 9-cis and 13-*cis*-β-carotene (Vásquez-Caicedo *et al.*, 2007). In regard to the effect of processing and isomerization of carotenoids in GLV, fruits and vegetables, 13-*cis*-β-carotene is the main product of geometric isomerization, 9-*cis*-β-carotene is formed when exposure to light (Lozano-Alejo *et al.*, 2007), while 13-*cis*-α- and β-carotene isomers are formed during storage (50 Tang and Chen, 2000). A study on the effect of β-carotene isomerization due to reflux heating has exhibited that degradation occurs to all-*Trans*-β-carotene, with a significant

Tables 7: Method Adopted for Processing of Foods

S. No	Method Name	Processing Type
1	Uncooked sample	Raw green leafy vegetables were processed and analyzed.
2	Boiling without lid	Food samples (GLV) were cooked in a vessel using little amount of water without closing the lid.
3	Boiling with lid	Food samples (GLV) were cooked in a vessel using little amount of water with lid closed.
4	Steaming process	Food samples (GLV) were cooked till they attained the required softness by steaming process.
5	Microwave cooking	GLV was taken in a fiber (microwavable) vessel and placed in a microwave and cooked, with addition of water.
6	Sautéing	Ground nut oil was preheated in a wok and GLV samples were placed in it and stir-fried.
7	Deep frying	About 1000 ml of ground nut oil was taken in an aluminum vessel and was preheated in a wok and GLV were fried until the samples completely dried.

Source: Sreenivasa Rao J et al., IJCH (2014) increase in 13-*cis*-β-carotene (Chen and Huang, 1998). Based on the structures of all-T*rans* β-carotenes, the double bonds can be relocated during heating and form several isomers (ESA, 2009). Marx *et al.* (2003) have revealed that in pasteurized and sterilized samples, 13-*cis*-β-carotene was the only isomer formed during pasteurization and sterilization of carrot juice, while 9-cis-β-carotene was probably formed during blanching of sterilized carrot juice. Moreover 9-*cis*- and 13-*cis*-β-carotenes were thought to originate independently from *cis* precursors by non-enzymatic isomerization of all-*trans* forms (Breitenbach and Sandmann, 2005).

GLV are rich sources of carotenoids (Raju *et al.*, 2007). It has been reported that available β-carotene from greens in India is 95%, and out of this 90% is contributed by GLV (Jayaprakasha *et al.*, 2001). It is hypothetically believed that carotenoids content was decreased significantly by the processing methods. Similarly, the β-carotene content of the GLV cooked by different cooking methods also decreased significantly. A pronounced retention of *Trans* isomerization of β-carotene using various processing methods were 80.77% in spinach by sautéing process, 77.57% in amaranth by microwave cooking, 66.74% in curry leaves by steaming and 82.60% in fenugreek leaves by steaming process respectively. The loss in β-carotene Tran's isomers during processing is because the Trans form changes to Cis form (Yoon *et al.*, 2002) which are not biologically active. This finding is supported by the result of the study conducted by (Zhang and Hamauzu, 2004) that both conventional and microwave cooking caused loss of total carotenoids in broccoli florets and stems (De Sa and Rodriguez-Amaya, 2004). The reduction of carotenoids concentration both in boiled and stir-fried were also observed in green vegetables.

In the present study a similar observation was found in all cooking treatments. The maximum retention of β-carotene Tran's isomers was observed in steaming, microwaving and sautéing process. The greatest loss of β-carotene Tran's isomers was found in all the GLV after cooking without lid, followed by cooking with lid respectively. This could well be attributed to water loss, which causes a drastic drop in β-carotene Tran's isomers content in steaming and sautéing procedures compared to that of deep frying with oil. (De Sa and Rodriguez-Amaya, 2004; Kidmose *et al.*, 2006; and Ferracane *et al.*, 2008). In terms of processing, Wasantwisut and others (Chavasit *et al.*, 2002) showed 89% to 90% retention of carotene with 5 minutes boiling of vegetables while Rahman and others (Jobson *et al.*, 1997) establish comparable preservation rates when vegetables were oven-dried or sundried. In the present study the processing methods like sautéing, microwave cooking and steaming was analogous with above literature. The other processing methods were shown consequence divergence.

β-carotene all Tran's isomers content of each raw GLV was significantly higher than cooked ones. Effect of cooking methods and the time taken for the same, altered the β-carotene Tran's isomers and cis isomers content of the GLV studied. Sautéing, microwave and steaming process had increased the retention of β-carotene all Tran's isomers in all GLVs because of added oil and water loss (De Sa and Rodriguez-Amaya, 2004). Changes in tissue morphology, which occur as a result, allow greater penetration of extracting solvents into the cells and enhance release of β-carotene as well as the common chloroplast carotenoids of green vegetables like lutein that are resistant to heat treatment (Bernhardt and Schlich, 2006). Vimala and others (Vimala *et al.*, 2011) showed that yellow-fleshed cassava had retained 51.3-81% and 44.1-83.9% of β-carotene after boiling and stir-frying respectively. Cooking at appropriate temperature and time, would cause the cell walls to disrupt more readily and yield more extractable β-carotene (Santos and Silva, 2008). Variation of β-carotene retention could be due to type of vegetables, the cooking method, and the interaction between type of vegetable and cooking method (Santos and Silva, 2008; and Vimala *et al.*, 2011). Hence, these factors could affect β-carotene retention in the studied vegetables.

Analysis of cooked vegetables is different from those of raw samples. Cooking softens the cell walls and makes the extraction of carotenoids easier. However, incorporation of oil and the formation of degradation products during cooking may pose some analytical difficulties (De Sa and Rodriguez-Amaya, 2004). The time-temperature relationship is important for all types of food preparation employing heat, but the impact varies with different cooking methods and products (Ferracane *et al.*, 2008). Leskova *et al.* (2006) pointed out that continual changes of nutrient content in GLV during culinary processes have not been sufficiently investigated. Thus, further research on kinetic parameters describing vitamins decomposition, should be undertaken to promote the development of reasonable processes in the field of cooking various foods especially green leafy vegetables. It would be necessary to carry out a greater number of analyses for each GLV, from different geographical sources and at different times of the year so that the data produced would be holistic (Khachick *et al.*, 1992; and Santos and Silva, 2008). The limitations of the study were as follows the data presented does not represent the GLV consumed throughout India. Besides, GLV from different places may have different composition of minerals and vitamins, affected by the usage of fertilizers and herbicides as well as the soil quality. This selection may help consumers on the choice of cooking practices to improve the nutritional quality of foods, as well as their overall acceptability. According to (38), more investigations are needed to provide a better understanding of the oxidative phenomena of carotenoids during cooking since different vegetables have different chemical and physical characteristics.

CONCLUSION

Most of the data accessible on the provitamin A content of foods refer to the unprocessed materials. It is apparent, however, that data connecting to the form in which the foods are consumed by the population are urgently needed and the influence of processing and storage on provitamin A levels has to be determined. This type of information will help consumers and processors choose the processing and storage conditions that favor provitamin A retention. Effect of cooking on the β-carotene all Tran's isomers content of green leafy vegetables depend on, types of cooking method and communication between cooking method. In home processing methods, retention of vitamins and bioactive compounds decreases in the following order: microwaving > steaming > boiling. For the fat-soluble carotenoids, boiling is less harmful than sautéing. Deep-frying, prolonged cooking, combination of several preparation methods all result in substantial losses. Whatever the cooking or processing method used, retention decreases with longer cooking time, higher cooking temperature and peeling/ cutting of the food. Nevertheless, while frying and sautéing is clearly the most studied cooking method, data regarding these new methodologies is still scarce. In conclusion, the current study clearly showed that all cooking treatments, except sautéing, microwave cooking and steaming caused great losses of β-carotene all Tran's isomers. Stability studies highlighted the defensive effect of food matrix on dietary carotenoids compared to pure molecules from commercial origin. Household cooking processes were useful in improving the retention of β-carotene all Tran's isomers in GLV. Stability and retention studies offer harmonizing consequences for food researchers, nutritionists as well as health and dietetic practitioners to formulate precious strategies to fight against micronutrient deficiencies in Indian context. With the emerging of novel technologies such as short-time processing and high-pressure treatment of food, investigations of their effects on stability and isomerization of carotenoids appears necessary. Furthermore, the development of methods for the isolation of pure carotenoids to be used as standards for analytical purposes and for bioavailability studies is urgently required.

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REFERENCES

- Aman R, Schieber A and Carle R (2005), "Effects of Heating and Illumination on Trans-cis Isomerization and Degradation of -carotene and Lutein in Isolated Spinach Chloroplasts", *J. Agric. Food Chem*, Vol. 53, pp. 9512-9518.
- Anjali Verma (2015), "Food Fortification: A Complementary Strategy for Improving Micronutrient Malnutrition (MNM) Status", *Food Science Research Journal*, Vol. 6, No. 2, pp. 381-389, eISSN-2230-9403 Visit us: *www.researchjournal.co.in*, DOI: 10.15740/ HAS/FSRJ/6.2/381-389.
- Bernhardt S and Schlich E (2006), "Impact of Different Cooking Methods on Food Quality: Retention of Lipophilic Vitamins in Fresh and Frozen Vegetables", *Journal of Food Engineering*, Vol. 77, No. 2, pp. 327-333.
- Block G, Patterson B and Subar A (1992), "Fruit, Vegetable, and Cancer Prevention: A Review of the Epidemiological Evidence", *Nutr. Cancer*, Vol. 18, pp. 1-29.
- Breitenbach J and Sandmann G (2005), "β-Carotene *cis* Isomers as Products and Substrates in the Plant Poly*cis* Carotenoid Biosynthetic Pathway to Lycopene", *Planta*, Vol. 220, pp. 785-793.
- Britton G (1998), "Overview of Carotenoid Biosynthesis", in *Carotenoids: Biosynthesis and Metabolism*, Britton G, Liaaen-Jensen S and Pfander H (Eds.), pp. 13-147, Birkhäuser Verlag, Basel, Switzerland.

- Calvo M M (2005), "Lutein: A Valuable Ingredient of Fruits and Vegetables", *Crit Rev Food Science Nutrition*, Vol. 45, pp. 671-696.
- Castenmiller J J and West C E (1998), "Bioavailability and Bioconversion of Carotenoids", *Annual Review of Nutrition*, Vol. 18, No. 1, pp. 19-38.
- Chavasit V, Pisaphab R, Sungpuag P, Jittinandana S and Wasantwisut E (2002), "Changes in β Carotene and Vitamin A Contents of Vitamin A Rich Foods in Thailand During Preservation and Storage", *Journal of Food Science*, Vol. 67, No. 1, pp. 375-379.
- Chen B H and Huang J H (1998), "Degradation and Isomerization of Chlorophyll a and β-Carotene as Affected by Various Heating and Illumination Treatments", *Food Chemistry*, Vol. 62, pp. 299-307.
- Delia B Rodriguez-Amaya and Mieko Kimura (2004), "HarvestPlus Handbook for Carotenoid Analysis", Copyright HarvestPlus.
- De Sa M C and Rodriguez-Amaya D B (2004), "Optimization of HPLC Quantification of Carotenoids in Cooked Green Vegetables Comparison of Analytical and Calculated Data", *Journal of Food Composition and Analysis*, Vol. 17, No. 1, pp. 37-51.
- During A, Smith M K, Piper J B and Smith J C (2001), "β-Carotene 15,15'-dioxygenase Activity in Human Tissues and Cells: Evidence of an Iron Dependency", *J. Nutr. Biochem*, Vol. 12, pp. 640-647.
- ESA (2009), *Carotenoid Isomers*, ESA Application Note, 5600A, ESA Inc: Chelmsford, MA, USA, http://www.esainc.com (accessed on October 7, 2009).
- Fawzi W W, Herrera M G, Willett W C, Amin A E, Nestel P, Lipsitz S, Spiegelman D and Mohamed K A (1993), "Vitamin A Supplementation and Dietary Vitamin A in Relation to the Risk of Xerophthalmia", *Am. J. Clin. Nutr.*, Vol. 58, pp. 385-391.
- Ferracane R, Pellegrini N, Visconti A, Graziani G, Chiavaro E, Miglio C and Fogliano V (2008), "Effects of Different Cooking Methods on Antioxidant Profile, Antioxidant Capacity, and Physical Characteristics of Artichoke", *Journal of Agricultural and Food Chemistry*, Vol. 56, No. 18, pp. 8601-8608.
- Gayathri G N, Platel K, Prakash J and Srinivasan K (2004), "Influence of Antioxidant Spices on the Retention of β-carotene in Vegetables During Domestic Cooking Processes", *Food Chemistry*, Vol. 84, No. 1, pp. 35-43.
- Giovannucci E, Ascherio A, Rimm E B, Stampfer M, Jm Colditz G A and Willett W C (1995), "Intake of Carotenoids and Retinol in Relation to Risk of Prostate Cancer", *Natl J. Cancer Inst*, Vol. 87, pp. 1767-1776.
- Guo W H, Tu C Y and Hu C H (2008), "Cis-trans Isomerizations of β-carotene and Lycopene: A Theoretical Study", *J. Phys. Chem.*, Vol. 112, pp. 12158-12167.
- Herden E, Mulokozi G and Svanberg U (2002), "Estimation of Carotenoid Accessibility from Carrots Determined by an Invitro Digestion Method", *Eur. J. Clin. Nutr.*, Vol. 56, pp. 425-430.
- Ismail A and Fun C S (2003), "Determination of Vitamin C, β-carotene and Riboflavin Contents in Five Green Vegetables Organically and Conventionally Grown", *Malaysian Journal of Nutrition*, Vol. 9, No. 1, pp. 31-39.
- Jayaprakasha G K, Singh R P and Sakariah K K (2001), "Antioxidant Activity of Grape Seed Extracts on Peroxidation Models in Vitro", *Food Chemistry*, Vol. 73, No. 3, pp. 285-290.
- Jobson D J, Rahman Z U and Woodell G A (1997), "A Multiscale Retinex for Bridging the Gap Between Color Images and the Human Observation of Scenes, Image Processing", *IEEE Transactions*, Vol. 6, No. 7, pp. 965-976.
- Khachick F, Goli M B, Beecher G R, Holden J, Lusby W R, Tenorio M D and Barrera M R (1992), "Effect of Food Preparation on Quantitative and Qualitative Distribution of Major Carotenoid Constituents of Tomatoes and Several Green Vegetables", *Journal of Agricultural and Food Chemistry*, Vol. 40, pp. 390-398.
- Kidmose U, Yang R Y, Thilsted S H, Christensen L P and Brandt K (2006), "Content of Carotenoids in Commonly Consumed Asian Vegetables and Stability and Extractability During Frying", *Journal of Food Composition and Analysis*, Vol. 19, No. 6, pp. 562-571.
- Kohlmeier L and Hastings S B (1995), "Epidemiologic Evidence of a Role of Carotenoids in Cardiovascular Disease Prevention", *Am. J. Clin. Nutr.*, Vol. 62, pp. 1370S-1376S.

- Kuki M, Koyama Y and Nagae H (1991), "Triplet-Sensitized and Thermal Isomerization of All-*trans*, 7-*cis*, 9-*cis*, 13-*cis* and 15-*cis* Isomers of β-carotene: Configurational Dependence of the Quantum Yield of Isomerization via the T1 State", *J. Phys. Chem.*, Vol. 95, pp. 7171-7180.
- Lešková E, Kubíková J, Kováèiková E, Košická M, Porubská J and Holèíková K (2006), "Vitamin Losses: Retention During Heat Treatment and Continual Changes Expressed by Mathematical Models", *Journal of Food Composition and Analysis*, Vol. 19, No. 4, pp. 252-276.
- Liu R S H and Asato A E (1985), "The Primary Process of Vision and the Structure of Bathorhodopsin: A Mechanism for Photoisomerization of Polyenes", *Proc. Natl. Acad. Sci. USA*, Vol. 82, pp. 259-263.
- Lozano-Alejo N, Carrillo G.V, Pixley K and Palacios-Rojas N (2007), "Physical Properties and Carotenoid Content of Maize Kernels and its Nixtamalized Snacks", *Innov. Food Sci. Emerg. Technol.*, Vol. 8, pp. 385-389.
- Marx M, Stuparic M, Schieber A and Carle R (2003), "Effects of Thermal Processing on *trans*–*cis*isomerization of β-carotene in Carrot Juices and Carotene-Containing Preparations", *Food Chemistry*, Vol. 83, pp. 609-617.
- Masrizal M A, Giraud D W and Driskell J A (1997), "Retention of Vitamin c, Iron, and β carotene in Vegetables Prepared Using Different Cooking Methods", *Journal of Food Quality*, Vol. 20, No. 5, pp. 403-418.
- Mayne S T (1996), "Beta-Carotene, Carotenoids, and Disease Prevention in Humans", *FASEB. J.*, Vol. 10, pp. 690-701.
- Mertz C, Brat P, Caris-Veyrat C and Gunata Z (2010), "Characterization and Thermal Lability of Carotenoids and Vitamin C of Tamarillo Fruit (*Solanum betaceum* Cav.)", *Food Chem.*, Vol. 119, pp. 653-659.
- Michaela Marx, Andreas Schieber and Reinhold Carle (2000), "Quantitative Determination of Carotene Stereoisomers in Carrot Juices and Vitamin Supplemented (ATBC) Drinks", *Food Chemistry*, Vol. 70, pp. 403-408.
- Micronutrient Deficiency Information System (1995), "Global Prevalence of Vitamin A Deficiency: MDIS", Working Paper 22, WHO, Geneva, Switzerland.
- Mozaffarieh M, Sacu S and Wedrich A (2003), "The Role of the Carotenoids Lutein, Zeaxanthin, in Protecting Against Age Related Macular Degeneration: A Review Based on Controversial Evidence", *J. of Nutrition*, Vol. 2, pp. 20-28.
- Murphy E W, Criner P E and Gray B C (1975), "Comparisons of Methods for Calculating Retentions of Nutrients in Cooked Foods", *Journal of Agricultural and Food Chemistry*, Vol. 23, No. 6, pp. 1153-1157.
- National Nutrition Monitoring Bureau (NNMB, 2006), "Diet and Nutrition Status of Populations and Prevalence of Hypertension Among Adults in Rural Areas", Technical Report No 24, National Institute of Nutrition, ICMR.
- Nelis H J C F and Leenheer De A P (1983), "Isocratic Nonaqueous Reversed-Phase Liquid Chromatography of Carotenoids", *Analytical Chemistry*, Vol. 55, No. 2, pp. 270-275.
- Niedzwiedzki D M, Sandberg D J, Cong H, Sandberg M N, Gibson G N, Birge R R and Frank H A (2009), "Ultrafast Time Resolved Absorption Spectroscopy of Geometric Isomers of Carotenoids", *Chem. Phy.*, Vol. 357, pp. 4-16.
- Qiu D, Chen Z R and Li H R (2009), "Effect of Heating on Solid β-carotene", *Food Che.*, Vol. 112, pp. 344-349.
- Rahman M M, Wahed M A and Ali M A (1990), "Carotene Losses During Different Methods of Cooking Green Leafy Vegetables in Bangladesh", *Journal of Food Composition and Analysis*, Vol. 3, No. 1, pp. 47-53.
- Raju M, Varakumar S, Lakshminarayana R, Krishnakantha T P and Baskaran V (2007), "Carotenoid Composition and Vitamin A Activity of Medicinally Important Green Leafy Vegetables", *Food Chemistry*, Vol. 101, No. 4, pp. 1598-1605.
- Rickman J C, Bruhn C M and Barrett D M (2007), "Nutritional Comparison of Fresh, Frozen, and Canned Fruits and Vegetables II: Vitamin A and Carotenoids, Vitamin E, Minerals and Fiber", *J. Sci. Food Agric.*, Vol. 87, pp. 1185-1196.
- Santos M S, Meydani S N, Leka L, Wu D, Fotouhi N, Meydani M, Hennekens C H and Gaziano J M (1996), "Natural Killer Cell Activity in Elderly Men is Enhanced by β-carotene Supplementation", *Am. J. Clin. Nutr.*, Vol. 64, pp. 772-777.

- Santos P H S and Silva M A (2008), "Retention of Vitamin C in Drying Processes of Fruits and Vegetables—A Review", *Drying Technology*, Vol. 26, No. 12, pp. 1421-1437.
- Schieber A and Carle R (2005), "Occurrence of Carotenoid *cis*-isomers in Food: Technological, Analytical, and Nutritional Implications", *Trends Food Sci. Technol.*, Vol. 16, pp. 416-422.
- Schieber A and Carle R (2005), "Occurrence of Carotenoids Isomers in Food: Technological, Analytical, and Nutritional Implications", *Trends in Food Science & Technology*, Vol. 16, No. 9, pp. 416-422.
- Shi J, Yi C, Ye X, Xue S, Jiang Y, Ma Y and Liu D (2010), "Effects of Supercritical CO_2 Fluid Parameters on Chemical Composition and Yield of Carotenoids Extracted from Pumpkin", *LWT–Food Sci. Technol.*, Vol. 43, pp. 39-44.
- Sommerburg O, Keunen J E E, Bird A C and Van Kuijk F J G M (1998), "Fruits and Vegetables that are Sources for Lutein and Zeaxanthin: The Macular Pigment in Human Eyes", *Br. J. of Opthalmol.*, Vol. 82, pp. 907-910.
- Speek A J, Speek-Saichua S and Schreurs W H (1988), "Total Carotenoid and β-carotene Contents of Thai Vegetables and the Effect of Processing", *Food Chemistry*, Vol. 27, No. 4, pp. 245-257.
- Stahl W and Sies H (2005), "Bioactivity and Protective Effects of Natural Carotenoids", *Biochem Biophys Acta*, Vol. 1740, pp. 101-107.
- Tang Y C and Chen B H (2000), "Pigment Change of Freeze-Dried Carotenoid Powder During Storage", *Food Chemistry*, Vol. 69, pp. 11-17.
- The World Health Report (2002), "Reducing Risks, Promoting Healthy Life: Overview", *World Health Organization*, WHO/WHR/02.1, Geneva.
- Thurnham D I (2007), "Bioequivalence of Beta Carotene and Retinol", *Journal of Science of Food and Agriculture*, Vol. 87, pp. 13-39.
- Vásquez-Caicedo A L, Schilling S, Carle R and Neidhart S (2007), "Effects of Thermal Processing and Fruit Matrix on Beta-Carotene Stability and Enzyme Inactivation During Transformation of Mangoes into Purée and Nectar", *Food Chem.*, Vol. 102, pp. 1172-1186.
- Vásquez-Caicedo A L, Sruamsiri P, Carle R and Neidhart S (2005), "Accumulation of All-trans-β-carotene and its 9-*cis* and 13-*cis* Stereoisomers During Postharvest Ripening of Nine Thai Mango Cultivars", *J. Agric. Food Chem.*, Vol. 53, pp. 4827-4835.
- Vimala B, Nambisan B and Hariprakash B (2011), "Retention of Carotenoids in Orange-Fleshed Sweet Potato During Processing", *Journal of Food Science and Technology*, Vol. 48, No. 4, pp. 520-524.
- West C E and Castenmiller J J M (1998), "Quantification of the SLAMENGHI Factors for Carotenoids Bioavailability and Bioconversion", *Int J. Vit. Nutr.*, Vol. 68, pp. 371-377.
- World Health Organization (2009), "Vitamin—A Deficiency in Indian Rural Preschool Aged Children", Vol. 2, No. 1, pp. 11-14.
- Yadav S K and Sehgal S (1995), "Effect of Home Processing on Ascorbic Acid and β-carotene Content of Spinach (*Spinacia oleracia*) and Amaranth (*Amaranthus tricolor*) Leaves", *Plant Foods for Human Nutrition*, Vol. 47, No. 2, pp. 125-131.
- Yoon H S, Hackett J D and Bhattacharya D (2002), "A Single Origin of the Peridinin-and Fucoxanthin-Containing Plastids in Dinoflagellates Through Tertiary Endosymbiosis", *Proceedings of the National Academy of Sciences*, Vol. 99, No. 18, pp. 11724-11729.
- Zepka L Q and Mercadante A Z (2009), "Degradation Compounds of Carotenoids Formed During Heating of a Simulated Cashew Apple Juice", *Food Chem.*, Vol. 117, pp. 28-34.
- Zhang D and Hamauzu Y (2004), "Phenolics, Ascorbic Acid, Carotenoids and Antioxidant Activity of Broccoli and their Changes During Conventional and Microwave Cooking", *Food Chemistry*, Vol. 88, No. 4, pp. 503-509.

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