ORIGINAL ARTICLE

Retention based bio accessibility of carotenoids in green leafy vegetables: effect of different Indian culinary practices

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Abstract

Back ground: Green Leafy Vegetables (GLV) is pigment-rich and nutritionally relevant functional food sources with unique phytochemical constituents that include carotenoids which are precursors for vitamin A and protect cells from oxidation and cellular damage. 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. Rationale: Studies pertaining to the effect of cooking methods on dietary carotenoids bio accessibility and their retention percent are scarce, particularly in an Indian Diasporas. Objective: Present study was to determine the carotenoids retention based bio accessibility in GLV such as amaranth (*Amaranthus gangeticus*), spinach (*Spinacia oleracea*) 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. Method: The retention based bio accessibility of carotenoids were quantified by rapid separation liquid chromatography (RSLC) using RP-C-18 column (150mm×4.6µ) with 70% acetonitrile, 20% dichlomethane and 10% methanol for 20 minutes at flow rate of 0.5 ml/min. Results: The maximum retention based bio accessibility of total carotenoids and β-carotene were observed with micro wave cooking, steaming and sautéing methods. (Spinach: 57.88% and 55.92%, Amaranth: 56.15% and 57.49%, Curry leaves: 50.55% and 52.66% respectively). Conclusion: The reduction in the contents of carotenes in GLVs in correlation to various cooking methods are discussed which would be valuable for food researchers, nutritionists as well as health practitioners and dietitians, in developing and promoting nutritionally balanced diets and minimize vitamin A deficiency in Indian context.

Key Words

Vitamin A; green leafy vegetables; retention; carotenoids; bio availability; cooking methods

Introduction

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, mostly in African and South-East Asian developing countries (WHO).

Green Leafy Vegetables (GLV), fruits and other vegetables have been cited as a potential source of micronutrients, particularly carotenoids that can be absorbed and converted to vitamin A in the human body (1, 2). Epidemiological studies indicate that increased intake of GLV and vegetables are associated with decreased risk towards certain cancers, cardiovascular disease, cataract, macular degeneration, and other age-related ailments (3). In addition to serving as a critical source of micronutrients, leafy vegetables are also rich in many carotenoids (4, 5). More than 700 carotenoids have been identified in nature. The most commonly studied include lutein, zeaxanthin, lycopene, β-carotene, α-carotene, and β-cryptoxanthin (6). Besides the well-known provitamin A activity of some carotenoids (7), they also function as antioxidants and enhancers of the immune response, and as such are associated with lowering the risk of developing degenerative diseases (8, 9).

In lower income countries about 82% of vitamin A must be obtained from a variety of carotenoids in plants (10, 11). 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 (12). Improving vegetable processing and preparation that allow optimal retention of vitamin A activity (13, 14) is important to enable consumers and processors to choose the processing and storage conditions that favor retention of carotenoids 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 preference, rather than on nutrient relevance. Researchers have reported that 5 to 78% of beta carotene degraded when vegetables were prepared using different cooking methods (15, 16, 17). Considerable quantities of carotenoids needed by individuals may be lost during household cooking of GLV and vegetables (18). Thus, information on the possible losses of carotenoids from green leafy vegetables and vegetables, during traditional cooking methods, is of major importance (19). Several reports have documented the losses of beta carotene after boiling, frying, bleaching, and pressure cooking (17, 20).

However most of the reported data are on raw foods, although many are ingested after cooking. Hence this will lead to an over estimation or under estimation of nutrient intake in dietary assessment of the population in community studies. The best means of obtaining data on cooked or processed foods is through laboratory analysis. It is crucial 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. It can then be recommended to minimize losses of nutrients in foods (21). Hence this will provide guidelines for a healthy diet preparation that did not deplete nutrient content of vegetables (22). Indirectly this also contributes in recommending healthy cooking methods to the general public.

Several factors affect the bioavailability of carotenoids, including beta carotene, from foods (23). Among these factors, the matrix in which the carotenoids are embedded plays a crucial role. Vegetables such as tomato and green leafy vegetables are rich sources of β-carotene and are grown and consumed in India. Recently there has been an increasing interest in assessing carotenoids bio accessibility and bio availability from GLV and fruits because of the multi-dimensional health benefits associated with carotenoids consumption (24). A few studies have reported differences in the carotenoids content of different varieties of GLV and vegetables. Information on the carotenoids and their bio accessibility from GLV is still scanty.

Aims & Objectives

1. The purpose of the present study was to quantify the major carotenoids and to determine the effect of different cooking practices on carotenoids retention in commonly consumed Indian leafy vegetables.
2. Carotenoids were chosen for the present study since they are contributory to the health benefits of Vitamin A in humans.
3. The study was also aimed to compare the effect of cooking methods on carotenoids bio accessibility, between raw and cooked Indian leafy vegetables

Material and Methods

Materials: Three varieties of green leafy vegetables were selected, based on popular consumption by Indians. The GLV were Amaranth (*Amaranthus gangeticus*), Spinach (*Spinacia oleracea*) and Curry leaves (*Murraya koenigii*). Samples were purchased from local whole sale markets of twin cities of Hyderabad and Secunderabad.

Sample Preparation: Upon arrival at the laboratory, the fresh and healthy samples were immediately washed under tap water and excessive water drained off. The samples were pat dried with towel. Edible portions were mixed well after inedible parts and stems were removed. One portion of this sample was retained raw while the other portions were cooked using different cooking methods. The cooked samples were homogenized using stainless steel mixer grinder and transferred in to an air-tight container and stored at -200C, till carotenoids analysis. All procedures were carried out carefully without much exposure to light, oxygen and atmospheric pressure (25). Precautions (samples in ice, darkened room) were taken not to destroy the nutrients while performing analytical procedures (26). 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, cooking 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 100ml 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 N2 and kept in polyethylene bags at −80 °C. Parts from these frozen samples were freeze-dried for carotenoids bio accessibility assay.

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 (27). 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: Total Carotenoids and β-carotene contents were determined according to the method of Nelis et al (28). 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. At 452 nm absorbance, the total carotenoids content were measured spectrophotometrically. For determination of β-carotene content, the extract was passed through a column containing activated alumina. β-carotene band formed in the column was eluted and made up to volume with 3% acetone in petroleum ether. There after the absorbance was measured spectrophotometrically.

Analysis of β-carotene in leafy vegetables by HPLC method: Raw and cooked green leafy vegetables 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 HPLC consisted of a computer data system, an auto sampler maintaining samples at 100C, a column heater at 250C, and a programmable diode array detector (DAD). The separation was performed isocratically on RP C-18 waters spherisorb 5µ*150mm ODS2 column. The mobile phase consisted of 70% acetonitrile, 10% methanol and 20% dichloro methane maintained at flow rate of 0.5 ml/min. The injection volume of the extract was 2 µl with 15 min run time. A DAD was programmed to measure carotenoids at 450 nm. Linear calibration curves were prepared consisting of five concentrations of analytes which scanned the levels of carotenoids commonly found in GLV. Calibrates included α-and β-carotene. Quantification and quality control were performed by external standard calibration using peak areas. Single injections were performed and compared with spectrophotometric measurements for accuracy and consistency (28).

Quantification of β-carotene was done by plotting the peak area of authentic standard versus concentration. Five concentrations of 10, 20, 40, 80 and 120 µg/ml 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 checked with regard to accuracy, linearity and precision. The recovery and reproducibility was checked by 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).

In vitro bio accessibility of carotenoids: Determination of in vitro bio accessibility of ά-carotene and β-carotene was carried out according to in vitro digestion method (29). The in vitro method simulates digestion in the gastro intestinal tract and gives an estimation of the maximum amount of carotenoids released from the food matrix that at optimal physiological and dietary conditions would enable absorption in to the human mucosa.

Statistical Analysis: Descriptive statistics like Mean and SD Value was calculated for all the variables.

Mean value when compared across the methods by one way ANOVA with post hoc comparison. Level of significance as 0.05. SPSS version 19.0 was used for statistical analysis.

Results

The present study highlights various processing methods for GLV cooking and the retention of carotenoids content shown in Table 1. Different green leafy vegetables (GLVs) such as spinach, amaranth 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 and bio-accessibilities of carotene and total carotenoids contained in them.

The weight basis yield factors for different GLVs showed maximum values of 105.4, 108.6 and 103.0 % with regard to cooking by steaming of spinach, amaranth and curry leaves respectively. Least yield of 45, 58 and 47 % were noted in spinach, amaranth and curry leaves respectively cooked by deep frying. Table 2

In Spinach, the retention concentration of total carotenoids was found to be maximum in uncooked form at the rate of 96.6 μg/g while the bio accessibility concentration was 48.2 μg/g. Among various cooking methods followed, maximum retention of 77.4μg/g was observed in spinach cooked by sautéing. Microwave cooking and sautéing of spinach also showed maximum bio accessibilities of 27.9 and 27.7 μg/g among various cooking methods adopted. With respect to β-carotene as well, the maximum retention concentration and bio accessibility concentrations of 30.9 μg/g and 15.2 μg/g were observed in uncooked form. Among various cooking methods maximum retention bio accessibility concentrations of 23.5 μg/g and 8.55 μg/g were observed respectively in spinach cooked by sautéing. Cooking without lid was found to cause least retention and bio accessibility of 52.9 and 23.9 μg/g of total carotenoids. Even for β-carotene, least retention and bio accessibility of 18.5 and 7.7 μg/g was observed by cooking without lid. A significant difference in total carotenoids retention concentrations was observed among fresh and different cooking methods while the bio accessibilities of total carotenoids in steaming, microwave cooking and sautéing were comparable. Beta carotene retentions were similar among steaming and sautéing as well as microwave and deep frying methods. β-carotene bio accessibilities were similar among steaming and microwaving as well as among sautéing and deep frying Table 3.

In Amaranth, among various cooking methods, maximum retention concentration of 169.2 μg/g total carotenoids was observed by steaming while maximum bio accessibility concentration of 61.2 μg/g was observed in sautéed form. With respect to β-carotene, among various cooking methods, maximum retention concentration of 61.3 μg/g and bio accessibility concentration of 23.4 μg/g were both observed in sautéed forms. Maximum retention and bio accessibilities of 219.6 and 109.2 μg/g of total carotenoids was found in fresh form while with respect to β-carotene maximum retention and bio accessibilities of 83.3 and 40.7 μg/g were also noted in fresh forms. Among various methods of cooking studied, the method of cooking without lid caused least retention and bio accessibility of total carotenoids with values of 134.6 and 55.2 μg/g respectively and that of β-carotene with values of 51.2 and 20.3 μg/g respectively. Total carotenoids were significantly different among fresh and cooked forms of amaranth while that of total carotenoids bio accessibilities were similar among sautéing and deep frying. β-carotene retention were similar among steaming and microwaving method while bio accessibilities were similar among cooking with and without lid as well as among steaming, microwaving and deep frying. Table 4

In curry leaves, similar to the observation in other GLVs, the fresh samples contained maximum retention and bio accessibility concentrations of 218.5 and 100.3 μg/g of total carotenoids. Among the various cooking methods, sautéing yielded maximum retention and bio accessibility concentrations of 160.5 and 50.7 μg/g of total carotenoids respectively. However, the cooking method of maximum beta carotene retention of 55.8 μg/g was noted with steaming while the maximum bio accessibility values of 23.4 μg/g was observed in sautéed form. Cooking of curry leaves without lid yielded least retention and bio accessibilities of 212.5 and 47.3 μg/g respectively for total caroetnoids and 45.8 and 20.3 μg/g for beta carotene respectively. Total carotenoid retentions did not vary significantly among microwave and deep frying while bioaccessibilities of total carotenoids were similar among cooking without and with lid as well as among steaming, microwaving, sautéing and deep frying. Table 5.

Carotenoids retention percent in GLV after different cooking processes and retention effect on carotenoids bio availability were tabulated in Table 6. It has been observed that among the various cooking methods that have been studied, a maximum retention of total carotenoids of 80.37%, 70.78% and 73.51% was found in Spinach-sautéing, Amaranth- deep frying and curry leaves- sautéing respectively. However the minimum retentions of total carotenoids were 54.93%, 61.35% and 55.58% which were observed when cooked without lid in all the GLV studied. Amaranth-cooking without lid and Curry leaves- sautéing respectively. With respect to the retention of β-carotenes it has been observed that among the various cooking methods, a maximum retention of 76.38%, 73.50% and 76.78% was recorded in case of Spinach-sautéing, Amaranth-sautéing and Curry leaves- steaming respectively. Cooking without lid of all the three GLV yielded comparatively a minimum retention as witnessed in (Spinach, Amaranth and Curry leaves) 59.87%, 61.39% and 62.91% respectively.

Among the GLV studied the maximum bio accessibility of total carotenoids of 57.88% was observed in micro wave cooked spinach, while a minimum bio accessibility of 47.16% was noted in Curry leaves cooked without lid. Among the GLV maximum bio accessibility of total carotenoids with 57.88%, 56.15% and 49.65% were noted in micro wave cooked spinach, sautéed amaranth and steamed curry leaves respectively. On the other hand a minimum bio accessibility of total carotenoids with values of 49.59%, 49.55% and 47.16% were observed when cooked without lid in all the GLVs. The maximum bio accessibility of β-carotene was recorded in sautéed spinach, amaranth and curry leaves respectively the percentages being 55.92, 57.49 and 52.66 respectively. The method of cooking without covering lid was observed to yield least bio accessibility of β-carotene with values of 50.66%, 49.88% and 47.93% in the case of spinach, amaranth and curry leaves respectively.

Discussion

Weight yield of vegetables: Weight yield of the processed green leafy vegetables are given in Table 1. 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 total carotenoids and ß-carotene retention in GLV were investigated in the present study.

GLV are rich sources of carotenoids (30). It has been reported that available β-carotene from greens in India is 95%, and out of this 90% is contributed by GLV (31). 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 total carotenoids 80.37%, 77.16%, 73.51%, and β-carotene content 76.38%, 73.50%, 76.79% was found in Spinach, Amaranth and Curry leaves respectively. The loss in β-carotene during processing is because the Trans form changes to Cis form (32) which are not biologically active. This finding is supported by the result of the study conducted by (33) that both conventional and microwave cooking caused loss of total carotenoids in broccoli florets and stems. (21) Also noted a reduction of carotenoids concentration, both in boiled and stir-fried green vegetables.

In the present study a similar observation was found in all cooking treatment. The maximum retention of carotenoids was observed in steaming, microwaving and sautéing process. The greatest loss of carotenoids 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 carotenoids content in steaming and sautéing procedures compared to that of deep frying with oil. (21, 34, 35). In terms of processing, Wasantwisut and others (36) showed 89% to 90% retention of carotene with 5 minutes boiling of vegetables while Rahman and others (37) found similar retention rates when vegetables were oven-dried or sundried. Destruction of β-carotene and lutein has been shown to be higher when these processing methods were applied.

Total carotenoids and β-carotene content of each raw GLV was significantly higher than cooked ones. Effect of cooking methods and the time taken for the same, altered the carotenoids content of the GLV studied. Sautéing and deep frying had increased the retention of β-carotene in all GLVs because of added oil and water loss. (21). Absorption of water by the vegetable during boiling did not cause dilution of the carotenoids. 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 (38).

Vimala and others (17) 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 (39). 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 (17, 39). 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 (21). The time-temperature relationship is important for all types of food preparation employing heat, but the impact varies with different cooking methods and products (35). Leskova et al (22) 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 (39, 40).

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

There were variations in total carotenoids and β-carotene retention in the GLV studied due to cooking methods and type of green leafy vegetable. Future research on kinetic parameters describing vitamins decomposition during cooking should be carried out. Effect of cooking on the carotenoids content of green leafy vegetables depend on the GLV, types of cooking method and interaction between cooking method and types of vegetable. In conclusion, the current study clearly showed that all cooking treatments, except sautéing and steaming, caused great losses of carotenoids. Stability studies highlighted the protective effect of food matrix on dietary carotenoids, compared to pure molecules from commercial origin. Domestic cooking processes were useful in improving the bio accessibility of carotenoids in GLV. Stability and bio accessibility studies give complementary results for food researchers, nutritionists as well as health and dietetic practitioners to formulate valuable strategies to fight against micronutrient deficiencies in Indian context

Recommendation

This research may give directions to consumers to derive the health promoting benefits of carotenoids green leafy vegetables, fruits and vegetables while using different cooking practices in the context of changing dietary patterns.

Relevance of the study

Stability and bio accessibility studies give complementary results for food researchers, nutritionists as well as health and dietetic practitioners to formulate valuable strategies to fight against micronutrient deficiencies in Indian context

Authors Contribution

Both authors are contributed the research work and manuscript preparation and data compilation.

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Tables

TABLE 1 METHODS ADOPTED FOR PROCESSING OF FOODS

S. No	Name of the Method	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.

TABLE 2AVERAGE COOKED YIELD (%) OF VEGETABLES FOR DIFFERENT COOKING METHODS

S. No.	GLV	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	Curry Leaves	80.1	82.0	103.0	75.0	74.3	47

TABLE 3EFFECT OF DIFFERENT COOKING METHODS ON RETENTION OF CAROTENOIDS AND CAROTENOIDS BIO ACCESSIBILITY-(µG/G) IN SPINACH

Sample Name	Method of Cooking	Total. Carotenoids	β-carotene	Total carotenoids bio accessibility	β-carotene bio accessibility
Spinach	Uncooked	96.6a±0.3	30.9a±0.4	48.2a±0.2	15.2a±0.2
	Cooking without lid	52.9b±0.2	18.5b±0.2	23.9b±0.5	7.7b±0.1
	Cooking with lid	70.6c±0.2	20.8c±0.4	24.7c±0.3	7.8c±0.06
	Steaming	73.8d±0.3	23.4d±0.3	27.3d±0.4	8.43de±0.07
	Microwave cooking	66.9e±0.4	22.6e±0.3	27.9d±0.5	8.3d±0.06
	Sautéing	77.4f±0.6	23.5d±0.4	27.7d±0.4	8.55e±0.04
	Deep frying	72.4g±0.6	22.1e±0.4	26.8e±0.4	8.44e±0.06
	P Value	0.00	0.00	0.00	0.00

Values are ± SD for five determinations on fresh weight basis.
Values with different superscripts are only significant (P<0.05) ANOVA

TABLE 4EFFECT OF DIFFERENT COOKING METHODS ON RETENTION OF CAROTENOIDS AND CAROTENOIDS BIO ACCESSIBILITY-(µG/G) IN AMARANTH

Sample Name	Method of Cooking	Total. Carotenoids	β-carotene	Total carotenoids bio accessibility	β-carotene bio accessibility
Amaranth	Fresh Sample	219.6a±0.6	83.3a±0.6	109.2a±0.6	40.7a±0.21
	Cooking without lid	134.6b±0.5	51.2b±0.4	55.2b±0.4	20.3b±0.45
	Cooking with lid	151.7c±0.6	55.5c±0.4	56.6c±0.25	20.5b±0.57
	Steaming	169.2d±0.5	59.4d±0.3	60.4d±0.31	21.7c±0.6
	Microwave	144.5e±0.4	60.1d±0.5	59.6e±0.25	22.1c±0.55
	Sautéing	152.5c±0.4	61.3e±0.45	61.2f±0.45	23.4d±0.35
	Deep frying	155.2f±0.5	58.5f±0.35	60.6ef±0.25	22.5c±0.4
	P Value	0.00	0.00	0.00	0.00

Values are ± SD for five determinations on fresh weight basis.
Values with different superscripts are only significant (P<0.05) ANOVA

TABLE 5EFFECT OF DIFFERENT COOKING METHODS ON RETENTION OF CAROTENOIDS AND CAROTENOIDS BIO ACCESSIBILITY-(µG/G) IN CURRY LEAVES

Sample Name	Method of Cooking	Total. Carotenoids	β-carotene	Total carotenoids bio accessibility	β-carotene bio accessibility
Curry leaves	Fresh Sample	218.5a±0.4	72.8a±0.3	100.3a±0.45	40.7a±0.21
	Cooking without lid	121.5b±0.4	45.8b±0.6	47.3b±0.45	20.3b±0.45
	Cooking with lid	150.2c±0.7	50.8c±0.6	48.6c±0.25	20.5bc±0.57
	Steaming	151.6d±0.36	55.8d±0.45	49.8d±0.5	21.7cd±0.6
	Microwave	154.2e±0.41	53.5e±0.4	49.3c±0.5	22.1cd±0.55
	Sautéing	160.5f±0.7	53.3e±0.45	50.7e±0.40	23.4c±0.35
	Deep frying	154.3e±0.55	51.8f±0.55	49.2cd±0.30	22.5d±0.4
	P Value	0.00	0.00	0.00	0.00

Values are ± SD for five determinations on fresh weight basis.
Values with different superscripts are only significant (P<0.05) ANOVA

TABLE 6CAROTENOIDS RETENTION PERCENTAGES OF GREEN LEAFY VEGETABLES USING DIFFERENT COOKING METHODS

Cooking Method	Spinach				Amaranth				Curry Leaves			
	TC	BC	TC-Bio Acc	BC Bio Acc	TC	BC	TC-Bio Acc	BC Bio Acc	TC	BC	TC-Bio Acc	BC Bio Acc
Raw sample	100	100	50.05	48.72	100	100	49.55	49.57	100	100	46.22	46.75
Cooking without lid	54.93	59.87	49.59	50.66	61.35	61.39	50.64	49.88	55.58	62.91	47.16	47.93
Cooking with lid	73.31	67.31	51.24	51.97	69.14	66.79	52.02	50.37	68.71	69.78	48.45	49.41
Steaming	76.64	75.73	56.64	55.26	77.16	71.22	55.41	53.32	69.4	76.79	49.65	50.89
Microwave	69.47	73.46	57.88	54.61	65.91	72.18	54.68	54.3	70.49	73.63	49.15	52.07
Sautéing	80.37	76.38	57.47	55.92	69.55	73.5	56.15	57.49	73.51	73.35	50.55	52.66
Deep Frying	75.39	71.52	55.6	55.26	70.78	70.26	55.61	55.28	70.59	71.15	49.05	50.59

TC- Total Carotenoids, BC- Beta Carotene
TC-Bio Acc- Total Carotenoids Bio Accessibility
BC-Bio Acc- Beta Carotene Bio Accessibility