Short communication

Beta-carotene retention as retinol activity equivalent at different cooking and storage variants

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Abstract

Provitamin A carotenoids from various foods have been shown to have approximately eight-fold difference in β-carotene conversion factors (on a weight basis) ranging from 3.6:1 to 28:1, thereby yielding different values in terms of vitamin A. The major factor that affects the conversion of plant provitamin A to vitamin A is the nature of the food matrix. Therefore, to study the effect of different food processing on a selected food matrix (carrot was chosen in the present work) as retinol precursor, three boiling methods namely open flame boiling in open containers (gas oven; OF), open flame boiling in closed containers (pressure cooker; PC) and microwave irradiated boiling (MW) were selected. The differently boiled carrots were refrigerated for 24 and 48 h followed by reheating by open flame and microwave irradiation. Results showed that the characteristic three peak spectrum of all *trans*-β-carotene has not been altered much irrespective of different treatments, times and temperatures. Spectrum of raw carrot and all types of boiled products showed no peak for bio-available *cis*-isomer of β-carotene. Refrigeration for 24 and 48 h of the boiled mass (all types) resulted in the formation of *cis*-isomer. Reheating post 24 h refrigeration revealed total loss of *cis*-isomer while reheating post 48 h refrigeration revealed partial retention of *cis*-isomer in cases of MW and OF boiled products only. For PC boiled carrot, reheating post 24 and 48 h refrigeration resulted in total loss of *cis*–isomer. The results suggest that while the MW boiled carrot yielded the highest vitamin A (in IU), further refrigeration and reheating (in either way) lessened the bio-availability. For future application, the amount of carrot required to meet the daily value (DV) of vitamin A can be projected based on the loss/gain effect of boiling types demonstrated in the present work, provided the rate of consumption of carrot is known.

Introduction

The most important provitamin A carotenoid is β-carotene. Other provitamin A carotenoids include α-carotene and β-cryptoxanthin. The body converts these plant pigments into vitamin A. Both provitamin A and preformed vitamin A must be metabolised intracellularly to retinal and retinoic acid (the active forms of vitamin A) to support the vitamin-induced biological functions (Braumann *et al.*, 1982; Britton,1995). Vitamin A is an essential vitamin for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues and embryonic development (Underwood and Arthur, 1996). The disease-preventing activity of β-carotene and other provitamin A carotenoids could be ascribed either to their conversion into retinoid or to their activity as intact molecules. In nature, carotenes exist primarily in the most stable all *trans*-isomeric configuration but small amounts of *cis*-isomers also occur (during processing they can change to *trans*-form). The conversion of dietary β-carotene to vitamin A might relate to preformed vitamin A in the diet (Lemke *et al.*, 2003); that is, the conversion might be less efficient when vitamin A has been provided from other dietary sources. The issue of the efficiency of conversion of provitamin A carotenoids into retinol due to different cooking and storage conditions is therefore of interest in the present work.

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For a healthy population, the major factors that affect the bio-availability of food carotenoids and the bio-conversion of food provitamin A carotenoids to vitamin A in humans are food matrices, food preparation, and the fat content of a meal (Furr and Clark, 1997). Several data indicated that the bio-conversion of β-carotene to vitamin A was not as efficient as expected, and as a result, the estimated efficiency factor for the conversion of dietary β-carotene to vitamin A was revised from 6:1 by weight (National Research Council, 1989) to 12:1 by weight (Trumbo et al., 2001). However, this new conversion ratio must be regarded as temporary and could well change, as more data become available. Review articles that have evaluated the factors that affect the conversion of β-carotene to vitamin A have been published by Castenmiller and West (1998), van Het Hof et al. (2000) and Yeum and Russell (2002). Currently, vitamin A is listed on food and supplement labels in international units (IUs) even though nutrition scientists rarely use this measure. Conversion rates between mcg RAE (retinol activity equivalents) and IU are as follows (Otten et al., 2006): 1 IU retinol = 0.3 mcg RAE, 1 IU β-carotene from dietary supplements = 0.15 mcg RAE,1 IU β-carotene from food = 0.05 mcg RAE, 1 IU α-carotene or β-cryptoxanthin = 0.025 mcg RAE. The mcg (millicentigram) is equal to 10 μg. An RAE cannot be directly converted into an IU without knowing the source(s) of vitamin A. For example, the RDA of 900 mcg RAE for adolescent and adult men is equivalent to 3,000 IU if the food or supplement source is preformed vitamin A (retinol). However, this RDA is also equivalent to 6,000 IU of β-carotene from supplements, 18,000 IU of β-carotene from food, or 36,000 IU of α-carotene or β-cryptoxanthin from food. So a mixed diet containing 900 mcg RAE provides between 3,000 and 36,000 IU of vitamin A, depending on the types of foods consumed.

Vitamin A deficiency is common in many developing countries. People from these countries often depend on starch-based foods as a result of poverty and limited access to foods containing preformed vitamin A like fruits and vegetables. Daily Value. (DV) were developed by the U.S. Food and Drug Administration (USFDA) to help consumers compare the nutrient contents of products within the context of a total diet. The DV for vitamin A is 5,000 IU for adults and children age 4 and older. Foods providing 20% or more of the DV are considered to be high sources of a nutrient. As the dietary provitamin A carotenoids are a major source of our vitamin A needs, the present work attempted to find out the effect of different cooking processes and subsequent reheating conditions that might result in optimisation and bio-availability of carotenoids; as well as to ascertain the benefit of carrot as a potent dietary source of Vitamin A.

Materials and methods

Chemicals and raw materials

Fresh carrots (orange coloured variety) were purchased from local market. Acetone AR Grade was bought from Merck (Germany) while β-carotene standard from Sisco Research Laboratories Pvt. Ltd. (India).

Sample preparation

Locally purchased fresh carrots were washed thoroughly, cut into pieces, and boiled separately in potable water using three different methods commonly practiced at household level: (1) open flame boiling in open container, (2) open flame boiling in pressure cooker, and (3) boiling using microwave irradiation. In each treatment, the carrot pieces were boiled to a standard called "al dente" (to the tooth), and then homogenised using a mixer grinder. The homogenised mass (individually obtained from three different types of boiling) were equally divided into three portions. The first portion was immediately tested to observe the changes induced by the heat applications. The second and third portions were stored in refrigerator for the next 24 and 48 h, respectively and examined to observe the effect of refrigeration. Following refrigeration, reheating is a common practice to make the food consumable. Here, the second and third portions were further divided into two segments, and reheated (after thawing the mass in room temperature) with adequate stirring for a calculated period of time using open flame and microwave irradiation, respectively.

Extraction method

Acetone was used as the extracting solvent for β-carotene. After each treatment and/or interval, samples were divided into two portions; one for moisture determination and the other for determining the β-carotene content, yield of the carotenoid and the spectral study.

For moisture determination, a known amount of each treatment (≈ 2 g) was placed into a dried and weighed container, and was oven-dried at 103-105°C until a constant weight was obtained. This moisture content was used for calculating the actual weight of the samples (on dry weight basis) taken for the determinations of β-carotene content, yield of the carotenoid and the spectral study. The moisture
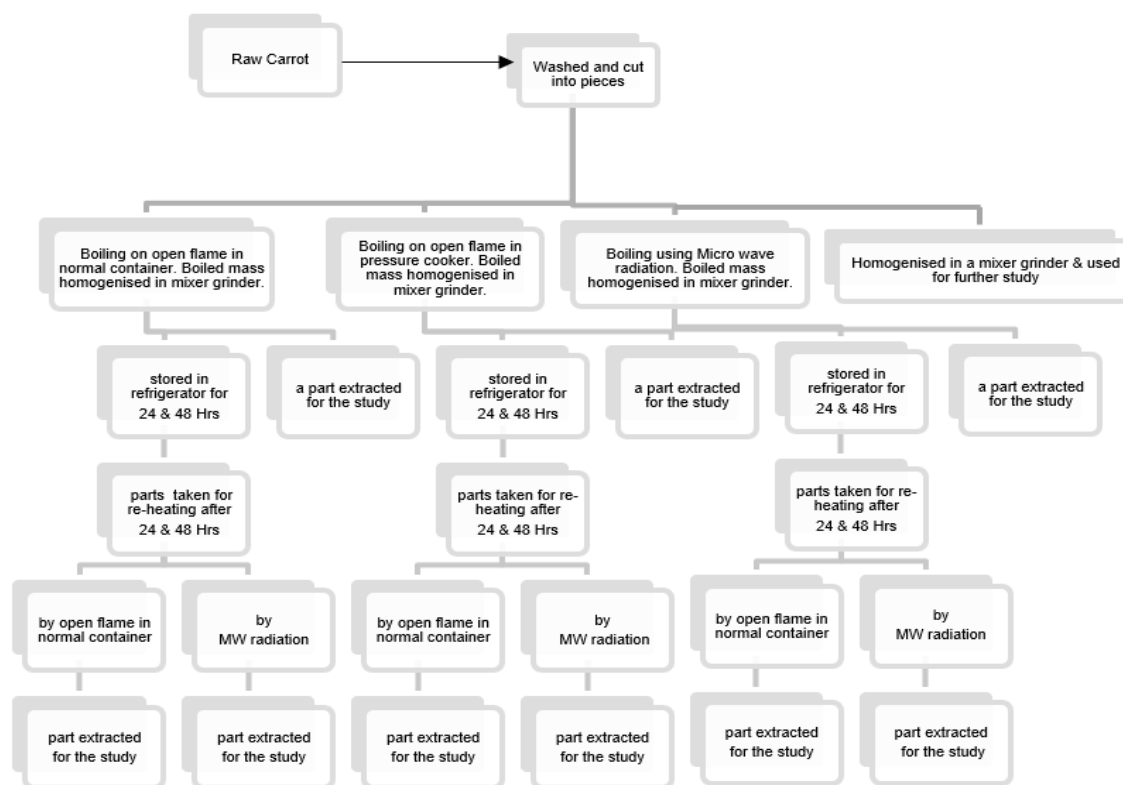
Figure 1. The work flow to study the β-carotene in carrots.

content of the samples varied from 40 to 70% at different treatment stages, while 92% for the fresh carrot sample.

For the determination of β-carotene content, yield of the carotenoid and the spectral study, a known amount of each treatment ($\approx$ 2 g) was treated with acetone (20 mL) and stirred at room temperature for about 30 min using a magnetic stirrer to extract the β-carotene. Following stirring, the contents were allowed to stand for a few minutes and most of the homogeneous supernatant liquid layer was carefully decanted off to another container. The extraction process was repeated several times till the supernatant liquid part appeared almost colourless. The liquid parts containing β-carotene were combined and filtered through a cotton bed (to remove traces of insoluble particles present) and then, most of the organic solvent in the filtrate was evaporated out by keeping the container for several hours on a thermostatically controlled water bath at temperature not more than 45-50°C. Thick liquid mass obtained was lyophilised to remove water, and the dry solid mass was weighed to determine the carotenoid yield percentage gravimetrically. Subsequently, each portion was re-dissolved in acetone, and was taken in a known volume of volumetric flask to determine the β-carotene content (in ppm) using a standard beta-β calibration curve at 454 nm, and also scanned to note the spectral data within the wavelength range from

325-600 nm using a spectrophotometer (Unicam 300, Thermospectronics).

Fresh sample

A known weight of the mashed sample (without boiling; control) was taken for the determinations of total carotenoids yield, β-carotene content, spectral study as well as the moisture content.

The work flow of the present work is depicted in Figure 1.

Result and discussion

Boiling is a common thermal-process applied to raw foods by introducing moist heat to soften the food matrix. Three ways of boiling were selected in the present work; open flame boiling in open containers (gas oven: OF), open flame boiling in closed containers (pressure cooker: PC) and microwave irradiated boiling (MW). Since cooked foods are not all consumed in one time, the leftovers are usually refrigerated, and reheated when the time comes for re-consumption. This was re-enacted in the present work's experimental design.

The UV-vis spectrum with λ_{max} values for each extract revealed the presence of β-carotene. Further, to confirm the presence of β-carotene by the spectral fine structure, the %III/II ratio analysis was done i.e., height of longest-wavelength absorption peak (III) to that of the middle absorption peak (II), taking the

Table 1. Variations of β-carotene content (ppm) of carrot during boiling processes.

Before Treatment (fresh)	Treatment Process (boiling)	Time required for complete boiling (100 g)	Initial β-carotene content	Refrigerated Storage and Re-heating			
						Reheating	
					Freezing	Micro Wave	Open Flame
963.13	Microwave irradiated boiling	15 min	1393.95 (+44.73)	24 h	708.21 (-49.19)	459.36 (-67.05)	918.13 (-34.19)
				48 h	949.89 (-31.86)	865.73 (-37.89)	944.72 (-32.23)
	Open flame boiling	18 min	1281.29 (+33.03)	24 h	1029.17 (-19.68)	564.35 (-55.95)	639.19 (-50.11)
				48 h	1092.69 (-14.72)	1037.38 (-19.04)	1137.82 (-11.20)
	Pressure cooker boiling	12 min	1275.32 (+32.41)	24 h	1114.73 (-12.59)	1110.22 (-12.95)	757.35 (-40.61)
				48 h	1015.87 (-20.34)	1174.29 (-7.92)	972.90 (-23.71)

Parentheses denote the loss/gain % of β-carotene content after treatments.

Table 2. Variations of wavelength (UV-vis spectrum) of β-carotene during treatments with % III/II ratio of absorption peaks.

Before Treatment (fresh)	Treatment Process (boiling)	Time required for complete boiling (100 g)	Refrigerated Storage and Re-heating			
			Initial	Freezing	Reheating	
					Microwave	Open Flame
428, 450, 476 (17.9)	Microwave irradiated boiling	15 min	427, 450, 474 (15.6)	24 h 345, 426, 449, 474 (13.0)	423, 449, 476 (16.7)	425, 450, 477 (16.7)
				48 h 345, 426, 449, 475 (16.7)	343, 429, 449, 474 (13.6)	342, 427, 449, 475 (13.6)
	Open flame boiling	18 min	427, 450, 474 (15.2)	24 h 344, 425, 448, 474 (11.5)	425, 449, 477 (9.4)	424, 449, 477 (9.4)
				48 h 343, 427, 449, 474 (11.8)	345, 429, 449, 475 (14.6)	345, 429, 449, 475 (14.3)
	Pressure cooker boiling	12 min	427, 450, 474 (15.8)	24 h 345, 425, 449, 473 (18.2)	425, 449, 477 (7.9)	424, 449, 477 (8.8)
				48 h 345, 428, 449, 475 (13.9)	427, 448, 473 (9.4)	427, 449, 472 (10.71)

Light grey denotes the λ_{max} of *trans*-β-carotene
Dark grey denotes the λ_{max} of *cis*-β-carotene
Parentheses denote the %III/II ratio, which depicts the height of longest-wavelength absorption peak (III), to that of the middle absorption peak (II), taking the min between the two peaks as the baseline, multiplied by 100.(ref: % III/II β-carotene in acetone as solvent = 15; Briton,1995 and Davis (1996)

minimum between the two peaks as the baseline, multiplied by 100.(ref: % III/II β-carotene in acetone as solvent = 15; Briton,1995). The results revealed the ratio to be around 15 for most of the samples, though some were below or above the prescribed value (7.9-18.2). The absence of other unsubstituted carotenes was established on the basis of this %III/II ratio values. The absence or non-formation of epoxy carotenoids, hydroxy carotenoids or apocarotenoids was ascertained using the spectroscopic data and TLC studies.

Initial boiling of carrot revealed remarkable

increase in the β-carotene contents (Table 1) with a usual spectral characteristic (Table 2) of all *trans*-β-carotene. The basic concept of carotenoid retention and further carotenogenesis lies in the fact that blanching or any kind of thermal stimulation results in an increase in the β-carotene content, perhaps because of greater chemical extractability due to inactivation of certain oxidative enzymes which results in the breakdown of some structures leading to a higher bio-availability of β-carotene. It has been shown that (Anderson *et al.*, 1978; Braumam et al. 1982; Grimme and Brown 1984; Guerra–Vargas *et al.*, 2001) carotenoids in plants are bound by protein. Heat treatment such as blanching, cooking and steaming help to release bound carotenoids and render them easily extractable hence the β-carotene content increased in the present work as summarised in Table 1.

The effects of OF and PC boiling on β-carotene content were almost comparable (+33.03 and +32.41% increment, respectively: Table 1) while the MW boiled samples were found to have greater β-carotene concentration (+44.73%; Table 1). As the working principle of microwave is to heat up the food molecule wholly, the efficacy of electro-magnetic microwave to intervene interstitial space of food matrix and to denature the protein is more profound than the other two methods.

Although the β-carotene bio-availability in carrot from raw to final consumption yielded variable results (Table 1 and Table 2); the nature of UV-vis spectra remained almost unchanged (Table 2). The MW boiling of raw sample yielded maximum β-carotene (1,393.95 ppm) from the initial value of 963.13 ppm. Post refrigeration products yielded lesser values. It further decreased on first day reheating (459.36 ppm).

The β-carotene contents yielded better results on reheating due to OF (Table 1). The OF boiled product showed lesser degradation in two days' refrigeration. While, in this case, MW reheating depicted lesser values and OF reheating yielded good result post 2-day refrigeration (1,137.82 ppm). The OF pressure cooked product showed almost comparable values following refrigeration and MW reheating but lesser values after further reheating by OF. Therefore, MW boiling followed by refrigeration and reheating by OF is recommended because while the open flame boiling product retained the β-carotene to the maximum after reheating by OF, the MW assisted reheating is favourable in case of PC boiled sample (Table 1).

The leftover cooked foods are most of the time refrigerated for future consumption. The aim of the present work was also to monitor the changes induced by refrigeration after 24 and 48 h to differently boiled carrot samples. A clearly comparable result, as compared to previous study (Dey *et al.*, 2015) was found in the present work (Table 2). Reheating following refrigeration is essential prior to consumption. The results obtained (Table 1) show a remarkable degradation of β-carotene in all the samples. The loss during MW reheating following 24 h refrigeration was more profound in the case of MW and OF boiled samples. During reheating the sample, the action of MW and OF became more prominent on the samples which might be due to the lack of surface water layer which used to be there while boiling for the first time (initial treatment). The structural degradation might have contributed to greater loss of β-carotene. The lipid soluble carotenoid fractions remained complexed with the protein in the carrot samples which might get condensed during

Table 3. Variations of approximate Vitamin A content (IU) per gram of carrot during boiling processes.

Before Treatment (fresh)	Treatment Process (boiling)	Time required for complete boiling (100 g)	Initial	Refrigerated Storage and Re-heating			
				Freezing		Reheating	
						Micro Wave	Open Flame
1608.21	Microwave irradiated boiling	15 min	2327.90	24 h	1182.71	767.13	1533.28
				48 h	1586.32	1445.77	1577.68
	Open flame boiling	18 min	2139.75	24 h	1718.71	942.46	1067.45
				48 h	1824.79	1732.42	1900.16
	Pressure cooker boiling	12 min	2129.78	24 h	1861.60	1854.07	1264.77
				48 h	1696.50	1961.06	1624.74

Vitamin A: 1 IU is the biological equivalent of 0.3 mcg retinol, or of 0.6 mcg β-carotene. Based on NIH, Office of Dietary Supplements, Dietary Supplement Ingredient Database, USDA

continuous refrigeration for two consecutive days. The application of heat on refrigerated sample might have freed the lipid fraction from the protein complex by denaturing it thereby increasing its concentration in the food matrix. Throughout the entire work the characteristic three peak spectrum of all *trans*-β-carotene (having a λ_{max} value of 450 nm) has not been altered much, following different techniques, time and temperature employed in the experiment (Table 2) (Figure 2).

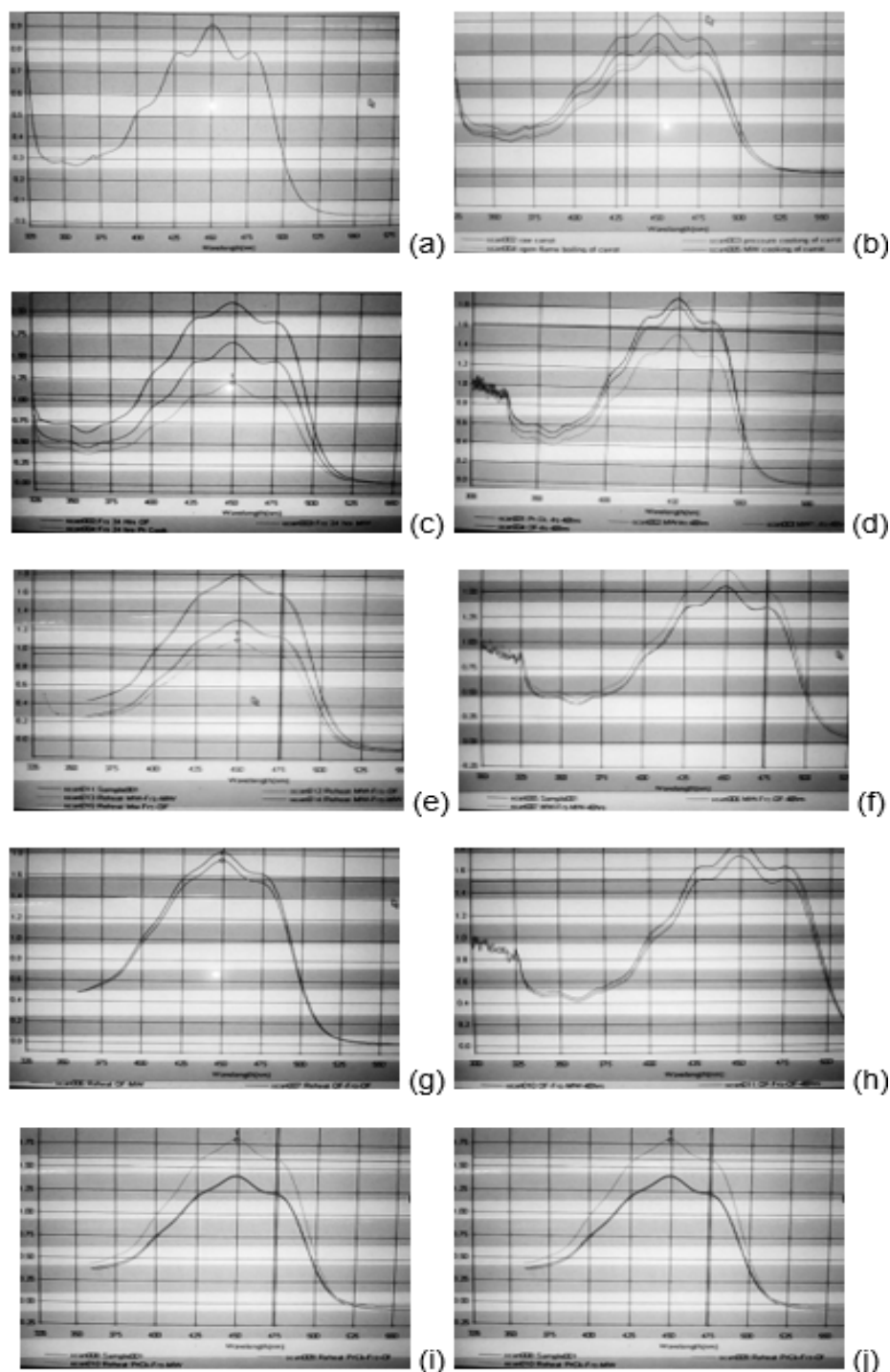The U.S. Food and Drug Administration has established a vitamin A Daily Value (DV) of 5,000

Figure 2. UV-vis spectrum of β-carotene after several treatments: (a) standard curve of β-carotene, (b) raw carrot and initial boiling by PC, MW, OF, (c) 24 h refrigeration, (d) 48 h refrigeration, (e) Reheating of MW boiled product after 24 h refrigeration, (f) Reheating of MW boiled product after 48 h refrigeration, (g) Reheating of OF boiled product after 24 h refrigeration, (h) Reheating of OF boiled product after 48 h refrigeration, (i) Reheating of PC boiled product after 24 h refrigeration, and (j) Reheating of PC boiled product after 48 h refrigeration.

IU from a varied diet of both plant and animal foods required for adults and children aged 4 years and older. The following table (Table 3) furnishes the available vitamin A (in IU) from carrot due to β-carotene conversion, as a result of several cooking and reheating conditions. While the MW boiled carrot sample yielded the highest vitamin A (in IU), further refrigeration and reheating lessened its bio-availability.

The spectrum of carrot extract of raw and the initial boiling showed similar nature (Figure 2b) with all *trans*-peak detected at 449 nm. The spectrum of β-carotene showed slight development of *cis*-peak after 48 h refrigeration and reheating of MW and OF boiled samples after 48 h which pointed out that the bio-availability of β-carotene started to decrease after prolonged refrigeration and reheating (Figure 2c to Figure 2h). The spectral analysis of reheated sample did not depict the development of *cis*-peak in case of PC boiled carrot sample (Figure 2i to Figure 2j).

Conclusion

Provitamin A carotenoids from various foods have been shown to have an almost 8-fold difference in β-carotene conversion factors (on a weight basis) ranging from 3.6:1 to 28:1 and thus have different values in terms of vitamin A nutrition. The major factor that affects the vitamin A value of plant provitamin A carotenoids is the food matrix. It should be noted that human subjects might have different abilities to convert provitamin A carotenoids to vitamin A. These differences in conversion efficiency might be due to the genetic variability in β-carotene metabolism of individual human subject. Therefore, provitamin A carotenoids might not be a good vitamin A source for those subjects of the poor converter phenotype. However, the present work suggests that, while the MW boiled raw carrot yielded the highest vitamin A (in IU), further refrigeration and reheating lessened its bio-availability. Dietary provitamin A-carotenoids to vitamin A conversion factors can be used in the development of dietary guidelines in well-nourished populations, and ultimately used to help combat vitamin A deficiency worldwide. Future studies on various plant-based foods, which include staple foods rich in provitamin A carotenoids, will be needed to both discover and evaluate vitamin A rich sources of plant foods.

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