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Carotenoids retention in leafy vegetables based on cooking methods

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Abstract

Green leafy vegetables are good sources of carotenoids. Generally, food composition databases related to raw foods are available, but data on cooked foods in Malaysia are still lacking. Since carotenoids are prone to degradation during cooking processes, the present study was undertaken to evaluate the extent of nutrient loss in vegetables subjected to two conventional cooking methods, which were boiling and stir-frying with cooking durations of 4 and 8 minutes. The vegetables selected were Chinese cabbage (*Brassica Pekinensis* var. *cephalata*), swamp cabbage (*Ipomoea aquatica*), spinach (*Spinacia oleracea*), Ceylon spinach (*Basella rubra*), red spinach (*Amaranthus gangeticus*), white spinach (*Amaranthus viridis*) and tapioca shoots (*Manihot utilissima*). Percentage losses of nutrients after cooking treatment were calculated based on retention factors. Results obtained showed that stir-frying had reduced lutein content for all vegetables ranging from 8-89% while the effect of boiling for lutein varied (0-428%) with different vegetables at both cooking durations of 4 and 8 min. Boiling for 8 min increased retention of β-carotene in all vegetables ranging from 18-380% except for Chinese cabbage and spinach compared with 4 min, while stir-frying generally increased the retention of β-carotene for all vegetables 2-3 times except for spinach. Cooked vegetables have variations in carotenoids composition brought by varying cooking conditions (time and temperature), type of vegetables and the interaction between cooking methods and type of vegetables.

Introduction

Many vegetables and fruits contain significant amounts of β-carotene and other provitamin A carotenoids that can be absorbed and converted to vitamin A in the human body. In many developing countries, the largest contribution of vitamin A intake comes from the provitamin A carotenoids in plant foods, which may contribute up to 82% of the total vitamin A intake, whereas the contribution from fish and meat is of minor importance, because these foods are expensive and/or are not accessible (van den Berg *et al*., 2000). Malaysians mostly consume green vegetables such as Chinese mustard leaves, Chinese kale, lettuce, spinach and swamp cabbage (Amin and Cheah, 2003; Wen *et al*., 2010). According to the Nutrient Composition of Malaysian Foods (Tee *et al*., 1997), these green vegetables have been found to contain about 1825-4760 μg of β-carotene/100g edible portion. Food balance sheet data for Malaysia showed that per capita of vegetables supply per year was 37.60 kg (FAO, 2005). Mubarik (1996) reported that Malaysians consumed 4.65 kg cabbage, 2.90 kg swamp cabbage, 2.45 kg spinach and 0.77 kg kale in 1991.

Commonly, vegetables are prepared at home on the basis of convenience and taste preference rather than nutrient losses. Researchers have reported that 5 to 78% of the β-carotene degraded when vegetables were prepared using different cooking methods (Speek *et al*., 1988; Rahman *et al*., 1990; Vimala *et al*., 2011). Considerable quantities of carotenoids needed by individuals may be lost during household cooking of vegetables (Masrizal *et al*., 1997). Thus, information on the possible losses of carotenoids from vegetables, during traditional cooking methods, is of major importance (Gayathri *et al*., 2004). Several reports have documented the losses of β-carotene after boiling, stewing, frying, blanching, and pressure cooking (Sehgal and Yadav, 1995; Vimala *et al*., 2011).

It is often emphasized that databases on nutrient

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content should be on foods as consumed. However, most of the reported data are on raw foods, although many are eaten cooked. Hence, this will lead to overestimation or underestimation 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. However, this approach is expensive, difficult and time-consuming, and need trained analysts. Thus, methods of estimating the concentrations of nutrients or other food components from the raw food data have been proposed (Rodriguez-Amaya and de Sa, 2004). Data on weight yield after preparation of dish are mostly not included in the cookbook recipes (Bognar and Piekarski, 2000). As it is hard to analyze every dish and other prepared food items, calculation on nutrient content is a preferred method of choice (Bognar and Piekarski, 2000).

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 verify the factors that enhance or reduce degradation. It can then be recommended to minimize losses of nutrients in foods (Rodriguez-Amaya and de Sa, 2004). Hence, this will provide guidance in healthy food preparation methods that did not deplete nutrient content in vegetables (Leskova *et al*., 2006). Indirectly, this also contribute in recommending healthy cooking methods to the general public.

The objective of the present study was to determine the effect of different domestic cooking methods on carotenoids retention in commonly consumed Malaysian leafy vegetables. Lutein and β-carotene were chosen for the present studies since they form the main carotenoids contributing to the health benefits of Vitamin A in human. The study was also aimed to compare the effect of boiling and stir-frying at different cooking durations (4 and 8 min) on raw and cooked Malaysian leafy vegetables.

Materials and Methods

Plant material

Seven types of green leafy vegetables were selected based on popular consumption among Malaysians. They were Chinese cabbage (*Brassica Pekinensis* var. *cephalata*), swamp cabbage (*Ipomoea aquatica*), spinach (*Spinacia oleracea*), Ceylon spinach (Basella rubra), red spinach (*Amaranthus gangeticus*), white spinach (*Amaranthus viridis*) and tapioca shoots (*Manihot utilissima*). These specimens were authenticated by a taxonomist and voucher specimens were preserved in our laboratory. Five kilogram of each type of vegetables was purchased from two stalls located in the retail market of Selangor state, near to the campus of Universiti Putra Malaysia.

Sample preparation

Upon arrival at the Department of Nutrition and Dietetics laboratory, the fresh and healthy vegetables were immediately washed under tap water and excessive water dripped off. The vegetables were pat dried with towel. Edible portions (100 g) of the vegetables were mixed well after the inedible parts and stems were removed. One kilogram was taken and divided into three portions with 300 g for each application. One portion was retained raw and others were cooked in different methods, which were boiled and stir fried. The edible portion was homogenized using a blender (National; model MX-291N) for 3 min. The homogenized sample was transferred into an air-tight container and kept at -20°C before vitamin analysis. All procedures were carried out carefully without much exposure to light, oxygen and atmospheric pressure (Amin and Cheah, 2003). Precautions (samples in ice, darkened room) were taken not to destroy the nutrients during all analyses (Masrizal *et al*., 1997). All the chemicals and reagents used were of analytical grades or otherwise stated.

Cooking methods

Stir-frying

Five gram of palm oil was placed on a non-stick pre-heated wok. Then, vegetables were put into the wok, stir-fried for 20s, covered for 10s. The procedure was repeated sequentially until 4 or 8 min. Then, the samples were cooled to room temperature, drained off for 30s and the weight was recorded. Then, the samples were stored at -20°C before analysis.

Boiling

Hundred gram of vegetable was added to 500 ml of water that has just reached the boil in a stainless steel pot, cooked for 4 or 8 min. After that, the samples were cooled to room temperature, drained off for 30s and the weight was recorded. The samples were stored at -20°C before analysis. The duration of 4 and 8 min were chosen so that the vegetables won't subject to undercooking or over cooking.

Extraction procedures for carotenoids

The β-carotene and lutein were extracted according to the method AOAC (1984) with some modifications as described by Tee *et al*. (1996). The sample (10 g) was added with 40 ml of 100% ethanol and 10 ml of 100% (w/v) potassium hydroxide, with 0.1% BHT (butylated hydroxytoluene) as antioxidant and homogenized for 3 min. For the extraction step,

the mixture was transferred into a separation funnel and 50 ml of n-hexane was added. The funnel was inverted, vented and then shaken vigorously for a few seconds, and the layers of organic solvent was re-extracted twice, each time with 50 ml of n-hexane. Then, the upper layer was pooled and washed with distilled water at least 5 times until free of alkali. Phenolphthalein solution (1%) was used to check for any alkali present. The presence of alkali turns this indicator into pink. The extract was then filtered through anhydrous sodium sulphate to remove any water residue. The hexane residue was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Germany). The resulting extract was diluted to 10 ml with dichloromethane. All samples were carried out at least in duplicate.

Analysis of β-carotene and lutein in leafy vegetables by HPLC method

Raw and cooked vegetables were homogenized 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) technique at 21°C. A Hewlett Packard HPLC Agilent Series 1100, (Santa Clara, USA) equipped with degasser, quaternary pump, autosampler and diode array detector was used. An YMC C-30 column (5.0μm, 250 × 4.6 mm I.D.) (Tokyo, Japan) was used to determine the carotenoids. The solvent used was 1-butanol : acetonitrile (30:70) (A) and dichloromethane (B) with gradient elution 99% A and 1% B with the following gradient elution: 99% A and 1% B initially, increased to 4% B in 20 min, 10% B in 50 min and returned to 1% B in 55 min. The flow rate was 2.0 ml/min with detection at 450 nm. Acetonitrile contained 0.05% or triethylamine functioning as a modifier, as recommended by Hart and Scott (1995), to improve carotenoids recovery from the chromatographic column. A UV-visible photodiode-array detector was used, detection being at the wavelengths of maximum absorption.

Quantification of β-catotene was done by plotting the peak area of authentic standard versus concentration. Five concentrations of 5, 10, 15, 25 and 100 μg/ml of all-trans-β-carotene (Sigma Co. Chemical, St. Louis, USA) were used to prepare the standard curve. Duplicate analyses were performed and the mean value was determined. The regression equation and correlation coefficient (r^2) were obtained using Microsoft Excel 2007 software. A high correlation coefficient (r^2 >0.99) was achieved for all-trans-β-carotene (y=19.908x – 10.73). Lutein concentrations were calculated by using the formula proposed by Rodriguez-Amaya and Kimura (2002):

$$C_x\ (\mu g/g) = [A_x \times C_s\ (\mu g/ml) \times \text{total volume of extract (ml)}] / [A_s \times \text{sample weight (g)}]$$

where C_x is the concentration of lutein/β-carotene, A_x is the peak area of lutein/β-carotene, C_s is the concentration of the standard and A_s is the peak area of the standard. The peak of β-carotene and lutein of the samples were identified based on two techniques: comparing the retention time and spiking test with that of all-trans-β-carotene and all-trans-lutein (Sigma, Co. Chemical, St. Louis, USA).

Quality control of the analytical methods

Method validation was checked with regard to accuracy, linearity and precision. The recovery was checked by addition of known amounts of carotenoid standards to the studied vegetable extract. To determine the reproducibility, the vegetable sample was extracted at least two to three times for the two methods. The coefficient of variance (CV) was below 10%. CV for determination of carotenoids between the the two independent extractions was below 10% for all the samples analyzed. The recovery analysis was carried out by addition of 100 μg/ml all-trans-β-carotene and 10 μg/ml all-trans-lutein separately to the raw vegetable samples, which all carotenoids were removed earlier, where the vegetable sample was bleached by using hexane and dichloromethane. The recovery percentage of β-carotene was calculated using the formula below:

$$\%\ \text{Recovery} = [CF/CA] \times 100\%$$ (Lin and Chen, 2003)

where CF = concentration of carotenoids measured, CA = concentration of carotenoids added

True retention of carotenoids and nutrient losses

The true retention (TR) defines 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. It is therefore adjusted for the weight loss or gain during processing in the calculations (Murphy *et al*., 1975). The true retention was calculated as the following formula

$$\%\ TR = [(\text{nutrient content per g of cooked food} \times \text{g of food after cooking}) / (\text{nutrient content per g of raw food} \times \text{g of food before cooking})] \times 100$$

The value for vitamin A activity in the total cooked portion of vegetables was adjusted to 100 gram vegetable material accounting the weight loss during cooking, but not the possible effect of oil or water absorbed into the vegetable material. The

values for the cooked samples per 100 gram vegetable material were comparable to the raw samples. The nutrient content was then corrected by taking into account nutrient losses for carotenoids (Kidmose *et al*., 2006).

The weight of the vegetables subjected to cooking was weighed using a commercial food weighing instrument. The weight yield factor was calculated using the formula as

weight yield factor = [(weight of sample after cooking / weight of raw sample)] × 100

Statistical analysis

Data were expressed as mean value ± standard deviation of which all experiments were repeated three times. SPSS version 17 (SPSS Inc., Chicago, IL, USA) was used to perform one way analysis of variance (ANOVA) followed by Bonferroni's test for comparison, as a post hoc test, at a 95% confidence level ($p<0.05$) to determine differences among groups.

Results and Discussion

Weight yield of vegetables

Weight yield of the cooked vegetables is given in Table 1. The yield values of each vegetable varied, significantly ($p < 0.05$), by cooking method and the type of vegetables. Boiled vegetables had the highest mean yield values, due to absorption of water in boiling while stir-fried vegetables had the lowest mean yield values, due to loss of water through evaporation after stir-frying. Spinach, swamp cabbage and tapioca shoot showed an increment in weight yield while Chinese cabbage, white spinach and red spinach showed reduction of weight yield after boiling for 8 minutes. All these could be due to the different leafy matrices of respective vegetables. The weight yield factor for each vegetable sample was also shown in Table 1.

Lutein and β-carotene contents of vegetables

The percentage of mean recovery of β-carotene for all the raw vegetable samples analyzed using HPLC were reported as 83.3%, 80.1%, 82.6%, 87.4%, 90.1%, 94.3% and 92.7% while the percentage of mean recovery of lutein were reported as 70.2%, 74.3%, 78.7%, 79.3%, 84.7%, 85.6% and 86.1% for swamp cabbage, Chinese cabbage, Ceylon spinach, tapioca shoots, red spinach, spinach and white spinach respectively.

Table 2 and 3 showed the lutein and β-carotene contents of the selected vegetables, after boiling and stir-frying for 4 and 8 minutes respectively. Spinach was a good source of lutein while other vegetables showed very low values. Spinach, Ceylon spinach and tapioca shoots are good sources (>1000 µg/100g) of β-carotene. Rodriguez-Amaya and de Sa (2004)

Table 1. Weight yield, percentage of weight loss or gain and weight yield factor of various vegetables after cooking treatments

Vegetables (100g)	Treatment	Lutein (µg/ 100g edible portion) [a]	Concentration change (%CC)	True Retention of lutein (%)
Chinese Cabbage (*Brassica pekinensis var. cephalata*)	Raw	70 ± 1.2		
	SF4	139 ± 6.1*	97.6	138.3
	SF8	14 ± 0.7*	-80.2	9.9
	B4	6 ± 0.0*	-91.9	11.8
	B8	Trace*	-100	0
Swamp Cabbage (*Ipomoea aquatica*)	Raw	53 ± 0.5		
	SF4	61 ± 0.2*	15.7	94.5
	SF8	26 ± 0.0*	-51.8	34.5
	B4	31 ± 1.5*	- 40.7	78.8
	B8	90 ± 1.3*	68.9	241.5
Spinach (*Spinacia oleracea*)	Raw	495 ± 1.4		
	SF4	154± 2.8*	-68.9	28
	SF8	124 ± 1.9*	-74.9	20
	B4	428 ± 0.5*	-13.5	99.4
	B8	140 ± 2.4*	-71.8	35.3
Ceylon Spinach (*Basella rubra*)	Raw	107 ± 0.0		
	SF4	98 ± 0.0	-8.42	73.3
	SF8	32 ± 3.2	-69.9	19.5
	B4	21 ± 1.3	-80.5	14.7
	B8	134 ± 20.6	24.6	58.4
Red Spinach (*Amaranthus gangeticus*)	Raw	74 ± 0.5		
	SF4	8 ± 0.9*	-88.7	6.8
	SF8	50 ± 11.6*	-31.6	27.3
	B4	103 ± 1.5*	40.1	168.1
	B8	153 ± 6.6*	107.7	226.4
Tapioca Shoots (*Manihot utilissima*)	Raw	134 ± 1.9		
	SF4	26 ± 0.1*	-80.8	34.9
	SF8	99 ± 0.0 *	-26.4	44.2
	B4	230 ± 0.7 *	71	213.8
	B8	388 ± 0.4*	188.8	418.8
White Spinach (*Amaranthus viridis*)	Raw	59 ± 1.3		
	SF4	125 ± 19.4*	111.4	153.5
	SF8	28 ± 0.5	-52.8	18.8
	B4	110 ± 0.0*	86.8	242.8
	B8	235 ± 21.5*	297.9	417.9

SF4 = stir-fried 4 minutes; SF8 = stir-fried 8 minutes; B4 = Boiling 4 minutes; B8 = Boiling 8 minutes.
↑ Increment , ↓ Reduction

Table 2. Lutein content of vegetables and its retention after cooking

Vegetables (100g)	Treatment	Lutein (µg/ 100g edible portion) [a]	Concentration change (%CC)	True Retention of lutein (%)
Chinese Cabbage (*Brassica pekinensis var. cephalata*)	Raw	70 ± 1.2		
	SF4	139 ± 6.1*	97.6	138.3
	SF8	14 ± 0.7*	-80.2	9.9
	B4	6 ± 0.0*	-91.9	11.8
	B8	Trace*	-100	0
Swamp Cabbage (*Ipomoea aquatica*)	Raw	53 ± 0.5		
	SF4	61 ± 0.2*	15.7	94.5
	SF8	26 ± 0.0*	-51.8	34.5
	B4	31 ± 1.5*	- 40.7	78.8
	B8	90 ± 1.3*	68.9	241.5
Spinach (*Spinacia oleracea*)	Raw	495 ± 1.4		
	SF4	154± 2.8*	-68.9	28
	SF8	124 ± 1.9*	-74.9	20
	B4	428 ± 0.5*	-13.5	99.4
	B8	140 ± 2.4*	-71.8	35.3
Ceylon Spinach (*Basella rubra*)	Raw	107 ± 0.0		
	SF4	98 ± 0.0	-8.42	73.3
	SF8	32 ± 3.2	-69.9	19.5
	B4	21 ± 1.3	-80.5	14.7
	B8	134 ± 20.6	24.6	58.4
Red Spinach (*Amaranthus gangeticus*)	Raw	74 ± 0.5		
	SF4	8 ± 0.9*	-88.7	6.8
	SF8	50 ± 11.6*	-31.6	27.3
	B4	103 ± 1.5*	40.1	168.1
	B8	153 ± 6.6*	107.7	226.4
Tapioca Shoots (*Manihot utilissima*)	Raw	134 ± 1.9		
	SF4	26 ± 0.1*	-80.8	34.9
	SF8	99 ± 0.0 *	-26.4	44.2
	B4	230 ± 0.7 *	71	213.8
	B8	388 ± 0.4*	188.8	418.8
White Spinach (*Amaranthus viridis*)	Raw	59 ± 1.3		
	SF4	125 ± 19.4*	111.4	153.5
	SF8	28 ± 0.5	-52.8	18.8
	B4	110 ± 0.0*	86.8	242.8
	B8	235 ± 21.5*	297.9	417.9

[a] Mean ± standard deviation. *For each vegetable sample, value indicated are significantly different ($p <0.05$).
SF4 = stir-fried 4 minutes; SF8 = stir-fried 8 minutes; B4 = Boiling 4 minutes; B8 = Boiling 8 minutes.

Table 3. β-carotene content of vegetables and its retention after cooking

Vegetables (100g)	Treatment	β-carotene (µg/ 100g edible portion)[a]	Concentration change (%CC)	True Retention (%TR) β-carotene
Chinese Cabbage	Raw	356 ± 1.4		
(*Brassica*	SF4	1263.5 ± 2.1*	254.9	248.4
pekinensis var.	SF8	210.5 ± 4.5*	-40.9	41.4
cephalata)	B4	25 ± 0.5 *	-93	10.1
	B8	64.75 ± 1.8*	-81.8	18.2
Swamp Cabbage	Raw	138.4 ± 5.8		
(*Ipomoea aquatica*)	SF4	309.3 ± 2.3*	123.5	178.8
	SF8	619 ± 1.4 *	347.2	313
	B4	336.35 ± 6.2*	143	315.9
	B8	376.35 ± 12.9*	171.9	380.7
Spinach (*Spinacia*	Raw	6176.5 ± 65.8		
oleracea)	SF4	2206.5 ± 28.9*	-64.3	32
	SF8	1448 ± 1.4*	-76.5	18.8
	B4	3123 ± 1.4*	-49.4	58.2
	B8	6193 ± 349.3	0.27	125.3
Ceylon Spinach	Raw	1623.5 ± 4.9		
(*Basella rubra*)	SF4	1276 ± 2.8	-21.4	58
	SF8	1177.75 ± 1.3	-27.4	54.2
	B4	1424.5 ± 2.1	-12.3	61.3
	B8	834.5 ± 767.2	-48.6	84.8
Red Spinach	Raw	631 ± 11.3		
(*Amaranthus*	SF4	85.8 ± 9.2*	-86.4	8.2
gangeticus)	SF8	784.55 ± 130*	24.3	49.7
	B4	440.6 ± 0.0*	-30.2	83.8
	B8	596.5 ± 22.8	-5.47	104
Tapioca Shoots	Raw	3265.5 ± 0.7		
(*Manihot*	SF4	602.8 ± 20.9*	-81.54	14.8
utilissima)	SF8	10521 ± 2.8*	222.2	193.3
	B4	1386.8 ± 8.1*	-57.53	53
	B8	2704.5 ± 21.9*	-17.2	120
White Spinach	Raw	499.4 ± 14.7		
(*Amaranthus*	SF4	1260.2 ± 12.9*	152.3	176.6
viridis)	SF8	583.9 ± 1.6	16.92	46.8
	B4	781 ± 35.4*	56.4	187.7
	B8	1163.5 ± 91.2*	132.98	244.6

[a] Mean ± standard deviation. *For each vegetable sample, value indicated are significantly different ($p < 0.05$). SF4 = stir-fried 4 minutes; SF8 = stir-fried 8 minutes; B4 = Boiling 4 minutes; B8 = Boiling 8 minutes

reported that carotenoids in raw samples, were more difficult to extract, hence the values are lower. The lutein content was in the order of spinach > tapioca shoots > Ceylon spinach > red spinach > Chinese cabbage > white spinach > swamp cabbage.

β-carotene was in the order of spinach > tapioca shoots > Ceylon spinach > red spinach > white spinach > Chinese cabbage > swamp cabbage. Our results are in contradictory with reports of Tee and Lim (1991) probably due to change in stage of maturity, growing conditions, season, agricultural and post-harvest handling practices (Hart and Scott, 1995; Rodriguez-Amaya and de Sa, 2004). According to Hart and Scott (1995), analysis of carrot showed that the outer part contained twice as much β-carotene as the inner part. The outer leaves of a savoy cabbage were found to contain 150 times more lutein and up to 200 times more β-carotene than the average for the inner portion. Consequently, the carotenoid content will depend on the extent of the 'outer' material discarded during preparation (Hart and Scott, 1995).

Effects of cooking on lutein and β-carotene contents and their retention in leafy vegetables

Lutein content in spinach and Ceylon spinach had reduced from 4 to 8 min in stir-frying. An increment in lutein content was observed in white spinach, Chinese cabbage and swamp cabbage at 4 min, then, it reduced at 8 minutes. Surprisingly, red spinach and tapioca shoots showed reduction at 4 min, then, increased at 8 minutes in stir-frying. This could be due to the conditions in stir-frying may be more drastic compared with boiling as well as leaching of carotenoids to the oil used for cooking (Rodriguez-Amaya and de Sa, 2004; Kidmose *et al*., 2006; Miglio *et al*., 2008). Similar as boiling treatment, the lutein content had increased from 168-419% for red spinach, white spinach and tapioca shoots. Boiled tapioca shoots, red spinach and white spinach were found good sources of lutein. Retention of lutein was high for all vegetables except Ceylon spinach, spinach and Chinese cabbage after boiling at 8 min. This shows a good stability of lutein during boiling, as indicated by Rodriguez-Amaya and de Sa (2003). Hart and Scott (1995) demonstrated that boiled spinach had higher lutein content than the raw ones. Granado *et al*. (1992) showed that spinach and amaranth had retention of 103-409% after boiling. Findings of the present study were in agreement with those reported by previous reports.

β-carotene content of each raw vegetable prepared at different methods was significantly higher ($p<0.05$) than cooked ones for swamp cabbage, spinach and Ceylon spinach. Effect of cooking methods and time on β-carotene in the studied vegetables were varied. Stir-frying had increased the retention of β-carotene in all vegetables 2-3 times than raw ones except for spinach and Ceylon spinach. Because of water loss, stir-frying may concentrate the carotenoids, giving higher β-carotene content per unit weight of vegetable (Rodriguez-Amaya and de Sa, 2003). Boiling for 8 min had increased retention of β-carotene in all vegetables from 18-380% except for Chinese cabbage and spinach compared with boiling for 4 minutes. 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 in green vegetables like lutein that are resistant to heat treatment (Khachick *et al*., 1992; Bernhardt and Schlich, 2006).

Masrizal *et al*. (1997) demonstrated that spinach and swamp cabbage had retained 57-79 % and 65-80 % of β-carotene respectively after boiling for 3.0-5.5 min and stir-frying for 3.0-9.42 minutes. Granado *et al*. (1992) showed that various vegetables had retained 101-344 % of β-carotene after boiling while 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. Miglio *et al*. (2008) reported that both lutein and β-carotene were negatively affected by frying while boiling resulted increase of β-carotene but 11% losses of lutein. Hence, they concluded that β-carotene had a higher thermal stability than lutein. Thus cooking at appropriate temperature and time, the cell wall might disrupt more readily and yield more extractable β-carotene (Sungpuag *et al*., 1999; Bernhardt and Schlich, 2006). The discrepancy with the other data could be due to varietal differences and cooking conditions (Rodriguez-Amaya and de Sa, 2003).

In a study carried out by Aman *et al*. (2005a) on the effects of heating on trans-cis isomerization, retention values of 84.7 and 83.5% for β-carotene and lutein were obtained. Heating significantly increased 9-cis-β-carotene content from 14.2% to 18.8% and those of 9'cis-lutein from 7.1% to 15.0% respectively, whereas the levels of 13-cis isomers remained unchanged. Heating caused increase in conversion of trans isomer to cis-isomers of β-carotene and lutein by 50% and 25% respectively. These results implied that lutein was less prone to isomerization than β-carotene. This result disagrees with other researchers who studied on pure carotenoids, which mainly suffer from 13-cis isomerization after heat treatment (Watanabe *et al*., 1999; Chen and Tang, 1998). Aman *et al*. (2005a) pointed out that the different isomeric profiles of β-carotene and lutein after heating may result from the interactions of chlorophyll in the chloroplast enhancing the formation of the 9-cis isomers. 9-cis- and 13-cis carotenoid isomers were predominantly detected in processed vegetables (Marx *et al*., 2003; Aman *et al*., 2005b).

Guo *et al*. (2008) showed that the rate of isomerization of lycopene exposed to heat and light are higher than β-carotene due to the presence of β-ionone ring in β-carotene which reduces the electron delocalization even though both lycopene and β-carotene possess 11 conjugated π-bonds. By using quantum chemistry computations, they predicted that the thermal isomerization reaction rate of mono-cis isomers and all-trans-β-carotene was in the order of 9-cis < 7-cis < 13-cis < 15-cis < 11-cis, where 9-cis isomers was the most commonly found and the most stable isomer *in vivo* or in various foods after heat processing. The stability of 9-cis isomers of β-carotene and lycopene might have important physiological implications due to their antioxidative ability to prevent oxidation.

A variation in lutein and β-carotene retention could be due to type of vegetables, cooking method, and the interaction between type of vegetable and cooking method (Kidmose *et al*., 2006; Vimala *et al*., 2011). Hence, these factors could affect β-carotene retention in the studied vegetables. More than 100% true retention values obtained did not mean true increases of carotenoid content. These values showed the difficulty in evaluating retention of carotenoids during cooking (Rodriguez-Amaya and de Sa, 2004). The overestimation of the retention of carotenoids during cooking or processing of foods was due to the greater ease with which carotenoids are extracted from cooked or processed samples (Rodriguez-Amaya, 1999). Published data on carotenoid composition of vegetables and fruits are limited and absolute comparison with other published reports is difficult because of the variations. This includes the reliability of the data (Hart and Scott, 1995).

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 (Rodriguez-Amaya and de Sa, 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 (Sungpuag *et al*., 1999; Miglio *et al*., 2008).

The limitations of the study were as followed: the data presented does not represent the vegetables consumed throughout Malaysia. Besides, vegetables 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. Other than that, spices or flavorings were not added as it might pose some analytical problems. Hence, it should be added in future research so that the results would be representative of the common practices in the general public. Due to the unique characteristics of each vegetable, there are different choices for the cooking process and each one has certain characteristics that will affect the cooking behavior and the final foodstuff quality and functionality (Miglio *et al*., 2008). 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 Santos and Silva (2008), 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. Leskova *et al*. (2006) pointed out that continual changes of nutrient content in food 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 vegetables. Besides that, Heredia *et al*. (2009) conducted microscopic observation of fresh and osmodehydrated tomato tissue in order to analyze the effect of structural changes on carotenoids distribution. Perhaps this technique should be practice to understand the carotenoids changes more effectively in future studies. Difficulties in analysis are due to qualitative and quantitative variations of the carotenoid composition of foods, wide concentration range among carotenoids in a given food, uneven distribution of carotenoids within a sample and between samples of a given food and variation in the nature of the matrix (Rodriguez-Amaya, 1999). The studied data on nutrient contents in vegetables will be included in the national food composition tables. It would be necessary to carry out a greater number of analyses for each vegetable, from different geographical sources and at different times of the year so that the data produced will be representative (Khachick *et al*., 1992).

Comparison of analyzed and calculated carotenoid concentrations in cooked vegetables

The carotenoids content of the raw and cooked vegetables, the percentage of true retention (% TR), and yield factors are shown in table 2 and 3. Values obtained by multiplying the concentration of the raw vegetable with the % CC were the same as those obtained from laboratory analyses. Furthermore, by dividing the carotenoids value of raw vegetable from multiplication with % TR by yield factors will provide the same carotenoid concentrations resulting from analyses (USDA, 2003).

According to the USDA (2002), the nutrient content of cooked food (V_c) can be obtained by multiplying the nutrient content of the raw food (Vr) by the nutrient retention factor (RF) and yield factors, using the following formula : $V_C = (V_r \times RF) / Y_C$. Y_C is the yield of cooked food and is equal to the weight of cooked food divided by the weight of raw food (Rand *et al*., 1991). Since RF is true retention proposed by Murphy *et al*. (1975), dividing by Yc will cancels out weight of the food after cooking and weight of the food before cooking in the formula for RF, the resulting formula being equal to % CC in the present study.

The present study shows that raw vegetable data are correctly transformed to cooked vegetables data using the % CC or the % TR together with yield factors. Calculation of the nutrient concentrations in cooked foods from the raw sample data is thus a valid alternative to laboratory analysis, provided that accurate raw sample data and representative average percentages of concentration change (or retention percentages and yield factors) are available.

Conclusions

There were variations between lutein and β-carotene retention in the vegetables studied with cooking methods and type of vegetable. The present study exhibits direct evidence that data from raw vegetable were correctly transformed to cooked vegetables after considering the percentage of concentration changes (% CC) or the percentage of true retention (% TR) together with yield factors. The % TR was the correct index for assessing degradation of carotenoids during cooking or processing. Future research on kinetic parameters describing vitamins decomposition during cooking should be carried out besides microscopic observation of fresh and cooked vegetables. Cooking affected the carotenoids content of vegetables depend on the vegetable, types of cooking method and interaction between cooking method and types of vegetable.

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