

Annals Of Agric. Sc., Moshtohor.
Vol. 44(2): 601-614, (2006).

**INFLUENCE OF RECIPE COMPONENT DURING COOKING FOR
SOME VEGETABLES ON THE RETENTION OF B-CAROTENE AND IN
VITRO DIGESTABILITY.**

BY

El-Sherefa, F.A. A.

Food Techno. Res. Inst. Agric. Res. Center, Giza, Egypt.

ABSTRACT

The objective of the present study was to evaluate the effect of cooking on the retention and the in vitro accessibility (available for absorption) of β -carotene and lycopene from tomatoes and tomato products (paste, ketchup and sauce). Also to measure the influence of the common ingredients in the diet (tomato, onion, garlic and oil) on the retention and the amount of bioaccessible of β -carotene from four vegetables dishes (Jew's mallow, green peas, carrot and snap beans,) cooked in open pan boiling cooking. The in vitro method used simulated the digestion process in the gastrointestinal tract; β -carotene and lycopene were quantified using HPLC.

The raw tomato had 412 and 360 $\mu\text{g}/100\text{g}$ (fresh weight) of β -carotene and lycopene, respectively. Boiling tomato juice for 10 min elevated lycopene content in tomato juices (increases of 195%); although loss of β -carotene was observed (retention of 77%). Whereas, boiling tomatoes in oil was found to be associated with an increase in β -carotene and lycopene released after digestion when compared to the released from processed tomato juice without oil. Also, β -carotene and lycopene accessibility from a single dose of tomato products (206 & 180 μg , respectively) was greater than that from an equal doses from fresh tomatoes.

The percentage amount of β -carotene available for absorption from various types of vegetables (Jew's mallow, green peas, carrot, and snap beans) cooked without any additives was 20, 16, 11 and 10%, respectively. Tomatoes, onion and garlic generally improved the retention of β -carotene in all four vegetables studied during cooking. Jew's mallow cooked without any additives induced a larger amount of in vitro accessible β -carotene than carrot, green peas and snap beans. Jew's mallow cooked with garlic showed a significant increase in amount of in vitro accessible β -carotene than carrot, green peas and snap beans ($p < 0.05$). Vegetables cooked with oil had 2-4 times higher amount of in vitro accessible β -carotene than vegetables cooked without any additives.

INTRODUCTION

Vitamin A deficiency has been recognized as a common public health problem in many parts of the world and it is a serious problem in many

developing countries (Ye *et al.*, 2000). Regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases such as cancer, coronary heart disease, diabetes, Alzheimer's disease, cataracts, and age-related functional decline (Temple, 2000, and Wertz *et al.*, 2004).

Provitamin A carotenoids, in particular β -carotene in fruits and vegetables, are the major source of vitamin A (retinol) for a large proportion of the world's population (Bafidu *et al.*, 1995). Recent epidemiological findings that the consumption of tomatoes and tomato products is strongly correlated with a reduced risk of certain cancers (e.g., prostate, gastrointestinal, and epithelial cell) and cardiovascular disease (Giovannucci, 1999), the antioxidant activity and, in particular, the carotenoid content of various tomato products are of interest. The protective effect of consuming tomato products has been attributed to lycopene, the major carotenoid in tomatoes. Lycopene is responsible for the red color of tomato fruit and generally represents more than 80% of its total carotenoid content, lycopene appears to be relatively stable during food processing and cooking (Martinez-Valverde, *et al.* 2002). It was shown to be the strongest biological quencher of singlet molecular oxygen (Willcox, *et al.*, 2003 and Sanchez-Moreno, *et al.*, 2006) and, therefore, presumably the most effective in protecting against oxidative damage from free radicals.

Several reports have documented the losses of β -carotene from vegetables during cooking procedures such as boiling, stewing, frying, blanching, and pressure cooking, etc. (Sweeney & Marsh, 1971; Sood & Bhat, 1974; Ogulensi & Lee, 1979; Akapa-punam, 1984; Onayemi & Bafidu, 1987; Park, 1987; Pad-mavathi, *et al.*, 1992; Bafidu *et al.*, 1995; Yadav & Sehgal, 1995,1997; Abdel Latif *et al.*, 1999; Gayathri *et al.*, 2004; Van Jaarsveld, *et al.*, 2006).

Bioavailability is defined as the fraction of an ingested nutrient from food that is available for absorption in the intestine and metabolic process and storage (Jackson, 1997). Bioavailability of pro-vitamin A carotenoids in leafy vegetables has been described as low, as a consequence of the matrix (De Pee *et al.*, 1995). A number of factors have been identified that may affect the bioavailability of carotenoids from foods, e.g. the matrix in which the carotenoids are incorporated, the content of dietary fiber and fat, the particle size, and the food processing method applied. In carrots the carotenoids are present in crystalline form or associated with proteins embedded in chromoplasts (Byrant *et al.*, 1992), which limit their release during the digestion process. Cooking may enhance the carotenoid release by softening or breaking down the cell walls and by dissociating the protein complex (Poor *et al.*, 1993; Zhou *et al.*, 1996; Rock *et al.*, 1998). Another way of reducing matrix effects is by homogenization or particle size reduction (Gartner *et al.*, 1997. Castenmiller *et al.*, 1999; van het Hof *et al.*, 1999). Dietary fat, zinc, and vitamin E have a documented positive effect on bioavailability of carotenoids (Shiau *et al.*, 1994; Jalal *et al.*, 1998; Noh & Koo, 2003; Mulokozi, *et al.*, 2004; Agte *et al.*, 2006).

The relative bioavailability of β -carotene from vegetables compared with purified β -carotene ranges between 3 and 6 % for green leafy vegetables, 19 and 34%

for carrots and 22 and 24% for broccoli (Brown *et al.*, 1989; Micozzi *et al.*, 1992; De Pee *et al.*, 1995; Torronen *et al.*, 1996; Castenmiller *et al.*, 1999 and van het Hof *et al.*, 1999). In one study, broccoli and green peas induced a larger β -carotene response in plasma than whole-leaf and chopped spinach, despite a 10 times lower β -carotene content in the former vegetables (Van het Hof *et al.*, 1999).

The objective of the present study was to measure the influence of the common ingredients in the diet (tomato, onion, garlic and oil) on the retention and the amount of bioaccessible (available for absorption) of β -carotene from four vegetables dishes (carrot, snap beans, Jew's mallow and green peas) prepared in open pan boiling cooking as well as to evaluate the effect of boiling cooking and processing on the carotenoids content (lycopene & β -carotene) of tomato juice & tomato products (paste, ketchup and sauce), respectively.

MATERIALS AND METHODS

Materials:

Tomato (*Lycopersicon esculentum*), Jew's mallow, snap beans, pods, (*Phaseolus vulgaris*), green peas (*Pisum sativum*) yellow carrot (*Daucus carota*), onion (*Allium cepa*), garlic (*Allium sativum*), sunflower oil, common salt, sucrose and spices were bought from the local markets in Cairo.

All chemicals used were analytical grade. Pepsin (from porcine pancreas) and bile extract (porcine) were obtained from Sigma Chemical Co.

Food sample preparation.

All samples were cleaned, washed and prepared as follows:

Tomato: the sample was extracted by electric mixer to obtain the juice, then was divided to obtain six different products: Fresh juice (raw), tomato juice-cooked by boiling either without oil or with 3% sunflower oil. Tomato products: tomato paste (TSS 28.39%), tomato ketchup (T.S.S. 29.07%) and tomato sauce (T.S.S. 29.58%). Samples were cooked by boiling in flasks fitted with air condensers for 10 min (in the case of juices) or concentrated in open pan boiling (in other tomato products), whereas, tomato ketchup was prepared by added sugar 4.5% and salt 1.6% at the beginning of the process while vinegar 11% (at concentration of 3%) was added at the end of the processing. Spices, clove (0.045%), cayenne (0.05%), cinnamon (0.05%), garlic (2.0%) and onion (1.1%) were chopped in a blender and pocked in a separate pouch. The pouches were put into the kettle containing concentrated juice. At the end, pouches were squeezed and removed. Tomato ketchup (29.07% T.S.S.) was filled hot into 200 ml clean glass bottles with screw caps. Tomato sauce is the same general character as ketchup, but was made from peeled and cored tomatoes without removing seeds; it contains more sugar (15%) and onions (20%) and sometimes was made hotter in flavor than ketchup by using more cayenne pepper (0.15%).

Carrot was diced to an unformed size of 5 mm thickness, while Jew's mallow, the stems and ribs were removed, and the edible portion, was finely chopped. The test vegetable (carrot, Jew's mallow, snap beans or green peas)

were prepared in the same way they are prepared for consumption, according to a traditional Egyptian recipe, in the following combinations.

- (1) Test vegetable alone (20g) + water 50g (uncooked).
- (2) Test vegetable alone (20g) + water 50g (cooked)
- (3) Test vegetable alone (20g) + tomato juice (7g) + water (43g) (cooked).
- (4) Test vegetable alone (20g) + onion fresh (5g) + water (45g) (cooked).
- (5) Test vegetable alone (20g) + garlic fresh (2g) + water (48g) (cooked).
- (6) Test vegetable alone (20g) + oil (2g) + water (48g) (cooked).
- (7) Test vegetable alone (20g) + tomato juice (7g) + onion fresh (5g) + garlic fresh (2g) + oil (2g) + water (34g) (cooked).

Samples were put in flat bottom flasks fitted with air condensers to prevent water loss during boiling cooking for 10 min.

HPLC determination of lycopene and β -carotene:

Extraction:

The vegetables were prepared for analysis in the same way they are prepared for consumption. All extractions were carried out under subdued light. All the glassware wrapped in aluminum foil. The entire fresh or cooked food sample was mixed with acetone (60 ml), blended in a mixer for 5 min, and filtered through No. 2 Whatman paper on a Büchner funnel under vacuum. The residue was again blended with acetone. This process was continued until the residue was colorless. The extract was made up to 150 ml with acetone. Fifty millimeters of acetone extract were placed in a separator funnel and agitated with petroleum ether (60-80 °C) and left to stand. Extraction was repeated with further portions of petroleum ether. Petroleum ether extract was filtered over anhydrous sodium sulphate on a Whatman No. 1 filter paper (Ranganna, 1977). The extract was made up to a known volume and filtered through a 0.45- μ m nylon filter prior to HPLC analysis. Lycopene and β -carotene concentrations in samples were calculated by extrapolation on the calibration curve. Peak identification of lycopene and β -carotene in the sample extracts were based on retention time and chromatography of authentic lycopene and β -carotene standard (Sigma Co.).

HPLC conditions:

Analysis were carried out using a Hewlett Packard 1050 liquid chromatograph (U.S.A.), equipped with a model HP1050 pump and UV detector (VWD) HP1050 adjusted at 475 nm, the samples were injected by HP1050 auto sampler. Data were stored and analyzed by computer system (hp, HPLC Chem Station, software). The solvents used were HPLC reagent grade. Samples were injected (20 μ L) and isocratic separation was achieved on C-18 (5 μ) column (4.6mmX25 cm). Mobile phase was methanol: THF: water (67:27:6), flow rate 2ml/min, (Sadler, *et al.*, 1990).

In vitro digestion:

The in vitro digestion procedure developed by Hedren *et al.*, (2002), which simulates digestion in the gastrointestinal tract, was to determine the bioaccessibility of β -carotene in the vegetable relishes and/or β -carotene & lycopene in the tomatoes and tomato products. Briefly, digestion was carried out

by acidifying the sample to pH 2 with HCl (2 mol/l), followed by treatment with porcine pepsin solution and incubation at 37 °C in a shaking water bath for 1 hour. The pH was adjusted to 7.5 and mixture of a pancreatin (4g/l) and bile salt (2.5g/l) solution was added. The sample was further incubated for 30 min. The digested was centrifuged and the aqueous fraction was extracted with petroleum ether that was evaporated to dryness. The residue was dissolved in mobile phase solvent and filtered through a 0.45 µm pore size cellulose membrane filter before HPLC analysis.

Statistical analysis:

All data were expressed as the mean ± standard deviations for the means (S.D.). Significant differences among the groups were determined according to Snedecor and Cochran (1967) by one-way ANOVA using MSTAT-C, (Michigan state university version 1.42, computer software), Duncan’s multiple range tests was performed if differences were identified between means at P<0.05.

RESULTS AND DISCUSSION

β-carotene and lycopene content and in vitro accessibility in tomatoes and tomatoes products:

The raw tomato had 412 and 360 µg/100g of β-carotene and lycopene, respectively. With thermal cooking, the β-carotene content was decreased to 322 and 318 µg/100g of tomato juice cooked without or with oil, respectively. Lycopene content was increased to 603 µg/100g of tomato juice cooked without oil and to 591 µg/100g of tomato juice cooked with oil (increases of 195 and 190%), respectively (Table 1). Tomato paste had 1873 and 4211 µg/100 g of β-carotene and lycopene, respectively; there was gain in content of both β-carotene and lycopene in ketchup and sauce, compared with tomato paste, (increases of 103&104% and 103&105%), respectively.

Table (1): β-carotene and lycopene of tomatoes products (on fresh wet. Basis as mean of 3 replicates ± SD).

Ingredient	Carotenoids			
	β-carotene % (µg/100g) Retention		Lycopene % (µg/100g) Retention	
Tomatoes:				
Tomato juice - Fresh (raw)	412 ± 0.38 ^a	100	360 ± 0.06 ^a	100
Tomato juice - Cooked	322 ± 0.74 ^b	80	603 ± 0.59 ^b	195
Tomato juice + Oil (cooked)	318 ± 0.96 ^b	77	591 ± 0.16 ^b	190
Tomato products:				
Tomato Paste (TSS 28.39%)	1873 ± 5.91 ^c	100	4211 ± 10.35 ^c	100
Tomato ketchup (TSS 29.07%)	1920 ± 6.06 ^c	103	4371 ± 6.98 ^c	103
Tomato sauce (TSS 29.58%)	1943 ± 12.37 ^c	104	4306 ± 9.16 ^c	105

^{abc} Values on the same column not sharing the same superscript were significantly differed (p<0.05).

Both tomato juices and tomato products were submitted to an in vitro simulation of human digestion and absorption, and the β -carotene and lycopene were analyzed. The in vitro accessibility of a single dose β -carotene (206 μ g) in tomatoes and tomato products (paste, ketchup and sauce) is presented in Table 2. The percentage of in vitro accessible β -carotene was 7% in fresh tomato juice, while in the tomato juice cooked without oil the percentage in vitro accessibility of β -carotene varied between 12-21%. The amount of accessible β -carotene increased four times (increases of 46%) when tomato juice cooked with oil, as compared with juice cooked without oil.

Table (2): The in vitro accessibility of a single dose β -carotene in tomatoes products * (mean of 3 replicates \pm SD).

Ingredient	β -carotene (μ g)	In vitro accessibility	
		(μ g)	(%)
Tomato juice - Fresh (raw) (50g)	202	13.97 \pm 2.08 ^a	7
Tomato juice - Cooked (64g)	204	24.15 \pm 5.32 ^b	12
Tomato juice + Oil (cooked) (64g)	203	93.68 \pm 5.45 ^c	46
Tomato Paste (TSS 28.39%) (11g)	205	39.56 \pm 4.29 ^d	19
Tomato ketchup (TSS 29.07%) (11g)	202	41.69 \pm 6.09 ^d	21
Tomato sauce (TSS 29.58%) (11g)	204	40.98 \pm 2.98 ^d	20

* Digestibility from a single dose of 206 μ g β -carotene.

^{abcd} Values on the same column not sharing the same superscript were significantly differed ($p < 0.05$).

The lycopene intake of fresh tomato juice in a single dose lycopene (180 μ g) was 17.58 μ g (Table 3). The amount of lycopene available for absorption from cooked tomato juice was 37.76 μ g. However, the amount of lycopene available for absorption from single dose of lycopene from cooked tomato juice with oil was significantly ($p < 0.05$) higher (139.09 μ g). The amount of in vitro accessible lycopene ranged from 54.96 to 55.06 μ g/ single dose (180 μ g) in tomato products (paste, ketchup and sauce). These studies support the findings of Giovannucci *et al.* (1995), that the association between consumption of various tomato products and the risk of prostate cancer depends on the bioavailability of lycopene. That is, an association was found with the consumption of tomato paste or sauce and with consumption of minimally processed tomato juice.

Boiling cooking significantly increased the content of bioaccessible lycopene and carotene in tomatoes. The increase in bioaccessible lycopene and carotene content may primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction. It was reported that food processing such as cooking or grinding might improve lycopene bioavailability by breaking down cell walls (Garther *et al.*, 1997 & Shi and Maguer, 2000). Most lycopene is located in the outer pericarp and the skin attached to the insoluble fiber portion of the tomatoes (Shi and Maguer, 2000). Thermal processing disrupts the cell membranes and cell walls and releases lycopene from the insoluble portion of the tomatoes, which increase the pool of

bioaccessible lycopene and improves lycopene absorption. Lycopene and β -carotene bioavailability from tomato juice cooked with oil was significantly ($p < 0.05$) higher than that from fresh tomatoes (Table 2 and 3) it may be attributable to extraction of lycopene and β -carotene into the lipophilic phase during the boiling process. Carotenoids are known to be readily absorbed from lipophilic matrixes (Brown, *et al.*, 1989 and Zhou, *et al.*, 1996).

Table (3): The in vitro accessibility of a single dose lycopene in tomatoes products* (mean of 3 replicates \pm SD).

Ingredient	Lycopene (μ g)	In vitro accessibility	
		(μ g)	% Retention
Tomato juice - Fresh (raw) (50g)	178	17.58 \pm 4.08 ^a	10
Tomato juice - Cooked (30g)	179	37.76 \pm 7.09 ^b	21
Tomato juice + Oil (cooked) (30g)	181	139.09 \pm 8.22 ^c	77
Tomato Paste (TSS 28.39%) (4g)	177	54.96 \pm 6.99 ^d	31
Tomato ketchup (TSS 29.07%) (4g)	180	54.38 \pm 10.41 ^d	30
Tomato sauce (TSS 29.58%) (4g)	177	55.06 \pm 12.89 ^d	31

Digestibility from a single dose of 180- μ g lycopene.

^{abcd} Values on the same column not sharing the same superscript were significantly differed ($p < 0.05$).

β -carotene content and in vitro accessibility in vegetable relishes prepared by boiling cooking:

Retention and in vitro digestibility of β -carotene in Jew's mallow prepared according to a traditional Egyptian recipe are shown in Table 4. The loss of β -carotene from Jew's mallow was greater when the vegetable was boiled in water for 10 min (60%) with or without oil, than in boiling with tomato, onion or garlic, (29%). Tomato, onion and garlic improved the retention of β -carotene in Jew's mallow during boiling cooking. Tomato improved the retention of β -carotene to 72%. Onion and garlic had a similar beneficial influence on Jew's mallow by boiling (71, 70%) retention, respectively.

The percentage changes in the in vitro accessibility of β -carotene in Jew's mallow during cooking are also present in Table 4. In the relishes cooked without oil the percentage in vitro accessibility of β -carotene in cooked Jew's mallow without oil ranged from 18 to 31%. Jew's mallow with garlic or oil had a significantly higher in vitro accessibility (31 and 45%) of β -carotene, respectively ($p < 0.05$).

Table 5 shows the effect of cooking on the β -carotene content of the green peas. Cooking reduced the β -carotene content of the green peas relish. Tomato, onion and garlic caused greater retention of β -carotene than in boiled cooking. The oil did not improve the retention of β -carotene in boiling-cooked green peas. The amount of β -carotene available for absorption from green peas relish, which contained oil, was significantly higher (25 μ g/100g). While, the amount of in vitro accessible β -carotene ranged from 10 to 11 μ g/100g in green peas relishes cooked with tomato, onion and garlic.

Table (4): Retention and in vitro digestibility of β -carotene in chopped Jew's mallow during boiled cooking for 10 min (mean of 3 replicates \pm SD).

Ingredient	β -carotene (μ g/100g)	% Retention	In vitro accessibility	
			μ g/100g	% Retention
Jew's mallow - Uncooked	1054 \pm 0.57 ^a	100	NA	NA
Jew's mallow - Cooked	421 \pm 0.77 ^b	40	85 \pm 3.12 ^a	20
Jew's mallow + Tomato	757 \pm 0.98 ^{ca}	72	136 \pm 4.09 ^b	18
Jew's mallow + Onion	751 \pm 1.22 ^c	71	143 \pm 1.98 ^b	18
Jew's mallow + Garlic	745 \pm 0.95 ^c	70	230 \pm 6.78 ^c	31
Jew's mallow + Oil	409 \pm 2.09 ^b	39	184 \pm 5.41 ^d	45
Jew's mallow + Tomato + Onion + Garlic + Oil	761 \pm 1.69 ^a	72	23 \pm 5.21 ^e	31

NA = not analyzed.

^{abcde} Values on the same column not sharing the same superscript were significantly differed ($p < 0.05$).

Table (5): Retention and in vitro digestibility of β -carotene in green peas relish during boiled cooking for 10 min (mean of 3 replicates \pm SD).

Ingredient	β -carotene (μ g/100g)	% Retention	In vitro accessibility	
			μ g/100g	% Retention
Green peas -Uncooked	79 \pm 2.58 ^a	100	NA	NA
Green peas - Cooked	63 \pm 3.44 ^b	80	10 \pm 1.06 ^a	16
Green peas + Tomato	72 \pm 6.35 ^c	91	10 \pm 0.89 ^a	14
Green peas + Onion	73 \pm 4.89 ^c	92	11 \pm 2.01 ^a	15
Green peas + Garlic	71 \pm 5.09 ^c	91	11 \pm 3.88 ^a	15
Green peas + Oil	64 \pm 3.88 ^d	81	25 \pm 2.56 ^b	39
Green peas + Tomato + Onion + Garlic + Oil	74 \pm 4.52 ^c	94	35 \pm 2.19 ^c	47

NA = not analyzed.

^{abcd} Values on the same column not sharing the same superscript were significantly different ($p < 0.05$).

Table 6 presents data on the loss of β -carotene in carrot during cooking. As in case of green peas, cooking caused a higher loss of β -carotene from carrot (20%). Tomato, onion and garlic produced a greater retention of β -carotene in cooked carrot. The percentage changes in in vitro accessible β -carotene concentrations differ in the processed carrot without oil vs. carrot with oil.

Data on the influence of the presence of additives during cooking on retention of β -carotene in the snap beans are presented in Table 7. Boiling snap beans in water for 10 min resulted in greater loss of β -carotene (19%). This loss was minimized to a considerable extent by the presence of tomato juice, onion and garlic.

Table (6): Retention and in vitro digestibility of β -carotene in carrot relish during boiled cooking for 10 min (mean of 3 replicates \pm SD).

Ingredient	β -carotene (μ g/100g)	% Retention	In vitro accessibility	
			μ g/100g	% Retention
Carrot - Uncooked	2034 \pm 4.21 ^a	100	63 \pm 0.59 ^a	3
Carrot - Cooked	1626 \pm 4.89 ^b	80	179 \pm 0.79 ^b	11
Carrot + Tomato	1913 \pm 5.98 ^c	94	188 \pm 1.08 ^b	10
Carrot + Onion	1838 \pm 7.25 ^d	90	184 \pm 0.88 ^b	10
Carrot + Garlic	1848 \pm 3.81 ^d	91	186 \pm 0.92 ^b	10
Carrot + Oil	1629 \pm 4.86 ^b	80	576 \pm 0.76 ^c	35
Carrot + Tomato + Onion + Garlic + Oil	1928 \pm 4.91	95	690 \pm 1.63 ^d	36

^{abcd} Values on the same column not sharing the same superscript were significantly differed ($p < 0.05$).

The amount of β -carotene available for absorption from snap beans relish, which contained oil, was significantly higher (19 μ g). The corresponding amount of β -carotene available for absorption from snap beans cooked with tomato, onion and garlic, was between 6 and 7 μ g.

Jew's mallow is a common vegetable in Egyptian diet. The vegetables used in the present study are often cooked with tomato, onion, garlic or oil. Among the β -carotene values of the four vegetables examined in this study, were similar to the values reported by (Abdel latif *et al.*, 1999). The high sensitivity of β -carotene to light and heat is well recognized and its loss is therefore expected during heat processing. Higher losses of β -carotene occurred during cooking of Jew's mallow leaves compared with green peas, carrot and snap beans could be attributed to higher oxidative destruction of leafy vegetables. The loss of β -carotene from leafy vegetables-Jew's mallow during boiling for 10 min in the present study was 60% as compared with a loss of 20% in green peas, carrot and snap beans during boiling in water for the same duration. It may be recalled here that boiling resulted in a greater loss of β -carotene from the leafy vegetables (Jew's mallow) than observed with the green peas, carrot and snap beans.

β -carotene retention during boiling cooking of vegetables was examined in the presence of tomato juice, onion or garlic. Tomatoes, onion or garlic generally improved the retention of β -carotene in all four vegetables studied; this may be due to the higher destruction of the active ingredients (lycopene, flavonoids, anthocyaned and α -tecopherol) of tomatoes, onion and garlic responsible for β -carotene retention during cooking.

Despite reduction in β -carotene content due to cooking, heat processing has a potential of increasing the bioavailability of carotenoids in cooked vegetables (Mulokozi. *et al.*, 2004). In the present study in vitro 3% of the β -carotene content was released from raw carrots in pieces, which is in agreement

with results obtained by Hedren *et al.*, (2002). In our study the heat treatment improved the release of β -carotene from carrots about four times that associated with the raw from green leafy vegetable (Jew's mallow) induced a larger amount of in vitro accessible β -carotene than carrot, green peas and snap beans.

Table (7): Retention and in vitro digestibility of β -carotene in snap beans relish during boiled cooking for 10 min (mean of 3 replicates \pm SD).

Ingredient	β -carotene (μ g/100g)	% Retention	In vitro accessibility	
			μ g/100g	% Retention
Snap beans – Uncooked	60 \pm 3.25 ^a	100	NA	NA
Snap beans – Cooked	49 \pm 2.55 ^b	81	5 \pm 2.80 ^a	10
Snap beans + Tomato	56 \pm 1.98 ^c	93	7 \pm 3.26 ^a	12
Snap beans + Onion	55 \pm 2.54 ^c	92	6 \pm 4.32 ^a	11
Snap beans + Garlic	56 \pm 5.23 ^c	91	6 \pm 2.68 ^a	11
Snap beans + Oil	48 \pm 6.28 ^b	80	19 \pm 1.05 ^b	40
Snap beans + Tomato+ Onion+ Garlic+ Oil	57 \pm 2.16 ^c	95	23 \pm 0.96 ^b	

NA = not analyzed.

^{abcd} Values on the same column not sharing the same superscript were significantly differed ($p < 0.05$).

In the present study in vitro, addition of tomato, onion or garlic to the vegetables samples had no improving effect on accessible β -carotene except cooking Jew's mallow with garlic, 31% of β -carotene content in Jew's mallow become available for absorption after cooking with garlic, this may be due to interactive effect of antioxidant micronutrients in garlic like α -tocopherol. Agte *et al.*, (2006) used the plasma response method on humans and showed that green leafy vegetables (spinach) supplemented with vitamin E resulted in significant increase of plasma β -carotene (40%).

In our study depending on vegetable species, 35-45% of the β -carotene content became available for absorption after cooking with oil. Huang *et al.*, (2000) used the plasma response method on humans and showed that 13-37% of the β -carotene from stir-fried (with oil) water convolvulus leaves was absorbed after a single ingestion. Our results on in vitro accessibility are thus about one and halve to two times higher. Ingestion of fat along with carotenoids is thought to be crucial in the absorption process (Prince & Frisoli, 1993). Fat provides a hydrophobic environment for the released carotenoids that would be dissolved into small lipid droplets facilitated by mechanical mixing. With addition of bile salts these lipid droplets are transformed into mixed bile salt micelles from which the carotenoids are thought to be absorbed in the intestine.

In conclusion, the in vitro digestion method described in the present paper appears to be useful for rapid screening of carotenoids accessibility in plant foods, it has been shown that cooking is more important for release of carotenoids in vegetables, and addition of oil is more effective in enhancing accessibility of

carotenoids. If open pan boiling is the chosen method of cooking, adding onion and garlic as well as tomatoes can minimize β -carotene losses.

REFERENCES

- Abdel Latif, S.H.; Mahmoud, A.H. and Basyony, A.E. (1999): Beta carotene in some vegetables, influenced by different processing methods. 6th Arabic conference on food science and technology, Cairo- Egypt, PP, 369-383.
- Agte V., Jahagirdar, M. and Chiplonkar, S. (2006): GLV supplements increased plasma beta-carotene, vitamin C, zinc and hemoglobin in young healthy adults. *Eur. J. Nutr.*, 45 (1): 29-36.
- Akapa-punam, M. A. (1984): Effects of wilting, blanching and storage temperatures on ascorbic acid and total carotenoids content of Nigerian fresh vegetables. *Plant Foods for Human Nutrition*, 34, 177-180.
- Bafidu, G.I.O.; Akapapunam. M. A., and Mybemere, V. N. (1995): Fate of β -carotene in processed leaves of fluted pumpkin a popular vegetable in Nigerian diet. *Plant Foods for Human Nutrition*, 48, 141-147.
- Brown E.D.; Micozzi, M.S.; Craft, N.E.; Bieri, J.G. Beecher, G.; Edwards, B.K.; Rose, A.; Taylor, P.R. and Smith, J.C. (1989): Plasma carotenoids in normal men after a single ingestion of vegetables or purified β -carotene. *Am. J. Clin. Nutr.*, 49, 1258-1265.
- Byrant, J.D.; McCord, J.D.; Unlu, L.K. and Erdman, J.W. (1992): Isolation and partial characterization of α - and β -carotene-containing carotenoprotein from carrot (*Daucus carota L.*) root chromoplasts. *J. Agric. Food Chem.*, 40, 545 -549.
- Castenmiller, J.J.M.; West, C.M.; Linssen, J.P.H.; Van het Hof, K.H., and Voragen, A.G.J. (1999): The food matrix of spinach is a limiting factor in determining the bioavailability of β -carotene and to a lesser extent of lutein in humans. *J. Nutr.*, 129, 349 - 355.
- De Pee, S.; West, C.E.; Muhilal, Daryadi, D. and Hautvast, J.G.A.J. (1995): Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. *Lancet*, 346, 75-81.
- Garther, C.; Stahl, W. and Sies, H. (1997): Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *Am. j. Clin. Nutr.*, 66,116-122.
- 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*, 84, 35-43, PP. 35-43.
- Giovanucci, E.; Ascherio, A.; Rimm, E.B.; Stampfer, M.G.; Colditz G., and Willett W.S. (1995): Intake of carotenoids and retinal in relation to risk of prostate cancer. *J. Natl. Cancer Inst.*, 87, 1767-1776.
- Giovanucci, E. (1999): Tomatoes, tomato-based products, lycopene, and cancer. Review of the epidemiologic literature. *J. Natl. Cancer Inst.*, 91, 317-331.
- Hedren, E.; Diaz, V., and Svanberg, U. (2002): Estimation of carotenoid accessibility from carrots determined by in vitro digestion method. *Eur. J. Clin. Nutr* 56: 425-430.
- Huang, C.J.; Tang, Y.L.; Chen, C.Y., Chen, M.L.; Chu, C.H. and HseuC-T (2000): The bioavailability of β -carotene in stir- or deep-fried vegetables in men determined by measuring the serum response to a single ingestion. *J. Nutr.*, 130,534-540.

- Jackson, M.J. (1997): The assessment of bioavailability of micronutrients: introduction. *Eur. j. Clin. Nutr.*, 51, SI-S2.
- Jalal, F.; Nesheim, M.C.; Agus, Z.; Sanjur, D., and Habicht, J.P. (1998): Serum retinol concentrations in children, affected by food sources of β -carotene, fat intake, and anthelmintic drug treatment. *Am. j. Clin. Nutr.*, 68, 623-629.
- Martinez-Valverde, I.; Periago, J.M.; Provan, G. and Chesson A. (2002): Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato. *J. Sci. Food Agric.*, 82: 323-330.
- Micozzi, M.S.; Brown, E.D.; Edwards, B.K.; Bieri, J.G.; Taylor, P.R.; Khachik, F.; Beecher, G.R., and Smith, J.C. (1992): Plasma carotenoid response to chronic intake of selected foods and β -carotene supplements in men. *Am. J. Clin. Nutr.*, 55, 1120-1125.
- Mulokozi, G., Hedren, E. and Svanberg, U. (2004): In vitro accessibility and intake of β -carotene from cooked green leafy vegetables and their estimated contribution to vitamin A requirements. *Plant Food for Human Nutrition*, 59: 1-9.
- Noh, S.K. and Koo, S.I. (2003): Low zinc intake decrease the lymphatic output of retinal in rats infused intraduodenally with beta-carotene. *J. Nutr. Biochem.*, 14(3): 147-53.
- Ogulensi, A.T. and Lee, C.Y. (1979): Effect of thermal processing on the stereo-isomerisation of major carotenoids and vitamin A value of carrots. *Food Chemistry*, 4, 311-318.
- Onayemi, O. and Bafidu, G.I.O. (1987): Effect of blanching and drying methods on nutritional and sensory quality of leafy vegetables. *Plant Foods for Human Nutrition*, 37. 291-298.
- Pad-mavathi, K.; Udipi, S.A., and Rao, M. (1992): Effect of different cooking methods on β -carotene content of vegetables. *J. of Food Science and Technology*, 29, 137-140.
- Park, Y.W. (1987): Effect of freezing, thawing, drying and cooking on carotene retention in carrots, broccoli, spinach. *J. of Food Science*, 52, 1022-1025.
- Poor, C.L.; Bierer, T.L.; Merchen, N.R.; Fahey, G.C. and Erdman, J.W. (1993): The accumulation of α - and β -carotene in serum and tissues of preruminant calves fed raw and steamed carrot slurries. *j. Nutr.*, 123, 1296-1304.
- Prince MR & Frisoli JK (1993): Beta-carotene accumulation in serum and skin. *Am. j. Clin. Nutr.*, 57, 175-181.
- Ranganna, S. (1977): Plant pigments. In: *Manual of analysis of fruit and vegetable products* (PP. 73-77): New Delhi: Tata Mc Graw-Hill.
- Rock, C.L.; Lovalvo, J.L.; Emenhiser, C.; Ruffin, M.T.; Flatt, S.W. and Schwartz, S.J. (1998): Bioavailability of β -carotene is lower in raw than in processed carrots and spinach in women. *j. Nutr.*, 128, 913-916.
- Sadler, G.; Davis, J. and Dezman, D. (1990): Rapid extraction of lycopene and β -carotene from reconstituted tomato past and pink grapefruit homogenates. *J. Food Sci.*, 55 (5), 1460-1461.
- Sanchez-Moreno, C.; Plaza, L.; De Ancos, B. and Cano, M.P. (2006): Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. *Food chemistry*, 98: 749-756.

- Snedecor, G. W. and Cochran, W. G. (1967): Statistical methods. 6th ed. Iowa, State Univ. Press. Ames. IA.
- Shi, J. and Maguer, M.L. (2000): Lycopene in tomatoes: chemical and physical properties affected food processing. *Crit. Rev. Food Sci. Nutr.*, 40, 1-42.
- Shiau, A.; Mobarhan, S.; Stacewicz-Sapuntzakis, M.; Mobarhan, S., Stacewicz-Sapuntzakis, M.; Benya, R.; Liao, Y., Ford, C.; Bowen, P.; Friedman, H. and Frommel, T.O. (1994): Assessment of the intestinal retention of beta-carotene in humans. *J. Am. Coll. Nutr.* 13,369-375.
- Sood, R. and Bhat, C.M. (1974): Changes in ascorbic acid and carotene content of green leafy vegetables on cooking. *J. of Food Science and Technology*, 11, 131-133.
- Sweeney, J.P. and Marsh, A. C. (1971): Effect of processing on provitamin A in vegetables. *J. of American Dietetics Association*, 59. 238-243.
- Temple, N.J. (2000): Antioxidants and disease: more questions than answers. *Nutr. Res.*, 20. 449-459.
- Torronen, R.; Lehmusaho, M.; Hakkinen, S.; Hanninen, O. and Mykkanen, H. (1996): Serum β -carotene response to supplementation with raw carrots, carrot juice of purified β -carotene in healthy non-smoking women. *Nutr. Res.*, 16, 565-575.
- Van het Hof, K.H.; Tijburg, L.B.M.; Pietrzik, K. and Westrate, J.A. (1999): Influence of feeding different vegetables on plasma levels of carotenoids folate and vitamin C. Effect of disruption of the vegetable matrix. *Br. J. Nutr.* 82, 203 -212.
- Van Jaarsveld, P.J.; Marais, D.W.; Harmse, E.; Nestel, P., and Rodriguez-Amaya, D.B. (2006): Retention of β -carotene in boiled, mashed orange-flashed sweet potato. *J. of food composition and analysis*, 19,4, 321-329.
- Wertz, K.; Seifert, N.; Buchwald Hunziker, P.; Riss, G.; Wyss, A.; Lankin, C. and Goralczyk, R. (2004): β -carotene inhibits UVA-induced matrix metalloprotease 1 and 10 expression in keratinocytes by a singlet oxygen-dependent mechanism. *Free Radical Biol. Med.*, 37; 654-670.
- Willcox, J.K.; Catignani, G.L. and Lazarus, S. (2003): Tomatoes and cardiovascular health. *Critical reviews in food science and nutrition*, 43 (1): 1-18.
- Yadav, SK and Sehgal, A. (1995): Effect of home processing on ascorbic acid and β -carotene content of spinach (*Spinachia Oleracia*) and amaranth (*Amarantus tricolor*) leaves. *Plant Foods for Human Nutrition*, 47. 125-131.
- Yadav, S.K., and Sehgal, A. (1997): Effect of home processing on ascorbic acid and β -carotene content of bathua (*Chenopodium album*) and fenugreek (*Trigonella foenumgraecum*) leaves. *Plant Food for Human Nutrition*, 50. 239-247.
- Ye X., Al-Babili, S.; Klott, A.; Zhang, J.; Lucca, P.; Beyer, P. and Potrykus, I. (2000): Engineering the provitamin A (β -carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, 287, 303-305.
- Zhou, J.R., Gugger, E.T., Erdman, J.W. (1996): The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of β - and α - carotene in the ferret model. *J. Am. Coll. Nutr.*, 15:84-91

تأثير نسب مكونات بعض الخضروات أثناء الطهي
على المتبقي من البيتا كاروتين وقابليته للهضم.

فؤاد على عبد الجليل الشريفة

معهد بحوث تكنولوجيا الأغذية-مركز البحوث الزراعية-الجيزة-مصر.

يهدف البحث إلى تقييم تأثير المعاملة الحرارية على كمية البيتاكاروتين والليكوبين المتبقية وأيضا القابلة للامتصاص في الطماطم ومنتجاتها (الصلصة، والكاتشب والصوص) وأيضا تقدير تأثير مكونات الطعام الشائمة (الطماطم والبصل والثوم والزيت) على الكمية المتبقية والكمية القابلة للامتصاص من البيتاكاروتين أثناء طبخ كلا من الملوخية والبسلة الخضراء والجزر والفاصوليا الخضراء بالخلي في حقل مفتوحة. واستخدم الطريقة الأنزيمية لمحاكاة عملية الهضم في الجهاز الهضمي وتم تقدير البيتاكاروتين والليكوبين بجهاز HPLC.

وجد أن الطماطم الطازجة تحتوى على ٤١٢، ٣٦٠ ميكروجرام/١٠٠ جرام (وزن طازج) بيتاكاروتين والليكوبين، بالترتيب. على عصير الطماطم لمدة ١٠ دقيقة زاد من كمية اليكوبين بها (نسبة الزيادة ١٩٥%)، بينما انخفض كمية البيتاكاروتين (المتبقي ٧٧%). على الطماطم في وجود زيت زاد من كمية كل من البيتاكاروتين والليكوبين المنفردة بعد عملية الهضم بالمقارنة بالطماطم المغلية بدون زيت وأيضا وجد أن الكمية القابلة للامتصاص لكل من البيتاكاروتين والليكوبين في منتجات الطماطم اكبر من مثيلتها في العصير الطازج وذلك عند مقارنة كمية محددة من البيتاكاروتين والليكوبين (٢٠٦، ١٨٠ ميكروجرام على الترتيب).

كانت النسبة المئوية للبيتاكاروتين القابلة للامتصاص في كلا من الملوخية والبسلة والجزر والفاصوليا الخضراء المطبوخة بدون أي إضافات ٢٠، ١٦، ١١، ١٠% على الترتيب. ووجد أن كمية البيتاكاروتين القابلة للامتصاص من الملوخية المطبوخة بدون أي إضافات اكبر من مثيلتها في باقي الخضروات. وجد أن إضافة الثوم إلى الملوخية أثناء طبخها زاد من كمية البيتاكاروتين القابلة للامتصاص بالمقارنة بالجزر والبسلة والفاصوليا الخضراء. ويزيد وجود الزيت مع الخضروات المطبوخة من كمية البيتاكاروتين القابلة للامتصاص وذلك بالمقارنة بمثيلتها من الخضروات المطبوخة دون إضافة زيت.