International Food Research Journal 23(4): 1531-1540 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of cooking methods and extraction solvents on the antioxidant activity of summer squash *(Cucurbita pepo)* vegetable extracts

*Baljeet, S.Y., Roshanlal, Y. and Ritika, B.Y.

Department of Food Technology, Maharshi Dayanand University, Rohtak (Haryana) India-124001

Article history

Received: 27 April 2015
Received in revised form: 28 November 2015
Accepted: 4 December 2015

Keywords

Antioxidant activity
Cooking methods
Total phenolics
Radical scavenging
Extraction solvents

Abstract

The effects of cooking treatments (pressure cooking, microwave cooking and frying) and extracting solvents (absolute methanol, absolute ethanol and butanol) was investigated on the antioxidant activity of extracts of fruits of *Cucurbita pepo*. In general, the recovery of total phenolics and tannins was the best in methanol whereas, flavonoids showed their highest presence in ethanol. The average antioxidant activity of the methanolic extracts as measured by FTC, TBA and DPPH assays was the highest. The methanolic and butanolic extracts of all types of cooked vegetable showed decreased content of total phenolics and flavonoids whereas increased concentration of total phenolics and flavonoids was observed in ethanolic extracts of microwaved and fried sample. Pressure cooking emerged as most effective cooking treatment in lowering the antioxidant activity. HPTLC analysis of the methanolic extracts revealed the presence of chlorogenic acid, cinnamic acid, catechin, rutin and quercetin as the prominent phenolic compounds.

Introduction

Antioxidant compounds contribute to the health potential of foods, and their intake has been correlated to lower incidence for cardiovascular diseases, cancer, aging, and age-related degenerative processes (Pandey and Rizvi, 2009). Among the phytochemicals found in plants, polyphenolic compounds have been considered as most important and ubiquitous compounds in the plant kingdom. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers (Chang *et al*., 2007). Summer squash *(Cucurbita pepo)*, which belongs to the cucurbitaceae family is available throughout India and consumed as vegetable in various parts of the world also. Extensive investigations have led to isolation of several bioactive compounds from *C. pepo* which includes phenolic compounds such as chlorgenic acid, benzoic acid, quercetin, luteolin and kaempferol (Iswaldi *et al.*, 2013), syringic acid (Dragovic-Uzelack *et al*., 2005), p-coumaric acid, sinapic acid (Pericin *et al.*, 2009), β carotene (Veda *et al*., 2006), and tannin (Silveira *et al.*, 1996).

Previous studied conducted on various vegetables including gourd vegetables showed that total polyphenol content and antioxidant activity of the cooked vegetables could be higher or lower in comparison to fresh vegetables. Boiling and stir frying have been reported to reduce the total phenolic content but cause increase in the free radical scavenging activity of the cooked samples of pumpkin *(C. moschata)* (Azizah *et al*., 2009). Saikia and Mahanta (2013) have reported both positive and negative impact of cooking on the phytochemicals and antioxidative activity of bottle gourd and teasle gourd. Jimenez-Monreal *et al.* (2009) have reported that free radical scavenging activity of boiled and fried *C. pepo* reduced considerably over its raw counterparts. Variable effects of different cooking treatments have been reported by Sengul *et al.* (2014) on various vegetables including beet root, turnip, cabbage and broccoli. Thermal treatment decreased the total phenolic content and antioxidant activity in kale, spinach, cabbage and shallots (Ismail *et al*., 2004). Moreover, the extraction mechanism and type and polarity of extracting solvents also have been reported to exert significant effects on extractability of phytochemicals and hence the *in vitro* antioxidative acitivity of plant extracts (Naczk and Shahidi, 2006). Therfore, the present study was undertaken to investigate and quantify the effects of different cooking methods and extarcting solvents on the total phenol content and antioxidant activity of *C. pepo* extracts.

*Corresponding author.
Email: *baljeetsingh.y@gmail.com*
Tel: +91-9896360766

Materials and Methods

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid, thiobarbituric acid, β carotene, Tween 20, linoleic acid, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), phenolic acids (p-coumaric, tannic, benzoic, gallic, ellagic, chlorogenic acids), flavonoid standard (quercetin and rutin,) were purchased from Himedia (India). Folin–Ciocalteu reagent, HPLC-grade methanol, chloroform, hexane, formic acid, acetonitrile and HPLC grade water were purchased from Merck (Darmstadt, Germany). Phenolic acids (vannilic acid and caffeic acid) and flavonoid standards (kaempferol, myricetin, catechin, leutolin and apigenin) were purchased from Sigma-Aldrich fine chemicals.

Cooking of vegetables

Cooking conditions were optimized by preliminary experiments carried out for each vegetable. For all cooking treatments, the minimum cooking time to reach a similar tenderness for an adequate palatability and taste, according to the Indian eating habits, was used.

Pressure cooking

Small pieces (3x 0.5 x0.5 cm) of vegetable (500 g) were placed in pressure cooker (134 mm diameter, Hawkins) containing 100 ml water. The vegetables were cooked at low flame for 5 min after the pressure existed in the cooker.

Microwave cooking

The cut and pinched pieces were placed on a glass dish and cooked at 110^{o}C for 8 min in a microwave oven (IFB model: 25SC3).

Frying

Vegetable pieces of approx 0.25 cm thickness were placed in a frying pan (170^{o}C) containing 80 ml of soya refined oil and stirred until the vegetable became crisp - tender. The excess oil was drained off and the vegetable sample was recovered.

Preparation of vegetable extracts

The macerates of vegetable samples were extracted with absolute methanol, ethanol and n-butanol separately for 6 days at room temperature with intermediate shaking. The extract after filtration and centrifugation was concentrated using rotary evaporator at 45^{0}C under reduced pressure (97.3 kPa) until the weight becomes constant and the extract was stored in dark at -20^{o}C for further analysis.

Phytochemical screening

Phytochemicals screening of the extracts was performed to identify the various constituent components as described by Sofowora (1996); Trease and Evans (1989); Harborne (1984).

Determination of total phenol content

The total phenolic content of the extracts was determined using Folin–Ciocalteu reagent (Zhou and Yu, 2006) using the regression equation of the standard curve for gallic acid ($Y = 4.262x + 0.043$; $R^2 = 0.997$).

Determination of total flavonoids

The method as reported by Meda *et al.* (2005) along with minor modifications was used for determination of flavonoids. Quercetin was used as the standard and results were calculated from the regression equation of $Y = 14.32x + 0.047$; $R^2 = 0.990$) as obtained from the standard curve and expressed in terms of quercetin equivalent (mg of QE/ 100g dwb).

Determination of tannin content (Vanillin –HCl method)

Condensed tannins were determined by slight modification of the vanillin method (Burns, 1971). The regression equation $Y= 0.5523x + 0.0273$; $R^2= 0.998$ as obtained from the standard curve of catechin was used to express the results as mg equivalents of catechin/100 g dwb.

Determination of β- carotene

The method as reported by Santra *et al.* (2005) along with some modifications was used for determination of β-carotene. Sample extract (0.1 g) was mixed with 10 ml of water saturated n-butanol and kept in the dark for 16-18 h for extraction of β-carotene. The contents were centrifuged at 1990 g for 10min and the absorbance of collected supernatant was measured at 440 nm. The amount of β- carotene was calculated using regression equation $Y = 0.1398x + 0.0463$ ($R^2 = 0.9809$) as obtained from the standard curve.

Determination of antioxidant activity of extracts

Ferric thiocyanate (FTC) method

FTC method was adapted as described by

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Thiobarbituric acid (TBA) method

The samples prepared for FTC method were also used to measure the percent inhibition by TBA method as described by Kikuzaki and Nakatani (1993). Antioxidant activity was recorded on the final day of the FTC assay and the % inhibition was measured by the formula as used in FTC method.

Ferric reducing antioxidant power (FRAP) method

FRAP assay was adapted as described by Moyer *et al.* (2002). Based on the measured absorbance, the concentration of $FeSO_4$ was measured (mM $FeSo_4$/100 g dwb) from the regression equation of the standard curve of $FeSO_4$ (Y = 0.5783x 0.0042; $R^2 = 0.9991$).

Evaluation of the free radical scavenging activity by DPPH assay

DPPH free radical scavenging activity assay used by Chan *et al.* (2007) was adapted with slight modifications. The scavenging activity of each extract on DPPH radical was calculated using the

$$\text{Percent scavenging activity} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

required for 50% scavenging of DPPH).

HPTLC analysis of phenolic acids and flavonoid compounds

A densitometric HPTLC analysis was performed for the analysis of phenolic acids and flavonoids. The standard stock solution (0.1 mg/ml) and sample (100 mg/ml) were prepared in HPTLC grade methanol. These solutions were filtered through 0.2 µ syringe filter before loading in the sample syringe (Hamilton, Bonaduz, Switzerland). The sample (8 µL) was applied with a 100 µL sample syringe using automatic Linomat-5 system (CAMAG, Switzerland). The size of spot was 6 mm, distance from lower edge 8 mm and distance from the left side 15 mm. The stock solutions of the reference compounds were prepared in methanol at different concentration levels (2-8 µL). The plates were developed in a vertical glass chamber (CAMAG) until the respective mobile phase i.e. solvent system I (6.4 chloroform: 3.9 hexane: 2.0 methanol: 0.5 formic acid) for detection of gallic acid, caffeic acid, quercetin, apigenin, kampferol and chlorogenic acid; solvent system II (4 chloroform: 1 hexane: 1 methanol: 1 formic acid) for detection of p-coumaric acid, leutolin, myricetin, catechin and ellagic acid and solvent system III (4.5 acetonitrile: 1.0 methanol: 0.5 water) for detection of ferulic acid, benzoic acid, cinnamic acid and vanillic acid) respectively rose to 80% of the plate height. The developed plates were dried on TLC plate heater at 120^0C for 5 min and cooled at room temperature. The densitometric evaluation was performed with a TLC scanner 3 (CAMAG) at wavelength of 254 nm. The plate image was documented by TLC visualizer documentation system (CAMAG). The quantification and documentation was done by win-CAT software. The concentration of each compound was determined by using calibration curve prepared by plotting the peak area versus concentration of standard compound.

Statistical analysis

All observations were taken in triplicate and the results were reported as means ± standard deviation (SD). Analysis of variance was performed by one way ANOVA analysis (SPSS 19.0) followed by Tukey's HSD post hoc comparison test at $p < 0.05$. Pearson correlation coefficients (r) among various antioxidant assays and phytochemicals were performed using SPSS 19.0 software by selecting the bivariate correlation with two tailed test of significance. The confidence limits used in pearson correlation were based on 95% ($p < 0.05$) and 99% ($p < 0.01$).

Results and Discussion

The phytochemicals screening of various extracts *C. pepo* showed the presence of considerable amounts of flavonoids and tannins in most of the extracts of the raw as well as cooked forms of the vegetable (results not shown). Saponins were however tested positive only in the ME and EE of *C. pepo.* Interestingly, the saponins were absent in raw state of ME but after the pressure cooking and microwave cooking the presence was observed. This was probably due to increase in saponins consisting aglycone and glycoside forms during heating (Chaturvedi *et al*., 2012). Terpenoids were also present in all the extracts except the ME of fried sample. Previous studies have established the presence of various phytochemicals including flavonoids, alkaloids, tannin, saponin etc. in raw form of *C. pepo* (Wattermen, 1992; Chonoko *et al*., 2010).

Total phenol content

The total phenol content (TPC) of raw and cooked *C. pepo* vegetable samples is reported in Table 1. ME revealed the highest concentration of TPC (393.3 mgGE/100g dwb) followed by EE (390.7

Table 1. Total phenols, flavonoids, and tannins content of various extracts of raw and cooked vegetable of *C.pepo*

Treatments	Total phenol (mg GE/100g)				Total Flavonoids (mg QE/100g)				Tannin Content (mg CE/100g)				Carotenoid content (mg β-carotene/100g)			
	ME	EE	BE	Mean	ME	EE	BE	Mean	ME	EE	BE	Mean	ME	EE	BE	Mean
Raw	519.8±2.35^{dC}	336.2±1.52^{bB}	272.1±1.57^{dA}	376.1^{c}	64.4±1.37^{dC}	56.8±0.59^{bB}	45.0±0.90^{dA}	55.4^{b}	18.1±4.80^{bB}	7.7±1.47^{aA}	6.9±3.36^{abA}	10.9^{b}	3.8±0.08^{bC}	1.2±0.1^{aA}	2.4±0.08^{aB}	2.8^{a}
Pressure cooked	403.6±2.78^{cC}	282.6±2.57^{aB}	259.6±3.04^{cA}	315.3^{b}	50.2±0.83^{cC}	45.9±0.59^{aB}	25.1±0.57^{aA}	40.4^{a}	16.6±2.85^{bB}	8.2±2.32^{aA}	9.8±2.38^{bcA}	11.5^{b}	8.9±0.14^{cB}	1.9±0.29^{bA}	12.9±0.12^{dC}	7.9^{c}
Microwave cooked	376.6±2.33^{bB}	453.8±2.98^{cC}	102.4±0.93^{aA}	310.9^{a}	22.5±0.75^{aA}	70.3±0.69^{cC}	28.4±0.34^{bB}	40.4^{a}	6.1±2.59^{aA}	11.8±2.88^{aB}	2.9±1.72^{aA}	6.9^{a}	2.4±0.11^{aA}	10.2±0.17^{cC}	2.8±0.07^{bB}	5.1^{b}
Fried	273.1±1.58^{aB}	490.2±2.05^{dC}	162.7±1.13^{bA}	308.7^{a}	41.1±0.60^{bB}	101.1±0.49^{dC}	30.0±0.34^{cA}	57.4^{c}	6.2±1.76^{aA}	9.8±3.63^{aA}	12.0±3.05^{cB}	9.3^{ab}	9.9±0.14^{aD}	17.5±0.31^{dB}	9.9±0.09^{cA}	12.5^{d}
Mean	393.3^{C}	390.7^{B}	199.2^{A}		44.5^{B}	68.6^{C}	32.1^{A}		11.77^{B}	9.37^{AB}	7.90^{A}		6.3^{A}	7.7^{C}	7.0^{B}	
	All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 31315.9 at df = 2, p=0.000 F-Statistics for cooking treatments = 1981.6 at df = 3, p=0.000 F-Statistics for cooking treatments & extracted solvent= 7332.3 at df = 6, p=0.000				All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 7871.9 at df = 2, p=0.000 F-Statistics for cooking treatments = 1480.2 at df = 3, p=0.00 F-Statistics for cooking treatments & extracted solvent= 2002 at df = 6, p=0.000				All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 5.53 at df = 2, p=0.01 F-Statistics for cooking treatments = 4.52 at df = 3, p=0.01 F-Statistics for cooking treatments & extracted solvent= 8.84 at df = 6, p=0.000				All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 250.4 at df = 2, p=0.000 F-Statistics for cooking treatments = 6476.6 at df = 3, p=0.000 F-Statistics for cooking treatments & extracted solvent= 2720.4 at df = 6, p=0.000			

Values are presented as mean ±SD (n=3) and referred to the dry weight. Small and capital alphabets in superscripts indicate significant differences ($p<0.05$) among cooking methods and extracts respectively
GE= gallic acid equivalent, QE= quercetin equivalent, CE= catechin equivalent
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

mgGE/100g dwb) and BE (199.2 mgGE/100g dwb). F- value statistics (31315.9 at df = 2, p=0.000) also indicated highly significant impact of extraction solvent on the extraction yield of total phenols. Previous studies have reported TPC of 8.6 mgGE/g (fwb) (Hamissou *et al*., 2013) and 5684 mgGE/100g (dwb) (Oloyede, 2012) in *C. pepo* extracts. The main effect of the cooking method was also highly significant (F=1981.6 at df = 3, p=0.000). The total phenolic content decreased in cooked vegetable samples with frying showing the most pronounced effect. There was significant interaction effect between the extracting solvent and the heat treatment on the TPC (F=7332.3 at df = 6, p=0.000). However, this effect was inconsistent and the results showed a decreased concentration of TPC in the cooked samples except EE of microwaved and fried samples where an increase in the TPC was observed. The maximum reduction in TPC was obtained in case of BE of the microwaved sample. The reduction TPC could be ascribed to the breakdown of some heat labile phenolic compounds (Hunter and Fletcher, 2002), temperature induced chemical oxidation (Rawson *et al*., 2013) and release of oxidative and hydrolytic enzymes that could destroy the antioxidant compounds in vegetables (Chism and Haard, 1996). The increase in TPC of EE of microwaved and fried samples may be because of availability of bound phenolic substances in ethanol from the complex vegetable matrix, which possibly reacted better with the FC reagent (Sultana *et al*., 2008). The results of the present study on the reduction in TPC after cooking treatments are comparable with the previous studies carried out in different vegetables (Zhang and Hamauzu, 2004; Ismail *et al*., 2004). In contrast, increase in the phenolic content after cooking of different vegetables including garlic has been observed in the previous studies also (Kim *et al*., 2013; Turkment *et al*., 2005).

Total flavonoids

The concentration of total flavonoids content of raw and cooked vegetables is as shown in Table 1. The average flavonoids content of the raw samples was 55.4 mg QE/100g (dwb), whereas in cooked samples this value significantly varied from 40.4 to 57.4 (F=1480.2 at df = 3, p=0.000) suggesting the inconsistent effect of cooking methods. Pressure cooking and microwave cooking decreased the flavonoid content whereas frying resulted in increase of the flavonoids content. The main effect of extracting solvents was also significant (F=7871.9 at df = 2, p=0.000) and the flavonoids content was highest in EE (68.6 mg QE/100g). The interaction effect of cooking method and the extraction solvent was also significant (F= 2002 at df = 6, p=0.000). The results showed a maximum reduction of 65% in the flavonoids content of ME of microwaved sample followed by BE of pressure cooked (44.2%) and microwave cooked sample (36.9%). As also observed in case of TPC, the EE of microwave cooked and fried samples showed significant increase in the flavonoids content. It has been reported that heat treatment increased the level of free flavonols (Stewart *et al*., 2000). Previous studies have established the presence of various phytochemicals including flavonoids

Table 2. Antioxidant activity of various extracts of raw and cooked vegetable of *C.pepo* as determined by FTC, TBA, FRAP assays

Treatments	FTC (%Inhibition)				TBA (%Inhibition)				FRAP (mM $FeSO_4$/100g)				IC-50 (mg/ml)			
	ME	EE	BE	Mean	ME	EE	BE	Mean	ME	EE	BE	Mean	ME	EE	BE	Mean
Raw	30.4 ± 0.73^{cC}	16.7 ± 1.27^{bA}	19.0 ± 0.87^{dB}	22.1^{d}	40.5 ± 0.44^{cC}	28.0 ± 0.39^{bA}	30.2 ± 0.39^{dB}	32.9^{d}	973.8 ± 19.56^{cC}	714.8 ± 16.20^{bB}	440.1 ± 16.27^{cA}	709.6^{c}	0.36	0.13	0.37	0.28
Pressure cooked	25.7 ± 0.70^{bC}	5.1 ± 1.17^{aA}	15.8 ± 0.76^{cB}	15.5^{a}	36.4 ± 0.40^{bC}	16.9 ± 0.54^{aA}	28.6 ± 0.62^{cB}	27.3^{a}	758.7 ± 29.39^{bC}	612.9 ± 25.53^{aB}	456.5 ± 21.7^{cA}	609.4^{b}	0.26	0.08	0.33	0.22
Microwave cooked	23.7 ± 0.57^{aC}	18.6 ± 0.58^{cB}	9.3 ± 1.17^{aA}	17.2^{b}	34.8 ± 0.38^{aC}	29.0 ± 0.38^{cB}	21.5 ± 0.42^{aA}	28.4^{b}	650.5 ± 28.52^{aB}	816.4 ± 22.49^{cC}	180.7 ± 7.51^{aA}	549.2^{a}	0.12	0.17	0.07	0.12
Fried	23.8 ± 0.87^{aB}	26.4 ± 1.07^{dC}	11.5 ± 1.41^{bA}	20.6^{c}	34.3 ± 0.44^{aB}	37.0 ± 0.64^{dC}	23.1 ± 0.51^{bA}	31.4^{c}	618.1 ± 20.63^{aB}	1693.3 ± 19.08^{dC}	365.3 ± 10.48^{bA}	892.2^{d}	0.10	0.26	0.27	0.21
Mean	25.9^{C}	16.7^{B}	13.9^{A}		36.5^{C}	27.8^{B}	25.8^{A}		750.3^{B}	959.4^{C}	360.6^{A}		0.21	0.16	0.19	
	All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 504.3 at df = 2, p=0.000 F-Statistics for cooking treatments = 85.5 at df = 3, p=0.000 F-Statistics for cooking treatments & extracted solvent= 127.6 at df = 6, p=0.000				All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 1748.3 at df = 2, p=0.000 F-Statistics for cooking treatments = 270.3 at df = 3, p=0.000 F-Statistics for cooking treatments & extracted solvent = 499.6 at df = 6, p=0.000				All data are reported as mean ± SD (n=3) F-Statistics for extracted solvent = 2571.1 at df 2, p=0.000 F-Statistics for cooking treatments = 470.7 at df = 3,p=0.000 F-Statistics for cooking treatments & extracted solvent = 767.4 at df = 6, p=0.000				

Values are presented as mean ±SD (n=3) and referred to the dry weight. Small and capital alphabets in superscripts indicate significant differences ($p < 0.05$) among cooking methods and extracts respectively
FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19
IC_{50} value of Ascorbic acid=0.005 mg/ml
ME= methanolic extract, EE= etanolic extract, BE= butanolic extract

(Ibrahim *et al.*, 2011; Irshad *et al.*, 2010) and phenolic acids (Kubola and Siriamornpun, 2008) in gourd vegetables. Flavonoids are considered as most popular natural antioxidants and effective secondary metabolic products (Gyamfi and Aniya, 2002).

Tannins

The tannin content in cooked vegetable samples varied from 6.9 to 11.5 mg CE/100g in comparison to 10.9 mg CE/100g of the raw vegetable sample. Microwave cooking exerted most pronounced decreasing effect on the tannin content. Methanol was most effective solvent in recovering the tannins followed by ethanol and butanol. The significant interaction effect (F=8.84 at df = 6, p=0.000) of the cooking method and the extraction solvent suggested that the tannin content of the ME of the cooked samples decreased up to 66.2%. In contrast, except BE of microwave cooked sample, an increase in the tannin content of EE and BE was observed in case of all the cooked samples. The results of present investigation are in agreement with observations of Zhang and Hamauzu (2004) and Racchi *et al*. (2002) who reported the loss of tannic acid after different cooking process. During the thermal treatment most important reactions are hydrolysis, oxidation, polymerisation and interaction of composition and reactions of thermal decomposition (Rakic *et al*., 2004) due to which reduction in tannins content is possible.

Total carotenoids

The carotenoid content measured as beta carotene of raw and cooked samples of *C. pepo* is presented in Table 1. The carotenoid content of cooked vegetable samples varied from 5.1 to 12.5 mg CE/100g (dwb) in comparison to 2.8 mg CE/100g (dwb) in the raw vegetable sample. Results showed that after cooking carotenoid content increased significantly ($p < 0.05$) in order of fried > pressure cooked > microwave cooked. In general, ethanol was most effective solvent in extracting carotenoids (7.7 mg CE/100g, dwb) followed by butanol and methanol. The most abrupt increase in the carotenoid content was observed in fried samples, being maximum in EE (14.6 times) followed by BE and ME. The previous study regarding the green leafy vegetables also observed a decrease in the retention of carotenoids after cooking (Chandrika *et al*., 2006). The higher value of carotenoids in the fried samples could be ascribed to the fact that carotenoids are fat soluble compounds and can solubilise readily in oil during frying, and thus the availability of carotenoids increased in the extract. Several authors also observed that cooking may increase carotenoids content because of its better extractability and enhanced bioavailability from heat treatment (Mazzea *et al*., 2011).

Total antioxidant activity as measured by FTC and TBA assays

The antioxidant activity of different samples in terms of measurement of inhibition of peroxidation

Table 3. Pearson correlation coefficients (r) between phytochemicals and antioxidant activity as determined by different assays

	Phenol	Flavonoids	Tannin	Carotenoids	FTC	TBA	FRAP	DPPH
Phenol	1.000							
Flavonoids	0.733**	1.000						
Tannin	0.616*	0.372	1.000					
Carotenoids	0.236	0.415	0.265	1.000				
FTC	0.759**	0.446	0.497	0.292	1.000			
TBA	0.737**	0.411	0.483	0.303	0.997**	1.000		
FRAP	0.828**	0.893**	0.385	0.511	0.617*	0.599*	1.000	
DPPH	0.303	0.195	0.597*	0.306	0.431	0.453	0.202	1.000

*Significantly correlated at $p < 0.05$, n=12
** Significantly correlated at $p < 0.0$, n=12

as measured by FTC and TBA methods is as shown in Table 2. The extracts from different samples of vegetable inhibited 5.1–30.4% peroxidation of linoleic acid after incubation for 96 h as measured by FTC assay. Nevertheless, the antioxidant activity of all extracts was significantly ($p < 0.05$) lower than BHT (88.8%) and vitamin E (74.6%) as standards. In general, ME revealed significantly higher antioxidant activity followed by EE and BE ($F= 504.3$ at df = 2, $p=0.000$). The percent inhibition decreased after cooking and highest loss of antioxidant activity was observed in pressure cooked samples followed by microwaved and fried samples ($F=85.5$ at df = 3, $p=0.000$). A high correlation between FTC and total phenols (0.759, $p < 0.01$) as given in Table 3 suggested that phenols were the main compounds responsible for the antioxidative activity therefore, the decrease in TPC in cooked sample extracts resulted in decreased percent inhibition as measured by FTC method. These findings are in agreement with the previous studies which have reported the positive correlation between phenol content and total antioxidant activity (Kubola and Siriamornpun, 2008). Interestingly, although the flavonoids content of the EE of the vegetable samples was the highest followed by ME, but still the antioxidant activity of the ME was higher than EE. This observation in the present study suggested a wide variation in the nature and kind of flavonoids compounds recovered in the different solvents and hence differences in the antioxidative potency of the extracts. The effectiveness of phenolics and flavonoids as antioxidants is not only because of their composition or relative amount but also influenced by the degree of polymerization, concentration and interaction of their diverse chemical structures to the colorimetric assays (Moure *et al.*, 2001).

During the oxidation process, peroxide is gradually decomposed to malondialdehyde, which was measured by TBA method on the final day of the incubation period. As determined by TBA method all the extracts revealed lower antioxidant activity than the standard BHT (90.9%) and vitamin E (78.3%). Percent inhibition of various extracts of raw and cooked *C. pepo* samples as measured by TBA method revealed highest average percent inhibition in ME (36.5%) followed by EE (27.8%) and BE (25.8%). As also observed in case of FTC method, a highly significant and positive correlation of the TBA results with phenols content ($r= 0.737$, $p < 0.01$) was observed indicating that phenols was the main phytochemicals which inhibited malondialdehyde formation and responsible for the antioxidant activity of *C. pepo* as described by TBA method. A very high correlation ($r =0.997$, $p < 0.01$) between FTC and TBA assay showed that the increase in peroxide level caused formation of malondialdehyde compounds (Zin *et al.*, 2002).

FRAP assay

As indicated by the results of FRAP assay given in Table 2, significant differences were observed in the FRAP values among all the cooking methods in their respective extracts ($p < 0.05$). The average FRAP values for all the selected cooking methods varied from 360.6 to 959.4 mM $FeSO_4$/100g being highest in EE followed by ME and BE. Pressure cooking significantly ($p < 0.05$) decreased the reducing power by 22% and 14.2% in ME and EE respectively while in case of BE it was found to increase by 3.7%. However, microwave cooking and frying exhibited a significant ($p < 0.05$) decrease in reduction power of all the extract except EE. The

Table 4. Phenolic acids/flavonoid compounds (µg/ml of extract) in methanolic extracts of raw and cooked vegetable of *C.pepo* as determinesd with HPTLC

Compounds	Raw	Pressure Cooked	Microwave cooked	Fried
Phenolic acid				
Gallic acid	nd	nd	nd	nd
Caffeic acid	nd	nd	19.32	Nd
Chlorogenic acid	303.34	nd	nd	Nd
p-Coumaric acid	nd	nd	nd	Nd
Vanillic acid	nd	nd	nd	Nd
Ferulic acid	nd	nd	nd	Nd
Cinnamic acid	8.58	10.70	7.18	0.14
Benzoic acid	nd	192.25	180.61	Nd
Ellagic acid	nd	nd	nd	Nd
Flavonoids				
Quercetin	nd	31.87	45.95	Nd
Myrecetin	53.11	nd	nd	42.09
Apigenin	3.78	2.69	2.91	Nd
Kaempferol	nd	nd	nd	4.96
Leutolin	nd	nd	nd	Nd
Rutein	727.89	500.75	552.76	470.98
Catechin	263.85	59.18	59.39	72.61

nd= not detected

phenolic content and antioxidant activity have a strong relationship between them (Velioglu *et al*., 1998). A highly significant and positive correlation of the FRAP value with phenols content ($r = 0.828$, $p < 0.01$) and flavonoids content ($r = 0.892$. $p < 0.01$) as indicated in Table 3 concluded that lower activity of processed sample may be due to the loss of phenols and flavonoids after the cooking process. In contrast, the increase in FRAP value might be attributed to the fact that the processing treatments bruised the tissue and expose the antioxidant components. In addition, increase in the reducing power could be attributed to the possible breakdown of tannins content (Oboh, 2005). The increased in reducing power after assorted cooking treatments were earlier reported in various studies (Ng *et al*., 2011; Lin and Chang, 2005).

Free radical scavenging activity

Free radical scavenging activity for DPPH radicals was expressed as IC_{50} value in samples (Table 2). It is evident from the results that the effect of cooking method and the extraction solvent was inconsistent. The ME of all the cooked samples revealed lower IC_{50} values in order of pressure cooked > microwaved > fried samples suggesting that antioxidant activity of the ME of the cooked samples was higher in comparison to the raw sample extract. The EE of microwaved and fried samples however, showed reduced antioxidant activity in comparison to the raw sample. Generally, the cooking treatments reduced the IC_{50} value, thus increasing the antioxidant activity. This increase in antioxidant capacity after heat treatment may be due to the suppression of oxidation by antioxidants due to thermal inactivation of oxidative enzymes (Yamaguchi *et al*., 2000). Moreover, enhanced antioxidant activity could also be witnessed due to the production of novel compounds due to maillard reaction (Morales and Babel, 2002). The earlier study showed that antioxidant capacity of different vegetables increased during cooking procedure compared to the fresh vegetables (Faller *et al*., 2009; Turkmen *et al*., 2005). In contrast, antioxidant capacity was also reported to decrease after thermal treatment of various vegetables (Ismail *et al*., 2004). The higher antioxidant activity of cooked samples even after their lower total phenolic content indicated that radical-scavenging activity differed not only by the concentration of phenolic compounds but also with the nature and kind of phenolic compounds. Therefore, no correlation was found between phenolic compounds and antioxidant activity measured by DPPH assay.

Identification of phenolic acids and flavonoids by HPTLC

Considering the fact that the average value of phytochemicals and antioxidant activity of ME of *C. pepo* was reasonably higher than the EE and BE and the methanol being considered as universal extraction solvent, the ME were subjected to HPTLC analysis. The Rf value of different phenolic acids and flavonoid standard compounds were: 0.08 (chlorogenic acid); 0.29 (gallic acid); 0.63 (quercetin); 0.72 (caffeic acid); 0.87 (kaempferol); 0.90 (apigenin) in solvent system I while 0.13 (rutin); 0.22 (ellagic acid); 0.37 (catechin); 0.52 (myricetin); 0.79 (leutolin); 0.91 (p-coumaric acid) in solvent system II and 0.73 (vanillic acid); 0.74 (ferulic acid); 0.75 (benzoic

acid); 0.94 (cinnamic acid) in solvent system III. The HPTLC analysis revealed the presence of phenolic acids and flavonoids in ME as presented in Table 4 and Figure 1 showed the HPTLC profile. Cinnamic acid was identified as phenolic acid in extracts of raw as well as cooked samples but in varying concentrations being maximum to 10.7 µg/ml in pressure cooked sample extract. Chlorogenic acid (303.3 µg/ml) in raw sample extract and benzoic acid in pressure cooked sample extract (192.25 µg/ml) and microwaved sample extract (180.61 µg/ml) were identified as other prominent phenolic acids. Rutin (727.89 µg/ml) and catechin (263.85 µg/ml) were identified as prominent flavonids in *C. pepo* extracts. However, their concentration decreased drastically in the heat processed samples. Quercetin was although not identified in the raw sample extract, but it was detected in the extracts of pressure cooked (31.87 µg/ml) and microwaved (45.95 µg/ml) samples. Presence of chlorgenic acid and benzoic acid as phenolic acids along with other phenolic compounds and flavonoids including quercetin, luteolin, kaempferol has been reported in *C. pepo* extracts (Iswaldi *et al*., 2013). Rutin has been studied for its antioxidant, anti-inflammatory and protective effects against hepatotoxicity (Koda *et al*., 2008). The results presented here showed that heat processing treatments could make the phenolic acids and flavonoids different from that of uncooked form. The formation of new phenolic compounds after heat treatments was due to availability of precursors formed by non-enzymatic inters conversion between phenolic molecules (Vega-Galvez *et al*., 2009). The most destructive effect of heat processing was on cholorgenic acid, which was not identified at all in the cooked samples. The degradation in polyphenolic compounds may also due to the epimerization, dimerization, hydrolysis, oxidative and polymerization reaction (Okumura *et al*., 2008).

Conclusions

It could be concluded from the results that cooking method as well as extraction solvent could have considerable effects on recovery of phenols and flavonoids present in *C. pepo.* Methanol was most effective solvent in extracting phenolics and tannins whereas flavonoids were extracted best in ethanol. The antioxidant activity of the methanolic extracts as measured by FTC, TBA and DPPH assays was the highest. From the results it could be inferred that cooking had both positive and negative impact on the concentration of phytochemicals and their antioxidant activity. In general, pressure cooking emerged as most effective cooking treatment in lowering the antioxidant activity. However, from the observations of quantitative testing and HPTLC profiles, it can also

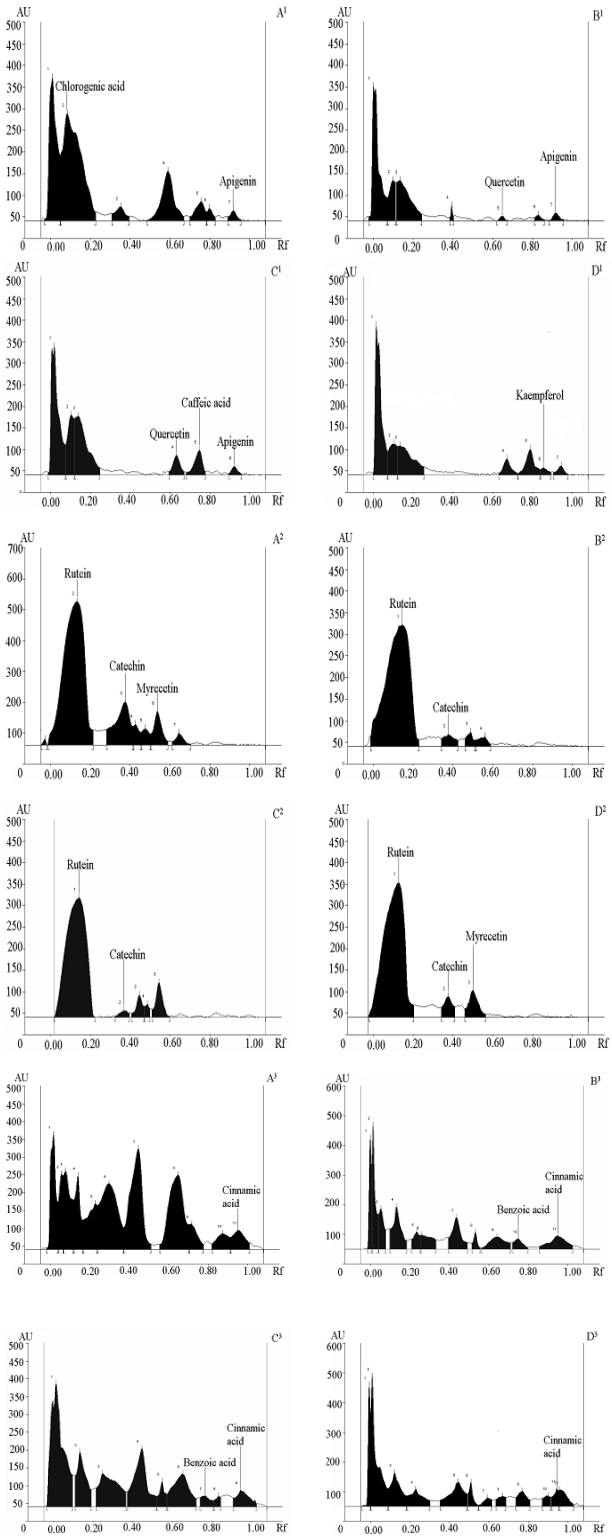

Figure 1. HPTLC profiles of ME of *C. pepo* (A) raw (B) pressure cooked (C) microwave cooked (D) fried; numeric superscript on the alphabet represent (1) solvent system I (6.4 chloroform: 3.9 hexane: 2.0 methanol: 0.5 formic acid) (2) solvent system II (4.0 chloroform: 1.0 hexane: 1.0 methanol: 1.0 formic acid) (3) solvent system III (4.5 acetonitrile:1.0 methanol: 0.5 water)

be concluded that there could be even wider range of phytochemicals present in *C. pepo*. Therefore, more detailed qualitative and quantitative analyses of the phytochemicals in this gourd vegetable will be necessary to elucidate its antioxidant potential.

Acknowledgements

This study was financially supported by University Grants Commission, New Delhi.

References

Azizah, A.H., Wee, K.C., Azizah, O. and Azizah, M. 2009. Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin *(Cucurbita moschato)*. International Food Research Journal 16(1): 45-51.

Burns, R.E. 1971. Method for estimation of tannin in grain sorghum. Agronomy Journal 63: 511-512.

Chan, E., Lim, Y. and Omar, M. 2007. Antioxidant and antibacterial activity of leaves of *Etlingera* species *(Zingiberaceae)* in Peninsular Malaysia. Food Chemistry 104(4): 1586-1593.

Chandrika, U.G., Svanberg, U. and Jansz, E.R. 2006. In vitro accessibility of beta-carotene from cooked Sri Lankan green leafy vegetables and their estimated contribution to vitamin A requirement. Journal of Science of Food and Agriculture 86(1): 54–61.

Chang, H.Y., Yu-Ling, H.O., Ming-Jyh, S., Yaw-Huei, L., Mu-Chuan, T., Sheng-Hua, W.U., Guan-Jhong, H. and Yuan-Shiun, C. 2007. Antioxidant and free radical scavenging activities of Phellinus merrillii extracts. Botanical Studies 48(4): 407-417.

Chaturvedi, S., Hemamalini, R. and Khare, S.K. 2012. Effect of processing conditions on saponin content and antioxidant activity of Indian varieties of soybean (*Glycine max* Linn.) Annals of Phytomedicine 1(1): 62-68.

Chism, G.W. and Haard, N.F. 1996. Characteristics of edible plant tissues. In Fennema, O.R (3rd Eds). Food Chemistry, p. 943–1011. New York: Marcel Dekker.

Chonoko, U.G. and Rufai, A.B. 2011. Phytochemical screening and antibacterial activity of *Cucurbita pepo* (pumpkin) against *Staphylococcus aureus* and *Salmonella typhi.* Bayero Journal of Pure and Applied Sciences 4(1): 145-147.

Dragovic-Uzelac, V., Delonga, K., Levaj, B., Djakovic, S. and Pospisil, J. 2005. Phenolic profiles of raw apricots, pumpkins, and their purees in the evaluation of apricot nectar and jam authenticity. Journal of Agricultural and Food Chemistry 53(12): 4836-4842.

Faller, A.L.K. and Fialho, E. 2009. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. Food Research International 42(1): 210-215.

Gyamfi, M.A. and Aniya, Y. 2002. Antooxidant properties of Thonningianin A. Isolated from the African medicinal herb, Thonningia sanguinea. Biochemical Pharmacology 63 (9): 1725-1737.

Hamissou, M., Smith, A.C., Carter, J.R.E. and Triplett, J.K. 2013. Antioxidative properties of bitter gourd *(Momordica charantia)* and zucchini *(Cucurbita pepo)*. Emirates Journal of Food and Agriculture 25(9): 641-647.

Han, X., Shen, T. and Lou, H. 2007. Dietary polyphenols and their biological significance. International Journal of Molecular Science 8 (9): 950-988.

Harborne, J.B. 1984. Phytochemical methods. 2nd edn. London: Chapman and Hall publications.

Hunter, K.J. and Fletcher, J.M. 2002. The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. Innovative Food Science and Emerging Technologies 3(4): 399–406.

Ismail, A., Marjan, Z.M. and Foong , C.W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry 87(4): 581–586.

Iswaldi, I., Gomez-Caravaca, A.M., Lozano-Sanchez, J., Arraez-Roman, D., Segura-Carretero, A. and Fernandez-Gutierrez, A. 2013. Profiling of phenolic and other polar compounds in zucchini (*Cucurbita pepo* L.) by reverse-phase high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Food Research International 50(1): 77-84.

Jimenez-Monreal, A.M., Garcia-Diz, L., Martinez-Tome, M., Mariscal, M. and Murcia, M.A. 2009. Influence of cooking methods on antioxidant activity of vegetables. Journal of Food Science 74(3): 97-103.

Kikuzaki, H. and Nakatani, N. 1993. Antioxidant effects of some ginger constituents. Journal of Food Science 58(6): 1407-1410.

Kim, J.S., Kanga, O.J. and Gweon, O.C. 2013. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. Journal of Functional Foods 5(1): 80-86.

Koda, T., Kuroda, Y. and Imai, H. 2008. Protective effect of rutin against spatial memory impairment induced by trimethyltin in rats. Nutrition Research 28(9): 629-634.

Kubola, J. and Siriamornpun, S. 2008. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. Food Chemistry 110(4): 881-890.

Lim, Y., Lim, T. and Tee, J. 2007. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry 103(3): 1003-1008.

Lin, C.H. and Chen, B.H. 2005. Stability of carotenoids in tomato juice during storage. Food Chemistry 90(4): 837-846.

Mazzea, T., N'Dri, D., Chiavaro, E., Visconti, A., Fogliano, V. and Pellegrini, N. 2011. Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetables. Food Chemistry 128(3): 627- 633.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina

Fasan honey, as well as their radical scavenging activity. Food Chemistry 91(3): 571-577.

Morales, F.J. and Babel, M.B. 2002 . Antiradical efficiency of Maillard reacting mixtures in a hydrophilic media. Journal of Agricultural and Food Chemistry 50(10): 2788–2792.

Moure, A., Cruz, J.M., Franco, D., Dominguez, J.M., Sineiro, J., Dominguez, H., Nunez, M.J. and Parajo, J.C. 2001. Natural antioxidants from residual sources. Food Chemistry 72(2): 145–171.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B. and Wrolstad, R.E. 2002. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. Journal of Agricultural and Food Chemistry 50(3): 519-525.

Oboh, G. 2005. Effect of blanching on the antioxidant property of some tropical green leafy vegetables. LWT-Food Science and Technology 38(5): 513-517.

Okumura, H., Ichitani, M., Takihara, T. and Kunimoto, K.K. 2008. Effect of cyclodextrins on the thermal epimerization of tea catechins. Food Science and Technology Research 14(1): 83-88.

Oloyede, F.M., Obisesan, I.O., Agbaje, G.O. and Obuotor, E.M. 2012. Effect of NPK fertilizer on chemical composition of pumpkin (*Cucurbita pepo* Linn.) seeds. The Scientific World Journal 2012: 1-6.

Pandey, K.B. and Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cell Longevity 2(5): 270-278.

Pericin, D., Krimer, V., Trivic, S. and Radulovic, L. 2009. The distribution of phenolic acids in pumpkin's hull-less seed, skin, oil cake meal, dehulled kernel and hull. Food Chemistry 113(2): 450–456.

Racchi, M., Daglia, M., Lanni, C., Papetti, A., Govoni, S. and Gazzani, G. 2002. Antiradical activity of water soluble components in common diet vegetables. Journal of Agricultural and Food Chemistry 50(5): 1272–1277.

Rakic, S., Maletic, R.O., Perunovic, M.N. and Svrzic, G. 2004. Influence of thermal treatment on tannin content and antioxidation effect of oak acorn *Quercus cerris* extract. Journal of Agricultural Science 49(1): 97-107.

Rawson, A., Hossain, M.B., Patras, A., Tuohy, M. and Brunton, N. 2013. Effect of boiling and roasting on the polyacetylene and polyphenol content of fennel *(Foeniculum vulgare)* bulp. Food Research International 50(2): 513-518.

Saikia, S. and Mahanta, C.L. 2013. Effect of steaming, boiling and microwave cooking on the total phenolics, flavonoids and antioxidant properties of different vegetables of Assam, India. International Journal of Food and Nutritional Science 2: 47-53.

Santra, M., Santra, D., Rao, V., Taware, S. and Tamhankar, S. 2005. Inheritance of β-carotene concentration in durum wheat (*Triticum turgidum* L. ssp. durum). Euphytica 144 (1-2): 215-221.

Şengul, M., Yildiz, H. and Kavaz, A. 2014. The Effect of Cooking on Total Polyphenolic Content and Antioxidant Activity of Selected Vegetables. International Journal of Food Properties 17(3): 481-490.

Silveira, N.A., Alvarez-Leite, J.I., Silva, M.E., Vieira, L.Q., Bambirra, E.A. and Vieira, E.C. 1996. Effect of high ingestion of dehydrated pumklin (*Cucurbita pepo* L.) on the cholesterol metabolism and on the hepatic histology in mice. Arquivos de Biologia e Tecnologia 39(4): 961-74.

Sofowora, A .1996. Research on medicinal plants and traditional medicine in Africa. Journal of Alternative and Complimentary Medicine 2(3): 365-372.

Stewart, A.J., Bozonnet, S., Mullen, W., Jenkins, G.I., Michae, E.J. and Crozier, A. 2000. Occurrence of flavonols in tomatoes and tomatobased products. Journal of Agricultural and Food Chemistry 48(7): 2663 2669.

Sultana, B., Anwar, F. and Iqbal, S. 2008. Effect of different cooking methods on the antioxidant activity of some vegetables from Pakistan. International Journal of Food Science and Technology 43(3): 560-567.

Trease, G.E. and Evans, W.C. 1989. Pharmacognosy. 13th edn. London: Balliere Tindall.

Turkmen, N., Sari, F. and Velioglu, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 93(4): 713–718.

Veda, S., Kamath, A., Platel, K., Begum, K. and Srinivasan, K. 2006. Determination of bioaccessibility of β-carotene in vegetables by *in vitro* methods. Molecular Nutrition and Food Research 50 (11): 1047-1052.

Vega-Galvez, A., Di Scala, K., Rodriguez, K., Lemus-Mondaca, R., Miranda, M., Lopez, J. and Perez Won, M. 2009. Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum*, L. var. *Hungarian*). Food Chemistry 117(4): 647-653.

Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Journal of Agricultural and Food Chemistry 46(10): 4113-4117.

Waterman, P.H. 1992. Searching for Bioactive compounds various Strategies. Journal of Natural Products 53(1): 13-22.

Yamaguchi, F., Ariga, T., Yoshimira, Y. and Nakazawa, H. 2000. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. Journal of Agricultural and Food Chemistry 48(2): 180-185.

Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 88(4): 503–509.

Zhou, K. and Yu, L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT - Food Science and Technology 39(10): 1155-1162

Zin, Z.M., Abdul-Hamid, A. and Osman, A. 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. Food Chemistry 78(2): 227-231.