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**EFFECT OF DOMESTIC COOKING METHODS ON THE NUTRITIVE
AND ANTIOXIDATIVE COMPONENTS OF MUSTARD LEAVES
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ABSTRACT

Effect of different cooking methods on the proximate composition, vitamin C, total phenolic content (TPC), total carotenoid, β -carotene content and antioxidant activity of mustard leaves, one of the most commonly consumed green leafy vegetables in India was studied. Cooking caused a significant change in some components of the proximate nutrients. Vitamin C content decreased significantly ($p \leq 0.05$) by all the cooking methods compared to raw. Carotenoid content decreased by all the cooking methods with the lowest retention in sautéed sample. Total phenolic content of the cooked and uncooked vegetable ranged from 62.62 mg GAE/100g FW in raw sample to 84.70 mg GAE/100g FW in sautéed sample. Total antioxidant activity of mustard increased upon cooking with the highest significant increase in sautéed sample.

Keywords: Antioxidant, Carotenoid, Cooking, Mustard, Phenolic, Proximate

INTRODUCTION

Epidemiological studies have provided evidence revealing the protective effect of fruits and vegetables consumption against the risk of cardiovascular diseases, neuro-degenerative diseases, certain cancers and other age related chronic diseases (Dauchet *et al.*, 2006; Hooper and Cassidy, 2006; Sahni *et al.*, 2008; Takachi, 2008; Stidley *et al.*, 2010). Fruits and vegetables are important components of a healthy diet and their intake is widely promoted worldwide (Hanif, 2006; Podsedek, 2007; Takachi *et al.*, 2008).

They contain a wide variety of biologically active, nutritive and non-nutritive compounds known as phytochemicals.

The protection that fruits and vegetables provide against diseases has been attributed to the various phytochemical antioxidants contained in them (Wang and Daun, 2004; Carlsen *et al.*, 2010; Song *et al.*, 2010). Their contributory factors are due to the presence of vitamins and pro-vitamins such as ascorbic acid, tocopherols and carotenoids and in addition to that they are also rich in a wide variety of phenolic substances (Cieslik *et al.*, 2006; Pourmorad *et al.*, 2006).

Green leafy vegetables (GLV) have a unique place among vegetables because of their colour, flavour and health benefits. They are rich source of many nutrients and antioxidant vitamins (Gupta *et al.*, 2005; Gupta and Prakash, 2009). Mustard leaves (*Brassica campestris*) is one of the most commonly consumed green leafy vegetables in India during the cold season. It is steamed or cooked in many different forms. The beneficial effects of *Brassica* vegetables on health improvement have been partly attributed to their complex mixture of phytochemicals possessing antioxidant activity. The most widespread and diverse group of polyphenols in *Brassica* species are the flavonoids (mainly flavonols but also anthocyanins) and the hydroxycinnamic acids (Cartea *et al.*, 2011).

In general, vegetables are prepared at home on the basis of convenience and taste preference rather than retention of nutrient and health-promoting compounds (Masrizal *et al.*, 1997). Cooking would bring about a number of changes in physical characteristics as well as chemical composition of the vegetables. Keeping this point in mind, the study was planned with an objective to evaluate the proximate

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composition and some of the antioxidant constituent of spinach beet so as to understand the best way to preserve the nutrients during processing and cooking.

MATERIALS AND METHODS

Plant Materials and Sample Preparation

Mustard leaves (*Brassica campestris*) were procured from Department of Vegetable Crops, Punjab Agricultural University, Punjab. The vegetable was washed with tap water, pat dried with paper towel and the inedible parts were removed. The edible portions were cut uniformly (1 cm), mixed well and divided into six equal portions. One portion was retained raw, others were cooked in five different methods (i.e., boiling, steaming, pressure cooking, microwaving and sautéing). Two hundred gram of each vegetable was utilized for each cooking method. The treatment time, amount of water and oil used are given in Table 1. Refined soybean oil was used for sautéing. Raw and cooked samples of the mustard leaves were then blended and stored in the freezer for further analysis.

Table 1: Treatment time (in min), amount of water and oil used (in ml)

Treatments	Time	Water	Oil
Boiling	13	300	-
Steaming	8	-	-
Pressure cooking	4	150	-
Microwave cooking	5	10	-
Sautéing	8	-	10

Proximate Composition Analysis

A portion of the raw and cooked samples was analyzed for moisture immediately after cooking. The rest of the samples were oven dried at 60-70°C, powdered and were analyzed for their proximate compositions using the AOAC (2000) method. The moisture content was determined by air-oven drying up to constant weight, and the crude protein contents by microKjeldah method. The lipid content was determined using petroleum ether (bp. 60-80°C) in a soxhlet extraction apparatus and crude fiber content by dilute acid and alkali hydrolysis. Total ash was determined by igniting the sample in a muffle furnace for 5-6 hours at about 600°C. Carbohydrate contents were calculated by difference of total contents from 100.

Determination of Ascorbic Acid

The vegetable samples were estimated for their ascorbic acid content by the Association of Vitamin Chemists (AOVC, 1996) method. The blue colour produced by the reduction of 2,6-dichlorophenyl indophenols dye by ascorbic acid is estimated colorimetrically.

Determination of Carotenoid Contents

Total Carotenoid and β -carotene contents were determined according to the method of Ranganna, (2002). Two grams of each vegetable samples were taken in a pestel and mortar, grinded using acetone. Extraction was repeated for 2-3 times until the extract becomes colourless. The extracts were pooled and filtered. The filtrate was transferred to a separating funnel and added 10-15 ml of petroleum ether. The pigments got transferred into the petroleum ether phase by diluting the acetone with water containing 5% sodium sulphate. Petroleum ether extracts were pooled and volume was made up to 25 ml with 3% acetone in petroleum ether. Absorbance at 452 nm was measured spectrophotometrically for total carotenoid content. For determination of β -carotene content, the extract was passed through a column containing activated alumina. β -carotene band formed in the column was eluted and made up the volume with 3% acetone in petroleum ether. Then the absorbance was measured spectrophotometrically.

Preparation of Extract

Two gram of homogenized sample was extracted with 50 ml 80% methanol for all the carrot samples for the determination of total phenolic content and total flavonoid content. The mixture was centrifuged at

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2200 rpm for 15 min at room temperature and the supernatant decanted into polypropylene tubes. The clear extracts were analyzed for the determination of phenolic content, flavonoid content and antioxidant activity.

Determination of Total Phenolic Content

The total phenol content of the extract was determined using the method reported by Singleton and Rossi (1965). A sample of methanolic extract (0.2 ml) was mixed with 1 ml of Folin–Ciocalteu reagent (ten fold dilutions). The mixture was allowed to stand for 5 min at room temperature before adding 0.80 ml of 20% Na₂CO₃ and then mixed gently. The reaction mixture was incubated for 40 min and the absorbance measured at 760 nm in spectrophotometer. The total phenolic content was calculated using gallic acid as standard.

Determination of Total Flavonoid Content

The total flavonoid content was measured using the Aluminium chloride colorimetric method modified from the procedure reported by Woisky and Salatino (1998). Two ml of the extract was mixed with 100µl of 10 percent AlCl₃, 100µl of 1 mol per litre potassium acetate and 2.8 ml water and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm. TFC was calculated by using the following formula (Kiranmai et al., 2011)

$$TFC (g/100g) = (R * D.F * V * 100)/W$$

Where R - Result obtained from the standard curve

D.F - Dilution factor

V - Volume of stock Solution

W - Weight of plant used in the experiment

Determination of Antioxidant Activity using DPPH Method

Total antioxidant activity was determined by the 2, 2,-di- phenyl-2-picryl-hydrazyl (DPPH) method of Liang Yu (2008). An aliquot of 0.1 ml of the samples was taken in a test tube and then 2.9 ml of 0.01mM DPPH reagent was added and vortexed and let to stand at room temperature in the dark for 30 min. The decrease in absorbance at 517nm was measured. Antioxidant activity (AA) was expressed as percentage inhibition of the DPPH radical and was determined by the following equation.

$$AA\% = (1 - A/B \times 100), \text{ Where, } A = \text{Absorbance of the sample, } B = \text{Absorbance of the blank}$$

RESULTS AND DISCUSSION

Table 2: Effect of cooking on the proximate composition of Mustard leaves

Nutrients	Raw	Boiling	Steaming	Pressure cooking	Microwave cooking	Sautéing
Moisture (g/100g FW)	87.9±0.03 ^a	91.8±0.08 ^b	91.2±0.23 ^c	90.7±0.10 ^c	86.4±0.16 ^d	79.1±0.33 ^e
Protein (g/100g DW)	33.1±0.01 ^a	32.8±0.21 ^a	33.1±0.69 ^a	33.4±0.89 ^a	33.9±0.66 ^a	32.8±0.16 ^a
Fat (g/100g DW)	2.7±0.01 ^a	2.8±0.00 ^{ab}	2.7±0.07 ^a	2.9±0.04 ^b	2.7±0.00 ^a	26.7±0.10 ^c
Crude fibre (g/100g DW)	6.5±0.06 ^a	6.4±0.06 ^a	6.3±0.07 ^b	6.5±0.06 ^a	6.4±0.02 ^a	6.2±0.32 ^b
Total ash (g/100g DW)	12.3±0.92 ^a	11.5±0.55 ^b	11.8±0.39 ^c	11.4±0.22 ^d	11.7±0.11 ^c	10.9±0.06 ^c
Carbohydrate (g/100g DW)	46.1±0.01 ^a	46.4±0.02 ^b	42.9±0.01 ^c	46.6±0.01 ^d	43.4±0.01 ^e	45.9±0.03 ^f

Mean±SE in rows followed by different superscripts differ significantly (p≤0.05)

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Proximate Composition

Data in Table 2 show the effect of cooking on the proximate compositions of mustard leaves. Moisture content of the raw and cooked sample ranged from 87.9 to 91.8 percent. There was a significant ($p \leq 0.05$) difference in the moisture content of raw and cooked samples. Moisture content was found highest in boiled (91.8 %), followed by steamed (91.2 %), pressure cooked (90.7 %), raw (87.85 %), microwave cooked (86.4 %) and the least in sautéed mustard (77.3%).

Kala and Prakash (2006) has reported that microwave cooking resulted in greater moisture loss compared to conventional cooking and pressure cooking. The protein content of raw, boiled, steamed, pressure cooked, microwave cooked and sautéed samples were 33.1, 32.8, 33.1, 33.4, 33.9 and 32.8 percent respectively. There was no significant ($p \leq 0.05$) difference in the protein content of the cooked from the raw mustard. The result is supported by Kala and Prakash (2006) that reported no significant difference in the protein content because of cooking.

The percent fat content did not differ significantly ($p \leq 0.05$) for raw (2.7g), boiled (2.8g), steamed (2.7g) and microwave cooked (2.7g). However, the fat content for pressure cooked (2.9g) and pressure cooked (26.7g) samples increased significantly.

Percent fat content was highest in the sautéed sample because of the oil used for sautéing. Crude fibre content of mustard leaves cooked by different cooking methods did not differ significantly ($p \leq 0.05$) from that of raw except for sautéed sample.

Similar findings were reported by Mepba *et al.*, (2007). However, the total ash and carbohydrate content differ significantly for all the samples from the raw.

Antioxidant Components

Table 3: Effect of cooking on the antioxidant components of Mustard leaves (FW)

Antioxidant components	Raw	Boiling	Steaming	Pressure cooking	Microwave cooking	Sautéing
Vitamin C (mg/100g)	30.56+0.09 ^a	8.51+0.07 ^b	9.58+0.05 ^c	8.39+0.08 ^b	9.61+0.07 ^c	8.29+0.08 ^b
Total carotenoid (mg/100g)	10.45+0.15 ^a	7.04+0.10 ^b	8.20+0.10 ^{cd}	8.05+0.15 ^{cd}	8.65+0.05 ^d	7.70+0.10 ^c
β- carotene (mg/100g)	1.68+0.03 ^a	1.04+0.01 ^b	1.02+0.04 ^b	0.83+0.03 ^c	0.88+0.02 ^{bc}	0.68+0.03 ^d
TPC mg GAE/100g	62.62+0.06 ^a	77.50+0.07 ^{bc}	77.44+0.56 ^c	75.38+0.15 ^b	79.39+0.30 ^c	84.70+0.70 ^d
TFC mg QE/100g	23.09+0.78 ^a	23.10+0.25 ^a	22.69+0.34 ^b	24.26+0.21 ^c	24.50+0.24 ^d	40.52+0.98 ^e
DPPH %	69.44+0.06 ^a	88.30+0.01 ^c	89.42+0.19 ^d	88.50+0.19 ^c	90.26+0.09 ^e	84.88+0.09 ^b

Mean±SE in rows followed by different superscripts differ significantly ($p \leq 0.05$)

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Table 4: Percent retention of antioxidant compounds in processed mustard leaves

Antioxidant components	Boiling	Steaming	Pressure cooking	Microwave cooking	Sautéing
Vitamin C (mg/100g)	30.8 ^a	30.8 ^a	30.9 ^a	26.6 ^b	20.3 ^c
Total carotenoid (mg/100g)	78.4 ^a	77.1 ^b	86.7 ^c	69.9 ^d	55.3 ^e
β- carotene (mg/100g)	68.5 ^a	59.7 ^b	55.6 ^c	44.3 ^d	30.4 ^e
TPC mg GAE/100g	137 ^a	121.5 ^b	134.5 ^c	107.1 ^d	101.4 ^e
DPPH %	140.8 ^a	126.5 ^b	143.4 ^c	109.8 ^d	91.7 ^e

Mean±SE in rows followed by different superscripts differ significantly (p≤0.05)

The result of the vitamin C content of spinach beet cooked by different cooking methods is shown in Table 3. The vitamin C content in raw sample was 30.56 mg/100g FW, the value is in range with what Gupta and Prakash (2009) has reported for the ascorbic acid content in some green leafy vegetables. However, cooking caused a significant ($p \leq 0.05$) decrease in the vitamin C content with the lowest retention in sautéed sample (20.3 %) followed by microwave cooking (26.6 %), boiling (30.8 %), steaming (30.8 %) and pressure cooking (30.9 %) Table 4.

The reduction in vitamin C content of cooked vegetable is in line with the study conducted by Oboh (2005) on the effect of blanching on the vitamin C content of some green leafy vegetables which reported 47.5 to 82.4 percent loss. The loss in vitamin C content during the different cooking method could be attributed to the fact that vitamin C is very soluble in water and not stable at high temperatures (Adefegha and Oboh, 2011).

GLVs are rich sources of carotenoids (Raju *et al.*, 2007). It has been reported that available β-carotene from greens in India is 95%, and out of this 90% is contributed by GLVs (Singh *et al.*, 2001). Total carotene content of raw, boiled, steamed, pressure cooked, microwave cooked and sautéed mustard were 10.45, 7.40, 8.20, 8.05, 8.65 and 7.70 mg/100g FW respectively (Table 3). Carotenoid content decreased significantly ($p \leq 0.05$) by all the cooking methods. Similarly, the β-carotene content of the vegetable cooked by different cooking methods decreased significantly ($p \leq 0.05$). The retention of Carotenoids ranged from 30.4 to 86.7 percent depending on the cooking methods used (Table 4). This finding is supported by the result of the study conducted by Zhang and Hmauzhu (2004) that both conventional and microwave cooking caused loss of total carotenoids in broccoli florets and stems. De Sa and Rodriguez-Amaya (2004) also noted a reduction of carotenoid concentration both in boiled and stir-fried green vegetables.

Findings of the study conducted by Ismail *et al.*, (2004) indicated that phenolic compounds were very sensitive to heat treatment even in a short period of cooking. Total phenolic content of the cooked and uncooked vegetable ranged from 62.62 mg GAE/100g FW in raw sample to 84.70 mg GAE/100g FW in sautéed sample (Table 3). The TPC of the cooked vegetable were in the following order: sautéed > microwave cooked > Boiled > steamed > pressure cooked > raw. TPC of mustard were found to increase

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significantly ($p \leq 0.05$) by all the cooking methods utilized. The TPC retention in differently cooked vegetable was found to be in the range of 101.4 to 137 percent, which indicates an increased in the TPC was highest in boiled sample (Table 4). A significant ($p \leq 0.05$) increase in the total phenol content was reported by Oboh (2005) in some tropical green leafy vegetables and Turkmen *et al.*, (2005) in spinach. Contradictory findings were reported by Bunea *et al.*, (2008) for spinach and Podsedek (2007) for kale, cauliflower and broccoli.

Total antioxidant activity as determined by the 2, 2,-di- phenyl-2-picryl-hydrazyl (DPPH) method increased in the following order: raw < sautéed < boiled < pressure cooked < steamed < microwave cooked (Table 3).

Raw vegetable had the total antioxidant activity of 53.31 percent which significantly ($p \leq 0.05$) increased upon cooking by different cooking methods. Similarly, Turkmen *et al.*, (2005) reported a significant increase in the antioxidant activity of vegetables cooked by boiling, steaming and microwaving. The formation of novel substances, such as products of the Maillard reaction, during processing could also increase the antioxidant capacity (Monzocco *et al.*, 2001).

Conclusion

Antioxidant constituents- vitamin C and carotenoids were found to reduce by the different cooking methods. On the other hand TPC and total antioxidant activity increased by the heat treatments. The present study indicates that processing of vegetables, particularly cooking can enhance antioxidant potential by inhibition of enzyme activity and transformation of antioxidants into more active compounds on contrary to the common belief that the antioxidant concentrations and activities in processed vegetables are lower than those of the corresponding raw samples. However, the extent of increase depended on the cooking methods employed.

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