Impact of blanching and drying on antioxidant contents and antioxidant activity of Indian mustard leaves (Brassica juncea)

Sumitra Kumari Meena and Mukta Agrawal

1Krishi Vigyan Kendra, Durgapur, MPUAT, Udaipur, Rajasthan, India
2Department of Home Science, University of Rajasthan, Jaipur- 302004, Rajasthan, India
*Corresponding author: sumitra_dr@yahoo.com
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Abstract
Leafy vegetables contain significant amount of bio-active health promoting compounds which act as antioxidants that can impart health benefits beyond basic nutrition. Blanching and dehydration by drying are processes commonly used in preservation of leafy vegetables. The present investigation was planned to study the effect of blanching and drying on antioxidant contents (total phenols, flavonoids, Vitamin C and β carotene) and antioxidant activity of mustard leaves. In the present investigation the fresh mustard leaves were collected in a lot, washed thoroughly and divided into three portions, i.e. raw, blanched and dried to know the impact of processing (i.e. blanching and drying) on the antioxidant contents and antioxidant activity. Raw blanched and dried samples were analyzed for antioxidant contents and antioxidant activity through standard methods. Results of the study revealed that fresh mustard leaves contained total phenol 48.37 ± 0.45mg/100g GAE , flavonoids 9.17 ± 0.30 mg/100g QE, vitamin C 12.65 ± 6.01 mg/100g and β carotene 6.30±0.20 mg/100g and antioxidant activity was 46.50 ± 2.90 mg/100g AAE. The antioxidant contents and antioxidant activity of mustard leaves were found influenced by processing treatments (blanching and drying), Blanching caused loss while drying increased the concentration of antioxidant contents.

Key words: Antioxidants, blanching, drying, Indian mustard

Introduction
Vegetables play an important role in human diets, as they provide essential nutrients and health benefits to our body. Leafy vegetables are widely used for food, because of the rich assortment of nutrients found in them. They are rich source of carotene, ascorbic acid, minerals and dietary fiber. Leafy vegetables contain significant amount of bio-active health promoting compounds which act as antioxidants that can impart health benefits beyond basic nutrition. Mustard leaves (Brassica juncea) is a winter season vegetable, also known as Indian mustard, Chinese mustard and leaf mustard belongs to the Cruciferae family and widely used for both its edible green leaves and cooking oil, which is extracted from its seeds. Mustard leaves contain many bio-active compounds that have health promotional and disease prevention properties. Mustard leaves are rich source of vitamin C, phenolics, flavonoids, carotenes, lutein and zeaxanthin (Lin and Harnly, 2010). Recently special attention has been paid towards green leafy vegetables especially those that are rich in bio active compounds which act as antioxidants, and there is now increasing interest in antioxidant activity of such bio-active compounds, present in the diet and effect of processing on them. Thus present study was set to determine the antioxidant contents and antioxidant activity of mustard leaves and influence of blanching and drying treatments.

Materials and Methods
The mustard green leaves were purchased from local market of Sanganer, Jaipur, and Rajasthan in a single lot. The collected fresh leave samples (in triplicate) were washed thoroughly in running tap water to remove dust and dirt etc. and tender part of stems and seeds and foreign material and edible portion were separated. Each sample was divided
into three portions, *i.e.* raw, blanched and dried to know the impact of processing (*i.e.*, blanching and drying) on the antioxidant activity, vitamin C, beta carotene, total phenol and flavonoid content in the samples.

**Blanching:** The fresh leave samples were blanched by emersion in boiling water for 2 minutes. After blanching the extra water was drained and vegetables stored in airtight container and kept at -18°C ± 5°C till analysis is done.

**Drying:** The fresh green leaves (100g) were dried using a cabinet dryer at 60°C for 10-12 hours until samples became crisp and brittle to touch. After drying the samples were powdered (1.0 mm mesh) and stored in an airtight container and kept in a refrigerator for further analyses. The green leaves were also analyzed in raw and fresh form for which they were stored in airtight container and kept at -18°C ± 5°C till analyses was done.

**Antioxidant content extraction and determination:** Total phenols and flavonoids were extracted with methanol according to the method described by Parekh and Chanda (2007) with some modifications. For extraction 10 g of crushed sample from each vegetable was taken with 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 hrs. After 24 hrs, the extract was filtered through eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min., supernatant was collected and the solvent was evaporated and the dry extract was stored at 4°C in air tight bottles.

Total phenol content in the sample extract was determined using UV spectrophotometer by folin-ciocalteu method (Olajire and Azeez, 2011). The flavonoid content in extracted samples was estimated by aluminum chloride (AlCl₃) method (Jagadish *et al*., 2009) using UV spectrophotometer. The antioxidant activity was analysed using FRAP method (Oyaizu, 1986) in sample extract. UV spectrophotometer was used for reading the absorbance. The content of vitamin C was estimated by titrametric method using 2, 6 dichlorophenol indophenol (DCPIP) described by Rao and Deshpande (2006). β-carotene was analyzed according to the method of the Association of Official Analytical Chemists (AOAC, 1980). Experiments were performed in three replicates and obtained data was elaborated by ANOVA.

**Results and Discussion**

**Impact of blanching and drying on antioxidant contents and antioxidant activity**

**Total Phenol (mg GAE/100g):** Phenolic compounds are secondary plant metabolites and possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expression (Marinova *et al*., 2005). The total phenol content of fresh mustard leaves was 48.4±0.45 mg GAE/100g. The total phenol content in samples of blanched mustard leaves was 46.2±0.85 mg GAE/100g whereas in dried leaves it was 302.3±0.60 mgGAE/100 g (Table 1). The difference in total phenol content of fresh versus blanched, fresh versus dried, and blanch versus dried mustard leaves were statistically significant (Table 1).

**Flavonoids (mgQE/100g):** Flavonoids are an antioxidant group of compounds composed of flavonols, flavanols, anthocyanins, isoflavonoids, flavanones and flavones. The flavonoid content of the fresh leaves was 9.2±0.30 mgQE/100g. Yadav *et al*. (2013) also reported 23.4 GAE/100gfw (fresh weight basis) flavonoid in mustard leaves. The flavonoid content in blanched and dried samples of mustard leaves was 7.7 ± 0.50 mgQE/100g and 82.5 ± 0.81mg QE/100g respectively (Table 1). The flavonoid content reduced by 16.4% due to blanching whereas it increased by 799.7% due to drying.

**Vitamin C (mg/100g):** The importance of vitamin C as an antioxidant is indispensable in biological system. This vitamin is reputed for scavenging the harmful free radicals produced in the body and also enhance the antioxidant defense mechanism in body. The vitamin C content in fresh mustard leaves sample was 12.7±6.01 mg/100g. Gopalan *et al*. (2010) also reported 33 mg/100g vitamin C in mustard leaves. In blanched and dried samples of mustard leaves the vitamin C content was 8.4 ±0.04 mg/100g and 18.3±0.44 mg/100g respectively (Table 1). Blanching caused 4.2% decrease in vitamin C
content when compared with fresh leaves. While Vitamin C content was significantly increased by 139.4% after drying.

**β-carotene (mg/100g):** β-carotene commonly occurring in nature including α, β and γ carotene, lycopene and cryptoxanthin which is widely distributed in green leafy vegetables, yellow orange fruits and some other vegetables (Padmavati and Udupi, 1992). Besides being a precursor of vitamin A it also acts as an antioxidant capable of protecting the body against many diseases. The mean β-carotene content of mustard leaves in fresh and raw samples was 6.30±0.20 mg/100g. On blanching treatment it significantly decreased by 46.4% whereas on drying β-carotene content significantly increased (Table 2) the value of β-carotene in dried sample was observed 22.55±0.30 mg/100g. Blanched and dried samples were also differed significantly at 5% in β-carotene content (Table 1).

**Antioxidant activity (mg AAE/100g):** Antioxidant activity shows the overall antioxidant power of the particular food item. Antioxidant activity analyzed in fresh mustard leaves was 46.5±2.9 mgAAE/100g, in blanched sample 40.2±3.3 mgAAE/100g and in dried sample it was 461.5±2.5 mgAAE/100g (Table 1). Blanching caused 6.3 % loss in the antioxidant activity. In case of dried samples an increase in antioxidant activity by 892% was observed when compared with fresh leaves. Statistically at 5 per cent level of significance difference was observed among all three i.e. raw, blanched and dried mustard leaves (Table 1).

Similarly Yadav et al. (2013) studied antioxidant activity by FRAP method among different green leafy vegetables and reported that 15.2mg /100g ascorbic acid equivalent (AAE) in spinach 28.3mg /100g AAE in chenopodium leaves and 46.5mg/100g AAE in mustard leaves on fresh weight basis. Similarly the antioxidant activity as reported by Ali et al. (2011) studied in mustard leaves 48.7 mg/100g trolox equivalent (TE).

**Effect of blanching:** The blanching treatment reduced the antioxidant contents which may be due to leaching out of antioxidant content in water. Destruction of vitamins due to thermal processing may be other reason for reduction in vitamin C, and β-carotene content. Thermal treatment also caused destruction in total phenol and flavonoids content and antioxidant activity.

Kala and Prakash (2004) reported 9.4mg/100g vitamin C in spinach and found significant loss (95%) in conventional cooking. The significant decrease was reported in the vitamin C content of selected leafy vegetables [fresh (43.5–148.0 mg/100 g), blanched (15.8–27.3 mg/100 g)] by (Oboh, 2005). Gupta et al. (2008) reported that retention of ascorbic acid was reduced as the blanching time and temperature increased in commonly consumed green leafy vegetable and conclude that 80 °C for 1 min and chemical media are most ideal for blanching greens with maximum nutrient retention. Masrizal et al. (1997) demonstrated that spinach and swamp cabbage had retained 57-79 % and 65-80 % of β-carotene respectively after blanching for 3.0-5.5 min. Oboh (2005) reported 5.8 to 51.5% reduction in antioxidant activity of some tropical green leafy vegetables due to blanching.

### Table 1: Effect of processing on antioxidant contents and antioxidant activity in mustard leaves

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant Activity (mg/100g AAE)</th>
<th>Total Phenol (mg/100g GAE)</th>
<th>Flavonoid (mg/100g QE)</th>
<th>Vitamin C (mg/100g)</th>
<th>β-carotene (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>46.5 ± 2.9</td>
<td>48.4 ± 0.5</td>
<td>9.2 ± 0.3</td>
<td>12.7 ± 6.0</td>
<td>6.3±0.2</td>
</tr>
<tr>
<td>Blanched</td>
<td>40.2 ± 3.3</td>
<td>46.2 ± 0.9</td>
<td>7.7 ± 0.5</td>
<td>8.4 ± 0.04</td>
<td>3.4 ±0.1</td>
</tr>
<tr>
<td>Dried</td>
<td>461.5 ± 2.5</td>
<td>302.3 ± 0.6</td>
<td>82.5 ± 0.8</td>
<td>18.3 ± 0.4</td>
<td>22.6±0.3</td>
</tr>
<tr>
<td>CD 5%</td>
<td>5.8</td>
<td>1.3</td>
<td>1.2</td>
<td>6.96</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Raw and blanched green leaves were analysed on wet basis
*Dried leaves were analysed on dry weight basis

± Standard deviation
Table 2: Comparative assessment of antioxidant contents and antioxidant activity of raw and processed mustard leaves

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant Activity (mg/100g AAE)</th>
<th>Total Phenol (mg/100g GAE)</th>
<th>Flavonoid (mg/100g QE)</th>
<th>Vitamin C (mg/100g)</th>
<th>β-carotene (mg/100g)</th>
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<tr>
<td>Raw vs Blanched*</td>
<td>*</td>
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<td>Raw vs Dried</td>
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<tr>
<td>Blanched vs Dried</td>
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</table>

*-Significant   NS- Non Significant

Effect of drying: Drying is a very useful technique to extend the shelf-life of green leafy vegetables and to produce dried leaves with high antioxidants and a potent antioxidant activity.

Drying cause removal of water and in turn reduces the volume of food stuff. If any nutrient is assessed results are expressed on dry weight basis the values are very high. Zoro et al. (2016) studied the antioxidant properties of shade dried leafy vegetables and found increase in antioxidant activity which ranged from 34.4% to 52.0% after 15 days of shadow drying. Oboh and Akindahunsi (2004) reported that sun-drying caused a significant increase in total phenol content of green leafy vegetables. Capecka et al. (2005) reported that air-drying of peppermint at 25 to 32°C resulted in significant increases in total phensols.

Conclusion

Mustard leaves are rich source of antioxidant contents and antioxidant activity. Blanching cause a decrease in the total phenol, flavonoids, vitamin C, beta carotene and antioxidant activity while drying caused concentration of nutrients and increase in all assessed antioxidant contents. Thus the dried leaves then can be easily incorporated in different traditional recipes at acceptable levels. They can also be used as a natural fortificants in our daily diet for enhancing the micronutrient content.

Reference


Oboh G and Akindahunsi AA. 2004. Change in the ascorbic acid, total phenole and antioxidant activity of sun dried commonly consumed green
leafy vegetables in Nigeria. *Nutr Health* **18**: 29-36.


