Influence of blanching on antioxidant profile and phytochemical constituents of four edible flowers collected from West Bengal, India

Soma Sinha, Sanchita Bhattacharjee and Sauryya Bhattacharyya*

Department of Food & Nutrition, Sarada Ma Girls’ College, Talikhola, Barasat, Kolkata 700126, India.

*Corresponding author
Email – sauryya.b@gmail.com
Tel – 91 9830346992

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Abstract
The present study was designed to delineate the influence of blanching on four edible flowers viz. Sesbania grandiflora, Cucurbita maxima, Moringa oleifera and Brassica nigra regarding in vitro antioxidant profile and contents of phytochemicals. Common blanching methods like soaking at hot water at 80°C for 4 minutes and heating in a microwave for 90 seconds were engaged. The assays performed were DPPH radical decolorization assay, estimation of total polyphenols, flavonoids and monomeric anthocyanins. The study indicated that blanching by normal heating did not alter DPPH radical scavenging ability of Sesbania grandiflora and Cucurbita maxima but reduced in case of the other two. Blanching by normal heating also reduced total phenolic contents and flavonoid contents significantly in case of all four. Blanching using microwave reduced radical scavenging activities of Sesbania grandiflora and Cucurbita maxima but remained unaltered in case of the other two. This method also reduced total phenolic contents and flavonoid contents significantly in case of all four. However, anthocyanins contents improved in case of Sesbania grandiflora and Brassica nigra after blanching. The study indicated that common blanching processes had a moderate deteriorating effect on antioxidant potential and phytochemical constituents of the subject edible flowers.

Key words: Antioxidant, Blanching, Edible flowers, Polyphenolics, DPPH, Anthocyanin.

1. INTRODUCTION
Vegetables act as a source of natural antioxidants as they help in removing potentially damaging free radicals that cause various human diseases [1]. Epidemiological evidences indicate an association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain cancers, probably due to contributions from vitamins, minerals, dietary fiber and polyphenols [2, 3, 4]. Among vegetables, edible flowers are becoming more popular as evidenced by an increase in the number of edible flower cookbooks, culinary magazine articles, and television shows [5]. Flowers have traditionally been used in many types of cooking such as European, Asian, East Indian, Victorian English, and Middle Eastern. Recently, it was reported that edible flowers are good sources of minerals [6]. It is believed that consumption of these flower vegetables can cure illness and diseases, although not much work has been done in this field.

Blanching of foods involves mild heating in water with the objective of exposing vegetables or fruits to high temperatures for a short period [7]. It is commonly used in food processing to inactivate enzymes and destroy microorganisms [8]. Typically, blanching is carried out by treating the vegetables with steam or hot water for 1-10 min at 75-95°C, the time-temperature combination being dependent on the type of vegetable [9]. The process can decrease or increase antioxidant activity of agricultural products [10]. Pressurized blanching of Sesbania grandiflora L. Pers flower decreased the anthocyanin and vitamin C content [11]. However, studies with wheat reported that pressurized blanching at 100°C increased the total phenolic content in the wheat powder [12]. Blanching can have negative effect on nutrients, such as vitamins and phenolic compounds, which are relatively unstable when subjected to thermal treatments [13]. To summarize with, it could be said that blanching has some permanent effect on...
vegetables before it is consumed by humans.

Among edible vegetables, flowers of *Sesbania grandiflora*, *Cucurbita maxima*, *Moringa oleifera* and *Brassica nigra* are four very important edible delicacies in the state of West Bengal, India, not only for their typical tastes and flavors, but also for their potent pharmacological activities. *Sesbania grandiflora* (family – *Leguminosae*) flowers has been used in many pharmacological adverse conditions like libido, night blindness, ulcer, antioxidant, cardiac problems, obesity, oedema, dim vision, gout and bronchitis. Anxiolytic, anticonvulsant, hepatoprotective and anthelmintic activity of the flowers are also reported [14]. *Cucurbita maxima* (pumpkin) belongs to the family *Cucurbitaceae*. Many studies demonstrated that pumpkin has extensive bioactivities, such as hepatoprotection, anti-diabetes, anti-cancer, and anti-obesity properties [15]. *Moringa oleifera* is another plant that has been identified to contain natural antioxidants as a whole [16]. Traditionally, the plant is used as antispasmodic, stimulant, expectorant and diuretic. It also has antioxidant, anti-inflammatory, anticancer, hepatoprotective and fertility enhancing activities [17]. Black Mustard (*Brassica nigra*, family – *Brassicaceae*) is an appetizer, digestive, diuretic, emetic, irritant and stimulant. Grounded seeds of it have some skin applications also [18]. However, apart from *Sesbania grandiflora*, flowers of the remaining three plants were not evaluated for their pharmacological activities extensively.

Usually, thermal treatments associated with cooking processes could affect the levels of nutritional and antioxidant factors of the food ingredient [19, 20, 21]. Hence, it is a necessity to keep blanching conditions at a level just sufficient to cause inactivation of the deleterious enzymes but with minimal effect on other beneficial attributes. However, since blanching requires exposure to high temperatures, there is a possibility of reduction of therapeutic activities of the vegetables. In view of above, the present study was designed in such a way that it resembled closely with the common blanching processes practiced in India and to ascertain the change in the antioxidant and phytochemical profile of the subject edible flowers. To our knowledge, it was one of the very few studies that dealt with the change in quality of human consumable edible flowers before and after blanching for their radical scavenging abilities, and probably the first with the four subject edible flowers. In this way, we would be able to know the appropriate blanching methods, which would retain the most effectiveness of the edible flowers for human consumption. The present study reports the achievement of the aim through some common *in vitro* antioxidant assays.

2. MATERIALS AND METHODS

2.1 Chemicals

2,2'-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Ascorbic acid and Folin-Ciocalteau’s reagent were obtained from Merck, India. Quercetin was obtained from SRL, India. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study.

2.2 Collection of samples

Fresh flowers of the four vegetables were collected from a local market of Barasat in 24 Parganas (N) district of West Bengal. The samples were checked for dirt or any visible damages, and were discarded if found.

2.3 Blanching and extraction

The flowers were washed well with water before blanching. About 5 gms of samples were suspended in water with a solid-to-solvent ratio of 1:3 (w/v). Blanching for each sample was done in quadruplicate. The following are the two common methods of blanching [22]—

Hot water method – The samples in water were heated at 80°C for 4 minutes. Then the water was drained off, the samples were dried with a hand drier and hot extracted with 60% aqueous methanol for 5 minutes. Solid-to-solvent ratio for extraction was maintained 1:10 (w/v). After extraction, the mixture was filtered through Whatman 1 and used for further studies.

Microwave method – The samples in water were heated at high power for 90 seconds in a commercial microwave oven. Then the water was drained off, the samples were dried with a hand drier and hot extracted with 60% aqueous methanol for 5 minutes. Solid-to-solvent ratio for extraction was maintained 1:10 (w/v). After extraction, the mixture was filtered through Whatman 1 and used for further studies.

2.4 *In vitro* assays

DPPH radical decolorization assay – The DPPH assay was performed using a previously described procedure [19]. 1 ml DPPH solution (3 mg DPPH powder in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC₅₀ of the samples. Gallic acid was used as positive control and comparing with its’ IC₅₀ and the results were expressed as µg of gallic acid per gram of fresh flower.

Total polyphenolics content assay – The assay was performed using a previously described procedure [20]. Briefly, 0.5 ml of sample was mixed with 1.5 ml Folin-Ciocalteau’s solution (1:10 v/v diluted with distilled water) and allowed to stand for 28±2°C for 5 min. Then 2 ml of 7% (w/v) aqueous sodium carbonate solution was added and the mixture were allowed stand for another 90 min and at darkness. The absorbance of the blue color that developed was measured at 725 nm using spectrophotometer (Systronics, Model – 2202). Gallic acid was used to prepare the standard curve (20–100 µg/ml) and the total phenolic concentration in the spice extract was expressed as mg of gallic acid per gram of fresh flower.

Estimation of total flavonoids content – Colorimetric aluminum chloride method was used for flavonoid determination following a published procedure [23]. Briefly, 0.5 ml extractive of each sample were mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1ml of 1 M potassium acetate solution, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV-Vis...
spectrophotometer (model – Systronics 2202). Total flavonoids content were calculated using a calibration curve of quercetin as standard and the results were expressed as µg quercetin equivalent/gm fresh flower. Estimation of monomeric anthocyanins content – Determination of monomeric anthocyanin content was conducted by pH-differential method [20]. Total monomeric anthocyanins were expressed as cyanidin-3-glucoside. Sample absorbance was read against a blank cell at 700 nm and 510 nm and at pH 1.0 and 4.5. The absorbance (A) of the sample was then calculated according the following formula:

\[ A = (A_{510} - A_{700})_{pH\, 1.0} - (A_{510} - A_{700})_{pH\, 4.5} \]

where A is the net absorbance of samples at the wavelengths mentioned in the subscript. The monomeric anthocyanin pigment content in the sample will be calculated according to the following formula:

\[
\text{Anthocyanin content (mg/L)} = \left( \frac{A \times MW \times DF}{\varepsilon \times l} \right)
\]

Where DF was dilution factor, MW was molecular weight of cyanidin-3-glucoside (449.2) and \(\varepsilon\) was molar absorptivity (26,900). The anthocyanin content in the sample extractive was converted into content per gram of fresh flower and expressed.

2.5 Statistical Analyses

The results are presented as mean ± SD. Significant differences between means were detected by one-way analysis of variance (ANOVA), followed by multiple comparisons using Tukey’s post-hoc test. Differences were considered significant when \(p < 0.05\). The analyses were done with the software ‘Prism 4.0’ (GraphPad Inc., USA).

3. RESULTS

3.1 DPPH radical decolorization assay

The results of this assay indicated that radical scavenging activities of flowers of Brassica nigra and Moringa oleifera were relatively higher than the rest two without blanching (figure 1). However, in case of Sesbania grandiflora and Cucurbita maxima flowers, the radical scavenging effect reduced significantly (\(p \leq 0.001\)) after microwave assisted blanching compared to control (0 min blanching). In case of the other two flowers, significant decrement was observed in comparison to control after heating at 80°C for 4 minutes (figure 1).

3.2 Total polyphenolics content

The results of this assay indicated that total phenolic contents of all the four flowers reduced significantly in comparison to control (0 min blanching, figure 2). Maximum reduction was observed in case of flowers of Brassica nigra after blanching, although it contained the maximum polyphenolics amongst the four on 0 min blanching conditions (i.e. control).

3.3 Total flavonoids content

The results of this assay indicated that flavonoid content of Brassica nigra flowers was maximum at 0 min blanching (i.e. control) among the four flowers (figure 3). However, contents reduced significantly in all the four flowers in comparison to control after blanching by both the methods (figure 3).

3.4 Monomeric anthocyanins content

The results of this assay indicated that total monomeric anthocyanin content of the three flowers, apart from flower of Cucurbita maxima, improved significantly after blanching by both the methods (figure 4). Only in case of Moringa oleifera flowers, the content remained almost same after blanching using microwave irradiation.
4. DISCUSSION

It is of great importance to consider that only a small amount of vegetables is consumed in the raw state and most of them needed to be processed for safety and quality. However, many food composition data bases never consider the fact that concentrations of nutrients and their activity might change through heating effects, such as blanching [24]. It has also been observed that blanching mostly reduced effectiveness of the vegetables, although in some cases, it improved the contents of a few antioxidative phytochemicals.

Since determination of antioxidant potential of plant based substances is still being an unresolved problem and not a single assay would be sufficient for the assessment of their overall efficacy [25], a few inter-related assays should be employed for the adjudication of change of quality of the edible flowers before and after blanching, in the present study. It has been observed that amounts of total phenolics and flavonoids fall considerably after common blanching procedures. However, significant improvement was observed in the contents of anthocyanins after blanching, with the exception of *Cucurbita maxima* flowers only. Improvement in anthocyanin contents after blanching was also observed by other researchers [8]. One plausible cause might be the hydrolysis of cyanidin glycosides upon heating in water, thereby releasing non-polar aglycones thereof. This hypothesis would be substantiated by the fact that DPPH radical scavenging abilities of some of the flowers were not diminished significantly after blanching (except for *Moringa oleifera* and *Brassica nigra*, where scavenging reduced significantly after blanching by normal heating) as the assay involves less polar medium (e.g. ethanol) for the reaction, where monomeric anthocyanin aglycones could solublize well. Better performance by the less polar phytochemicals in DPPH assay protocol was also reported earlier [19].

The common methods of blanching employed in the households of India have been shown to reduce radical scavenging abilities as well as in the levels of phytochemical antioxidants moderately of the subject edible flowers, as revealed by the present study. The present study thus lend credence to the fact that thermal stress produced during blanching might reduce antioxidative potential of food ingredients to some extent, which could affect their potential during human consumption. However, marginal change in DPPH radical scavenging ability in case of *Moringa oleifera* and *Brassica nigra* upon microwave assisted blanching, as well as improvement in monomeric anthocyanin contents would indicate that employment of milder blanching methods might retain the effectiveness of the subject flowers.

5. CONCLUSION

The major conclusion arising out of this research was that the radical scavenging potential and a few phytochemical contents of four edible flowers viz. *Sesbania grandiflora*, *Cucurbita maxima*, *Moringa oleifera* and *Brassica nigra*, collected from West Bengal, were diminished moderately by thermal processing methods that resemble blanching. DPPH radical scavenging activities of *Moringa oleifera* and *Brassica nigra* were almost unchanged after blanching by microwave irradiation, probably due to enhanced monomeric anthocyanin concentration in the extracts. However, total phenolic contents and flavonoids contents were diminished significantly after blanching. The present study revealed that common methods of blanching employed in the households of India reduced radical scavenging abilities as well as in the levels of phytochemical antioxidants moderately of the subject edible flowers. The study thus lend credence to the fact that thermal stress produced...
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