International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 689-693

Received: 19-07-2019
Accepted: 23-08-2019

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Changes of nutrients in different green leafy vegetables during processing

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Abstract
Green leafy vegetables are important protective foods and highly beneficial for the maintenance of health and prevention of diseases as they are good sources of micronutrients which can be utilized to build up and repair the body. In the present investigation, the changes in nutrient content of selected green leafy vegetables (such as tree lettuce (*Pisonia alba*), pasalai leaves (*Basella alba*), fenugreek leaves (*Trigonella foenum*), manathakkali leaves (*Solanum nigrum*) and moringa leaves (*Moringa olifera*)) subjected to different drying methods such as shade drying, cabinet drying and fluidized bed drying were studied. The results indicated that fluidized bed dried manathakkali leaves powder had highest β-carotene content (37,712µg/100g) followed by tree lettuce leaves powder (32,595µg/100g) pasalai leaves powder (29,800µg/100g), fenugreek leaves powder (26,628µg/100g) and moringa leaves powder (23,628µg/100g). The moisture content of GLV powders dried under different drying techniques ranged from 4.02 to 5.60 per cent. Also, tree lettuce leaves powder subjected to fluidized bed drying had significantly higher antioxidant activity (95.99%) compared to manathakkali leaves powder which had lowest (83.13%) antioxidant activity. The study revealed that the fluidized bed dried GLV powder samples subjected to pretreatment of blanching had higher nutrient content compared to respective GLV powder samples which were either dried under shade or cabinet dried. The results suggest that green leafy vegetables powder has high potential as micronutrient rich food contributing significant health benefits for women to combat micronutrient malnutrition.

Keywords: Moisture, antioxidant activity, beta carotene, vitamin C

Introduction
Green leafy vegetables (GLVs) are abundant sources of micronutrients and antioxidants and are highly perishable because of its high moisture content. Green leafy vegetables have a unique place among vegetables because they are inexpensive, easy to serve and most of all due to their colour, flavour and also since they are rich sources of β-carotene, ascorbic acid, folic acid, chlorophyll, calcium, iron, phosphorous, zinc and dietary fibre (Makobo *et al*. 2010) [13]. In addition, GLVs confer significant health benefits to human nutrition as they are made up of cellulose, hemi-cellulose and pectin substances that give them their texture and firmness (Mohammed and Sharif, 2011) [14]. They provide adequate amount of dietary fibers, minerals, vitamins and other nutrients to people in developing countries. Apart from the variety which they add to the menu (Asaolu *et al*., 2012) [6], they are valuable sources of nutrients especially in rural areas where they contribute substantially to mineral, vitamin, fiber, protein and other nutrient contents of the daily diet which are usually in short supply. They are important protective foods and useful for the maintenance of health and for prevention of various diseases (Mohammed and Sharif, 2011) [14]. Leafy vegetables have low energy density and are thus recommended for weight management (Nwanekezie and Obiakor, 2014) [15]. The main protective action of leafy vegetables has been attributed to the presence of antioxidants, including ascorbic acid, α-tocopherol, β-carotene and phenolics as these constituents are reported to fight degenerative diseases (Kaur and Kapoor 2002) [11]. The easiest and healthiest way to maintain body antioxidant status is by consumption of antioxidant rich GLVs. The dietary supply of minerals is inadequate to meet the dietary requirements of rapidly growing human population in the world (Mohammed and Sharif, 2011) [14] since minerals cannot be synthesized by humans and animals thus they must be provided through food and water. Leafy vegetables contain numerous minerals such as Calcium, iron, copper, phosphorous, zinc, chlorine, potassium and sodium which are vital for growth and metabolism (Angela *et al*., 2010) [3].

Antioxidants are defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative diseases (Gulcin *et al.*, 2010) [10]. The highly unstable free radicals (reactive oxygen species) are released into human body during different metabolic processes. These free radicals are responsible for the disruption of stability of other molecules by releasing electrons from their molecular structure. Overproduction of ROS, such as superoxide anion, hydroxyl radical, hydrogen peroxide and reactive oxygen, is associated with cellular and metabolic injury leading to cancer, cardiovascular diseases, neurodegenerative disease and inflammation. These harmful effects of free radicals can be minimized by the addition of sufficient amounts of natural antioxidants to human diet. These natural antioxidants play an important role in the reduction of the risk of chronic diseases such as cardiovascular diseases and cancers (Serafini *et al*. 2002) [19]. Antioxidants also help prevent macular degeneration and other serious eye disease and are critical for maintaining optimal cellular and systemic health and well-being. Hence, there is a need for dynamic balance, between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects (Wang et *al.*, 2006) [21].

Many green leafy vegetables of promising nutritive value are often underutilized and has the potential to nourish the ever increasing human population. They can be raised comparatively at lower management cost even on poor marginal lands, but have remained underutilized due to lack of awareness about its nutritional and health benefits and need for popularization of processing technologies for better utilization. For the processing of GLVs, blanching is an important primary step which helps to inactivate enzymes, retain colour, and modification of product texture (Ahmed *et al*., 2001) [2]. Dehydration is most suitable method for post-harvest management to increase the shelf-life and promote food security (Grabowski *et al*., 2003) [9]. Dried leafy vegetables are highly nutritious, light weight, tasty and easy to prepare, store and use (Yaldyz and Ertekyn 2001) [22]. Hence, the study was undertaken to assess the changes in nutrient content of different green leafy vegetables subjected to different drying techniques to establish the processing condition which confers better retention of nutrients and antioxidant activity.

Materials and Methods

Selection of green leafy vegetables

Green leafy vegetables viz., tree lettuce, pasalai leaves, fenugreek leaves, manathakkali leaves and moringa leaves are rich in micronutrients and antioxidants such as β-carotene, ascorbic acid, colour, crude fiber, lutein, total phenols, flavonoids, antioxidant activity other nutrients like calcium, iron and phosphorous etc., However, these GLVs are underutilized due to lack of awareness of the need for regular consumption of locally available nutrients rich GLVs. Hence, the aforementioned green leafy vegetables were selected for the development of the micronutrients rich green leafy vegetables powder. The selected green leafy vegetables were subjected to different processing methods and also analysed for chemical characteristics.

Processing of GLVs

Blanching

The selected green leafy vegetables were sorted, trimmed, washed and steam blanched for three minutes. The blanched GLVs were then dried into GLV powders using three different techniques viz., shade drying, cabinet drying and fluidized bed drying. The GLVs not subjected to steam blanching served as control.

Dehydration of GLVs

Shade drying

The blanched and unblanched samples (Control) of the selected GLVs were evenly spread on sterilized trays and placed under room temperature for six days to dry until crisp and brittle (Moisture content 4.00 to 6.00 %). The dried GLVs were further milled to powder form using pulverizer and sieved through BS 60 mesh sieve, packed in airtight containers and stored at room temperature for further analysis.

Cabinet drying

The blanched and unblanched samples (Control) of the selected GLVs were evenly spread on sterilized trays and placed in preheated cabinet drier at a temperature of 60º C for 4 to 6 hours to completely dried until crisp and brittle (Moisture content 4.00 to 6.00 %). The dried GLVs were further milled to powder form using pulverizer and sieved through BS 60 mesh sieve, packed in airtight containers and stored at room temperature for further analysis.

Fluidized bed drying

The blanched and unblanched samples (Control) of the selected GLVs were placed in the glass enclosure of the fluidized bed drier, and its mouth was closed using a muslin cloth and dried at temperature of 60º C for 40 minutes until crisp and brittle (moisture content 4.00 to 6.00%). The dried GLVs were further milled to powder form using pulverizer and sieved through BS 60 mesh sieve, packed in airtight containers and stored at room temperature for further analysis.

Analysis of the chemical constituents of the GLVs powder

Moisture

The moisture content of the sample were estimated by hot air oven method as per the procedure given in AOAC (1995) [5]. A known quantity (5g) of processed GLVs powder samples were dried at 110ºC till concordant values were obtained. The moisture content was expressed in percentage.

β-carotene

A known quality (5g) of the processed GLV powdered samples was ground in pestle and mortar using 10-15 ml of acetone until the residue becomes colourless. The acetone extract was transferred to a separating funnel, 20 ml of petroleum ether were added and mixed gently. Then 20 ml of 5 per cent sodium sulphate were added and shaken gently. The upper petroleum ether layer was removed and re-extracted the lower aqueous phase with 20 ml of petroleum ether. The petroleum ether extracts were pooled and washed once with distilled water and then 10g of anhydrous sodium sulphate were added to the petroleum ether and kept for 30 minutes. The petroleum ether extract was decantated into a 100ml volumetric flask and volume was made up with petroleum ether and read in spectrophotometer at 453 nm using petroleum ether as blank. The β-carotene was expressed as µg per 100g of the sample (Ranganna, 1995) [16].

Antioxidant activity

The radical scavenging activity of green leafy vegetables powder were determined by the 2, 2,-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH assay was performed as described in Lim *et al.* (2007) [12] with slight modification. DPPH is a purple coloured stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolorizes the DPPH solution. The degree of discolouration indicates the scavenging potential of the antioxidant compounds. Different aliquots (0.2 - 1 ml) of methanol extracts of each sample were pipette out into test tubes and made up the volume in each test tube to 1ml with methanol. Then 2 ml of freshly prepared DPPH solution (0.1 mM) in methanol was added. The tubes mixed thoroughly and allowed to stand in the dark at room temperature. The absorbance decrease was determined after 30 min at 517 nm using a spectrophotometer. Methanol (1 ml) replacing the plant extract serve as a negative control and methanol (2 ml) replacing the DPPH reagent serve as sample blanks. The percentage of radical scavenging activity (% RSA) or percentage inhibitions of DPPH of the methanolic extract of the samples were calculated by the following formula.

$$\% \text{ RSA} = \frac{\{A(C) - (A(S)\}}{A(C)} \times 100$$

Where A(C) - absorbance of negative control, A(S) - absorbance

Statistical analysis

The data were analyzed using AGRESS software three factors ANOVA analysis with $p \leq 0.05$ was performed to identify significant difference in all experimental parameters carried out in studies at triplicates. Factorial completely randomized design (FCRD) was applied for the analysis (Gomez and Gomez, 1984) [8].

Results and Discussion

Changes in chemical constituents of green leafy vegetables subjected to different drying techniques

The green leafy vegetables powder were evaluated for chemical characteristics such as moisture, β-carotene and antioxidant activity and the data are presented in Table 1 to 3.

Moisture

The moisture content (Table 1) of the GLV powders obtained from subjecting the selected GLVs to different drying techniques was in the range of 4.02 to 5.50 per cent. This is in agreement with the range of 5.10% to 5.50 per cent for moisture content of green leafy vegetables powder under shade dried condition. The lowest moisture content was found to be in the samples subjected to blanching pretreatment (4.02%) and maximum moisture content (4.32 per cent) was noticed the control GLVs powder samples dried by fluidized bed drying. The moisture content of the samples dried under cabinet drying condition ranged from 4.40% to 4.58 %. The difference in terms of moisture content between different greens leafy vegetables powder and different treatments was highly significant. Emelike and Ebere (2016) [7]. stated that in his study of GLVs using three different drying methods, that among the drying techniques, the value for moisture content ranged from 1.65% to 5.89% and the highest value was observed in shade dried (5.89%) sample than sun drying (4.25%) and oven dried (1.65%) samples. Singh and Sagar (2010) [20] reported a moisture content of 5.50% to 6.50 per cent in dehydrated drumstick leaves. Ankita and Prasad (2013) [4] reported that the moisture content of spinach powder was found to be 4.43% and 4.48 per cent in coarse and fine powder. Sanni *et al.*, (2006) [18] stated that the lower the moisture content of a product the better the shelf stability of such products. The low moisture content of the GLVs powder samples obtained in the present study is within the permissible moisture content which can contribute to good storage stability.

Table 1: Moisture (% DWB) contents in green leafy vegetables powder

Green leafy vegetables powders	Shade drying		Cabinet drying		Fluidized bed drying	
	Without blanching	With blanching	Without blanching	With blanching	Without blanching	With blanching
Tree lettuce powder	5.22	5.10	4.50	4.40	4.08	4.02
Pasalai leaves powder	5.32	5.20	4.53	4.42	4.32	4.13
Fenugreek leaves powder	5.44	5.28	4.58	4.45	4.22	4.11
Manathakkali leaves powder	5.40	5.30	4.56	4.43	4.10	4.06
Moringa leaves powder	5.50	5.30	4.50	4.47	4.12	4.08

β-carotene

From the results (Table 2), it could be deduced that, the unblanched (control) and blanched samples of lettuce leaves (T_1) subjected to shade drying had β-carotene content of 30,138 and 32,409 µg/100g respectively. The corresponding values for the cabinet dried samples were 30,129µg/100g and 32,312 µg/100g respectively, and for the samples subjected to fluidized bed drying, the respective values were 30,140µg/100g and 32,595µg/100g. The unblanched and blanched samples of pasalai leaves powder (T_2) had β-carotene content in the range of 27,390µg/100g to 29,510µg/100g respectively for the shade dried sample, 27,383µg/100g and 29,433µg/100g respectively in cabinet dried samples and 27,400µg/100g and 29,800µg/100g respectively for the respective samples dried by fluidized bed drying. The β-carotene content of the fenugreek leaves powder (T_3) samples dried by shade drying was 24,263µg/100g and 26,475µg/100g respectively for the unblanched and blanched samples. The corresponding values were 24,250µg/100g and 26,469µg/100g for the samples dried by cabinet drying compared to 24,272µg/100g and 26,628µg/100g of β-carotene content noticed in the respective samples dried under fluidized bed drying. For the manathakkali leaves powder (T_4) the corresponding β-carotene content for the unblanched and blanched samples subjected to shade drying was 35,255µg/100g and 37,540 µg/100g respectively, for cabinet dried samples, the respective values were 35,248µg/100g to 37,511µg/100g respectively and was 35,270µg/100g and 37,712µg/100g respectively under fluidized bed drying. For the unblanched and blanched moringa leaves powder (T_5) samples the corresponding values were 21,210µg/100g and 23,391µg/100g respectively under

shade drying, 21,198µg/100g and 23,340µg/100g under cabinet drying and 21,223µg/100g and 23,628µg/100g for the samples subjected to fluidized bed drying. The highest retention of β-carotene content was found in all the GLVs samples which were pretreated by blanching compared to the unblanched respective GLV samples. Amog the different drying methods, the fluidized bed drying technique conferred maximum retention of β-carotene content (37,712µg/100g).

Table 2: β-carotene (µg/100g DWB) contents in green leafy vegetables powder

Green leafy vegetables powders	Shade drying		Cabinet drying		Fluidized bed drying	
	Without blanching	With blanching	Without blanching	With blanching	Without blanching	With blanching
T_1	30,138	32,409	30,129	32,312	30,140	32,595
T_2	27,390	29,510	27,383	29,433	27,400	29,800
T_3	24,263	26,475	24,250	26,469	24,272	26,628
T_4	35,255	37,540	35,248	37,511	35,270	37,712
T_5	21,210	23,391	21,198	23,340	21,223	23,628

T_1-tree lettuce leave, T_2-pasalai leave, T_3-fenugreek leaves, T_4-manathakkali leave, T_5-moringa leaves

Antioxidant activity

All the green leafy vegetables subjected to blanching pretreatment showed significantly higher antioxidant activity compared to the unblanched control samples (Table 3). Tree lettce leaves powder had the highest antioxidant activity (95.99%) for the blanched samples subsequently fluidized bed drying condition and tree lettuce powder had the lowest (93.45%) antioxidant activity. The blanching pretreatment increased the antioxidant activity when compared to the respective unblanched control samples. Similar observations were made by Adefegha and Oboh, (2011) [1]. who stated that the cooking causes a significant ($P < 0.05$) increase in the DPPH radical scavenging activity [raw (15.7 - 61.8%), cooked (52.8 - 92.7 %)], and that cooking increased the antioxidant activity in all the selected GLVs (*Talinium triangulare, Ocimum gratissimum, Amaranthus hybridus, Telfairia occidentalis, Ipomea batata, Cnidoscolous aconitifolius, Baselia alba and Senecio biafrae leaves.* Saha *et al*. (2015) [17]. stated that all the green leafy vegetables had high antioxidant activity. Antioxidant activity was almost same for all the samples but *Brassica juncea* (95.55%) had the highest free radical scavenging activity and *Amaranthus viridis* had the lowest (92.95%) free radical scavenging activity.

Table 3: Antioxidant activity (% DWB) of green leafy vegetables powder

Green leafy vegetables powders	Shade drying		Cabinet drying		Fluidized bed drying	
	Without blanching	With blanching	Without blanching	With blanching	Without blanching	With blanching
Tree lettuce powder	93.78	95.42	93.45	95.20	93.84	95.99
Pasalai leaves powder	86.20	88.11	86.10	88.05	86.26	88.88
Fenugreek leaves powder	89.67	91.61	89.53	91.51	89.77	91.97
Manathakkali leaves powder	83.28	85.30	83.13	85.22	83.35	85.89
Moringa leaves powder	90.29	92.25	90.15	92.13	90.38	92.84

Conclusion

Among the different drying techniques studied for optimization of appropriate drying techniques for GLVs, fluidized bed drying was found to be most effective in conferring better retention of β-carotene and antioxidant activity of the selected green leafy vegetables powder. The green leafy vegetables powder contained appreciable quantity of nutrients and also the pretreated blanched green leafy vegetables powder recorded high amount of β-carotene and antioxidant activity compared to the unblanched greens powder.

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