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Studies on impact of different blanching treatments on nutritional quality attributes of green leafy vegetables

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Abstract
Present work have been undertaken to study the impact of different blanching treatments on nutritional quality attributes of green leafy vegetables Viz. fenugreek, amaranth and roselle leaves. Green leafy vegetables (GLVs) occupy an important place among the food crops, as these provide adequate amounts of many vitamins and minerals for humans. They are rich source of carotenoids, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus. Pre-processing of GLV includes cleaning, washing and detaching the leaves. The fresh and cleaned leaves were blanched by different methods like, treatment with hot water, 1% NaCl (Sodium chloride) solution, 0.5% SMS (Sodium meta bisulphate) and 0.2% MgO (Magnesium oxide). The minimal losses of nutrient in green leafy vegetables were observed in 0.5% sodium metabisulphate solution. From the research it was observed that decreases in moisture content, protein, fat, fiber and ash content and drastically increasing carbohydrate were found. The blanching of fenugreek leaves results in retention of vitamin C contents significantly. The impact of blanching on fenugreek leaves depicts the pattern of good retention of chlorophyll content. From the research it was concluded that blanching of green leafy vegetable with 0.5% sodium meta bisulphate showed better results with respect to nutritional quality and retention of ascorbic acid and chlorophyll content.

Keywords: Amaranth, blanching, fenugreek, green leafy vegetables, roselle

Introduction
Green leafy vegetables (GLVs) occupy an important place among the food crops, as these provide adequate amounts of many vitamins and minerals for humans. They are rich source of carotenoids, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus. In nature, there are many underutilized greens of promising nutritive value, which can nourish the ever-increasing human population.

Fenugreek (*Trigonella foenum-graecum* L.) commonly known as methi, is a self-pollinating, leguminous crop native to the Indian subcontinent and the Eastern Mediterranean region (Petropoulos, 2002) [17] It is currently widely cultivated in central Asia, central Europe, northern Africa, North America and parts of Australia, with India being the leading fenugreek producer in the world (Fotopoulos, 2002) [8]. A study on the quantification of phytochemicals in different extracts of seeds, leaves and stems of methi leaves indicates that green leafy vegetables are rich sources of phytochemicals (Sumayya *et al*., 2012) [22]. The biological and pharmacological properties of methi are accredited to the diversity of its constituents like poly-phenolic substances, volatile constituents, amino acids, etc (Mehrafarin *et al*., 2010) [13].

Amaranth (*Amaranthus hypochondriacus, A. tricolor*) is an herbaceous annual with upright growth habit, cultivated for both its seeds which are used as a grain and its leaves which are used as a vegetable or green. Amaranth has been used in China for over 400 years. Amaranth is a highly nutritious food. The leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables, with a main dish or by itself. Amaranth leaves contains moisture, protein, fat, total carbohydrate, fiber, calories, phosphorous, iron, potassium, vitamin A, riboflavin, niacin, vitamin C, thiamine, ash and calcium about 86.9 g, 3.5 g, 0.5 g, 6.5 g, 1.3 g, 36 kcal, 67 mg, 3.9 mg, 411 mg, 6100 IU, 0.16 mg, 1.4 mg, 80 mg, 0.08 mg and 2.6 g. per 100 g respectively. (Brien and Price, 2008) [5].

Roselle (*Hibiscus sabdariffa* L.) is popularly recognized as 'mesta' or 'chukur' in Indian subcontinent including Bangladesh (Halimatul *et al*. 2007; Rao 2008) [11, 19]. It is widely cultivated in Tropical Africa, Sudan, Egypt, Ethiopia, Mali, Nigeria, Chad, India, Indonesia, the Phillipines, Malaysia, Brazil, Australia, Mexico, Hawai and Florida of USA. Roselle is a miracle plant with various utilizations (Crane 1949) [7]. The leaves and calyx are used as vegetable in many countries of the tropics. Generally roselle is considered as traditional medicine for the remedy of diuretic, mild laxative, cancer, cardiac and nerve diseases. Every fraction of roselle plants including leaves, fruits, roots, seeds are utilized in various foods. Among them, red fleshy calyces are employed for making fresh beverage tastes like Ribena, juice, jam, jelly, syrup, gelatin, pudding, wine, cakes, ice-cream and flavors.

Green leafy vegetables are categorized into highly perishable food commodities because of content of high percentage of moisture content (85 to 90%). Drying of vegetable is one of the oldest and cheap methods to increase shelf life of food commodities. The green leafy vegetable acquires a problem of discoloration during drying. So to overcome such problems vegetables are blanched prior to drying so as to increase nutritional profile and retain color of the vegetables.

2. Material and Method
2.1 Material
2.1.1 Raw material
The green coloured green leafy vegetables i.e. fenugreek, amaranth and roselle were collected from local market of Parbhani, Maharashtra.

2.1.2 Chemicals
All the chemicals, organic solvents and acids used were of analytical grade. Chemicals required for processing of raw materials, preparation and analysis of formulated products were obtained from Department of Food Engineering, College of Food Technology, V.N.M.K.V, Parbhani, Maharashtra, India.

2.2 Methods
2.2.1 Blanching of green leafy vegetables

Selection of GLV (Fenugreek, amaranth and roselle)
↓
Washing/Cleaning
↓
Cutting
↓
Blanching
↓ Hot water | ↓ 1% NaCl | ↓ 0.5% SMS | ↓ 0.2% Mg004F
↓
Blanched GLV
↓
Packaging and storage

Flowsheet 1: Blanching of green leafy vegetables

2.2.2 Analysis of proximate composition
a) Determination of moisture content

About 2 g of the extract was weighed and placed in a crucible of constant weight. This was placed in an oven at 105^{0}C then dried; the weight was measured carefully to get a constant weight. The loss in weight indicates the moisture content (AOAC, 2005) [4]. The moisture content was calculated by,

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{final weight}}{\text{Total weight of sample}} \times 100$$

b) Determination of ash content

Crucible used for ash content determination was weighed and dried in a hot air oven at 110^{0}C to a constant weight. About 2 g of each extract was weighed and placed in the crucible and weight of the crucible and extract was taken. This was placed in a furnace and ignited for 3 h at 250-300^{0}C. The temperature was again raised to 550^{0}C. The weight of crucible with its ash content was recorded and the ash content was calculated and expressed as percentage of original sample (AOAC, 2005) [4].

$$\text{Total Ash (\%)} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Wight of sample (g)}} \times 100$$

c) Determination of crude protein content

The crude protein was determined by the Micro Kjeldhal's method as described in method no. 46-10 of (AACC, 2000) [1]. This is based on the fact that on digestion with concentrated sulphuric acid and catalysts, organic compounds are oxidized and the nitrogen is converted to ammonium sulphate. Upon making the reaction mixture alkaline, ammonia is liberated, removed by the steam distillation, collected and titrated.

Procedure

The nitrogen content of samples was determined by using micro Kjeldhal's method. The sample was first digested in digestion flask with H_2SO_4 in presence of digestion mixture for 3-4 hours till the contents of digestion flask get transparent colour. The samples were then diluted with distilled water up to 250 ml in a volumetric flask. The ammonia from the samples was liberated through distillation after adding 40%

NaOH solution and collected in flask containing 4% boric acid solution using methyl red as an indicator. The nitrogen content in the samples was determined by titrating against standard 0.1 N H_2SO_4 solution and the crude protein percentage was calculated by using following formula,

$$\% N = \frac{(\text{Sample-Blank}) \times N \text{ of } H_2SO_4 \times 0.014 \times D.F.}{\text{Aliquot taken} \times \text{Weight of sample (g)}} \times 100$$

Total Crude Protein = % Nitrogen x 6.25

d) Determination of crude fat content

The crude fat was determined by Soxhlet extraction as described in method No. 30-10 (AACC, 2000) [1]. Dried sample remained after moisture determination was taken in a thimble and placed in extraction tube of Soxhlet apparatus. About 250 ml of Hexane was added in 500 ml bottom flask connected to Soxhlet apparatus. The fat was extracted by running Hexane over the sample at the rate of 3-4 drops per sec for about 5 h. The solvent was recovered and the flask was kept in hot air oven for 10 min at 40-50^{0}C. The flask was cooled in desiccator and weighed. Fat percentage was calculated according to the following formula.

$$\text{Crude fat (\%)} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

e) Determination of carbohydrate content

The sample was weighed (0.5 g) accurately in test tube and kept in ice water bath for few minute followed by the addition of cold H_2SO_4 (72 per cent) with gentle stirring. The viscous paste was diluted with distilled water to obtain final concentration 2 N with respect to acid. It was then refluxed at 98^{0}C for 3-4 hours to achieve complete hydrolysis. The sugar content was estimated by Phenol-H_2SO_4method, using glucose as standard. The orange yellow colour was read at 480 nm on spectrophotometer. From the calibrated curve the concentration of sugar in hydrolysate was calculated and per cent total sugar in the sample was quantified (Ranganna, 2011) [18].

f) Determination of vitamin C (Ascorbic acid)

The method of Hussian *et al.* (2006) [12] was used for determination of vitamin C. 1g of each ground sample was weighed in a 25 ml conical flask. Then 10 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added and the mixture allowed standing for 24 h, to provide the required reaction time. After 24 h, the samples were filtered through 0.45 µm Whatman filter paper No.1. Then 2.5 ml of each sample was transferred to a separate 25 ml volumetric brown flask, after which 2.5 ml of the oxalic acid (0.05 M)-EDTA (0.02 M) solution was added. Subsequently, meta phosphoric acid was added separately with acetic acid (0.5 ml), sulphuric acid (5% v/v) solution (1 ml) and ammonium molybdate solution (2 ml) in each volumetric brown flask and the volume was made up to 25 ml with distilled water. The absorbance was measured at 760 nm in a visible spectrophotometer.

2.2.3 Estimation of total chlorophyll

Leaf sample of 1 g was weighed and was ground in pestle-mortar with 5 ml distilled water to a paste. The contents were transferred to a centrifuge tube and the total volume was made up to 10 ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5 ml of 80% acetone. The contents were centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at the following wavelengths 645 and 663 nm (Garg *et al.*, 2012) [10]. The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula given by Talreja, (2011) [23],

Total chlorophyll (mg/L) = 20.20 A_{645} + 08.02 A_{663}
Chlorophyll 'a' (mg/L) = 12.70 A_{663} – 02.69 A_{645}
Chlorophyll 'b' (mg/L) = 22.90 A_{645} – 04.68 A_{663}

These can be converted to chlorophyll content in mg/g dry weight as follows:

$$\textbf{Chlorophyll 'a' (mg/g)} = \frac{12.3 \times \text{O.D. at 663 nm} - 0.86 \times \text{O.D. at 645 nm} \times V}{a \times 1000 \times W}$$

$$\textbf{Chlorophyll 'b' (mg/g)} = \frac{19.3 \times \text{O.D. at 645 nm} - 3.6 \times \text{O.D. at 663 nm} \times V}{a \times 1000 \times W}$$

Total Chlorophyll (mg/g) = a + b

Here, O.D. = Optical Density
V = Final volume of chlorophyll extract in 80% acetone
W = Dry weight of plant material
a = the length of light path in the cell (usually 1 cm)

3. Results and discussion

3.1 Proximate composition of green leafy vegetables

Proximate composition generally represents the nutritional quality of product. It is necessary to determine the proximate composition of green leafy vegetable (GLV) leaves to judge its effect on final product after utilization as a novel ingredient. The results obtained were presented in Table 1.

Table 1: Average chemical composition of green leafy vegetables

S. No.	GLV	Moisture	Fat	Protein	Carbohydrate	Ash	Fiber
1	Fenugreek	85.23	0.91	2.78	8.17	1.29	1.62
2	Amaranth	89.21	0.24	2.98	5.43	1.36	0.78
3	Roselle	86.20	0.54	2.63	8.45	1.32	0.87
4	SE±	1.239	0.287	0.365	0.403	0.187	0.054
5	CD at 5%	3.982	0.904	0.980	1.221	0.692	0.187

***Each value is average of three determinations**

The data obtained from table 1. Showed the proximate composition of green leafy vegetables (GLV). The highest moisture content was observed in amaranth leaves i.e 89.21% followed by roselle (86.20) and fenugreek leaves (85.23). The presence of high moisture in leafy vegetables make them available for microbial growth and development resulting in spoilage of green leafy vegetables. Drying of leafy vegetable results in decreasing moisture content of leaves to large extent thereby making them unavailable for microbial growth and hence shelf life of dried products is get increased.

Highest fat content were observed in fenugreek leaves i.e. 091% followed by roselle and amaranth leaves i.e. 0.54 and 0.24% respectively.

The amount of protein present in lemongrass leaves indicates that the plant can form a part of human diet. The protein contents of fenugreek, amaranth and roselle leaves are 2.78, 2.98 and 2.63% respectively. The carbohydrate provides energy in the form of calories to mankind. The carbohydrate contents of fenugreek, amaranth and roselle leaves are 8.17, 5.43 and 8.42% respectively.

The fiber content of fenugreek, amaranth and roselle leaves are 1.62, 0.78 and .88% respectively. The ash is inorganic matter left after complete burning of food. The ash content decide the mineral content of the food products. The amaranth leaves contain high amount of ash (1.36%) as compare to roselle (1.32%) and fenugreek leaves (1.29%).
The similar result were obtained by research finding of Funke (2011) [9] who studied the nutritional composition of amaranth leaves which is cooked by different cooking methods. Ahmad *et al*. (2015) [2] concluded that nutritional value and biologically active compounds profile of fenugreek leaves unquestionably appreciated by medical science.

3.2 Blanching of green leafy vegetables

3.2.1 Chemical composition of blanched green leafy vegetables

The blanching of green leafy vegetables done by hot water, 1% NaCl solution, 0.5% sodium metabisulphate solution and 0.2% magnesium oxide solution. The data pertaining impact of blanching on chemical composition of green leafy vegetables are summarized in table 2.

Table 2: Effects of blanching on average chemical composition of green leafy vegetables (%)

S. No	Blanching methods	Moisture	Ash	Fat	Protein	Fiber	Carbohydrate
Blanched fenugreek leaves							
1	Control	85.23	1.29	0.91	2.78	1.62	8.17
2	Hot water	83.68	1.16	0.80	2.61	1.58	10.17
3	1% NaCl	84.11	1.18	0.82	2.69	1.60	9.60
4	0.5% SMS	84.89	1.24	0.88	2.75	1.60	8.64
5	0.2% MgO	84.36	1.21	0.86	2.59	1.57	9.41
6	SE±	1.140	0.180	0.279	0.358	0.042	0.315
7	CD at 5%	3.482	0.676	0.891	0.978	0.144	1.045
Blanched amaranth leaves							
1	Control	89.21	1.36	0.24	2.98	0.78	5.43
2	Hot water	86.38	1.18	0.17	2.58	0.53	9.16
3	1% NaCl	87.23	1.26	0.20	2.67	0.61	8.03
4	0.5% SMS	88.68	1.31	0.21	2.78	0.73	6.29
5	0.2% MgO	87.93	1.30	0.20	2.74	0.71	7.12
6	SE±	1.038	0.189	0.289	0.310	0.033	0.308
7	CD at 5%	3.298	0.603	0.928	0.965	0.010	0.965
Blanched roselle leaves							
1	Control	85.23	1.29	0.91	2.78	1.62	8.17
2	Hot water	83.68	1.16	0.80	2.61	1.58	10.17
3	1% NaCl	84.11	1.18	0.82	2.69	1.60	9.60
4	0.5% SMS	84.89	1.24	0.88	2.75	1.60	8.64
5	0.2% MgO	84.36	1.21	0.86	2.59	1.57	9.41
6	SE±	0.995	0.180	0.176	0.288	0.087	0.301
7	CD at 5%	3.107	0.599	0.555	0.901	0.030	0.943

*Each value is average of three determinations

The data presented in table 2. showed the impact of blanching on chemical composition of green leafy vegetables. The minimal loss of nutrient in fenugreek leaves were observed in 0.5% sodium metabisulphate solution. The highest loss of moisture was observed in hot water blanching of fenugreek leaves i.e. 83.68% while minimum loss of moisture were observed in blanching in solution of 0.5% SMS. The reduction of ash content was found from 1.29 to 1.16%. The slightly reduction of mineral was observed in fenugreek sample blanched by solution of 0.5% SMS. The slight differences in fat content of blanched fenugreek leaves were observed i.e. 0.91 to 0.80%. Change in protein content was occurring due to reaction of protein with heat treatment. Heating of vegetables results in inactivation of enzyme. The protein loss was observed from 2.78 to 2.59%. Loss of fiber was also observed decreased in value from 1.62 to 1.57%. The carbohydrate was increased from 8.17 to 10.17%. Thus from the research it was found that blanching of fenugreek leaves in solution of 0.5% sodium metabisulphate is superior than other blanching process, and hence this process was followed for blanching of fenugreek leaves prior to drying.
The amaranth leaves blanched by 0.5% sodium metabisulphate observed minimum loss of chemical constituents of leaves where as the amaranth leaves blanched by hot water had highest loss of nutrients and trend of slight difference in chemical composition were observed in 1% NaCl and 0.2% MgO methods of blanching. The loss of moisture, ash, fat, protein and fiber were observed from 98.21 to 86.38, 1.36 to 1.18, 0.24 to 0.17, 2.98 to 2.58 and 0.78 to 0.53% respectively. The increase in carbohydrate was observed from 5.43 to 9.16%. The roselle leaves blanched by 0.5% sodium metabisulphate observed minimum loss of chemical constituents of leaves where as the roselle leaves blanched by hot water had highest loss of nutrients and trend of slight difference in chemical composition were observed in 1% NaCl and 0.2% MgO methods of blanching. The loss of moisture, ash, fat, protein and fiber were observed from 86.20 to 84.46, 1.32 to 1.26, 0.54 to 0.43, 2.63 to 2.41 and 0.87 to 0.62% respectively. The increase in carbohydrate was observed from 8.45 to 10.82%. Hence it was found that blanching of vegetables obtained better results with 0.5% sodium metabisulphate solution.
The similar results were obtained by Singh *et al*. (2006) [21] who studied the effects of different pre-processing method on quality of dehydrated green leafy vegetables rich in vitamin A i.e. Amaranth, curry leaves, drumstick leaves, methi and palak Among 5different pre-treatments (blanching in water (T1)' blanching in 2% sodium chloride solution (T2)' blanching in

0.5% sodium meta-bisulphite (NaMS) solution (T3), blanching in 0.1% magnesium oxide (MgO) solution (T4) and control (Ts)' blanching in 0.5% sodium meta-bisulphite (NaMS) solution was found to be the best for the preparation of dehydrated leafy vegetables and retaining better nutrients ascorbic acid, carotene, chlorophyll content and rehydration ratio.

3.2.2 Vitamin C and total Chlorophyll content of blanched green leafy vegetables

The data pertaining to effects of blanching on ascorbic acid and total chlorophyll contents of green leafy vegetables are summarized in table 3.

Table 3: Effects of blanching on vitamin C and total chlorophyll content of green leafy vegetables (mg/100g)

S. No	Blanching method	Fenugreek leaves		Amaranth leaves		Roselle leaves	
		Vitamin C	Total chlorophyll	Vitamin C	Total chlorophyll	Vitamin C	Total chlorophyll
1	Control	80.69	154.65	96.32	135.09	71.34	98.43
2	Hot water	72.63	147.88	89.98	131.23	65.22	94.58
3	1% NaCl	85.34	160.65	108.10	148.00	79.41	105.94
4	0.5% SMS	107.80	176.55	121.22	154.62	93.10	126.87
5	0.2% MgO	103.63	170.13	116.01	149.29	87.00	120.67
6	SE±	1.254	1.148	0.985	1.025	0.887	0.934
7	CD at 5%	4.577	4.190	3.595	3.741	2.839	2.989

*Each value is average of three determinations

The data presented in table 3. showed the impact of blanching on vitamin C (Ascorbic acid) and total chlorophyll contents of green leafy vegetables. The ascorbic acid is good anti-oxidant and play important role in preventing scurvy like disease in human being. Ascorbic acid, the antioxidant vitamin, is heat labile and sensitive to light, oxygen and oxidizing agents. Though blanching is a prerequisite to inactivate enzymes, it is deleterious to the vegetables causing vitamin losses by thermal degradation, diffusion and leaching (Negi and Roy, 2000) [15]. The vitamin C present in fresh fenugreek leaves is 80.69 mg/100g. The blanching of fenugreek leaves results in retention of vitamin C contents significantly. The highest retention of ascorbic acid observed in fenugreek leaves blanched by 0.5% sodium metabisulphate i.e. 107.80 mg/100g followed by 0.2% MgO, 1% NaoH and Hot water blanching. The impact of blanching on fenugreek leaves depicts the pattern of good retention of chlorophyll content. The highest retention of chlorophyll content was observed in fenugreek sample blanched by 0.5% SMS. Patel *et al*. (2016) [16] studied the impact of different blanching methods on retention of ascorbic acid contents of green leafy vegetables and concluded that among the greens selected, spinach had maximum retention of ascorbic acid followed by fenugreek, drumstick and amaranth by all blanching treatments irrespective of time and temperature chemically treated samples showed better retention of ascorbic acid than their respective untreated samples irrespective of blanching method or greens.

From the table 3. Depicted that vitamin C retention was more in amaranth leaves blanched by 0.5% SMS (121.22 mg/100g) than 0.2% MgO blanching (116.01 mg/100g), 1% NaCl blanching (108.10 mg/100g) and hot water blanching (89.98 mg/100g). Hot water blanching results decrease in ascorbic acid contents than fresh amaranth leaves i.e. from 96.32 to 89.98 mg/100g. According to Miglio *et al*. (2008) [14] who reported that steam cooking of broccoli has less influence on vitamin C, whereas cooking in water significantly lowered its content due to the leaching. Among all chemically blanched broccoli samples the maximum retention of ascorbic acid content (602 mg/100g) was observed in KMS treated samples. From the research it was found that the blanching of amaranth leaves results in improvement in chlorophyll content of amaranth leaves. The highest retention of chlorophyll found in sample treated by 0.5% SMS and minimum retention were observed in amaranth sample blanched by hot water.

The similar pattern of retention of ascorbic acid found in roselle leaves blanched by 0.5% sodium metabisulphate solution (93.10 mg/100g) and minimum retention were observed by hot water blanching (65.22 mg/100g). The retention of total chlorophyll was increased during blanching. The maximum retention of chlorophyll of roselle leaves were observed in 0.5% SMS blanched roselle leaves sample (126.87 mg/100g) and minimum retention was observed in hot water blanching (94.58 mg/100g). From the research it was found that blanching with 0.5% sodium metabisulphate had better results over retention of ascorbic acid and chlorophyll contents of fenugreek, amaranth and roselle leaves. Hence blanching with 0.5% sodium metabisulphate was used for blanching prior to drying for obtaining better retention of ascorbic acid and green color.

The similar results were obtained by Patel *et al*. (2016) [16]. Saranya *et al*. (2017) [20] studied the impact of different processing method on anti-oxidant activity of green leafy vegetables and concluded that potassium metabisulphite showed better retention of vitamin C followed by sodium bicarbonate and sodium chloride.

Conclusion

Thus in light of scientific data of the present investigation, it may be concluded that green leafy vegetables contained macro and micronutrients. Blanching of green leafy vegetables *Viz*. fenugreek, amaranth and roselle in 0.5% sodium meta bisulphate results in better retention of ascorbic acid and chlorophyll and minimum loss of chemical constituents. And hence it this method is better to follow prior to drying of green leafy vegetables for retention of nutrient and green color.

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