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EFFECT OF PRE-TREATMENT ON REDUCTION OF OXALIC ACID ON SPINACH POWDER

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ABSTRACT

This research focus to see the effect of different pre-treatment on reduction of oxalic acid content of Spinach having the oxalate content as 0.937% on dry basis. Spinach was first dried at temperature of 60˚C, 70°C and 80°C. Dried samples were rehydrated for 2 hours before processing. Different treatments such as hot water blanching reduce the oxalate content upto 14.72%, NaCl treatment upto 23.79% and EDTA blanching which reduces the oxalate content upto 66.92%. EDTA blanching at 2mMol concentration was significantly superior ($p<0.05$); was subjected to chemical analysis. Raw spinach soup was found to be statistically superior ($p<0.05$) in terms of color but showed no significant difference ($p>0.05$) with EDTA blanched spinach powder soup.

Keywords: *Spinach, oxalic acid, EDTA and oxalate.*

I. INTRODUCTION

Spinach has a high nutritional value and ising extremely rich in antioxidants, especially when fresh, steamed, or quickly boiled. It is a rich source of vitamin A (and especially high in lutein), vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron, vitamin B_2, calcium, potassium, vitamin B_6, folic acid, copper, protein, phosphorus, zinc, niacin, selenium and omega-3fattyacids.[1] Polyglutamyl folate (vitamin B_9 or folic acid) is a vital constituent of cells, and spinach is a good source of folic acid and Vitamin B_9.[2]

Spinach has high calcium content. However, the oxalate content in spinach binds with calcium, decreasing its absorption. Calcium and zinc also limit iron absorption.[3] The calcium in spinach is the least bio-available of calcium sources.[4] Among different component present in the spinach, one of the major anti-nutritional factors present is oxalic acid. Oxalic acid in the human body when ingested in food, oxalic acid combines with certain metals, result in, i.e. oxalic acid + metal salt are called oxalates which lead to certain diseases like kidney damage, stones and gout problem, poisoning. Fruits and vegetables which contain high amount of vitamin C, consumption may lead to oxalic acid kidney stone formation. Oxalic acid is a product of the breakdown of vitamin-C which accumulates to form kidney stones. The human body can absorb only around 5% of the calcium in spinach.[2] Oxalic acid is excreted in the urine, and its crystals are commonly found in microscopic urinalysis. Too much oxalic acid in the urine will result in kidney or bladder stones. This is because metal ions like calcium form insoluble precipitates with oxalate (calcium oxalate). Calcium oxalate is responsible for 75% [5]and probably 80% of kidney stones.[6]

Without oxalic acid, foods such as spinach and kale would have a much higher, bio-available calcium content than they do because it is bound up with oxalic acid. This may be the cause of pain and other conditions such as cystic fibrosis, fibromyalgia, thyroid disease, and asthma. Even autism has been linked to high oxalate levels.[7]

Calcium oxalate crystals in the urine are the most common constituent of human kidney stones and calcium oxalate crystal formation is also one of the toxic effects of ethylene glycol poisoning. Even a small dose of calcium oxalate is enough to cause intense sensations of burning in the mouth and throat, swelling, and choking that could last for up to two weeks. In greater doses it can cause severe digestive upset, breathing difficulties, coma or even death. Recovery from severe oxalate poisoning is possible, but permanent liver and kidney damage may have occurred.[8] Commercial and residential water-softening units use ion exchange resins to remove the offending ions that cause the hardness. These resins are generated and regenerated using sodium chloride.[9] For removal of oxalic acid, blanching of fruits and vegetable (plunge it into boiling water), a part of the oxalic acid gets into the cooking water. Ethylenediaminetetraacetic acid, widely abbreviated as EDTA is widely used to dissolve limescale. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. In a similar manner, EDTA is added to some food as a preservative or stabilizer to prevent catalytic oxidative decoloration, which is catalyzed by metal ions. So for the removal of oxalic acid to a certain level EDTA acts as a binding agent with calcium magnesium and other metal.[10] This leaf is used in different medicinal aspect. Since plants are the primary producers in the food chain, it is efficient to use plant sources rather than using expensive and inefficient animal sources.[11]

In spite of the fact that, spinach has been an integral part of diet of the tribal community, scientific validation of traditional processing methods in terms of food quality and safety has not been attempted. Although a number of treatment methods like boiling, salt dipping, EDTA boiling and EDTA soaking are capable of reducing oxalic acid content of spinach, the information on comparative effectiveness of these methods are still the subject matter of research. The documentation of all such common treatments is also much important. Hence efforts to gear up the oxalic acid reduction by house hold treatments towards improving the nutritional status of spinach which is practiced worldwide is more than justified.

II. MATERIALS AND METHODOLOGY

The raw material Spinach was collected from Purano Bazaar at Dharan. The leaves which are used for treatment are of different grade. The photographs of the sample were taken for proper identification of species. The samples were then cleaned and sorted to remove dust, foreign matters and damaged parts. Cleaned samples were kept in separate air tight plastic bags. Samples were first dried in cabinet dryer at different temperature i.e. 60ºC, 70ºC and 80ºC. The samples were powdered with the help of the mixer grinder for different experiment in triplicate.

Rehydration of sample

Firstly, for each analysis 3 gram (on dry basis) of sample was taken. The sample was rehydrated for 2 hrs before the treatment. Here 3 g of sample was dipped in 100ml of distilled water for 2hrs to rehydrate the sample. Then the sample was drained with the help of filter paper. After that the analysis was carried out.

Hot water treatment (*Boiling the sample for 1min, 2 min and 5 min*)

From the representative lot of sample, 3 gram of rehydrated drained sample was taken out. The sample was dipped in 500 ml distilled boiling water for 1 minute, 2 minute and 5 minute respectively. The boiled sample was then drained out and free water removed using blotting paper. It was then labeled and kept for the determination of the oxalic acid.

Salt treatment i.e. Boiling in 1%, 2% and 5% NaCl

From the representative lot of sample, 3 gram of rehydrated sample was weighed out. The sample was dipped in 100 ml boiling salt solution of 1% , 2% and 5% concentration (1 g, 2 g and 5 g salt respectively in 100 ml distilled water) for 2 minutes only. The salt boiled sample was then drained out and free water removed using blotting paper. It was then labeled and kept safely for the determination of oxalic acid.

EDTA blanching treatment with 0.1, 0.2, 0.5, 1, 2 and 3 mMol

From the representative lot of sample, 3 gram of rehydrated sample was drained out. The sample was boiled in 500ml boiling water with 0.1 , 0.2, 0.5, 1, 2 and 3 mMol (equivalent to 0.0125g, 0.025g, 0.075g, 0.15g, 0.15g, 0.3g and 0.45 g respectively) of EDTA solution for 2 minutes only. The EDTA boiled sample was then drained out and free water removed using blotting paper. It was then labeled and kept safely for the determination of oxalic acid.

Flow chart for the Production of oxalic acid reduced Spinach powder

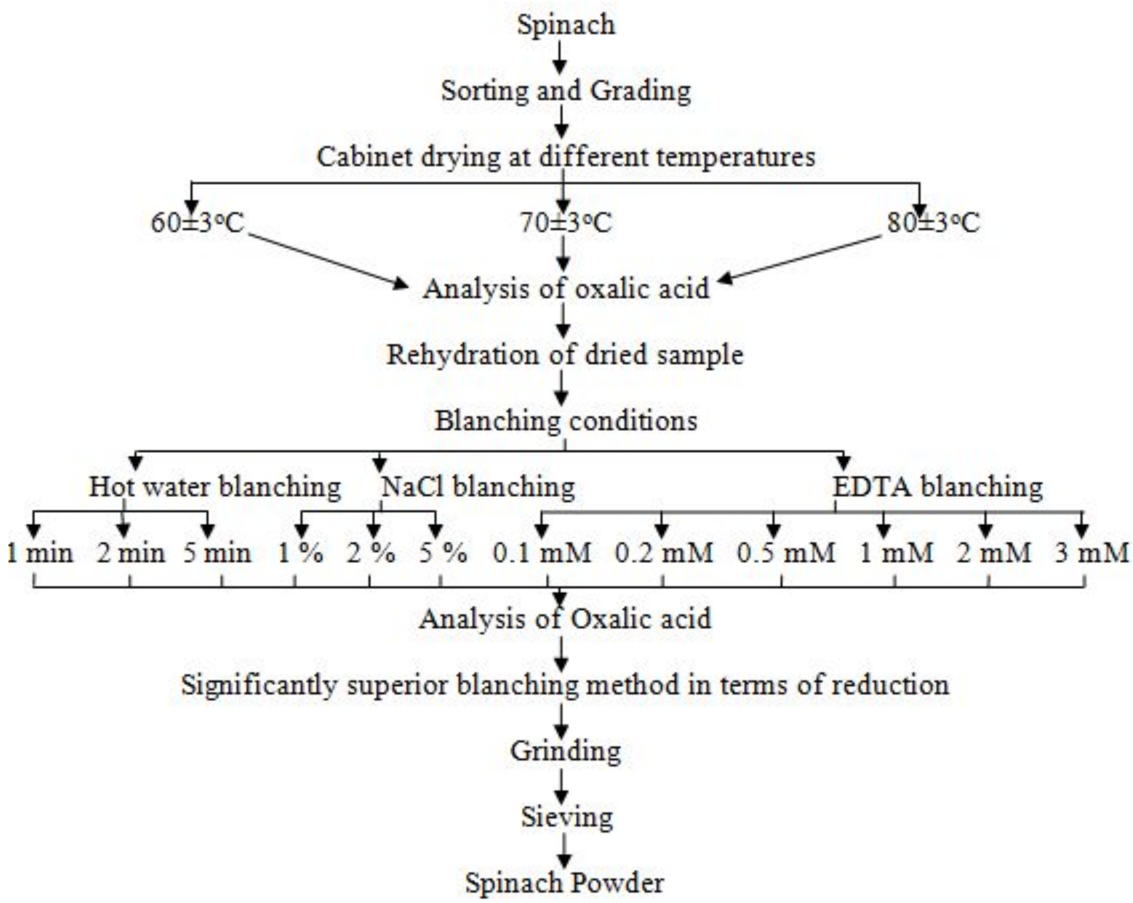

Fig. 1 Flow chart for Production of Oxalic acid reduced Spinach powder

III. ANALYTICAL METHODS

Moisture content, Total Ash, Calcium content**,** Chlorophyll and Crude fibre was determined as described.[12] Vitamin C was determined by method as described.[13] Oxalate as oxalic acid present in the sample was estimated according to the method of [10] and modified.[14]

Statistical analysis
All experiments were carried out in triplicates. Mean values with standard deviations were computed. Data were subjected to analysis of variance and read at 0.95 confidence level using statistical software GenStat Release 7.1 (Discovery Edition 3 developed by VSN International limited). Fisher's least significant differences (LSD) test was used to define differences between means at the 5% significance level ($p<0.05$).

IV. RESULTS AND DISCUSSIONS

The study was carried out to find out the nutritional component inherent in the *Spinacia oleracea* given in Table 1 and to decrease the oxalate content of *Spinacia oleracea* via (hot water, NaCl and EDTA solution).

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Table 4.1 Chemical composition of Spinach powder and EDTA blanched Spinach powder (on dry basis)

Constituents	Spinach Powder	EDTA blanched powder
Moisture	0.57±0.02 %[a]	0.62±0.03 %[a]
Total ash	15.83±0.02 %[a]	12.23±0.021 %[b]
Crude Fibre	10.54±0.032 %[a]	12.02 ±0.021%[b]
Calcium	19.11±0.012 mg/g[a]	22. 97±0.011mg/g[b]
Vitamin C	18.07±0.011 mg/g[a]	24.09±0.02 mg/g [b]
Chlorophyll	0.46±0.021 mg/g[a]	0.36±0.012 mg/g[b]
Oxalic Acid	0.937±0.005 g/100 g[a]	0.309±0.002 g/100 g[b]

Drying of sample (Spinach)

The leaves of *Spinacia oleracea* were dried in cabinet dryer at different temperature i.e, 60°C, 70°C and 80°C. But drying of spinach at higher temperature has no significant differences ($p<0.05$) on the concentration of oxalic acid. Hence, the sample was dried at 60°C and then the further process was carried out i.e. blanching at different conditions. Concentration of Oxalic acid on drying at different temperature is shown in fig. 2 :

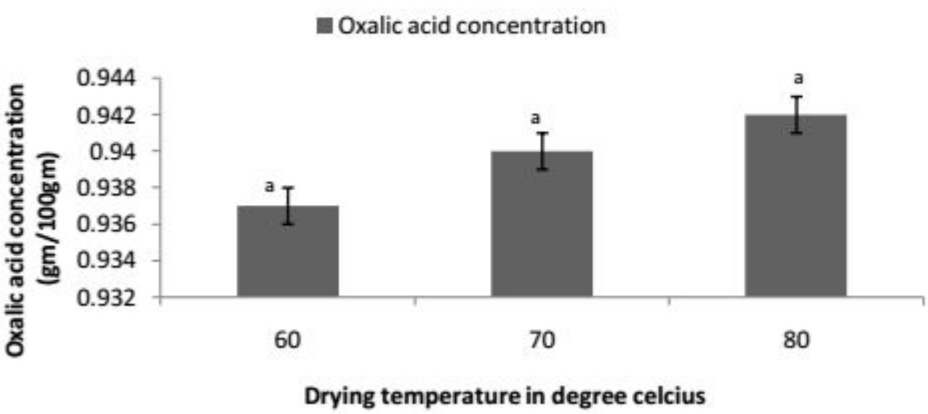

Fig 2 Concentration of oxalic acid at different drying temperatures*

*The values in the Fig. 2 are the means scores of concentration of oxalic acid on drying at different temperatures. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance.

Asian Journal of Food and Agro industry reported that drying of spinach at different temperature does not significantly reduce oxalate content present in spinach, hence drying results in loss of moisture and other components but not significant differences on concentration of oxalic acid. [3]

Hot water blanching

Blanching treatment used in the study was done by dipping the rehydrated leaves of *Spinacia oleracea* for 1 min, 2 min and 5 min respectively. % Reduction in Oxalic acid by hot water blanching for different time is shown in Table 2 :

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Table 2 Effect of blanching on oxalate content in Spinacia oleracea by hot water on (dry weight basis)*

Treatment	Oxalates (g/100g)	% change in oxalates	Remarks
Control	0.937±0.005		
1min	0.931±0.002	0.63	Reduction
2min	0.893±0.00	4.69	Reduction
5min	0.799±0.004	14.72	Reduction

* The values are mean values of triplicate determinations on a dry weight basis.
Mean values within a column and a row with different superscripts are significantly different at $P < 0.05$. Values with ± sign indicate standard deviation.

In the present study the leaves of *Spinacia oleracea* in boiling water for 1min does not show any significant differences ($p<0.05$) with the fresh sample on the amount of oxalic acid content. But when blanching is done for 2min and 5min respectively it shows significant differences ($p<0.05$) with the reduction of oxalic acid. Both hot water blanching with 2min and 5 min significantly ($p<0.05$) reduced the oxalate content up to 4.69 % and 14.72% respectively. The result shows that increase in the boiling time during blanching in hot water for 1min doesn't significantly ($p<0.05$) reduced the oxalate content but when boiling time is increased for 2min and 5 min it shows significant differences at ($p\leq0.05$) with reduction in oxalic acid up to 4.69 % and 14.72 % respectively.

NaCl blanching
Salt treatment used in the study was boiling in salt solution with the concentration of 1%, 2% and 5% respectively. Blanching the leaves of *Spinacia oleracea* in 1% NaCl solution significantly ($p<0.05$) resulted in reducing the oxalate content by 10.45 %. Increase in the concentration of NaCl to 2% and 3% during blanching show significant reduction in oxalate content up to 15.68% and 23.79% respectively. The reduction in oxalate during the blanching may be due to leaching of soluble oxalate. Higher % reduction of oxalate in 5% NaCl may be due to high concentration of NaCl which facilitates the leaching. The significant reduction in oxalate content by NaCl treatment may be due to the formation of soluble sodium oxalate which leached out easily during blanching.[5] % Reduction in Oxalic acid by NaCl blanching at different concentration is shown in Table 3:

Table 3 Effect of blanching on oxalate content in Spinacia oleracea by NaCl solution (dry weight basis)*

Treatment	Oxalates(g/100g)	% changes in oxalates	Remarks
Control	0.937±0.005		
1%NaCl	0.839±0.002	10.45	Reduction
2%NaCl	0.790±0.013	15.68	Reduction
5%NaCl	0.714±0.004	23.79	Reduction

* The values are mean values of triplicate determinations on a dry weight basis. Values with ± sign indicate standard deviation.
Mean values within a column and a row with different superscripts are significantly different at $P < 0.05$.

EDTA blanching
EDTA used in the study was boiling in EDTA solution with the concentration of 0.1mMol, 0.2mMol, 0.5mMol, 1mMol, 2mMol and 3mMol respectively. % Reduction in Oxalic acid by EDTA blanching at different concentration is shown in Table 4 :

Table 4 Effect of blanching on oxalate content in Spinacia oleracea by EDTA solution (dry weight basis)*

Blanching treatment	Oxalates(g/100g)	% change in oxalates	Remarks
Raw(control)	0.937±0.005		
0.1mMol	0.590±0.002	37.03	Reduction
0.2mMol	0.577±0.005	38.42	Reduction
0.5mMol	0.482±0.004	48.55	Reduction
1mMol	0.365±0.006	61.04	Reduction
2mMol	0.3097±0.002	66.94	Reduction
3mMol	0.3098±0.003	66.92	Reduction

* The values are mean values of triplicate determinations on a dry weight basis.
Mean values within a column and a row with different superscripts are significantly different at $P < 0.05$. Values with ± sign indicate standard deviation.

Blanching the leaves of *Spinacia oleracea* with EDTA solution for 2min with different concentration of EDTA as 0.1mMol, 0.2mMol, 0.5mMol, 1mMol, 2mMol and 3mMol reduced oxalate content up to 37.03%, 38.42%, 48.55%, 61.04%, 66.94%, 66.92% respectively. Here the oxalate content reduced significantly ($p<0.05$) as the concentration of EDTA is increased. But when the concentration is increased above 0.2mMol it shows a significant reduction in the oxalate content of the *Spinacia oleracea*..

Among this treatment blanching with EDTA solution was highly effective in reducing the level of oxalates in *Spinacia oleracea*. The EDTA concentration used in the study lies within 596.42ppm at 2mMol EDTA concentration. It means that it lies within the permissible limit and hence the residual EDTA in the production has no harmful effect. The reduction in the oxalate by EDTA treatment may be due to the possible conversion of calcium oxalate(insoluble) to sodium oxalate (soluble form) which may be leach out during blanching. EDTA is an excellent complexing agent.[15]

V. SENSORY EVALUATION

Three samples i.e. Raw spinach soup, Spinach powder soup and EDTA blanched spinach powder soup were taken for sensory evaluation in terms of Color, Aroma, Taste, Mouthfeel and Overall Acceptability by means of Hedonic Rating Test and the data obtained were statistically examined using Two-way ANOVA (no-blocking). The result of sensory analysis of three samples i.e. Raw spinach soup, Spinach powder soup and EDTA blanched spinach powder soup is shown in figure 3 :

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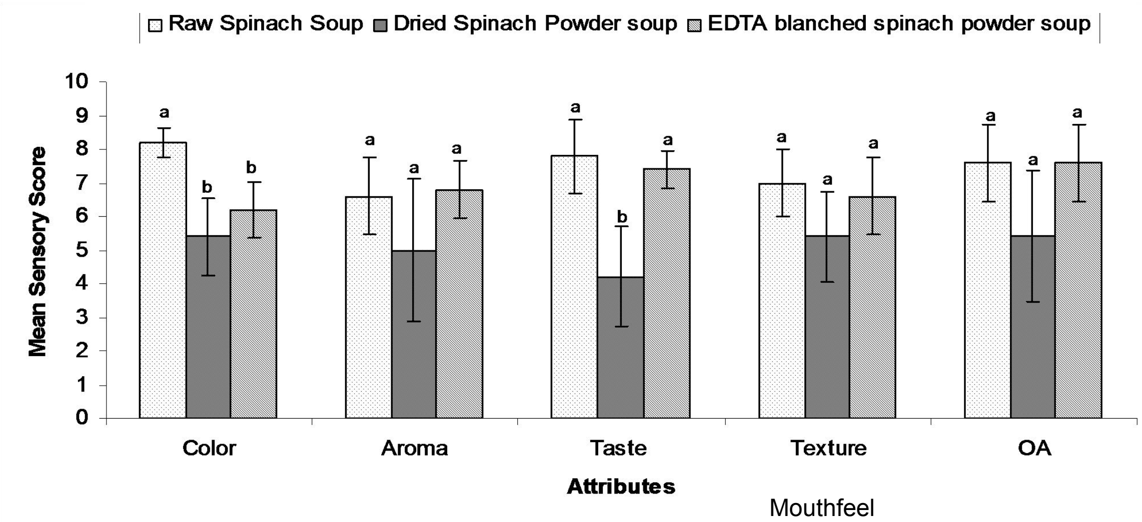

Fig. 3 Mean Sensory Score for three different samples

* Figures are arithmetic means of the scores. Figures in parenthesis are standard deviation. Figures in the rows having the same subscripts are not significantly different at 5% level of significance.

In terms of superiority (at 5% level of significance) of the samples with respect to Color, Aroma, Taste, Mouthfeel and Overall Acceptability, following conclusions can be drawn:

Color : Sample S_1 > Sample S_2 = Sample S_3
Aroma : Sample S_1 = Sample S_2 = Sample S_3
Taste : Sample S_1 > Sample S_2 < Sample S_3
Mouthfeel : Sample S_1 = Sample S_2 = Sample S_3
O.A : Sample S_1 = Sample S_2 = Sample S_3

Based on the frequency of occurrence as 'best' in each attribute type and the weightage on each attribute for describing sensory quality, Sample S_1 appears to be the best formulation.

VI. CONCLUSIONS

Spinach dried in different temperatures showed no significant difference in terms of concentration of oxalic acid, so it can be concluded that drying temperature doesn't reduce the oxalic acid so, 60^{o}C temperature was chosen for drying. Blanching *Spinacia oleracea* in NaCl solution for 2min with different amount of the concentration of NaCl shows significant differences with the reduction of oxalic acid up to 23.79%. After blanching *Spinacia oleracea* with EDTA solution for 2min with different concentration of EDTA reduced oxalate content up to 66.94%. It does not show the reduction when the concentration is increased above 2mMol. It may be concluded that among the method used EDTA treatment (blanching) appear to be more effective to reduced maximum amount of oxalate in *Spinacia oleracea* followed by blanching in hot water and NaCl solution. Product made from fresh spinach, dried spinach powder and EDTA blanched spinach powder showed no significant differences in terms of aroma, mouthfeel and overall acceptance but were found significantly different in terms of color and taste.

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