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Evaluation of Sanitizers for Amaranthus Leaves and Stem (*Amaranthus tricolor* L.) for Minimal Processing

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Abstract Sanitization is an important step in the process line of fresh cut vegetables. Different sanitizing agents, 30 ppm sodium hypochlorite, warm water (40°C), RO water (reverse osmosis), ozonised water (2 ppm), citric acid (1%), acetic acid (1%) and tap water for surface decontamination were evaluated for red amaranthus (*Amaranthus tricolor* L.) leaves and stem separately. Along with microbial analysis, influence of these treatments on anthocyanin, ascorbic acid content and visual parameters were also studied. The study revealed ozonised water (2 ppm) as the best sanitizer for fresh cut amaranthus leaves and stem with maximum nutrient retention and freshness.

Keywords Sanitization, Ozone, Fresh-cut amaranthus, Ascorbic acid, Anthocyanin.

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Introduction

Vegetables are rich source of vitamins, minerals and other bioactive compounds that play an important role in healthy human diet. Minimally processed products are one of the major growing segments in food retail establishments and are also called ready-to-use, fresh-cut or pre-cut produce. The market demand for fresh cut fruits and vegetables has undergone an important rise because of busy lifestyles, new consumers profile, increasing purchasing power. Amaranthus (*Amaranthus tricolor* L.) is one of the largely consumed leafy vegetables of South India, popularly called as poor mans spinach and well known for its high nutritional value. Ready to use amaranthus has high consumer demand and is easily available source of protective nutrients.

Vegetables in general contain more than 60% water and get spoiled very quickly and amaranthus has a short shelf life. Minimally processed vegetables, due to processing operations that alter the physical integrity, are more perishable than the original raw materials. Developing technologies that helps in reducing quality degradation with extended shelf life and to maintain quality during processing and distribution is highly needed [1]. High microbial load

Table 1. Effect of sanitizing treatments on microbial population (10^3 cfu g^{-1}) of amaranthus leaves. Figure in parentheses are logarithmic transformed values, TLTC - Too Less to Count, NS - Non-significant.

Treatments	Bacterial population 10^3 cfu g^{-1} Before treatment	After treatment	Reduction %	Fungal population Before treatment	After treatment
T_1	5.80	3.62	37.54 (1.57)	TLTC	TLTC
T_2	5.80	3.91	32.54 (1.51)	TLTC	TLTC
T_3	5.64	4.02	28.47 (1.44)	TLTC	TLTC
T_4	5.68	3.32	40.53 (1.60)	TLTC	TLTC
T_5	5.71	3.62	36.54 (1.56)	TLTC	TLTC
T_6	5.47	3.37	39.11 (1.59)	TLTC	TLTC
T_7	5.84	4.94	15.68 (1.19)	TLTC	TLTC
CD (0.05)	NS	0.124	6.023 (0.089)	TLTC	TLTC

Table 2. Effect of sanitizing treatments on ascorbic acid content (mg/100 g) of amaranthus leaves and stem. NS - Non-significant.

Treatments	Leaves Before treatment	After treatment	Stem Before treatment	After treatment
T_1	17.87	17.28	16.83	16.26
T_2	17.89	17.10	16.70	16.14
T_3	17.93	17.85	16.82	16.75
T_4	17.89	17.88	16.84	16.73
T_5	17.91	17.85	16.86	16.77
T_6	17.89	17.20	16.85	16.31
T_7	17.95	17.90	16.88	16.80
CD (0.05)	NS	0.054	NS	0.094

present in harvested vegetables can be substantially reduced through sanitization treatments which improved the shelf life of minimally processed produce. Bacteria, viruses and parasites are present in the harvested vegetables and are to be removed before processing. Washing with sanitizing chemicals is a most important step in destroying the microbial population and to reduce quality deterioration of minimally processed vegetables [2, 3].

Materials and Methods

Red amaranthus (var Arun) raised by direct sowing was harvested 25 to 30 days after sowing transported to the Postharvest Technology Laboratory of Department of Processing Technology without any delay where the processing operations are performed. In the laboratory, after removing roots, whole plant was immersed in sanitizing solutions viz. 30 ppm sodium hypochlorite, warm water (40°C), RO water (reverse osmosis), ozonized water (2 ppm), citric acid (1%), acetic acid (1%) and tap water for 5 minutes. Each treatment was taken in three replications. After the sanitization, excess water was drained off and for determining the effectiveness of sanitizing agents for surface decontamination, microbial population was enumerated before and after the treatments for amaranthus leaves and stem separately. For enumeration of microbial population by serial dilution spread plate technique was carried for quantitative assay of micro flora in pre and post treated samples. For enumeration of bacterial population, NA (Nutrient Agar) and for fungal population RB (Rose Bengal Agar) medium were used. Biochemical parameters, ascorbic acid and anthocyanin content, and visual parameters (color and texture) of amaranthus leaves were analyzed and the best sanitizing treatment was selected for further studies. Vitamin C content was estimated in amaranthus leaves by 2, 6-dichloro phenol indophenol (DCPIP) day method and expresed as mg/100 g.

$$\text{Ascorbic acid} = \frac{0.5\text{ mg}}{V_1\text{ ml}} \times \frac{V_2}{5\text{ ml}} \times \frac{100}{\text{weight of sample}}$$

For anthocyanin estimation, one gram of the sample from each treatment was extracted with ethanolic hydrochloric acid, filtered through a

Table 3. Effect of sanitizing treatments on anthocyanin content (mg/100 g) of amaranthus leaves and stem. NS - Non-significant.

Treatments	Leaves		Stem	
	Before treatment	After treatment	Before treatment	After treatment
T_1	41.82	41.26	26.90	26.20
T_2	41.84	41.23	26.82	25.13
T_3	41.87	41.75	26.86	26.80
T_4	41.81	41.66	26.82	26.80
T_5	41.84	41.71	26.91	26.81
T_6	41.81	41.24	26.91	26.80
T_7	41.80	41.78	26.89	26.87
CD (0.05)	NS	0.280	NS	0.183

Buchner funnel using Whatsman No. 1 filter paper. The filterate was then diluted with ethanolic hydrochloric acid to 50 ml to get optical density (OD) values within the optimum range of spectrophotometer at 535 nm. The anthocyanin content was then calculated using the following equation and the quantity was expressed as mg per 100 g of the sample.

$$\text{Total optical density per 100 g of sample (X)} = \frac{[(\text{Absorbance at 535 nm}) \times (\text{Volume made up of the extract used for color measurement}) \times (\text{Total volume}) \times 100]}{\text{Volume (ml) of the extract used} \times \text{weight of the sample taken}}$$

The absorbance of a solution containing 1 mg is equal to 98.2 (constant).
Therefore, Total anthocyanin in mg/100 g of the sample=X/98.2

Sanitized amaranthus leaves and stem were stored under room temperature (26 ± 2°C) and scoring for sensory parameters viz. color, texture and overall acceptability after one hour of the treatment was conducted by a semi trained panel of 30 members. Biochemical and microbial population were analyzed statistically in a completely randomized design and significance was tested using analysis of variance technique and sensory scores were analyzed statistically using the non-parametric ANOVA (Kruskall Wallis test) and mean ranks and critical values were calculated. Based on microbial biochemical and sensory parameters, the best sanitizing agent was selected for minimal processing of amaranthus leaves as well as stem.

Results and Discussion

Total microbial load

Enumeration of microbial population before and after the sanitization treatments for amaranthus leaves is depicted in Table 1. Bacterial population present in amaranthus leaves before sanitization ranged from 5.47 to 5.84 × 10^3 cfu g^{-1} and there was no significant variation among the treatments. Sanitization treatments helped in reducing the bacterial population in amaranthus leaves. Amaranthus leaves treated with 2 ppm ozonized water had the least bacterial population (3.32×10^3 cfu g^{-1}) with the highest reduction percentage of 40.53 which did not differ significantly with 1% acetic acid (3.37 × 10^3 cfu g^{-1}) treatment. Highest bacterial population was observed in amaranthus leaves treated with tap water (4.94× 10^3 cfu g^{-1}) with lowest reduction percentage of 15.68. Fungal population in amaranthus leaves was too less to count before and after the sanitization treatments. Bacterial population present in amaranthus stem before sanitization ranged from 5.20 to 5.24× 10^3 cfu g^{-1} and there was no significant variation among the treatments (Table 2).Amaranthus stem treated with 2 ppm ozonized water had the least bacterial populations (3.19× 10^3 cfu g^{-1}) with the highest reduction percentage of 39.15 and there was no significant difference with the treatment (30 ppm sodium hypochlorite) that recorded 35.38% of reduction in bacteria population. Sanitization with 30 ppm sodium hypochlorite and 1% acetic acid also did not show any significant difference in reduction of bacterial population. Sanitization with tap water recorded the highest bacterial population of 4.94× 10^3 cfu g^{-1} with the lowest reduction percentage of 5.53 only. Fungal population in amaranthus stem was too less to count before and after the sani-

Table 4. Effect of sanitizing treatments on visual parameters of amaranthus leaves.

	Color				Texture				Overall acceptability			
	Leaves		Stem		Leaves		Stem		Leaves		Stem	
Treatments	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
T_1	102.9	4.60	102.9	4.60	110.1	4.93	110.1	4.93	102.9	4.60	102.9	4.60
T_2	99.70	4.56	99.70	4.56	94.25	4.50	94.25	4.50	99.70	4.56	99.70	4.56
T_3	97.53	4.60	97.53	4.60	102.2	4.63	102.2	4.63	97.53	4.60	97.53	4.60
T_4	127.2	5.00	127.2	5.00	130	5.00	130	5.00	127.2	5.00	127.2	5.00
T_5	115.3	4.83	115.3	4.83	114.1	4.83	114.1	4.83	115.3	4.83	115.3	4.83
T_6	95.0	4.50	95.0	4.50	94.2	4.50	94.2	4.50	95.0	4.50	95.0	4.50
T_7	99.7	4.60	99.7	4.60	93.4	4.46	93.4	4.46	99.7	4.60	99.7	4.60
CV (0.05)						12.592						

tization treatments.

Similar results are found in fresh cut lettuce [4, 5] treated with ozone (2.5 ppm) and turnip greens [6]. Ozone at 3 ppm was more effective in inactivating bacteria, moulds and yeasts on vegetables [7]. Chinese cabbage treated with ozonated water (2.3 mg/l) for 60 minutes recorded more than 90% microbial reduction percentage [8]. Sanitization of amaranthus with 2 ppm ozonized water was found effective in reducing microbial population [9]. Influence of sanitization treatments on bio-chemical parameters viz. ascorbic acid and anthocyanin content of amaranthus leaves and stem is depicted in Tables 3 and 4.

Ascorbic acid

Ascorbic acid present in amaranthus leaves before sanitization ranged from 17.87 mg/100 g to 1.95 mg/100 g and there was no significant variation among the treatments. Ascorbic acid content of amaranthus leaves treated with RO water, 2 ppm ozonized water, 1% citric acid and tap water did not show any significant difference after the treatment and ranged from 17.85 to 17.90 mg/100 g. Warm water treated leaves recorded the lowest ascorbic acid of 17.10 mg/100 g. Ascorbic acid present in amaranthus stem before sanitization ranged from 16.70 to 16.88 mg/100 g and there was no significant variation among the treatments. Ascorbic acid control of amaranthus stem treated with RO water, 2 ppm ozonized water, 1% citric acid and tap water did not show any significant difference after the treatment and ranged from 16.73 to 16.80 mg/100 g. Warm water treated leaves recorded the lowest ascorbic acid of 16.14 mg/100 g.

Similar finding are reported by Kaur and Kapoor [10] to improve overall appearance and maximum antioxidant activity incorporation of ascorbic acid and citric acid in the dip water was effective. In leafy vegetables, antioxidant activity and ascorbic acid content was investigated as indicators of quality change by washing treatments and found that tap water, ozonated and chlorinated water did not influence the quality [11]. Studies revealed that Altunkaya and Gokmen [12] retention of both ascorbic acid and total anthocyanins in fresh lettuce with citric acid treatment. Fresh strawberry immersed in citric acid resulted in enhanced retention of ascorbic acid and total anthocyanin content [13]. Lettuce treated with different levels of ozonated water, the highest preservation of vitamin C was with 1 ppm ozonated water [14].

Anthocyanin content

Anthocyanin content present in amaranthus leaves

before sanitization ranged from 41.80 to 41.87 mg/100 g and there was no significant variation among the treatments. Anthocyanin content in amaranthus leaves treated with RO water, 2 ppm ozonised water, 1% citric acid and tap water showed no significant difference which ranged from 41.66 to 41.78 mg/100 g. Sodium hypochlorite 30 ppm warm water and 1% acetic acid recorded the lowest anthocyanin content 41.23 to 41.26 mg/100 g. Anthocyanin content present in amaranthus stem before sanitization ranged from 26.76 to 26.91 mg/100 g and there was no significant variation among the treatments. Anthocyanin content in amaranthus stem treated with the RO water, 2 ppm ozonized water, 1% citric acid and tap water did not show any significant difference after the treatment and ranged from 26.80 to 26.87 mg/100 g. Sodium hypochlorite 30 ppm and warm water recorded the lowest anthocyanin content in the range of 25.13 to 26.20 mg/100 g.

Similar result was reported in leafy vegetables [11]. Ascorbic acid and total anthocyanin retention was high in fresh lettuce with citric acid treatment [12] and similar result was obtained in strawberry [13].

Physical (visual) parameters

Visual parameters play an important role in consumer acceptability of leafy vegetables as they are more sensitive to wilting and loss of freshness. Sensory parameters viz. color, texture and overall acceptability of sanitized amaranthus leaves and stem are depicted in Table 4.

Amaranthus leaves sanitized with 2 ppm ozonised water recorded the highest mean score of 5.00 for color, texture and overall acceptability with highest mean rank 127.2. It was followed by the treatment 1% citric acid with a mean score of 4.83 for color, texture, overall acceptability. The lowest mean score was obtained for 1% acetic acid for color (4.50), texture (4.50) and overall acceptability (4.50). Amaranthus stem treated with 2 ppm ozonised water recorded the highest mean score of 5.00 for color, txcture and overall acceptability. It was followed by 1% citric acid with a mean score of 4.83 for color, texture, overall acceptability. The lowest mean score was obtained for 1% acetic acid for color (4.50), texture (4.50) and overall acceptability (4.50).

Highest acceptability for sensory qualities was reported with ozonized water treatment was reported by several researchers [4, 14—16].

On analyzing the effectiveness of sanitizing agents for surface decontamination and superiority in biochemical and physical (visual) parameters, 2 ppm ozonized water was selected as the best sanitizer for amaranthus leaves and stem.

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