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Evaluation of sanitization treatments for red amaranthus (*Amaranthus tricolor* L.)

■ GEOGY MARIAM GEORGE[1]*, P.R. GEETHA LEKSHMI[1], C. MINI[1], K.N. ANITH[2] AND R.V. MANJU[3]

[1]Department of Processing Technology, College of Agriculture, Vellayani, THIRUVANATHAPURAM (KERALA) INDIA
[2]Department of Microbiology, College of Agriculture, Vellayani, THIRUVANATHAPURAM (KERALA) INDIA
[3]Department of Plant Physiology, College of Agriculture, Vellayani, THIRUVANATHAPURAM (KERALA) INDIA
Email : gmaryg7@gmail.com; geetha_lekshmy@yahoo.co.in and minichandran123@gmail.com

*Author for Correspondence

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SUMMARY :

Amaranthus is known as 'poor man's spinach' which is most commonly used leafy vegetable rich in nutrients. Increased need of sanitization in postharvest handling of leafy vegetables has been reported due to outbreak of disease causing pathogens and also due to huge postharvest loss. In the present study amaranthus (var. Arun) were sanitized with different sanitizing agents *viz.*, ozonised water, sodium hypochlorite, brine and tap water (control). Effectiveness of these sanitizing agents for surface decontamination of harvested amaranthus was evaluated by analyzing microbial, physiological and visual parameters. The results revealed that amaranthus sanitized with 2 ppm ozonised water had highest microbial reduction (85.68%), lowest physiological loss in weight (25.63%), highest relative water content (63.11%) and highest score for visual parameters and overall acceptability which was followed by the treatment with 30 ppm sodium hypochlorite. Sanitization with 2 ppm ozonised water also extended the shelf-life of amaranthus upto 4 h of storage at room temperature.

KEY WORDS : Sanitization, Amaranthus, Ozonised water, Sodium hypochlorite, Shelf-life

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Amaranth is one of the ancient leafy vegetables which could play an important role in rural economic and nutritional security because its cultivation allows good yields of high quality leaves to be integrated in daily poor diet. A loss of 5 to 10 per cent of fresh weight would make leafy vegetables to appear wilted and become unusable. In recent years there is an increased need of sanitization in the chain of postharvest handling. Numerous cleaning compounds and processes have been developed to remove and destroy bacteria, viruses and parasites from vegetables. Washing with sanitizers is an important step in reducing the microbial population and quality deterioration (Kim, 2012). Use of sanitizers such as chlorine, peroxy acetic acid, hydrogen

peroxide, acidified sodium chloride or ozone can provide an additional reduction in the initial population of micro-organisms on the surface of fresh produce (Heard, 2000). Different protocols have been used for washing and disinfection of leafy vegetables and they comprise several washing times, kind of sanitizers and sanitizer concentrations and justifying its comparison (Oliveira *et al.*, 2012). Objectives of vegetable sanitization are reduction or elimination of micro-organisms from vegetables and guarantee it for consumption (Bachelli *et al*., 2013). Hence the present study was conducted for evaluating the effectiveness of different sanitization treatments and shelf-life for red amaranthus (var. Arun).

EXPERIMENTAL METHODS

The study was conducted at Department of Processing Technology, College of Agriculture, Vellayan, Kerala Agricultural University during the year 2013-2015. Freshly harvested Amaranthus (var. Arun) of uniform size without any visual defects were procured in the morning hours from VFPCK (Vegetable and Fruit Promotion Council Keralam) progressive farmer's field, Vellayani cultivated as per Kerala Agricultural University package of practices. Harvested Amaranthus (stem with leaves) of 25-30 cm long (after removing root portion) were taken and fully immersed in following sanitizing agents for five minutes. For each treatment 200 g samples were taken with five replications.

T_1 : Brine solution (2%)
T_2 : Sodium hypochlorite (30 ppm)
T_3 : Ozonised water (2 ppm)
T_4 : Tap water

After the treatments excess water was drained and samples were air dried. Effectiveness of sanitizing agents for surface decontamination was evaluated microbiologically before and after the treatment. Sanitized amaranthus were stored at room temperature (30±2°C) and observed for visual and physiological parameters. The data were analyzed statistically by using ANOVA (Completely Randomized Design).

Microbial count :

The quantitative assay of the micro flora in pre and post- treated samples was carried out by serial dilution spread plate technique. NA (nutrient agar) and RB (rose bengal agar) medium were used for the enumeration of bacterial and fungal population, respectively. Number of micro-organisms (bacteria and fungi) per 10 g of pre and post-treated samples was calculated as per the following formula:

$$\text{No.of colony for ming uints (CFU per gram of the sample)} = \frac{\text{Total number of colony formed x dilution factor}}{\text{Aliquote plated}}$$

Physiological parameters :

Physiological parameters *viz.*, physiological loss in weight and relative water content of sanitized amaranthus were recorded at hourly interval till the end of shelf-life. Amaranthus with cumulative physiological loss in weight of more than 25 per cent and relative water content less than 65 per cent with lower score for visual parameters and overall acceptability was considered as the end of shelf-life.

For determining physiological loss in weight, sample was weighed accurately at the time of storage (after treatment) and subsequently at an interval of one hour till the end of shelf-life. Cumulative physiological loss in weight was calculated using the formula :

$$\text{CPLW (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Relative water content (RWC) :

Percentage relative water content was estimated according to the method proposed by Smart and Bingham (1974). A composite sample of leaf discs is taken and the fresh weight is determined, followed by flotation in distilled water for up to 1 hour. The turgid weight is then recorded and the leaf tissue is subsequently oven dried to a constant weight at about 85°C. RWC is calculated in percentage by :

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - Dry weight)}}{\text{(Turgid weight - Dry weight)}} \times 100$$

Visual parameters :

Visual parameters like colour, texture, appearance, leaf wilting and defoliation/decay of sanitised amaranthus were taken initially and at an interval of one hour till the end of shelf-life by conducting a sensory evaluation performed by a 30 member semi-trained panel. A score card proposed by Swaminathan (1995) was used for assessing the sensory qualities with a 5 point hedonic scale with following scores.

Excellent- 5, Very good- 4, Good-3, Fair- 2, Poor - 1

The score given by 30 judges were statistically analyzed using the non-parametric ANOVA (Kruskall Wallis test) and mean ranks and critical values were calculated.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Enumeration of total microbial count :

Before the sanitization treatments, bacterial population of amaranthus in each treatment was assessed and was in range of 24.84 to 25.58×10^5 cfug^{-1}. No significant difference was found between the treatments before the sanitization and no fungal population was found.

After the sanitization treatments, the treatment T_3 (2 ppm ozonised water) recorded the lowest bacterial population of 3.24×10^5 cfug^{-1} followed by T_2 (30 ppm sodium hypochlorite) with 8.84×10^5 cfug^{-1}. The highest bacterial population was recorded in T_1 (2 % brine) with 15.51×10^5 cfug^{-1} and it showed no significant difference with the treatment T_4 (14.17×10^5 cfug^{-1}).

Surface sanitization of amaranthus (var. Arun) with 2 ppm ozonised water was found highly effective with 85.68 per cent reduction in microbial population followed by 30 ppm sodium hypochlorite with a reduction percentage of 64.26 (Table 1). Similar result was reported by Olmez and Akbas (2009) that application of 2 ppm ozonated water treatment for two minutes was the optimum processing conditions for ozone disinfection of green leaf lettuce, in terms of reducing the microbial load during cold storage. The sanitizing efficiency of ozone may be due to the fact that ozone destroys micro-organism by disruption or disintegration of cell envelope and internal cellular proteins by progressive oxidation causing rapid cell death (Komanapalli and Lau, 1996 and Kim, 2012). Ibrahim *et al.* (2012) reported that in turnip greens microbial population was reduced to 1.43 log cfug^{-1} when treated with 5 ppm ozone for five minutes. Chen *et al.* (2013) concluded that 3 ppm ozone is more effective in inactivating bacteria, moulds and yeasts on vegetables. Nath *et al.* (2014) observed that total bacterial count reduction was greater than 90 per cent in chinese cabbages treated with ozonated water (2.3 mg/l) for 60 minutes.

Surface sanitization with 30 ppm sodium hypochlorite reduced microbial population than brine and tap water. Similar result was reported by Varghese (2006) in fresh-cut vegetables like cowpea, okra, brinjal, ash gourd, pumpkin and elephant foot yam and Chandran (2013) in fresh-cut cabbage, beans, carrot and beetroot. Chlorine selectively destroys certain intracellular enzyme systems of micro-organisms causing rapid cell death that helped in reducing the microbial population (Karaca and Velioglu, 2007).

Surface sanitization of amaranthus with 2 per cent

Table 1 : Effect of sanitizing treatments on the microbial population of amaranthus

Treatments	Bacterial population $\times10^5$ cfug^{-1}			Fungal population
	Before treatment	After treatment	Reduction %	After treatment
T_1	25.58	15.51^a	39.39^c (6.26^c)	TLTC
T_2	24.84	8.84^b	64.26^b (8.02^b)	TLTC
T_3	24.84	3.24^c	85.68^a (9.24^a)	TLTC
T_4	24.84	14.17^a	42.91^c (6.54^c)	TLTC
C.D. (P=0.05)	NS	4.549	6.914 (0.444)	

Figure in parentheses is square root transformed values; TLTC- Too Less to count NS= Non-significant

Table 2 : Effect of sanitizing treatments on physiological loss in weight (%)

Treatments	After storage		
	2 h	3 h	4 h
T_1	12.55^a	28.95^a	31.04^a
T_2	8.23^c	21.36^c	27.53^c
T_3	6.88^d	11.17^d	25.63^d
T_4	10.07^b	22.53^b	29.12^b
C.D. (P= 0.05)	1.285	0.253	0.021

brine had highest microbial population (15.51×10^5 cfug^{-1}) with lowest reduction percentage of 39.39 per cent which was even lower than treatment with tap water (42.91%). This result is supported by the findings of Reddy (2010) in which brine (2%) dipping resulted in maximum decaying of rajagira leaves (*Amaranthus paniculatus*).

As salt acts as osmotic agent, draws water from the tissues leading to condition of moisture on inner surface of the film (Anonymous, 1999). Condensed moisture content inside the package adds to the acidity of the leafy vegetables which can end up as the cause of deterioration and growth of the microorganisms (King *et al.*, 1991; Ahvenainen, 1996 and Brackett, 1999).

Physiological parameters :

Physiological weight loss is a phenomenon of weight loss from produce during storage which in turn is related with shelf-life of vegetables whether whole or fresh-cut products. Amaranthus treated with 2 ppm ozonised water was found to have lowest physiological loss in weight of 6.88, 11.17 and 25.63 per cent at 2, 3 and 4 h of storage at room temperature (Table 2). This result was in agreement with the findings of Nadas *et al*. (2003) that fruits treated with ozonised water showed less weight loss than the non-treated samples after storage. Zhang *et al.* (2005) reported that fresh-cut celery treatment with ozonated water showed to be effective to reduce the population of micro-organisms and retard physiological metabolism. Sothornvit and Kiatchanapaibul (2009) reported that ozone treatment for fresh or fresh-cut vegetables extended the shelf-life by inhibiting growth of micro-organisms.

Relative water content was also found to be highest in the 2 ppm ozonised water treated amaranthus during 2, 3, and 4 h of storage as 82.70, 75.92 and 63.11 per cent, respectively (Table 3). Similarly, Beltran *et al.* (2005) reported that fresh visual appearance of fresh-cut lettuce and fresh-cut potato was maintained by ozonated water during storage.

It could be noted that there is a relation between physiological loss in weight, relative water content and shelf-life or freshness of leafy vegetable. Increase in weight loss decreases the water content and in turn reduces the freshness or shelf-life of leafy vegetables.

Amaranthus treated with 2 per cent brine were unable to sustain edibility. Highest physiological loss in weight of 12.55, 28.95 and 31.04 per cent was recorded by 2 per cent brine after 2, 3 and 4 h of storage, respectively. Lowest relative water content of 74.34, 60.24 and 53.64 per cent was observed in 2 per cent brine treated amaranthus, respectively during 2, 3 and 4 h of storage. Amoah *et al.* (2007) found that higher concentration of brine treatment greatly reduced the quality of the lettuce leaves. Reddy (2010) reported that brine (2%) used as dipping solution reduced the moisture significantly and could not extend the shelf-life of *rajagira* leaves with or without stem.

Table 3 : Effect of sanitizing treatments on relative water content (%)

Treatments	Before treatment	After treatment	After storage		
			2 h	3 h	4 h
T_1	83.12	82.03^b	74.34^d	60.24^d	53.64^d
T_2	83.22	86.22^a	77.88^c	65.11^b	58.43^b
T_3	82.69	87.41^a	82.70^a	75.92^a	63.11^a
T_4	82.93	87.28^a	80.58^b	64.38^c	55.23^c
C.D. (P=0.05)	NS	2.930	1.831	0.219	0.028

NS= Non-significant

Table 4 : Effect of sanitizing treatments on visual parameters after 2 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
T_1	34.25	3.25	30.40	3.60	66.00	2.10	59.55	1.45	26.50	3.00
T_2	52.40	3.55	43.65	4.00	31.35	1.15	40.50	1.00	38.30	3.35
T_3	60.65	4.00	51.45	4.25	29.4	1.10	34.50	1.00	60.05	3.95
T_4	39.75	3.35	36.50	3.80	35.25	1.25	52.50	1.15	37.15	3.30
C.V. (P= 0.05)	7.815									

Visual parameters :

Amaranthus is highly perishable in nature which leads to loss of freshness during storage. Amaranthus treated with 2 ppm ozonised water (T_3) recorded the highest mean score for visual parameters *viz.*, colour (4.00), texture (4.25) and overall acceptability (3.95) and lowest mean score for defoliation (1.00) and leaf wilting (1.1) (Table 4). It was followed by T_2 (30 ppm sodium hypochlorite) with a mean score for colour (3.55), texture (4.00), overall acceptability (3.35), defoliation (1.00) and leaf wilting (1.15). Minimum mean score was obtained for T_1 (2% brine) in colour (3.25), texture (3.6) and overall acceptability (3.00) and highest for leaf wilting (2.1) and defoliation (1.45) after 2 h of storage.

After 3 h of storage, amaranthus dipped in 2 ppm ozonised water (T_3) recorded the highest mean score for colour (3.55), texture (3.3) and overall acceptability (3.5) and lowest mean score for leaf wilting (2.00) and defoliation (2.05) which was followed by 30 ppm sodium hypochlorite (T_2) treated amaranthus with a mean score of 2.30, 2.80, 2.90, 2.25 and 2.55 for colour, texture, overall acceptability, leaf wilting and defoliation, respectively (Table 5). Zhang *et al.* (2005) revealed that the best preservation effect was found to be in treatment with ozonated water by which the microbial population was able to be lowered and nutritional and sensory quality of fresh-cut celery was maintained good for 9 days of storage at 4°C. Lowest mean score was observed for T_1 (2% brine) for colour (1.8), texture (2.00) and overall acceptability (2.2) and highest for leaf wilting (3.6) and defoliation (3.05).

After 4 h of storage, amaranthus dipped in 2 ppm ozonised water (T_3) showed highest mean score for colour (2.4), texture (2.75) and overall acceptability (2.00) and lowest for leaf wilting (2.3) and defoliation (2.65) which was followed by 30 ppm sodium hypochloride (T_2) (Table 6). Olmez and Akbas (2009) also reported similar results with application of 2 ppm ozonated water treatment for 2 minutes was the optimum processing conditions for ozone disinfection of green leaf lettuce, in terms of reducing the microbial load and maintaining the sensory quality during cold storage. Minimum mean score was obtained by amaranthus dipped in 2 per cent brine (T_1) for colour (1.85), texture (1.55) and overall acceptability (1.6) and maximum for leaf wilting (3.7) and defoliation (3.45). This could be due to the dehydrating nature of salt which renders to loss of moisture during storage which results in loss of freshness and turgidity of produce (Anonymous, 1999).

Freshness of a produce is related to the shelf-life of a produce. Amaranthus treated with ozonised water (2 ppm) had the longest shelf-life upto 4 h when compared to other treatments. Beltran *et al.* (2005) reported that the use of ozonated water applied to fresh-cut vegetables for sanitation purposes reduced the microbial populations and extended the shelf-life of some of these products. Ozone treatment is effective in inhibiting the growth of micro-organisms

Table 5 : Effect of sanitizing treatments on visual parameters after 3 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
T_1	21.85	1.80	22.55	2.00	66.10	3.60	54.70	3.05	19.80	2.20
T_2	35.60	2.30	47.55	2.80	28.37	2.25	36.67	2.55	43.60	2.90
T_3	67.35	3.55	62.15	3.30	20.72	2.00	19.37	2.05	60.75	3.50
T_4	37.20	2.35	29.75	2.25	46.80	2.80	51.25	2.90	37.85	2.75
C.V. (P=0.05)	7.815									

Table 6 : Effect of sanitizing treatments on visual parameters after 4 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
T_1	33.72	1.85	18.20	1.55	60.80	3.70	56.12	3.45	34.40	1.60
T_2	40.42	2.05	48.25	2.55	28.90	2.60	36.47	2.85	41.82	1.85
T_3	50.65	2.40	54.30	2.75	20.20	2.30	29.77	2.65	47.72	2.00
T_4	37.20	1.95	41.25	2.35	52.10	3.40	39.62	2.95	38.05	1.7
C.V. (P=0.05)	7.815									

and extends the shelf-life of fresh or fresh-cut products by Sothornvit and Kiatchanapaibul (2009).

Conclusion

Surface sanitization of red amaranthus (var.Arun) with different sanitizing agents had showed a significant reduction in microbial population. Amaranthus surface sanitized with 2 ppm ozone was found to be best the sanitizer with lowest microbial population and had highest quality parameters with prolonged shelf-life upto four hours during room temperature storage.

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