STANDARDIZATION OF MINIMAL PROCESSING OF AMARANTHUS

. (Amaranthus tricolor L.)

by

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(2014-12-133)

THESIS

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2016

DECLARATION

I, hereby declare that this thesis entitled "STANDARDIZATION OF MINIMAL PROCESSING OF AMARANTHUS (*Amaranthus tricolor* L.)" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

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%	Per cent
β	Beta
μg	Micro gram
μl	Micro liter
cfu	Colony forming units
O ₂	Oxygen
CO ₂	Carbon dioxide
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
et al.	And other co workers
Fig.	Figure
g	Gram
nm	Nano meter
h	Hours
LDPE	Low density polyethylene
PP	Poly propylene
1	Litre
ml	Milli litre
OD	Optical density
М	Molar
mg	Milli gram
viz.	Namely
ppm	Parts per million
рН	Negative logaritham of hydro carbon ions
rpm	Revolution per minute

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INTRODUCTION

1. INTRODUCTION

Vegetables are rich source of vitamins, minerals and other bioactive compounds that play an important role in healthy human diet. Minimally processed vegetables have gained consumer acceptance and are one of the growing segment in food retail establishments. The market demand for minimally processed fruits and vegetables has undergone an important rise because of busy lifestyles, new consumer profile 'rich in cash/poor in time' and health conscious consumers (Baldwin *et al.*, 1995). Minimally processed vegetables are also called ready-to-use, fresh-cut or pre-cut produce.

Minimal processing of fresh cut fruits and vegetables require preliminary steps, such as washing, peeling, shredding and cutting. These steps result cuts, bruises and injuries to internal tissues and can cause desiccation and wilting as well as microbial and enzymatic spoilage. These injuries fasten the respiration rate which further triggers the increased production of ethylene, senescence, and enzymatic browning (Bansal *et al.*, 2015).

Vegetables in general contain more than 60 per cent water and get spoiled very quickly. 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 (Maria, *et al.*, 2006). The minimally processed preservation techniques not only enables the storage of the products for a longer period, but should preserve flavour, nutritional qualities and prevent microbial spoilage.

Amaranthus (Amaranthus tricolor L.) is one of the largely consumed leafy vegetables of Kerala and is well known for its high nutritional value and ready to use amaranthus has high consumer demand. It is called as 'poor man's spinach' and also easily available source of protective nutrients and less expensive. For each fresh produce, tailor made minimal processing technology is to be developed and quality of minimally processed vegetables depend on colour, flavour, firmness, odour and nutritional quality brought about by physiological and microbiological factors (Ragaert *et al.*, 2007).

Factors that influence quality of minimally processed vegetables include method of preparation, packaging and storage conditions. Besides refrigeration, an improvement of the shelf life of ready-to-use fresh vegetables may be achieved by combining many of the classical preservation procedures such as chemicals (antioxidants, antimicrobials), pH reduction and modified atmospheres, using hurdle technology. Modified atmosphere packaging is the most commonly used technology for fresh cut vegetables and an important food preservation technique for maintaining natural quality and extending shelf life of minimally processed food (Ranjitha *et al.*, 2015)

As consumer's preferences have been changed significantly towards healthy and convenient food with freshness, great deal of research is focused on minimal processing of vegetables. By establishing an efficient and economic protocol for the development of fresh cut vegetables, consumption of vegetables in present day busy life schedule will increase which contribute to healthy diet. Hence the present study on "Standardization of minimal processing of amaranthus *(Amaranthus tricolor L.)*" was undertaken at the Department of Processing Technology, College of Agriculture, Vellayani with the objective to standardise minimal processing technology for the development of ready-to-use amaranthus with extended shelf life and nutritional quality.

REVIEW OF LITERATURE

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2. REVIEW OF LITERATURE

Leafy vegetables play an important role in healthy diet. Expanding urbanization and increase in working women population, has lead to the era of convenience in all aspects of life that demands for minimally processed food products. Green leafy vegetables need pre-processing and hence ready to use fresh cut vegetables are need of the hour.

Minimally processed vegetables are the products that contain living tissues or those that have slightly modified their fresh condition but keep their quality and character similar to those of fresh products (Wiley, 1994). Fresh-cut produce keep today's food market alive and remains at the top list of consumers (Lamikanra, 2002). According to International Fresh cut Produce Association (IFPA) (2004) fresh-cut products are fruit or vegetables that have been trimmed and/or peeled and/or cut into 100 percent usable product that is bagged or prepackaged to offer consumers high nutrition, convenience, and flavor while still maintaining its freshness. Kanlayanarat (2007) reported that loss of 5 to 10 % of fresh weight would make leafy vegetables to appear wilted and become unusable for consumption. 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 but it has short postharvest shelf life due to leaf wilting and it varied with genotypes (Suraweera et al., 2011). Leafy vegetables, due to high moisture content, are highly perishable in nature and susceptible to rapid depreciation of nutritive value soon after harvest and it is estimated that over 30 % of leafy vegetables are lost due to poor handling and storage conditions (Nyaura et al., 2014).

Thus the present study focus on standardization of minimal processing of amaranthus (Amaranthus tricolor L.) which involved standardization of different sanitisation and pre-treatments for reducing quality deterioration of fresh-cut amaranthus and development of suitable package and storage system for extended shelf life with minimum nutritional loss. This chapter describes the review of related research findings done in past years.

2.1. EVALUATION OF SANITIZING TREATMENTS

In recent years an increased interest in microbiological quality and safety of fresh produce linked to outbreaks of food borne pathogenic microbial illness was reported by Brackett (1999). The easiest and best method of decontamination could be done by thorough washing of leafy vegetables and addition of a chemical disinfectant to the wash water reduced the microbial load (Beuchat and Ryu, 1997; Sapers, 2001). Soilva *et al.* (2003) reported that high microbial load present in harvested vegetables can be substantially reduced through sanitization treatments which improved the shelf life of minimally processed produce. Oliveira *et al.* (2012) reported that different protocols were developed to wash and disinfect the leafy vegetables, and they comprise of several washing times, kind of sanitizers and sanitizer concentrations.

Regardless of the sanitizing solution used to dip fresh cut products, after immersion excess surface moisture should be removed to reduce potential quality deterioration caused by the presence of free water (Cantwell, 1992). Most processors and consumers have assumed that washing and sanitizing fresh vegetables will reduce the microbial load. However, although current techniques used by the fresh vegetable processing industry have improved the overall quality and extended the shelf life of these products, safety is still an issue of concern (Cliffe-Byrnes and O'Beirne, 2005). Jose and Vanetti (2015) revealed that washing with sanitizing solutions could reduce the microbial population from wegetables.

2.1.1. Sodium hypochlorite

Lee and Baek (2008) observed that sodium hypochlorite is most effectively used sanitizer in the fresh-cut industry. Sodium hypochlorite reduced micro flora to $1-1.3 \log$ cfu g⁻¹ in minimally processed products (Allende *et al.*, 2009).

Disinfections with different decontaminants viz. chlorinated, water, sodium hypochlorite, neutral electrolyzed water and peroxy acetic acid in fresh cut vegetables were studied by Martinez et al. (2009). Alvaro et al. (2009) and Kim et al. (2010) in their several studies reported that use of chlorine dioxide, hydrogen peroxide and sodium hypochlorite can reduce microbial load on outer surface of tomato, sweet pepper, cucumber and strawberry. (Shih et al. 2012) reported that use of sodium hypochlorite can provide effective antimicrobial and anti-browning treatments of fresh cut produce, including processed root vegetables.

Sodium hypochlorite was found active antimicrobial agent during washing for processed cabbage, carrot, onion and chinese cabbage by Dufkova (2000). Fantuzzi and Pushmann (2004) reported that reduction of microbial load was found in minimally processed cabbage after sanitization for ten minutes with sodium hypochlorite at 200 mg/l. Sodium hypochlorite fullfils many requirements as the ideal disinfectant (Fukuzaki, 2006). Varghese (2006) revealed that surface sanitization with 30 ppm sodium hypochlorite as the most effective sanitizer for preserving visual quality, reducing microbial contamination and enhancing shelf life of cut vegetables like cowpea, okra, brinjal, ash gourd, pumpkin and elephant foot yam. A study conducted by Amith (2012) standardized the effective concentration of sodium hypochlorite as 30 ppm, 60 ppm and 90 ppm sodium hypochlorite as the surface sanitizer for minimally processed pomegranate, mango, papaya and pineapple respectively. Oliveira et al. (2012) observed that lettuce treated with 200 ppm sodium hypochlorite for 15 minutes yielded an estimated decrease of 2 \log_{10} for the tötal microbial population. Sodium hypochlorite showed lower score for the count of mesophilic microorganisms in minimally processed lettuce when compared to other treatments during storage by Bachelli et al. (2013). Chandran (2013) reported that in fresh-cut vegetables viz. cabbage, beans, carrot and beetroot treated with 30 ppm sodium hypochlorite was found effective surface sanitization in reducing the microbial load. Hai and Uthaibutra (2015) reported that reduction of microbial

population on the surface of Longan fruit (*Dimocarpus longan* Lour.) by use of sodium hypochlorite.

2.1.2. Ozonised water

Ozone is applied in gaseous or aqueous form and also it is one of the most powerful oxidizing agents for sanitation purposes. Apples treated with ozone resulted in less weight loss and spoilage was observed by Bazarova (1982). The sanitizing efficiency of ozone may be due to destruction of microorganism by disruption or breakdown of cell envelope and interior cellular proteins by progressive oxidation causing quick cell death (Komanapall and Lau, 1996; Kim, 2012). A study was conducted by Sarig et al. (1996) showed that ozone (8 mg/l) exposed to grapes for 20 minutes had considerably enhanced the storage life and reduced the decay of grapes during cold storage. Suslow (1998) reported that at concentrations of 0.5 to 2 ppm ozone is a highly effective sanitizer and is reported to have 1.5 times oxidizing potential than chlorine and 3,000 times the potential of hypo-chlorous acid. Heard (2000) reported that reduction in the initial microbial load on the surface of fresh produce by use of sanitizers such as chlorine, peroxy acetic acid, hydrogen peroxide, acidified sodium chloride or ozone. The best preservation effect was found to be with ozonated water treatment by which the bacterial load was decreased and nutritional and sensory quality of fresh-cut celery were maintained good for 9 days of storage at 4°C (Zhang et al., 2005). Chinese cabbage treated with ozonated water (2.3 mg/l) for 60 minutes will reduce the total microbial load is greater than 90 % was observed by Nath et al. (2014). Najafi and Shaban (2015) reported that in lettuce the treatment with different levels of ozonated water increased shelf life and maximum retention of vitamin C was related to the treatment with 1 ppm ozonated water.

A study conducted by Song *et al.* (2000) reported that in onions treated with ozone greatly reduced the microbial population during storage without any change in biochemical composition and sensory quality. Khadre *et al.* (2001) reported that in atmosphere ozone is the natural substance and one of the most potent sanitizers against a wide spectrum of microorganisms. The use of ozone as

an antimicrobial agent for the treatment, storage and processing of foods in gas and aqueous phase in direct contact with foods, including raw and fresh cut fruits and vegetables was approved by Food and Drug Administration (FDA, 2001). A study was conducted by Garcia et al. (2003) to describe the efficiency of ozone (2.5, 5.0, and 7.5 ppm) on microbiological attributes of shredded lettuce and results showed 0.6 to 0.8 log reduction in microbial population after a 10 minute treatment. Guzel- Seydim et al. (2004) reported that fresh fruits and vegetables treated with ozone had been found to kill microorganisms by the progressive oxidation of vital cellular components and enhance the storage life of produce. Ozonated water had been applied to fresh-cut vegetables for sanitation purposes which decrease microbial load and increase the shelf life (Beltran et al., 2005). Rivera (2005) reported ozone as relatively safe fruit and vegetable sanitizer that is highly unstable in water, spontaneously decays into non toxic compounds, such as oxygen and does not leave any toxic residues. Treatment with ozone observed to have a beneficial effect in extending the storage life of fresh produce by Kim (2007) in cucumber, apple, grape, orange, pear, raspberries and strawberries by reducing microbial load. Application of 2 ppm ozonated water for two minutes was recorded as the best processing conditions for ozone disinfection of green leaf lettuce, in terms of reducing microbial load and maintaining sensory qualities (Olmez and Akbas, 2009). In food industry, application of ozone has received a commercial interest due to its effectiveness to increase the storage life of minimally processed products by hindering the microbial growth (Sothornvit and Kiatchanapaibul, 2009). Das and Kim (2010) reported that effectiveness of ozone against microbial growth depends on the amount applied, effectiveness of ozone delivery method, type of material, the target microorganisms and physiological state of bacterial cells at the time of treatment. Nature and composition of vegetable surfaces and the type and extend of microbial population affect overall performances of ozone treatment (Kim and Hung, 2012). Shredded carrot washed with 2 ppm ozonated water for twenty minutes maintained quality by hindering off-odour and high overall acceptable quality (Kim, 2012). Microbial decay of fresh and minimally processed produce was prevented by application of ozone

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(Ong et al., 2012). Chen et al. (2013) reported that 3 ppm O₃ is more effective than 3 ppm chlorine dioxide in deactivating microbial population on fruits and vegetables. Aguayo et al. (2014) reported that treating tomato slices with 0.4 mg/l ozonated water for three minutes achieved the best retention of firmness and microbial quality up to 10 days of storage at 5°C. Freshly harvested papaya fruit were exposed continuously to ozone fumigation (0, 1.5, 2.5, 3.5 and 5 ppm) for 96 h prior to room temperature storage at 25 ± 3 °C and 70 ± 5 % relative humidity (RH) for up to 14 days. The papaya fruit exposed to 2.5 ppm ozone had higher levels of ascorbic acid content (12.4 %) and antioxidant activity (30.9%), and also reduced weight loss (11.5 %) after ten days of storage compared to the control was observed by Ali et al. (2014). Fresh cut papaya was treated with ozone (9.2 \pm 0.2 µl/L) at 10, 20 and 30 min to evaluate its effect on biochemicals and microbial population and results obtained revealed that microbial population on minimally processed papaya could be decreased without reducing its key antioxidants (Yeoh et al., 2014). Dastan and Masoodi (2015) concluded that ozone treatment controls microbial population on the surface of fruits and vegetables and leads to more stable storage, minimum physiological loss in weight, and also retain best quality. George (2015) reported that amaranthus treated with 2ppm ozonised water for five minutes found that highest reduction of microbial population. Najafi and Shaban (2015) showed lettuces treated with citric acid and ozonated water especially in high concentrations and interaction of these treatments resulted in highest retention of vitamin C and decrease microbial population of minimally processed lettuces during storage.

2.1.3. Citric Acid

In frozen strawberries, citric acid gave the best colour and overall rating whereas calcium lactate and ascorbic acid improved firmness, suggesting that combinations might be the best (Hudson *et al.*, 1975). In leafy vegetables antioxidant activity and ascorbic acid content were investigated as indicators of quality change by washing treatments. In leafy vegetables biochemical *viz*.

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antioxidant and ascorbic acid showed no significant difference between tap water, ozonated and chlorinated water (Soh *et al.*, 2005). Retention of both ascorbic acid and total anthocyanin in fresh lettuce increased as increase in citric acid dosage as reported by Altunkaya and Gokmen (2009). Amith (2012) reported that a combination of potassium meta bisulphite and citric acid in fresh cut mango and papaya could give a storage life of five days. A study conducted by Magdy (2014) in fresh strawberry by dipping in different concentrations of citric acid and calcium lactate alone or in combination for five minutes revealed that citric acid helped in maintenance of ascorbic acid and total anthocyanin content.

2.1.4. Acetic acid

Studies conducted by Anderson *et al.* (1987); Dickson, (1992); Bell *et al.* (1997) revealed antimicrobial activity of acetic acid against *E. coli, L. monocytogenes, Salmonella typhimurium.* Growth of *Pseudomonas* sp. and *Moraxella* sp. was inhibited by 0.05% of acetic acid was observed by Sara *et al.* (1993). Akbas and Olmez (2007) reported that dipping of lettuce in organic acids primarily acetic acid, with concentration of more than 1.0% may influence the organoleptic quality of the samples. Park *et al.* (2011) reported that in apple and lettuce, *E. coli* O157:H7, *S.* Typhimurium, and *L. mono- cytogenes* was reduced by acetic acid treatment. Petri *et al.* (2015) reported that the effect of different sanitizers (acetic acid and chlorine dioxide) on the reduction of the foodborne pathogen *E. coli* O157:H7 on fresh-cut lettuce (*Lactuca sativa*) and carrots (*Daucus carota*).

2.1.5 Tap water

Washing vegetables with water is the most commonly used decontamination method at households, although its sanitization efficacy is very low. Adam *et al.* (1989) reported that a standard washing in tap water removed an average of 92.4 % of lettuce leaf microflora. Studies conducted by Nguyen and Carlin (1994) and Kim *et al.* (1999) revealed that washing of lettuce leaves with tap water reported to reduce the microbial population by approximately 1 log

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Washing the cauliflower florets in tap water brought about 25 % CFU/g. reduction in bacterial population was reported by Gomez et al. (2002). Allende et al. (2008) revealed that despite the initial differences, the produce washed with tap water or a sanitizing solution, showed a similar total bacterial count after storage. Attiq et al. (2010) shows that 1% CaCl₂ treatment did not show significant effect on quality parameters and was similar to the control of loquat. Temiz et al. (2011) reported that in parsley, maximum reduction in total mesophilic aerobic bacteria (0.83 log cfu/g) and total coliforms (0.98 log MPN/g) was observed when treated with tap water. The total bacterial count was reduced by 5.03 log CFU/g and 4.78 log CFU/g after 1 and 5 minutes of washing of turnip greens with tap water (Ibrahim et al. 2012). Lettuce leaves washed with tap water reduced total mesophilic aerobic microorganisms (0.67 log10 CFU/g) and the coliform population (1.09 log10 CFU/g) was reported by Oliveira et al. (2012). Reddy et al. (2013) reported that dipping in distilled water was found to be effective in extending the shelf life of rajagira leaves in modified atmosphere packaging. Feas et al. (2014) observed that tap water immersion of ready to eat lettuce was effective to reduce aerobic mesophiles under values less than 4 \log_{10} CFU/g.

2.2. EVALUATION OF PRE-TREATMENTS

Soliva and Martin (2003) reported that dipping treatments after peeling and/or cutting both reduce microbial loads and rinse of tissue fluids, and thus reduce growth of microorganisms. Use of pre-treatments consist of certain chemical preservatives such as citric acid, ascorbic acid and calcium chloride etc. at minimum level, alone or in combination during minimal processing have been found beneficial in minimizing the stress-induced metabolism, reducing the browning reaction, maintaining the firmness and improving the organoleptic quality of various produce along with extension in their shelf-life under modified atmosphere packaging at low temperature storage (Saxena *et al.*, 2008).

2.2.1. Calcium chloride

Tirmazi and Wills (1981) reported that respiration activity of shredded cabbage was retarded by calcium infusion which was attributed to inactivation of enzyme catalytic mechanism. The maintenance of firmness in calcium treated fruits might be due its accumulation in the cell walls leading to facilitation in the cross linking of the pectic polymers which increases wall strength and cell cohesion in plants was reported by White and Broadly (2003).

Lester and Grusak (1999) observed lower weight loss found in calcium treated musk melon fruits because calcium applications have known to be effective in terms of membrane functionality and integrity maintenance. Calcium chloride treatment helped in maintaining postharvest life of perishables including apple, mango, loquat and strawberry was reported by Souza *et al.* (1999); Akhtar *et al.* (2010); Hussain *et al.* (2012); Dhillon and Kaur, (2013). Luna-Guzman and Barrett (2000), Alzamora *et al.* (2005), Manganaris *et al.* (2007) reported forms of calcium used in the food industry as calcium lactate, calcium chloride, calcium phosphate, calcium propionate and calcium gluconate, which helped in longer shelf life and enhancement of the product firmness.

Chardonnet *et al.* (2003) reported that calcium chloride has been extensively used as preservative and firming agent in the fruits and vegetable industry for whole and fresh cut commodities. Calcium treatments can maintain or improve tissue firmness and crispness of minimally processed vegetables. Mahajan and Dhatt (2004) reported that pear fruit showed most effective reduction in weight loss when treated with CaCl₂ compared to non-treated fruit. This might be due to reduced rate of respiration transpiration and delayed senescence because of calcium. Calcium lactate treatment for fresh cut lettuce and carrots was found effective (Martin-Diana *et al.*, 2005). During storage, sensory analysis showed that use of calcium improved textural characteristic of apple (Anino *et al.*, 2006). In peaches, 62.5 mM of calcium chloride immersion treatment showed a potential postharvest treatment, since it reduced the risk of salt-related injuries, physiological disorders and increased tissue firmness (Manganaris *et al.*, 2007). Attiq *et al.* (2010) showed that CaCl₂ treatments had a significant effect on retaining ascorbic acid content and firmness in loquat fruit which might be due to the higher concentrations of CaCl₂ which delayed rapid oxidation of ascorbic acid. However, there had been differences in the recommended concentrations of calcium chloride appropriate for retaining quality and extending shelf life of tomato was reported by (Nirupama *et al.*, 2010).

To maintain freshness, quality and extended shelf life of fresh cut pomegranate and pineapple, one per cent calcium chloride was found to be effective (Amith, 2012). Chandran (2013) reported that shredded beans, beet root and cabbage treated with 1% calcium chloride scored the least percent leakage. Abdulaziz et al. (2015) showed that calcium chloride salt (1-2%) resulted in significant increase in quality parameters for canned and frozen artichoke, compared to the control. Babu et al. (2015) showed significant retention of firmness, ascorbic acid content and reduced weight loss with extended shelf life of loquat by dipping in 2 % and 3 % calcium chloride. The highest weight loss and the lowest firmness was recorded in control, while the lowest weight loss and the highest firmness was observed in loquats treated with 2% and 3% CaCl₂ as compared to the control. Bansal et al. (2015) reported that the calcium used to extend the shelf life of fruits and vegetables reacts with pectin present in the cell walls and form the salts like calcium pectate. Daundasekera et al. (2015) showed prolonged shelf life in calcium treated tomato with maximum retention of firmness and retardation of skin colour development. Shahi et al. (2015) reported the application of calcium chloride treatments of Ziziphus mauritiana fruit at refrigerated temperature retain the quality. Chepngeno et al. (2016) showed that weight loss and vitamin C were reduced by calcium chloride treatment in tomato, carrot and egg plants.

2.2.2. Sodium benzoate

Joshi and Sharma (1995) reported that wine treated with sodium benzoate had higher retention of total phenols and total anthocyanins. Bhagwan et al. (2000) reported that sodium benzoate treated tomato fruits exhibited improved shelf life and quality. Application of sodium benzoate in pasteurized mango pulp restricted growth of microbial population and maintained biochemical and sensory parameters (Hussain et al., 2003). Hashmi et al. (2007) observed that potassium sorbate, potassium metabisulphite, sodium benzoate separately or in combination with other chemical preservatives used for the improvement of sensory characteristics, control microbes and retain overall acceptability of mango. Saxena et al. (2008) reported that for extending the shelf life of fresh cut jackfruit (Artocarpus heterophyllus L.) a pre-treatment combination of calcium chloride, citric acid, ascorbic acid and with sodium benzoate was found effective. Gonzalez et al. 2010 reported that the shelf life of fruits and vegetables is largely represented from the perseverance of sensory parameters. The fruits and vegetables are prone to microbial spoilage since these are composed of enzymes, pectin and near acidic pH, and high water activity.

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Oms-Oliu *et al.* (2010) showed that the action of potassium sorbate, potassium metabisulphite and sodium benzoate, retains overall acceptability, nutrients stability and reduces microbial load in fresh cut fruits. Younis *et al.* (2011) and Biswas *et al.* (2015) revealed that sodium benzoate showed direct effect on the reduction of microbial population and also enhance the sensory and biochemical parameters of mango pulp. Bansal *et al.* (2015) reported that sodium benzoate can be added for preventing the growth of pathogens in minimally processed products. Kanchana *et al.* (2015) reported that sodium benzoate and benzoic acid exhibited antibacterial properties for fresh cut spinach leaves. Sarkar *et al.* (2015) found that addition of sodium benzoate minimized changes in chemical constituents in tomato pulp.

2.2.3. Ascorbic acid

Ascorbic acid is a moderately reducing agent, acidic in nature which is generally recognized as safe for use in fruits and vegetables (Alvarez and Chiralt, 2007).

A study was conducted by Rayon and Obeirne (1999) reported that retention of ascorbic acid is often followed when evaluating post-harvest storage effect on nutritional quality of vegetables. Lee and Kader (2000) and Jacobi et al., (2001) reported that the vitamin C contents during postharvest treatments were decreased by oxidation reaction occurred during storage. Ana and Luiz (2002) reported that among pre-treatments given to minimal processed kiwi fruit ascorbic acid provided by the treatment was efficiently absorbed by the tissues and maintained levels of vitamin C about 25% higher than other treatments. Soliva and Martin (2003) revealed that ascorbic acid was frequently proposed to reduce microbial populations. The antimicrobial action of acids is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid. Lettuce phenolics were protected from oxidation by ascorbic acid and cysteine and ascorbic acid and cysteine increased the total antioxidant activity of lettuce (Altunkaya and Gokmen, 2009). Ortega-Ramirez et al. (2014) reported that synthetic antimicrobial and antioxidant agents are approved in many countries as the use of natural safe and effective preservatives are in demand by the consumers and producers.

A study was conducted by Miguel *et al.* (2004) who found that anthocyanin content of minimally processed Assaria variety of pomegranate decrease significantly during 72 h storage at 4°C. Spinardi *et al.* (2010) reported that for spinach leaves stored at 4 or 6^0 C temperatures anthocyanins declined at both storage temperatures and higher reduction was found in leaves stored at 10° C.

2.2.4. Citric acid

Sara *et al.* (1993) reported inhibited growth of *Pseudomonas* sp. and *Moraxellas*p.in culture media by acetic and citric acids. Kim and Klieber (1997) reported that loss of ascorbic acid provides a useful index of oxidative deterioration and observed decrease in total ascorbic acid content in fresh cut cabbage during storage and exogenously added ascorbic acid, citric acid in the dip solutions reduce the loss and browning. Maria *et al.* (1999) observed decrease in total antioxidant activity of MAP stored spinach. Francis and Beirne (2002) reported that the solution of 1% citric acid for 5 minutes can reduce mesophilic bacteria on lettuce to about 1.5 log CFU g⁻¹. Soliva and Martin (2003) reported that citric was frequently proposed to reduce microbial populations and antimicrobial action is due to pH reduction in the environment, disruption of membrane transport, permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid.

Eswaranandam et al. (2004) reported that higher reduction of Salmonella was found by citric acid treatment. Jiang et al. (2004) reported that chinese water chestnut slices treated with citric acid showed higher contents of ascorbic acid, than that of the control. Application of citric acid was effective in increasing the shelf life and retaining quality of minimally processed chinese water chestnut slices during storage. A study was conducted by Uyttendaele et al. (2004) and Bari et al. (2005) chemical sanitizers such as organic acids have been described as potent antimicrobial agents against mesophilic and psychrophilic microorganisms in fresh cut fruit and vegetables. Barwal et al. (2005) revealed that cauliflower can be preserved by using different concentrations and combinations of potassium metabisulphite and citric acid in maintaining better physio-chemical, sensory qualities and checked microbial growth. Many other researchers showed that a combined ozone and citric acid treatment has a greater effect on reducing the microbial population of iceberg lettuce compared to single treatments (Yuk et al., Retention of both ascorbic acid and total anthocyanin's in fresh cut 2007). lettuce was obviously increased as increased citric acid concentration while

browning index was reduced due to inhibition of polyphenoloxidase as reported by Altunkaya and Gokmen (2009). Citric acid prevents the discoloration of fruits and vegetables through its inhibiting effects on the action of this enzyme and therefore extensively used on freshly processed fruits and vegetables (Olmez and Kretzschmar, 2009).

Immersion of iceberg lettuce in a 0.5% solution of citric or lactic acid for 2 minutes can be as effective in reducing microbial populations in freshly cut iceberg lettuce as effective as 100 ppm chlorine was reported by Olmez and Akbas (2009). Eleni and Theodoros (2011) in fresh cut cabbage showed better retention of colour, overall acceptance and higher organoleptic quality by treating with citric acid. (Amith, 2012) reported that citric acid could give a shelf life of five days for fresh cut mango and papaya. Fresh cut pineapple treated with ascorbic acid and citric acids were found with highest acceptable quality after seven days of storage at 5-7° C was observed by Daranagama et al. (2012). Devi and Joshi (2012) showed the best result for plum with 50% Ethanol + 0.2% citric acid treatment in terms of highest anthocyanin content and sensory score. A study conducted by Magdy (2014) on fresh strawberry immersed in various concentrations of citric acid and calcium lactate alone or in combination for five minutes, pre-treatment with citric acid enhanced the retention of ascorbic acid and total anthocyanin content. Najafi and shaban (2015) reported lowest weight loss in lettuce with citric acid treatment and the lowest microbial population and the highest colour and vitamin C retention were related to 1 g/lit of citric acid concentration and also treatment with 1 ppm ozonised water. Siriwardana et al. (2015) reported that pre-treatments with ascorbic acid (3%), citric acid (3%) and citric acid + ascorbic acid (1.5% each) were more successful in maintaining the quality of minimally processed cooking banana for one week in cold storage.

2.3. PRE-PACKAGING AND STORAGE

Reducing physical injury during transit and handling of vegetables by packaging leads to quality improvement was reported by Gast (1991). Kays (1991) reported that to maintain product quality during storage, temperature is a critical factor, to slow down evapo-transpiration and hence ensures a longer shelf life of vegetables. Ahvenainen (1996); Luo *et al.* (2004) and Ares *et al.* (2008) reported that packaging reduces the rate of physiological process and improve the shelf life. Different materials are used for pre-packaging of vegetables.

2.3.1 Polyethylene

Vegetables packed in low density polyethylene and stored in refrigerated temperature, retained vitamin C better than those stored in room temperature was reported by Weichmann (1987). Strawberries and custard apple packed in low density polyethylene recorded the lowest respiration which lead to less weight loss and maintain the quality of the fruits was observed by Li and Kader (1989) and Prasanna et al. (2000). Mohammed (1990) reported that capsicum packed in low density polyethylene (LDPE) and stored under refrigerated storage retained quality and shelf life up to 12 days of storage. Spinach and green bean packaged in polyethylene and stored at 10°C and 20°C reduced weight loss and retained chlorophyll and ascorbic acid (Ooraikul and Stiles, 1991). Naik et al. (1993) reported that packaging of tomato in 300 gauge polyethylene extended the shelf life to 42 days. Roy and Pal (1993) and Gonzalez et al. (1997) reported that polyethylene packaging of fruits and vegetables play a major role in preventing dehydration by generating a saturated micro-atmosphere around the perishables that decrease rate of transpiration by restricting the diffusion of gases and feedback mechanism.

Lee *et al.* (1995) and Zagory (1995) reported the property of low density polyethylene which exhibited good barrier to water vapour loss and also had the ability to decrease respiration rate of vegetables which in turn reduced moisture loss. Lowest weight loss was recorded in polyethylene packaged okra by Batu and Thompson (1998). Ladaniya and Singh (2001) and Ladaniya (2003) revealed that packaging with polyethylene films helped in maintaining higher ascorbic acid content in citrus fruits. Lu (2007) reported lowest weight loss of minimally processed Bok Choy (*Brassica chinensis* L.) in modified atmosphere packaging with highest sensory score compare to polypropylene after 10th day of storage. Boonyakiat *et al.* (2006) reported that packaging of minimally processed lettuce, beans and carrot with polyethylene was better than oriented polyethylene.

Babarinde and Fabunmi (2009) reported that okra packed in polyethylene and kept under refrigerated condition recorded the highest ascorbic acid, lowest weight loss and extended shelf life up to nine days. Polyethylene is widely acceptable packaging material because of its nature such as flexibility, transparency, low cost, easy processability and thermal stability was reported by Marsh et al. (2007) and Nobile et al. (2009). Reddy (2010) reported that in case of minimally processed fenugreek and kiraksali leaves, LDPE was found to be an effective package in maintaining highest moisture with better control of physiological loss in weight during storage. For storage of fresh cut cabbage LDPE packaging was found more suitable (Rinaldi et al., 2010). Polythene packaging helped in build-up of high relative humidity in the vicinity of vegetables and thereby moisture loss through transpiration is reduced which in turn reduced the physiological loss in weight (Koraddi and Devendrappa, 2011). Kumar et al. (2013) reported that drumstick leaves pre-packaged in 350 gauge LDPE maintained colour, vitamin C, beta carotene and lowest microbial load at the end of storage.

To extend the storage quality of *Amaranthus tricolor* leaves, polyethylene film packaging combined with low temperature storage was reported as an appropriate method by Suraweera *et al.* (2011). Nath *et al.* (2012) reported lowest weight loss in polyethylene packed fruits due to lesser availability of oxygen for respiration which delay the rate of respiration and thereby decreasing the moisture loss due to transpiration. Similar findings were reported by Singh *et al.* (2013). Ali *et al.* (2015) revealed that during storage of apricot, low density polyethylene played a major role for retaining maximum biochemical, higher antioxidant activity, lowest weight loss and lesser enzyme activities up to two weeks of storage under ambient condition. Gill *et al.* (2015) reported that Dashehari mango fruits packed in LDPE and kept under low temperature storage retain maximum firmness, sensory quality and overall acceptability up to three weeks under low temperature storage. Tomatoes stored in low density polyethylene bag (LDPE) at 15° C was best for increasing the shelf life by retaining the quality was reported by Hour *et al.* (2015). Kapsiya *et al.* (2015) reported that tomato fruits treated with CaCl₂ and packed in perforated polyethylene bags showed reduced fruit weight loss and maintain ascorbic acid as compared to other packaging systems.

Mohebbi *et al.* (2015) reported that cornelian cherry packed in low density polyethylene helped in retain of ascorbic acid, anthocyanin and decreased weight loss during the storage of fruit. Packaging of banana fruits in high density and low density polyethylene bags resulted in extended storage life and improved quality of the produce was observed by Prasad *et al.* (2015). Modified atmosphere packaging in LDPE of fresh cut bitter gourd had a lesser physiological loss in weight and bacterial population and maintained ascorbic acid with maximum score for sensory characters and overall acceptability (Preetha *et al.*, 2015). Polyethylene packaging was found better to maintain best quality characters in tomatoes during storage. In tomatoes, for better quality in terms of weight loss, colour and palatability (Randhawa *et al.*, 2015). Sonia *et al.* (2015) reported that tomato, cowpea and lady's finger packed in vented LDPE under low temperature storage recorded least physiological loss in weight. Kozuki *et al.* (2016) observed that spinach packaged in perforated polyethylene bags in refrigerated storage recorded lowest weight loss and retained good appearance and quality.

2.3.2. Polypropylene

Favell (1998) reported that leafy vegetables can be easily preserved by storing in polypropylene packages at 0° C temperatures. Leafy vegetables stored at 6° C lost 10 % of ascorbic acid in six days, while those held at room temperature lost 20% only in two days (Lee and Kader, 2000). Celery pre-packaged with polypropylene and stored at 4° C exhibited best quality after 15 days (Gomez and Arte, 2005). Minimally processed shredded cabbage packaged in polypropylene

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had extended shelf life up to three weeks with minimum colour change, weight loss and deterioration in sensory qualities (Roshita *et al.*, 2005). Radish packed in micro perforated oriented polypropylene film extended shelf life up to 6 days (Saito and Rai, 2005). Dulal *et al.* (2012) reported that quality and longer shelf life of the packaged produce is depended upon selection of suitable packaging film. Rajgira leaves and tender stem packed in 100 gauge polypropylene increased shelf life up to four days with 1.27 % physiological loss in weight, 86.32% moisture retention, 16.98 % yellowing and 14.52 % was reported by (Reddy *et al.*, 2013).

2.3.4. Modified Atmosphere Packaging

Vacuum packaging is a potential alternative to achieve inhibition of progress of deterioration of foodstuffs (Fleming *et al.*, 1983). The storage of fresh- cut carrot at 10° C or above allows most bacterial pathogens to grow rapidly (Francis *et al.*, 1997). Shelf life of fresh cut horticultural products could be extended by modified atmospheric packaging (Gorny *et al.*, 2000). Mattheis and Fellman (2000) reported that reduced rate of respiration, transpiration and ethylene evolution and reduced microbial growth ensuring better quality retention by use of modified atmospheric packaging during storage, transport and marketing. MAP helped in reducing the microbial population and maintaining the nutritional quality of fresh cut lettuce during preservation at low-temperature storage had been reported by (Prakash *et al.*, 2000).

Nitrogen (N_2) gas packaging for fresh cut vegetables like lettuce and cabbage had examined as a means of MAP for extending the shelf life of cut vegetables (Koseki and Itoh, 2002). To retard physiological processes and also minimize microbial infections in order to maintain optimum quality and extend the shelf life modified atmospheric packaging plays a major role (Das, 2004). Budu and Joyce (2005) reported that the effect of modified atmosphere conditions on the quality of minimally processed pineapple slices were determined and modified atmosphere of 2 per cent O₂ and 15 per cent CO₂ increased the shelf life for 14 days at 4.5° C storage. Phan and Magel (2005) reported that application of controlled atmosphere technique using sealed polyethylene bags for the storage of minimally processed carrot proved to be successful with 17 per cent O₂, 2.5-3 per cent CO₂ up to a shelf life of 15 days. Radish packed in micro perforated oriented polypropylene film extended shelf life up to 6 days (Saito and Rai, 2005). Boonyakiat *et al.* (2006) reported increased shelf life of fresh cut lettuce, beans and carrot when packaged in polythene bag with the addition of nitrogen gas. Ayhan *et al.* (2008) reported that minimally processed carrots MAP retained shelf life up to 14 days of storage with better retention of colour and texture. Reduction of qualitative changes like chlorophyll and ascorbic acid content in broccoli (*Brassica oleracea italica*) was reported under MAP in perforated polypropylene film packages (Rai *et al.*, 2008).

Increased shelf life of fresh cut vegetables in modified atmospheric packaging with increase in concentration of carbon dioxide was reported by Assumi *et al.* (2009). The rate of microbial growth also decreased under modified atmpspheric packaging (Rodriguez-Aguilera and Oliveira, 2009 and Sandhya, 2010). Goswami and Mangaraj (2011) revealed that functionality of treated minimally processed product is largely dependent on the bioactive compounds and antioxidant capacity and appropriate compositions of carbon dioxide or oxygen during modified atmosphere packaging avoid unfavourable changes. Dineshkumar *et al.* (2015) reported that modified atmosphere packaging technology offers the possibility to retard produce respiration rate and extend the shelf life of fresh cut pomegranate.

Esturk *et al.* (2015) reported that MAP can be used to maintain the quality of fresh cut carrots since it preserved the physicochemical characteristics and sensory quality up to 14 days of storage. Ranjitha *et al.* (2015) reported that the minimally processed green bell pepper packed in MAP helped to retain the visual marketability as judged by sensory analysis with vitamin C reduction and reduced microbial population till 9 days of storage at 8 °C. Roopa *et al.* (2015) reported that minimally processed and passive modified atmosphere packaging of breadfruit stored for up to 45 days at 6°C without incidence of pathogens. MAP is more useful for retaining the qualities of minimally processed litchi arils which reduced juice leakage and firmness and retarded microbial populations during storage (Punumong *et al.*, 2016).

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MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on "Standardization of minimal processing of amaranthus *(Amaranthus tricolor* L.)" was undertaken at Department of Processing Technology, College of Agriculture, Vellayani, during the period of 2014-2016, with the objective to standardise minimal processing technology for the development of ready-to-use amaranthus with extended shelf life and nutritional quality

In order to develop a protocol for fresh cut amaranthus, the study was carried out as three different continuous experiments under the following heads.

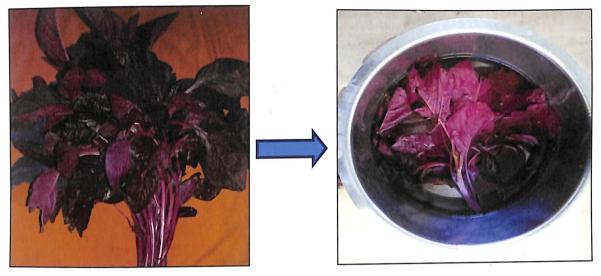
- 3.1 Evaluation of sanitizing treatments
- 3.2 Evaluation of pre- treatments
- 3.3 Pre-packaging and storage

3.1 EVALUATION OF SANITIZING TREATMENTS

Amaranthus (var. Arun) raised as per Kerala Agricultural University Packages of Practices was procured from progressive farmers of Vegetable and Fruit Promotion Council Kerala (VFPCK) at Pappanchani and Kalliyoor during morning hours. Amaranthus harvested at 25-30 days after sowing and of uniform size without any visual defects were used for the study. After removing the root portion, amaranthus leaves and stem were fully immersed in the following sanitizing solutions for five minutes in three replications.

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T₁- 30 ppm Sodium hypochlorite
T₂- Warm water (40^oC)
T₃- RO water (reverse osmosis)
T₄- Ozonised water (2 ppm)
T₅- Citric acid (1%)
T₆- Acetic acid (1%)
T₇- Tap water



Fresh amaranthus

Surface sanitization of amaranthus

Plate 1. SURFACE SANITISATION OF AMARANTHUS



Plate 2. OZONIZER

After the sanitization treatments, 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 leaves and stem separately. Biochemical parameters *viz*. ascorbic acid, anthocyanin content and visual parameters (colour and texture) of amaranthus leaves and stem were also analysed.

3.1.1. Enumeration of Total Microbial Load

Serial dilution spread plate technique was carried for quantitative assay of microflora 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.

Amaranthus leaves and stems were cut into pieces and 10 g from each treatment was suspended in 90 ml sterile distilled water and shaken thoroughly to get 10^{-1} dilution. 1 ml of the supernatant was accurately pipetted out into eppendroff tube containing 9 ml of sterile distilled water to get 10^{-2} dilution. This procedure was repeated to get 10^{-5} dilution. 100 µl each from 10^{-3} , 10^{-4} and 10^{-5} dilution was used for enumeration of total bacterial and fungal count by spread plating method. Bacterial and fungal count were noted 24 h and 48 h after inoculation. Number of microorganisms (bacteria and fungi) per 10 g of pre and post treated samples was calculated as per the following formula.

No. of colony forming units = <u>Total number of colony formed x dilution factor</u> (CFU per gram of the sample) Aliquote plated

The data were analyzed statistically using Completely Randomized Design

3.1.2. Ascorbic Acid

Ascorbic acid content was estimated by 2, 6-dichloro phenol indophenol (DCPIP) dye method (Sadasivam and Manickam, 1992) and expressed as mg/100g.

Ascorbicacid =
$$\frac{0.5mg}{V_1ml} \times \frac{V_2}{5ml} \times \frac{100}{\text{weight of sample}}$$

3.1.3. Anthocyanin Content

Estimation of anthocyanin was done as per the method described by Ranganna (1997). One gram of the sample from each treatment was extracted with ethanolic hydrochloric acid, filtered through a 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 100g of the sample.

Total Optical Density per 100 g of sample (X) =

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[(Absorbance at 535nm) x (Volume made up of the extract used for colour measurement) x (Total volume) x100]
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Volume (ml) of the extract used x weight of the sample taken

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

3.1.4. Physical (visual) Parameters

Sanitized amaranthus leaves and stem were analysed for visual parameters viz. colour, texture and overall acceptability after one, hour of the treatment by conducting a sensory evaluation by a semi trained panel of 30 members. 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 panel members were statistically analyzed using the non-parametric ANOVA (Kruskall Wallis test) and mean ranks and critical values were calculated.

Based on microbial, biochemical and visual parameters, the best sanitizing agent was selected for further studies.

3.2. EVALUATION OF PRE- TREATMENTS

For controlling decay and for reducing browning and retaining firmness in fresh cut vegetables, different chemical treatments were used. Effect of these chemical treatments in extending shelf life of minimally processed amaranthus was studied.

Amaranthus was surface sanitized using the best sanitizing agent selected from first part of the experiment (3.1.) and were given different pretreatments by immersing it in different solutions as given below.

- T_1 Calcium chloride (1%) for 3 minutes
- T_2 Calcium ascorbate (1%) for 3 minutes
- T₃ Sodium benzoate + ascorbic acid (0.1%) for 3 minutes
- T_4 Sodium benzoate + citric acid (0.1%) for 3 minutes
- T_5 KMS + ascorbic acid (0.1%) for 2 minutes
- T_6 KMS + citric acid (0.1%) for 2 minutes
- T₇-distilled water (control) for 3 minutes
- T_8 without any pre treatment

After pre-treatment, samples were drained and excess moisture was removed. Leaves with terminal soft stem and remaining stem were shredded into uniform size separately. The fresh cut samples of leaves (L_1) and stem (L_2) weighing 50 g each were stored under refrigerated condition in polystyrene trays in three replications.



Sanitization with 2ppm ozonized water



Pre-treatment of amaranthus



Fresh cut amaranthus leaves



Fresh cut amaranthus stem in Polystyrene tray



Fresh cut amaranthus leaves in polystyrene tray



Fresh cut amaranthus stem

Plate 3. PRE-TREATMENT OF FRESH CUT AMARANTHUS

Following physiological, physical and bio-chemical parameters and microbial analysis were done on alternate days till the end of shelf life.

3.2.1. Physiological Parameters

Physiological parameters of pre-treated amaranthus leaves and stem were recorded periodically till the product lost its quality.

3.2.1.1 Physiological Loss in Weight (PLW)

For determining physiological loss in weight, sample was weighed accurately at the time of storage and subsequently in alternate days till the end of shelf life and cumulative weight loss was calculated using the formula and expressed as percentage.

3.2.1.2. Relative Water Content (RWC)

Relative water content was estimated in percentage according to the method proposed by Barr and Weatherly (1962).

A composite sample of leaf discs (26 discs of 1 cm^2) was taken and the fresh weight was determined, followed by flotation in distilled water for up to one hour. The turgid weight was then recorded, and the leaf tissue was subsequently ovendried to a constant weight at about 85°C. RWC was calculated in percentage by the formula

(Turgid weight - Dry weight)

3.2.1.3. Membrane Integrity

Membrane integrity of samples were analysed in terms of percent leakage. Membrane integrity is inversely proportional to percent leakage. Fresh cut amaranthus were immersed in 20 ml distilled water for 3 hours and absorbance was read in a UV spectrophotometer at 273 nm. The immersed shreds were heated in water bath at 100° C for 20 minutes, filtered; filtrate was made up to 20 ml, the absorbance was read again in UV spectrophotometer at 273 nm. Percent leakage was calculated using the following formula

Percent leakage = <u>Initial absorbance value x 100</u>

Final absorbance value

3.2.2. Physical (visual) Parameters

Physical parameters viz. colour, texture and wilting of pre-treated amaranthus leaves and stem were evaluated on alternate days till the end of shelf life by a semi trained panel of 30 members using a 5 point hedonic scale as described in 3.1.4.

3.2.3. Biochemical Parameters

Following biochemical parameters of pre-treated amaranthus leaves and stem were recorded periodically till the end of shelf life.

3.2.3.1. Ascorbic Acid

Ascorbic acid content was calculated as per Sadasivam and Manickam, (1992).

3.2.3.2. Anthocyanin Content

Anthocyanin content was calculated as described in 3.1.4.

3.2.3.3. Antioxidant Activity

Total antioxidant activity of amaranthus leaves and stem was determined by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The scavenging effect of DPPH free radical was measured according to the procedure described by Sharma and Bhatt (2009).

1.0 ml sample was added to 2.0 mL mM DPPH solution, mixed thoroughly and left for 30 minutes at room temperature. The absorbance was read at 517nm. Scavenging effect was expressed as percent inhibition of DPPH as shown in the following equation:

% inhibition of DPPH =
$$(A_{\text{blank}} - A_{\text{sample}}) \times 100$$

A blank

Where,

 A_{blank} – Absorbance of DPPH solution without sample, read against ethanol blank A_{sample} – Absorbance of the test sample after 30 minutes.

3.2.4. Enumeration of Total Microbial Load

Microbial load on pretreated samples was calculated as described in 3.1.1.

3.3. PRE-PACKAGING AND STORAGE

Harvested amaranthus (var. Arun) after removing root portion was surface sanitized with the best treatment from first part of the experiment (3.1) followed by pre-treatment with the best treatment from second part (3.2), was shredded into uniform size. Hundred gram fresh cut samples of amaranthus leaves and stem were pre-packaged in different packages in two different proportions of leaves and stem as two proportions T_1 - 1:1 and T_2 - 1:2 (w/w). Pre-packaged minimally processed amaranthus were stored under two conditions *viz*. ambient storage (at room temperature of 30±2°C and RH 80-85%) and refrigerated condition (10±1°C and RH 90-95%) in two storage conditions

S₁- Room temperature

S₂- Refrigerated condition in two replications.

was (26.87 mg/100g), 2 ppm ozonised water (26.80 mg/100g) and 1 % citric acid (26.81 mg/100g) (Fig. 3.).

Similar result was reported by Soh *et al.* (2005) in leafy vegetables. Ascorbic acid and total anthocyanin retention in fresh lettuce with citric acid treatment was reported by Altunkaya and Gokmen (2009) and similar result was obtained in strawberry by Magdy (2014).

5.1.4. 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. In the present study, amaranthus leaves and stem sanitized with 2 ppm ozonised water recorded the highest mean score of 5.00 for colour, texture and overall acceptability. It was followed by the treatment 1 % citric acid with a mean score of 4.83 for colour, texture, overall acceptability. Highest acceptability for sensory qualities was reported with ozonised water treatment was reported by Song *et al.* (2000); Beltran *et al.* (2005); Zhang *et al.* (2005); Olmez and Akbas (2009) and Najafi and shaban (2015).

5.2. EVALUATION OF PRE-TREATMENTS

Fresh cut leafy vegetables have high rate of respiration, which generally leads to ageing of products by using reserve energy during oxidative – reduction process. Increased rate of respiration lead to decrease the storage life of fresh cut products and different pre-treatments can reduce the physiological changes and extend the shelf life. Gonzalez *et al.*, (2010) reported that different treatments are used to control undesirable changes that adversely affect quality of minimally processed products. In this study, different pre-treatments with 1% calcium chloride (T_1), 1% calcium ascorbate, 0.1% sodium benzoate + ascorbic acid, 0.1% KMS + citric acid, distilled water and without any pre-treatment as control were

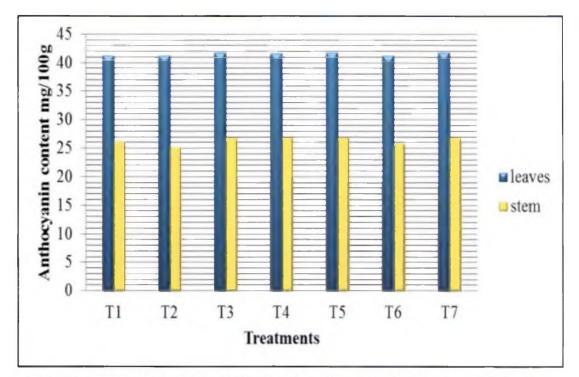
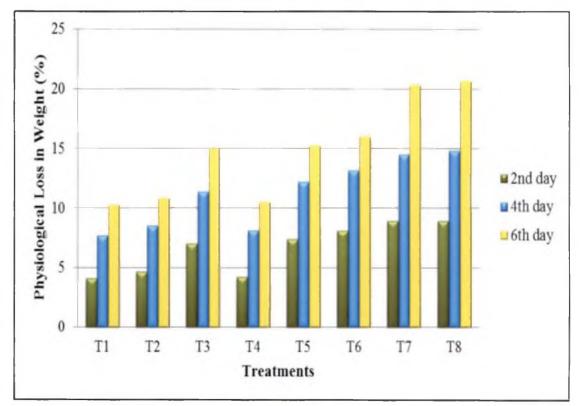


Figure 3. Effect of sanitizing treatments on anthocyanin content (mg/100g) of



amaranthus leaves and stem

Figure 4. Effect of pre-treatments on physiological loss in weight (%) of fresh cut amaranthus leaves

evaluated based on physiological, physical, biochemical and microbiological qualities.

5.2.1. Physiological Parameters

Physiological loss in weight (PLW), relative water content and membrane integrity were studied for pre-treated amaranthus leaves and stem. Shelf life of minimally processed vegetables shows a relation between PLW, RWC and membrane integrity. The highest weight loss, lowest relative water content and membrane integrity reduces freshness or shelf life of leafy vegetables.

Amaranthus leaves treated with (1% calcium chloride) was found to have lowest PLW of 4.12 %, 7.71 % and 10.31 % after 2nd, 4th and 6th day of storage (Fig. 4.) and for amaranthus stem, the lowest PLW of 3.66 % after two days of storage, 6.28 % after 4 days, and 10.45 % after 6 days of storage was recorded for the treatment with 1 % calcium chloride (Fig. 5.). Amaranthus leaves treated with (1% calcium chloride) was found to have highest relative water content of 80.72 %, 76.14 % and 69.98 % after 2nd, 4th and 6th day of storage respectively and amaranthus stem treated with 1% calcium chloride recorded the highest RWC of 71.29 %, 68.31 % and 65.86 % after 2nd, 4th and 6th day of storage. Membrane integrity expressed as percent leakage of pre-treated amaranthus leaves and stem also showed highest with 1% calcium chloride treatement. Pre-treatment with calcium ascorbate (1%) and sodium benzoate + citric acid (0.1%) also recorded lowest PLW, highest RWC and membrane integrity. Van-Buren (1979) reported that calcium treatments improve structural integrity to retain high relative water content. Tirmazi and Wills (1981) reported that respiration activity of shredded cabbage was retarded by calcium infusion which was attributed to inactivation of enzyme catalytic mechanism. A study conducted by Lester and Grusak (1999) showed lower weight loss in calcium treated melon fruits as calcium applications are effective in membrane functionality and integrity. This result were in agreement with findings of White and Broadley (2003) who reported that exogenously applied calcium stabilizes the plant cell wall and protects it from cell wall degrading enzymes. Extending the shelf life of fresh-cut jackfruit by a pre-

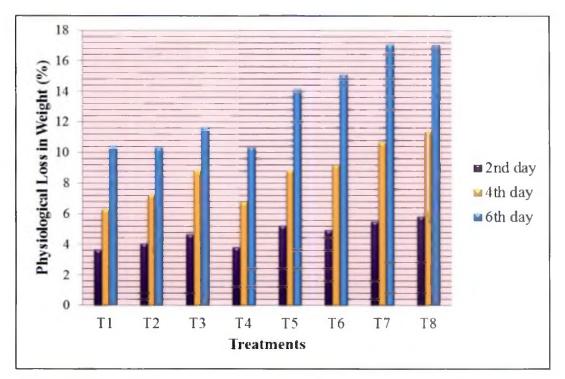


Figure 5. Effect of pre-treatments on physiological loss in weight (%) of fresh cut amaranthus stem

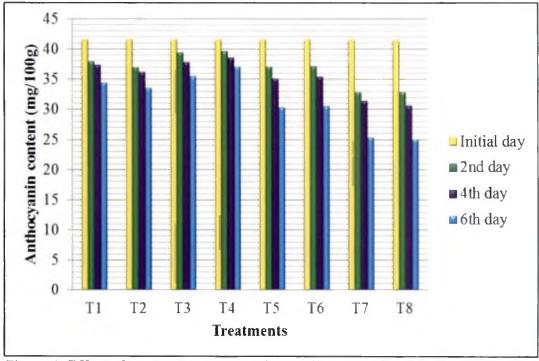


Figure 6. Effect of pre-treatments on anthocyanin content (mg/100g) of fresh cut amaranthus leaves

treatment with sodium benzoate showed a higher retention of quality was observed by Saxena *et al.* (2008). Babu *et al.* (2015) showed significant retention of firmness and ascorbic acid content and reduced the weight loss with extended the shelf life of loquat by dipping in 2 % and 3 % calcium chloride. Bansal *et al.* (2015) reported that sodium benzoate can be added for preventing the growth of pathogens to improve quality and shelf life of minimally processed fruits. Chepngeno *et al.* (2016) showed that weight loss was reduced by the use of calcium chloride in carrot and eggplant.

5.2.2. Physical (visual) parameters

Amaranthus is highly perishable in nature which leads to loss of freshness during storage. In the study, amaranthus leaves treated with sodium benzoate + citric acid (0.1%) recorded the highest mean score of 4.83, 3.90 and 3.25 for colour and highest mean score of 4.96, 4.16 and 2.63 for overall acceptability after 2nd, 4th and 6th day of storage. Similarly amaranthus stem treated with sodium benzoate + citric acid 0.1% recorded the highest mean score of 4.93, 4.33 and 2.33 for colour and 4.76, 4.10 and 2.33 for overall acceptability after 2nd, 4th and 6^{th} day of storage. This result was in agreement with findings of Kaur *et al.* (2001) who reported improved overall appearance in Indian cabbage by incorporation of ascorbic acid and citric acid in dip water. Hashmi et al. (2007) observed that sodium benzoate was effective for the improvement of sensory characteristics and retain overall acceptability of mango. Oms-Oliu et al. (2010) showed that the action of sodium benzoate retains overall acceptability, nutrients stability in perishables. Devi and Joshi (2012) reported best sensory qualities for plum treated with citric acid. Najafi and Shaban (2015) reported that highest colour and vitamin C retention was with 0.1% citric acid concentration and 1 ppm ozonated water during storage of lettuce.

Amaranthus leaves and stem treated with 1% calcium chloride recorded the highest mean score for texture with lowest wilting after 2^{nd} , 4^{th} and 6^{th} day of storage. Similar results are reported by Van-Buren (1979) where

calcium complexes increased structural integrity to retain the relative water content which resulted in better texture. Chardonnet *et al.* (2003) reported that calcium treatments helped in maintaining or improve tissue firmness and crispness of minimally processed vegetables. White and Broadley (2003) reported that exogenously applied calcium stabilizes the plant cell wall and protects it from cell wall degrading enzymes in plants. Anino *et al.* (2006) reported that calcium improved the textural characteristics and sensory qualities of apple. Nirupama *et al.* (2010) also reported that 1.5% calcium chloride helped in retaining quality and extending shelf life of tomato. The applications of calcium chloride to Ziziphus mauritiana fruit under refrigerated storage retain fruit quality (Shahi *et al.*, 2015).

5.2.3. Biochemical Parameters

Pre-treatment of amaranthus showed significant influence in retention of ascorbic acid, anthocyanin content and antioxidant activity. Pre-treatments with sodium benzoate helped in retention of these biochemical qualities in amaranthus leaves and stem than other treatments which reduced the quality substantially.

In amaranthus leaves, the highest ascorbic acid of 17.27 mg/100g after 2^{nd} day, 16.20 mg/100g after 4^{th} day and 15.49 mg/100g after 6^{th} day of storage was recorded for the treatment (sodium benzoate + citric acid 0.1%) and for amaranthus stem it was 15.42 mg/100g, 14.58 mg/100g and 13.74 mg/100g respectively after 2^{nd} , 4^{th} and 6^{th} days of storage. The highest anthocyanin content of 39.66 mg/100g, 38.63 mg/100g and 37.10 mg/100g was recorded for the pre-treatment with sodium benzoate + citric acid (0.1%) for leaves (Fig. 6.) and for amaranthus stem, 24.20 mg/100g, 23.20 mg/100g and 21.26 mg/100g after 2^{nd} , 4^{th} and 6^{th} days of storage (Fig. 7.).

The highest antioxidant activity of 88.48 %, 85.35% and 79.49 % was also recorded for the treatment sodium benzoate + citric acid (0.1%) for leaves and assay of 80.20 %. 73.87 % and 67.61 mg/100g for stem after 2^{nd} , 4^{th} and 6^{th} days

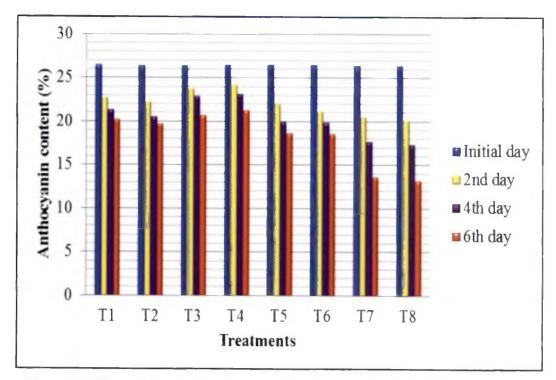
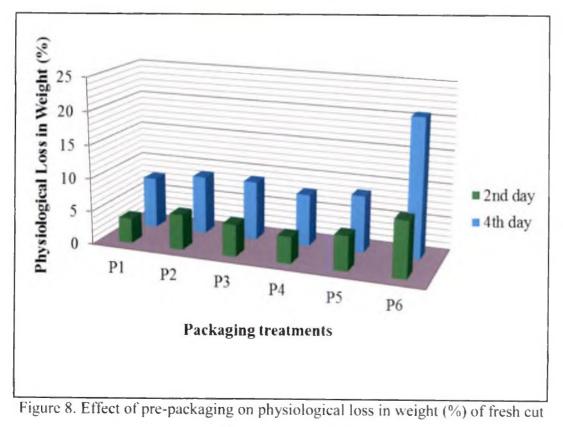


Figure 7. Effect of pre-treatments on anthocyanin content (mg/100g) of fresh cut amaranthus stem



amaranthus (1:1) under ambient condition

of storage. Thus the pre-treatment with sodium benzoate + citric acid (0.1%) was found effective in retaining ascorbic acid, anthocyanin and antioxidant activity.

Joshi and Sharma (1995) reported that incorporation of sodium benzoate in wine helped in higher retention of total phenols and anthocyanins. Bhagwan *et al.* (2000) observed that sodium benzoate treated tomato fruits recorded delayed ripening and improved shelf life and quality. Saxena *et al.* (2008) reported extended shelf life and better quality of fresh-cut jackfruit (*Artocarpus heterophyllus L.*) by pre-treatment with sodium benzoate. Sodium benzoate treatment to minimally processed fruits improved overall acceptability and nutrient stability (Oms-Oliu *et al.*, 2010).

Kim *et al.* (1999) reported that the loss of ascorbic acid in fresh cut chinese cabbage is reduced by pre-treatment with ascorbic acid, citric acid solutions and Kaur *et al.* (2001) observed that this treatment helped in maximum retention of antioxidant activity, ascorbic acid and total carotenoids and improved overall appearance of cabbage. Chinese water chestnut slices treated with citric acid showed higher ascorbic acid content than that of the control during storage was reported by Jiang *et al.* (2004). Barwal *et al.* (2005) observed that cauliflower could be preserved with better physio-chemical and sensory qualities by using citric acid. Altunkaya and Gokmen (2009) reported that both ascorbic acid and total anthocyanins in fresh lettuce increased with increase in citric acid concentration of pre-treatment. Devi and Joshi (2012) found that highest retention anthocyanin content of plum by the 0.2 % citric acid treatment and in strawberry high amount of ascorbic acid and total anthocyanin with citric acid dip for 5 minutes was reported by Magdy (2014).

5.2.4. Enumeration of Total Microbial Load

Pre-treatments also influenced microbial quality and shelf life of minimally processed amaranthus leaves and stem. The lowest bacterial population after the pre-treatments was observed for sodium benzoate + citric acid (0.1%) for amaranthus leaves (2.45x 10³ cfu g⁻¹) and stem (2.55 x 10³ cfu g⁻¹) after

the treatment. Bacterial population of fresh cut amaranthus leaves and stem increased with the storage and lowest population was reported for sodium benzoate + citric acid (0.1%) treatment and recorded 2.95 x 10^3 cfu g⁻¹ for leaves and 3 x 10^3 cfu g⁻¹ for stem after four days of storage. This result was in agreement with the findings of Oms-Oliu *et al.* (2010) who reported that the action of sodium benzoate retains nutrients stability and restricts the microbial load in fresh cut fruits. Kanchana *et al.* (2015) reported that sodium benzoate and benzoic acid were capable of suppressing the growth of major spoilage microorganisms of spinach leaves.

A study conducted by Sara *et al.* (1993) reported reduction of microbial population by acetic and citric acid treatment. Francis and Beirne (2002) reported that the solution of 1% citric acid for 5 minutes can reduce microbial population on lettuce about 1.5 log CFU g⁻¹. Uyttendaele *et al.* (2004); Bari *et al.* (2005) reported that citric acid treatment as potent antimicrobial agents against mesophilic and psychrophilic microorganisms in fresh-cut fruit and vegetables. Barwal *et al.* (2005) revealed that cauliflower treated with citric acid helped in checking microbial growth. Combined treatment of ozone and citric acid treatment helped in decreasing the microbial load of iceberg lettuce as compared to single treatment (Yuk *et al.*, 2007). Olmez and Akbas (2009) reported that iceberg lettuce treated with 0.5% solution of citric or lactic acid for 2 minutes was effective in reducing microbial populations in freshly cut iceberg lettuce. Najafi and shaban (2015) also reported highest microbial reduction during storage of lettuce with ozonated water and citric acid treatment.

5.3. PRE-PACKAGING AND STORAGE

Packaging has the ability to reduce weight loss, restrict movement of oxygen and carbon dioxide, lower respiration, inhibit ethylene production, seal in flavor volatiles, and retard discoloration (Ahvenainen, 1996; Luo *et al.*, 2004; Ares *et al.*, 2008). Packaging minimizes postharvest losses by protecting against mechanical damage, microbial spoilage and moisture loss by acting as a barrier

between the product and the environment and protects nutritional quality along with better appeal in addition to preventing pilferage (John 2008). Onyango (2010) revealed that leafy vegetables are highly perishable with an active metabolism of respiration and transpiration during storage period and higher water loss due to large surface area to volume ratio. Reddy (2013) reported that the shelf life can be extended by retarding the deteriorative processes that occurs after harvest. Packaging has an important role to play in creating a barrier between environment and food, in addition to ease of transport, handling and marketing.

Sanitised, pre-treated fresh cut amaranthus pre-packaged in different materials in two proportions of leaves and stem stored under room temperature and refrigerated condition were analysed for physiological, physical, biochemical and microbial qualities to assess the shelf life of ready to use amaranthus.

5.3.1. Physiological Parameters

Physiological loss in weight plays an important role in determining the shelf life of minimally processed vegetables. Micro ventilated PE recorded the lowest PLW of 3.43 % after 2^{nd} day and 6.08 % after 4^{th} day of storage (Fig. 8.) when fresh cut amaranthus is stored under room temperature and 6.87% after 8^{th} day of refrigerated storage (Fig. 9.). Storage temperature influenced physiological loss in weight and it was 10.82% at room temperature and 6.15% under refrigerated storage after 4^{th} day. Fresh cut amaranthus with leaves and stem proportion as 1:1 recorded the lowest PLW during storage as compared to 1:2 under both storage conditions. Minimally processed amaranthus with leaves and stem proportion as 1:1 packaged in 150 gauge micro ventilated PE were capable of retaining the quality in terms of lowest physiological loss in weight (6.38 %), highest relative water content of (75.35 %) and lowest percent leakage of (62.76 %) after eighth day of refrigerated storage.

Fresh cut amaranthus pre-packaged in 150 gauge micro ventilated PE had highest relative water content of 77.41 % after 2nd day and 74.81 % after 4th day of storage when fresh cut amaranthus is stored under room temperature and 74.81% after 8th day of refrigerated storage. Storage temperature influenced relative water

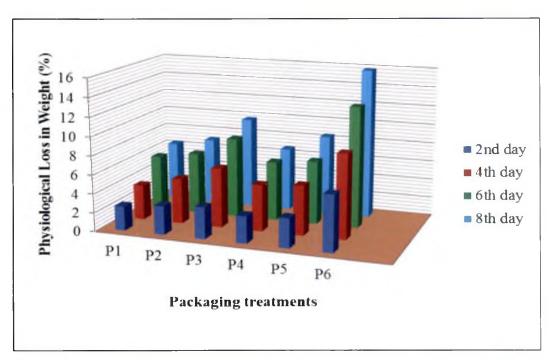


Figure 9. Effect of pre-packaging on physiological loss in weight (%) of fresh cut amaranthus (1:1) under refrigerated storage

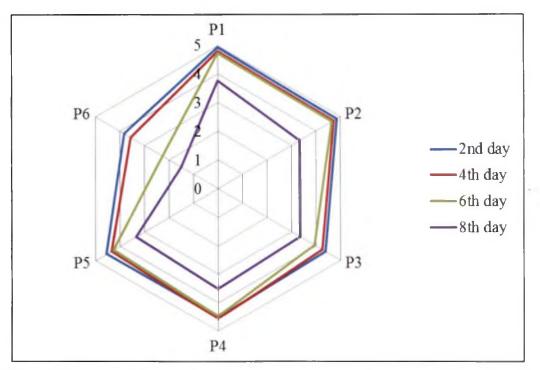


Figure 10. Effect of packaging on colour and texture of fresh cut amaranthus (1:1) under refrigerated storage

content and it was 64.71 % at room temperature and 73.52 % under refrigerated storage after 4th day. Fresh cut amaranthus with leaves and stem proportion as 1:1 recorded the highest RWC during storage as compared to 1:2 under both storage conditions. This result was supported by the data of membrane integrity which is expressed as percentage leakage.

Minimally processed amaranthus packaged in 150 gauge micro ventilated PE recorded the lowest percent leakage of 42.07 % after 2nd day and 51.55 % after 4th day of storage when stored under room temperature and 63.24 % after 8th day of refrigerated storage. Storage temperature influenced percent leakage and it was 68.60 % at room temperature and 52.85 % under refrigerated storage after 4th day of storage. Lower respiration rate at lower temperature and higher humidity inside the package might have resulted in minimum physiological loss in weight and water loss which is evident from high relative water content and membrane integrity.

The results are in agreement with the findings of Ooraikul and Stiles (1991) and Batu and Thompson (1998) who reported that reduced weight loss, better retention of chlorophyll and ascorbic acid of spinach and green bean was recorded in polyethylene packaging and stored at low temperature. Jacxsens et al. (2002) reported that pre-packaging of amaranthus under refrigerated conditions showed significant retention of water content and less physiological loss in weight during storage. Boonyakiat et al. (2006) revealed that to extend quality and shelflife of minimally processed head lettuce, common bean and carrot polyethylene packaging is better. Minimally processed Bok Choy (Brassica chinensis L.) in modified atmosphere packaging recorded lowest physiological loss in weight and highest sensory score compared to polypropylene (Lu 2007). (Assumi et al., 2009) revealed that packaging also helps to enhance shelf life by creating a modified atmosphere with an increase in concentration of carbon dioxide in the package. Babarinde (2009) reported that okra packed in polyethylene and kept under refrigerated condition, recorded the highest ascorbic acid, lowest weight loss and extended shelf life up to nine days of storage. Loss of relative water content is one of the major problems in amaranthus which finally resulted in visible wilting and reduction in produce quality which can be reduced with appropriate packaging and storage (Lokke *et al.*, 2010).

In case of minimally processed fenugreek and kiraksali leaves, LDPE was found to be an effective package in maintaining highest moisture with better control of physiological loss in weight during storage was observed by Reddy (2010). Koraddi and Devendrappa (2011) reported that high relative humidity built up in the vicinity of vegetables packaged in polyethylene retarded moisture loss through transpiration which in turn reduced the physiological loss in weight. Suraweera et al. (2011) revealed that to extend the storage quality of Amaranthus tricolor leaves by polyethylene film packaging, combined with low temperature storage could be recommended as an appropriate method. Rajagira leaves with tender stem packed in 100 gauge polypropylene with vents extended the shelf life up to four days with reduced moisture loss, diminished physiological loss in weight and low yellowing and decaying per cent was reported by Reddy et al. (2013). Kapsiya et al. (2015) reported that in tomato fruits treated with $CaCl_2$ and packed in perforated polyethylene bags were found to further reduce fruit weight loss and maintain ascorbic acid contents in the stored fruits when compared to other packaging systems. Sonia et al. (2015) revealed that tomato, cowpea and lady's finger packed in vented LDPE (Low Density Polyethylene) under low temperature storage conditions recorded least physiological loss in weight (PLW).

A minimum weight loss of 4.2% was observed in fresh cut bitter gourd packaged bin LDPE pouches after 15 days of storage at $8\pm2^{\circ}$ C was reported by Preetha *et al.* (2015). Tomatoes packaged in polyethylene retained better quality in terms of weight loss, colour and palatability as compared to the control as reported by Randhawa *et al.* (2015). Kozuki *et al.* (2016) reported that spinach packaged in perforated polyethylene bags kept in refrigerated storage recorded lowest weight loss and sensory qualities as compared to control. Modified atmosphere packaging helped in reducing the microbial population and maintaining the nutritional quality of fresh cut lettuce during low temperature storage was reported by Prakash *et al.* (2000). Hong and Gross (2001) observed better quality of fresh-cut tomato slices under MAP during cold storage with a shelf life of two weeks at 5 °C. Ayhan *et al.* (2008) reported that minimally processed carrots packed in polyethylene cover retained shelf life up to 14 days of storage with better retention of colour and texture.

Modified atmosphere packaging in polyethylene film was found beneficial in extending shelf life with better retention of nutritional qualities and sensory attributes was reported in fresh cut beetroot, carrot, beans and cabbage (Chandran, 2013), fresh cut bread fruit (Roopa *et al.*, 2014), minimally processed onion (Baskaran *et al.*, 2015), ready to use pomegranate (Dineshkumar *et al.*, 2015), fresh cut carrot (Esturk *et al.*, 2015), fresh cut bitter gourd (Preetha *et al.*, 2015), minimally processed green bell pepper (Ranjitha *et al.*, 2015), and in minimally processed litchi arils (Punumong *et al.*, 2016).

5.3.2. Physical (visual) Parameters

Amaranthus is highly perishable in nature which leads to loss of freshness during storage. In the study, fresh cut amaranthus with leaves and stem proportion as 1:1 were packed in different packages of which 150 gauge micro ventilated PE exhibited maximum freshness during storage with highest mean score for colour (3.76), texture (3.76) (Fig. 10.) and lowest mean score for wilting (2.5) during eighth day of refrigerated storage. Similar results are reported by Lu (2007) in minimally processed Bok Choy. Rathore *et al.* (2009) In chaunsa, mango variety modified atmosphere package was found to be effective in retaining the colour, texture and overall acceptability of fresh cut beetroot, carrot, beans and cabbage was reported by Chandran (2013). Kumar *et al.* (2013) reported that drumstick leaves pre-packaged in 350 gauge thick LDPE was found better in colour retention. Bansal *et al.* (2015) reported that the appearance, taste, color and

texture of fresh cut fruits and vegetables are the most appealing attributes to the consumers. Gill *et al.* (2015) reported that Dashehari mango fruits packed in LDPE and kept under low temperature storage; retain maximum firmness, sensory quality and overall acceptability up to three weeks under low temperature storage. Maximum score for sensory characters and overall acceptability of fresh cut bitter gourd packaged in LDPE was recorded by Preetha *et al.* (2015). Similar results of sensory qualities were observed in tomato by Randhawa *et al.*, (2015) and in spinach by Kozhuki *et al.*, (2016).

5.3.3. Biochemical Parameters

Amaranthus pre-packaged and stored at room temperature and refrigerated condition showed a significant difference in retention of ascorbic acid, anthocyanin and antioxidant activity.

Fresh cut amaranthus (1:1) pre-packaged in 150 gauge micro ventilated PE had highest ascorbic acid content of 15.48 mg/100g after 2nd day and 14.01 mg/100g after 4th day of storage (Fig. 11.) when fresh cut amaranthus is stored under room temperature and 14.46 mg/100g after 8th day of refrigerated storage (Fig. 12.). Storage temperature influenced ascorbic acid content and better retention was observed with refrigerated storage.

Fresh cut amaranthus (1:1) pre-packaged in 150 gauge micro ventilated PE recorded highest anthocyanin content of 34.30 mg/100g after 2nd day and 32.04 mg/100g after 4th day of storage (Fig. 13.) when fresh cut amaranthus is stored under room temperature and 32.83 mg/100g after 8th day of refrigerated storage (Fig. 14.). Storage temperature influenced anthocyanin content and it was 24.55 mg/100g after 4th day of storage at room temperature and 31.35 mg/100g under refrigerated storage.

Fresh cut amaranthus pre-packaged in 150 gauge micro ventilated PE had highest antioxidant activity of 79.55 % after 2^{nd} day and 74.19 % after 4^{th} day of storage when fresh cut amaranthus is stored under room temperature and 76.75 % after 8^{th} day of refrigerated storage. Storage temperature influenced antioxidant

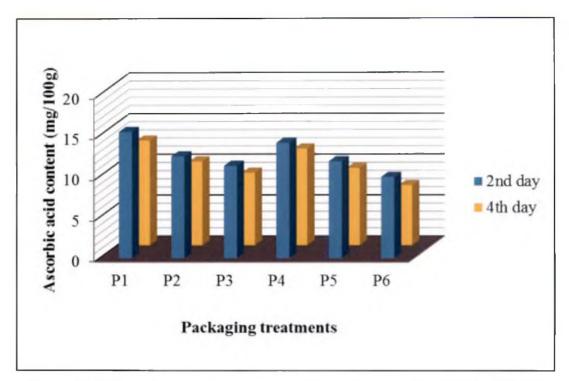


Figure 11. Effect of pre-packaging on ascorbic acid content (mg/100g) of fresh cut amaranthus (1:1) under ambient condition

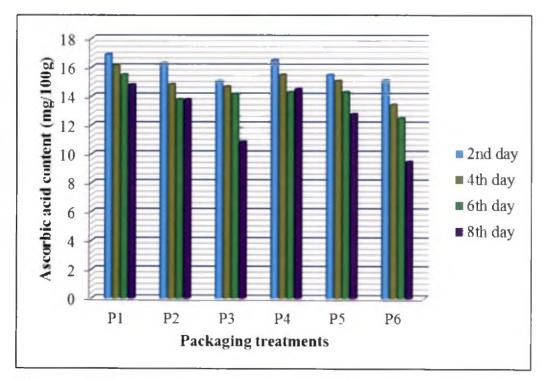


Figure 12. Effect of pre-packaging on ascorbic acid content (mg/100g) of fresh cut amaranthus (1:1) under refrigerated storage

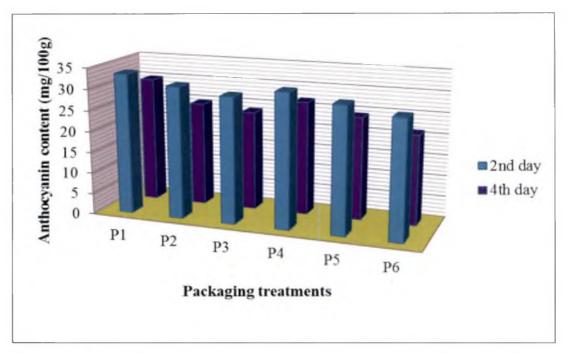


Figure 13. Effect of pre-packaging on anthocyanin content (mg/100g) of fresh cut amaranthus (1:1) under ambient condition

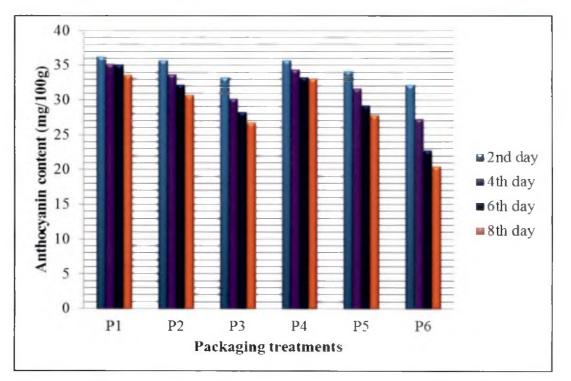


Figure 14. Effect of pre-packaging on anthocyanin content (mg/100g) of fresh cut amaranthus (1:1) under refrigerated storage

activity and it decreased with increase in storage temperature. Fresh cut amaranthus with leaves and stem proportion as 1:1 recorded the highest ascorbic acid, anthocyanin and antioxidant activity during storage under both storage conditions. Loss of nutritional parameters was greater with increasing storage temperature and duration and the results were supported by the findings of Yadav and Sehgal (1995).

Similar results are reported by Weichmann (1987) vegetables packed in low density polyethylene and stored in refrigerated temperature, which retained vitamin C better than those stored in room temperature. Ladaniya and Singh (2001) and Ladaniya (2003) revealed that the influence of polyethylene films on maintaining higher ascorbic acid content in citrus fruits. Babarinde (2009) reported that okra packed in polyethylene and kept under refrigerated condition, recorded the highest ascorbic acid. Drumstick leaves packaged in 350 gauge LDPE retained more vitamin C during storage (Kumar *et al.*, 2013)

Ali *et al.* (2015) revealed that during storage of apricot in low density polyethylene retained maximum biochemical, and higher antioxidant activity, up to two weeks of storage under ambient condition. Kapsiya *et al.* (2015) reported that in tomato fruits treated with CaCl₂ and packed in perforated polyethylene bags record higher ascorbic acid as compared to other packaging systems. Mohebbi *et al.* (2015) reported that cherry packed in low density polyethylene (LDPE) maintained higher ascorbic acid, anthocyanin during the storage. Ascorbic acid content of fresh cut bitter gourd under MAP decreased with storage and the reduction was less when stored at low temperature was reported by Preetha *et al.* (2015).

5.3.4. Enumeration of Total Microbial Load

Fresh cut amaranthus with leaves and stem proportion as 1:1 packaged in 150 gauge micro ventilated PE showed lowest bacterial population of 3.11×10^3 cfu g⁻¹ after 8th day of refrigerated storage. Microbial population was observed to

be higher in all pre-packaged fresh cut amaranthus than without pre-packaging during storage. This may be due to the high moisture condensation on the inner surface of the packaging film due to respiration and transpiration, which adds to the acidity of the leafy vegetable and can end up as the cause of deterioration and growth of microorganisms was reported by King *et al.* (1991); Ahvenainen, (1996); Brackett, (1999). Kumar *et al.* (2013) reported lowest microbial load i.e. fungi, bacteria was less at the end of storage period when drumstick leaves prepackaged in 350 gauge thick LDPE. Pre-packaging polyethylene bags reported lowest microbial population in fresh cut bitter by Preetha *et al.*, (2015) and fresh cut bread fruit by Roopa *et al.*, (2015)

5.3.5. Sensory Analysis of the Standardised Technology

Fresh cut amaranthus with standardised technology stored at room temperature for four days recorded a mean score of 4.53, for taste, 4.46 for colour and 4.68 for overall acceptability. After 8 days under refrigerated condition recorded a mean score of 4.65, 4.75 and 4.74 for taste, colour and overall acceptability respectively and freshly prepared one also recorded similar score for all sensory qualities. The findings are in conformity with the sensory quality of pre-treated, polyethylene packaged minimally processed carrot, beetroot and beans by Chandran (2013), in bok choy (Lu, 2007), carrot (Esturk *et al.*, 2015), fresh cut bitter gourd (Preetha *et al.*, 2015), bell pepper (Ranjitha *et al.*, 2015) and bread fruit sticks (Roopa *et al.*, 2015)

SUMMARY

6. SUMMARY

The study entitled "Standardization of minimal processing of amaranthus *(Amaranthus tricolor L.)*" was conducted at Department of Processing Technology, College of Agriculture, Vellayani, during 2014-16, with the objective to standardise minimal processing technology for the development of ready-to-use amaranthus with extended shelf life and nutritional quality. Major findings of the study are summarized below.

Protocol for fresh cut amaranthus preparation consist of removal of inedible portions, surface sanitization, pre-treatments, packaging and storage. Hence the present investigation was carried out as three different continuous experiments

- 1. Evaluation of sanitizing treatments
- 2. Evaluation of pre- treatments
- 3. Pre-packaging and storage

Amaranthus (var. Arun) harvested after 30 days of sowing were subjected to sanitisation treatments, after removing root portion and effectiveness of sanitising agents was determined for leaves and stem separately by analysing microbial, biochemical and visual parameters.

Effectiveness of different sanitizing treatments on surface decontamination of amaranthus leaves reported that surface sanitization with 2 ppm ozonised water recorded least bacterial population $(3.32 \times 10^3 \text{ cfu g}^{-1})$ with the highest reduction percentage of 40.53. Surface sanitization of amaranthus leaves with 1% acetic acid also reduced the microbial population up to 39.11%. Similarly in amaranthus stem, sanitized with 2 ppm ozonised water recorded the lowest bacterial population $(3.19 \times 10^3 \text{ cfu g}^{-1})$ with the maximum reduction percentage of 39.15. Surface sanitization of amaranthus stem with 30 ppm sodium hypochlorite recorded as the best sanitizer which reduced the microbial population to 35.38%.

Biochemical qualities of surface sanitized amaranthus were analyzed and found that sanitisation treatments *viz.* 2 ppm ozonised water. RO water, 1% citric acid and tap water treatment recorded the highest retention of ascorbic acid in amaranthus leaves (17.85 to 17.90 mg/100g) and amaranthus stem (16.73 to 16.80 mg/100g). Highest retention of anthocyanin content which ranged from 41.66 to 41.78 mg/100g for amaranthus leaves from 26.80 to 26.87 mg/100g in amaranthus stem.

Sensory analysis revealed that amaranthus leaves and stem treated with 2 ppm ozonised water recorded the highest mean score (5.00) for colour, texture and overall acceptability. Based on the effectiveness of sanitizing agents in reducing microbial population and maintaining the biochemical and sensory quality of amaranthus leaves and stem, 2 ppm ozonised water was selected as the best sanitizer.

After surface sanitisation of amaranthus with 2 ppm ozonised water, effect of different pre-treatments on physiological, physical and biochemical quality parameters of fresh cut amaranthus leaves and stem were evaluated separately along with their efficiency to control microbiological growth.

Amaranthus leaves pre-treated with sodium benzoate + citric acid 0.1 % and 1 % calcium chloride recorded the lowest physiological loss in weight of 4.12 % and 7.71 % after 2^{nd} and 4^{th} day of storage and it was 3.36 % and 6.28 % respectively for amaranthus stem. Relative water content of amaranthus leaves and stem treated with sodium benzoate + citric acid 0.1 % and 1 % calcium chloride recorded the highest percentage after 2^{nd} and 4^{th} day with highest membrane integrity. Amaranthus leaves recorded the highest relative water content of 80.72 % and 76.14 % after 2^{nd} and 4^{th} day of storage whereas stem recorded 71.29 % and 68.31 % respectively. The treatment noticed lowest percent leakage for amaranthus leaves as 45.66 % 2^{nd} day and 50.63 % after 4^{th} day of storage and it was 41.03 % and 48.63 % respectively for amaranthus stem.

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Physical parameters *viz.* colour, texture, wilting and overall acceptability of pre-treated amaranthus leaves and stem were evaluated. Fresh cut amaranthus leaves treated with sodium benzoate + citric acid 0.1 % recorded highest score for colour (3.9) and overall acceptability (4.16) and fresh cut stem recorded a score of 4.33 for colour and 4.10 for overall acceptability after 4th day of storage while highest score for texture and lowest score for wilting was noticed for the treatment with 1 % calcium chloride followed by sodium benzoate + citric acid 0.1 % for both amaranthus leaves and stem.

Ascorbic acid, anthocyanin content and antioxidant activity of pre-treated fresh cut amaranthus leaves and stem during storage were evaluated and found decreasing trend with storage.

In pre-treated amaranthus leaves, the highest ascorbic acid content of 17.27 mg/100g was recorded for the treatment sodium benzoate + citric acid 0.1% after two days of storage which decreased to 16.20 mg/100g after 4th day of storage. Similarly for fresh-cut amaranthus stem, the highest ascorbic acid content of 15.42 mg/100g after 2 days and 14.58 mg/100g after 4 days of storage was recorded for the treatment with 0.1% sodium benzoate + citric acid.

Highest anthocyanin content of 39.66 mg/100g for amaranthus leaves was recorded for the treatment sodium benzoate + citric acid 0.1% after two days of storage which decrease to 38.63 mg/100g after 4^{th} day of storage. For fresh-cut amaranthus stem, the highest anthocyanin content of 24.20 mg/100g after 2 days and 23.20 mg/100g after 4 days of storage was recorded for the treatment with 0.1% sodium benzoate + citric acid.

In pre-treated amaranthus leaves, the highest antioxidant activity of 88.48 % was recorded for the treatment sodium benzoate + citric acid 0.1% after 2 days of storage and 85.35 % after 4^{th} day of storage. Similarly for fresh-cut amaranthus stem, the highest antioxidant activity of 80.20 % after 2 days, 73.87 % after 4 days was recorded for the same pre-treatment.

In pre-treated amaranthus leaves, the lowest bacterial population of 2.45x 10^3 cfu g⁻¹ was recorded for the treatment sodium benzoate + citric acid 0.1% before storage. After 2nd, 4th and 6th days of storage the pre-treatment sodium benzoate + citric acid 0.1% showed the lowest bacterial population of 2.84 x 10³ cfu g⁻¹, 2.95 x 10³ cfu g⁻¹ and 3.25 x 10³ cfu g⁻¹ respectively. For fresh-cut amaranthus stem, the lowest bacterial population of 2.85 x 10³ cfu g⁻¹ after 2 days and 3 x 10³ cfu g⁻¹ after 4 days of storage for the treatment with 0.1% sodium benzoate + citric acid. Hence the pre-treatment with sodium benzoate + citric acid 0.1% was selected as the best treatment.

Amaranthus surface sanitized with 2 ppm ozonised water and pre-treated with sodium benzoate + citric acid (0.1%) were shredded in to uniform size and subjected to pre-packaging and storage studies. Fresh cut amaranthus as two different proportions of leaves and stem (1:1 and 1:2 as w/w) packaged in different packaging materials and stored at ambient and refrigerated conditions were analysed for physiological, physical, biochemical, microbial and sensory qualities.

Fresh cut amaranthus pre-packaged as 1:1 proportion and stored under refrigerated refrigerated condition recorded better shelf life, nutritional and sensory qualities with less microbial population. The results revealed that prepackaging and storage of sanitized and pre-treated fresh cut amaranthus (leaves : stem as 1:1) packaged in 150 gauge micro ventilated polyethylene had shelf life up to eight days with highest retention of freshness and nutritional qualities under refrigerated storage and four days shelf life when stored at room temperature.

Minimally processed amaranthus packaged in 150 gauge micro ventilated PE recorded lowest PLW (6.08 %), highest RWC (74.81 %) and lowest percent leakage (51.55 %) after 4th day of storage at room temperature and it was 6.87 %, 74.81 % and 63.24 % respectively after 8th day of refrigerated storage.

Effect of different pre-packaging treatments on visual parameters of fresh cut amaranthus was studied and results revealed that 150 gauge micro ventilated PE exhibited maximum freshness during storage with highest mean score for colour (3.76), texture (3.76) and lowest mean score for wilting (2.5) after eighth day of storage under refrigerated condition.

Minimally processed amaranthus pre-packaged in 150 gauge micro ventilated PE had highest ascorbic acid content of 15.48 mg/100g after 2nd day and 14.01 mg/100g after 4th day of storage under room temperature and 14.46 mg/100g after 8th day of refrigerated storage. Highest retention of anthocyanin and antioxidant activity was also reported for the same pre-packaging treatment.

The highest anthocyanin content of 34.30 mg/100g after 2^{nd} day and 32.04 mg/100g after 4^{th} day of storage was recorded when stored under room temperature and 32.83 mg/100g after 8^{th} day of refrigerated storage.

Minimally processed amaranthus pre-packaged in 150 gauge micro ventilated PE had highest antioxidant assay of 79.55 % after 2^{nd} day and 74.19 % after 4^{th} day of storage under room temperature and 76.75 % after 8^{th} day of refrigerated storage.

Micro ventilated PE recorded the lowest bacterial population of 2.57×10^3 cfu g⁻¹ after 2nd day and 3.01×10^3 cfu g⁻¹ after 4th day of storage when fresh cut amaranthus is stored under room temperature and 3.18×10^3 cfu g⁻¹ after 8th day of refrigerated storage. Fresh cut amaranthus with leaves and stem proportion as 1:1 packaged in 150 gauge micro ventilated polyethylene had lowest physiological loss in weight, highest relative water content, membrane integrity, mean score for visual parameters *viz.* colour, texture and overall acceptability with lower score for wilting, highest retention of ascorbic acid, anthocyanin, antioxidant activity and lowest microbial load under both room temperature and refrigerated conditions.

Sensory analysis of the standardised technology revealed that there was no difference in sensory attributes of the sanitised, pre-treated and pre-packaged ready-to-use amaranthus stored for four days at room temperature and eight days under refrigerated condition as compared to freshly harvested amaranthus. Surface sanitization of amaranthus (var. Arun) with 2 ppm ozonised water and pre-treatment with sodium benzoate + citric acid (0.1%) and pre-packaging in micro ventilated polyethylene could extend the shelf life of minimally processed amaranthus up to eight days when stored under refrigerated condition and four days under ambient conditions.

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APPENDICES

APPENDIX I

DATE: COLLEGE OF AGRICULTURE, VELLAYANI

Dept. of Processing Technology

Score card for visual and sensory parameters

Particulars	Τı	T ₂	T ₃	T4	T ₅	T_6	T ₇	Τ8
Coiour		<i>,</i>						
Texture						,,,,,,,,,,,_		
Wilting								
Overall							·	
acceptance								

<u>Score:</u>

Excellent - 5

Very good- 4

Good- 3

Fair-2

Poor-1

Name:

Signature:

Appendix II

Cost incurred for sanitized and pre-treated amaranthus (200g) pre-packaged in micro ventilated PE cover

Items	Market Price (Rs)	Quantity	Cost (Rs)	
Ozonised water	0.75/ppm	2 ppm	1.50	
Sodium benzoate	570/kg	0.5 g	0.28	
Citric acid	488/kg	0.5 g	0.24	
PE cover	180/kg	1 no.	0.65	
Miscellaneous	-	-	2.65	
Total		<u> </u>	5.32	

STANDARDIZATION OF MINIMAL PROCESSING OF AMARANTHUS (Amaranthus tricolor L.)

by AMBAREESHA K.N. (2014-12-133)

Abstract of the thesis Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN HORTICULTURE

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DEPARTMENT OF PROCESSING TECHNOLOGY COLLEGE OF AGRICULTURE VELLAYANI, THIRUVANANTHAPURAM-695522 KERALA, INDIA

2016

ABSTRACT

The study entitled "Standardization of minimal processing of amaranthus *(Amaranthus tricolor L.)*" was conducted in Department of Processing Technology, College of Agriculture, Vellayani, during 2014-16 with the objective to standardise minimal processing technology for the development of ready-to-use amaranthus with extended shelf life and nutritional quality.

Amaranthus (var. Arun) harvested after 30 days of sowing were subjected to sanitisation treatments, after removing root portion and effectiveness of sanitising agents was determined for leaves and stem separately by analysing microbial, biochemical and visual parameters. The study revealed that surface decontamination with 2 ppm ozonised water had the highest percentage of microbial reduction (40.53 per cent for leaves and 39.15 per cent for stem), highest retention of ascorbic acid, anthocyanin and mean score for visual parameters.

After surface sanitisation of amaranthus with 2 ppm ozonised water, effect of pre-treatments was analysed separately for leaves and stem and it was observed that pre-treatment with sodium benzoate + citric acid (0.1%) recorded the lowest physiological loss in weight, highest relative water content, membrane integrity, mean score for visual parameters, ascorbic acid, anthocyanin content, antioxidant assay and lowest microbial population for both amaranthus leaves and stem which was followed by calcium chloride (1%).

Amaranthus surface sanitized with 2 ppm ozonised water and pre-treated with sodium benzoate + citric acid (0.1%) were shredded in to uniform size and subjected to pre-packaging and storage studies. Fresh cut amaranthus, packed as two different proportions of leaves and stem (1:1 and 1:2 (w/w) in different packaging materials and stored at ambient and refrigerated conditions were analysed for physical, physiological, biochemical, microbial and sensory qualities. Fresh cut amaranthus packaged in 150 gauge micro ventilated polyethylene had lowest physiological loss in weight, highest relative water content, membrane integrity, mean score for visual parameters *viz.* colour, texture and overall acceptability with lower score for wilting, highest retention of ascorbic acid, anthocyanin, antioxidant activity and lowest microbial load for both proportions under ambient as well as refrigerated storage. The results revealed that pre-packaging and storage of sanitized and pre-treated fresh cut amaranthus (leaves : stem as 1:1) packaged in 150 gauge micro ventilated polyethylene had shelf life up to eight days with highest retention of freshness and nutritional qualities under refrigerated storage and four days shelf life when stored at room temperature. Sensory analysis of the standardised technology revealed that there was no difference in sensory attributes of the sanitised, pre-treated, pre-packaged ready-to-use amaranthus from the freshly harvested amaranthus.

Surface sanitization of amaranthus (var. Arun) with 2 ppm ozonised water and pre-treatment with sodium benzoate + citric acid (0.1%) and prepackaging in micro ventilated polyethylene could extend the shelf life of minimally processed amaranthus up to eight days when stored under refrigerated condition and four days under ambient conditions.

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