POSTHARVEST HANDLING FOR EXTENDING SHELF LIFE OF AMARANTHUS *(Amaranthus tricolor* L.)

by

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"POSTHARVEST HANDLING FOR EXTENDING SHELF LIFE OF AMARANTHUS *(Amaranthus tricolor* L.)"** is bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	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
l	Litre
ml	Milli litre
OD	Optical density
M	Molar
mg	Milli gram
viz.	Namely
ppm	Parts per million
pH	Negative logaritham of hydro carbon ions
rpm	Revolution per minute

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Introduction

1. INTRODUCTION

Recent developments in vegetable production technology have significantly contributed to improve production. However, efforts to prevent the vegetable losses between harvesting and consumption are meagre. Several factors influence the postharvest losses of vegetables. The losses due to physical, physiological, mechanical and microbial conditions are characterized by high metabolic activities. With a loss of about 10-15% in fresh weight, vegetables appear shriveled and stale lowering their market value and consumer acceptability considerably.

Vegetables hold an important position in balanced diet, especially, green leafy vegetables which are rich source of β-carotene, iron, calcium, magnesium, phosphorus, potassium, fiber and vitamins like, K, C, E and folic acid (Borah *et al*., 2008). Being rich in these nutrients, leafy vegetables have helped to combat micronutrients deficiencies.

India is the second largest producer of green leaves next to China with an estimated production of 96 million tons (Kumar *et al*., 2013). Amaranthus is the most popular leafy vegetable of Kerala playing an important role in food and nutritional security. It is less expensive and easily available source of protective nutrients, also called as 'Poor man's spinach'. But it is highly perishable due to high moisture content and susceptible to rapid depreciation of nutritive value soon after harvest (Makobo *et al*., 2010). Hence amaranthus doesn't stand storage for more than a few hours under normal condition and also at cool temperature in super markets. Thus, proper packaging and storage is needed for increasing its shelf life and maintaining nutritive quality.

Leafy vegetable quality is mainly based on visual attributes like freshness, typical colour, turgid or not wilted, free of defects such as rot, physical damage, yellowing etc. Packaged and stored leafy vegetables are prone to microbial spoilage leading to postharvest losses. Hence proper sanitization of leafy vegetables like amaranthus is needed before packaging and storage.

Wilting due to water loss, senescence associated discoloration, high respiration rate, and decay or rotting are the main causes of quality deterioration and postharvest loss of leafy vegetables. These causes of quality loss are physiological, pathological, and mechanical in nature. Water loss also induces degradation of nutritional value and imposes stress that increases respiration and ethylene production. Physiological processes can be retarded by different pretreatments and suitable packaging. Modified atmosphere packaging is very effective in retaining freshness and extending shelf life of fresh produce by maintaining the colour, inhibiting water loss, reducing loss due to product respiratory heat, and maintaining the natural fresh taste of produce.

Tropical leafy vegetable like amaranthus is highly perishable and suitable postharvest handling practices can enhance the shelf life by preserving its nutritional quality and there by extend the availability for domestic and overseas market. Hence the present study on postharvest handling practices like sanitization, pretreatments, prepackaging and storage of amaranthus had been undertaken at Department of Processing Technology, College of Agriculture, Vellayani with the objective to extend the shelf life of amaranthus (var. Arun) with minimum nutritional loss through postharvest handling practices.

Review of literature

2. REVIEW OF LITERATURE

Leafy vegetables, due to high moisture content, are highly perishable in nature and susceptible to rapid depreciation of nutritive value soon after harvest. It is estimated that over 30% of leafy vegetables are lost due to poor handling and storage conditions (Nyaura *et al.*, 2014). Special processing treatments are required to prevent postharvest losses of leafy vegetables (Makobo *et al*., 2010).

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. It has short postharvest shelf life due to leaf wilting and it varied with genotypes (Suraweera *et al*., 2011). A loss of 5 to 10 % of fresh weight would make leafy vegetables to appear wilted and become unusable for consumption (Kanlayanarat, 2007).

Thus the present study focus to extend shelf life of amaranthus (var. Arun) with minimum nutritional loss through postharvest handling practices. This chapter describes the review of related research findings done in past years.

2.1. SURFACE SANITIZATION

In recent years an increased interest in the microbiological quality and safety of fresh produce linked to outbreaks of food borne pathogenic microbial illness has been reported (Brackett, 1999). The simple step of thorough washing of leafy vegetables reduces pathogens that may be present as a result of contamination at any point in postharvest chain.

The addition of a chemical disinfectant to the wash water reduces the microbial load from vegetables (Beuchat and Ryu, 1997; Sapers, 2001). Different protocols have been used for washing and disinfection of leafy vegetables, and they comprise several washing times, kind of sanitizers and sanitizer concentrations and justifying its comparison (Oliveira *et al.*, 2012).

2.1.1. Brine

The most common disinfectants used in households are salt in addition to bleach followed by potassium permanganate and water. As salt acts as osmotic agent draws water from the tissues and lowers the water activity of a system and thus renders conditions less favorable to microbial life (Anon., 1999). Bacteria are more inclined to accumulate certain amino acids when the water activity is lowered, thus salt inhibits their growth (Lee, 2004).

Barwal *et al.* (2005) reported that cauliflower preserved in 15 % salt solution containing 0.2 % potassium meta bisulphite to be effective in extending shelf life up to 180 days followed by samples steeped in 10 per cent salt solution containing 0.2 per cent potassium meta bisulphite. Colour and texture of the samples was scored above seven even after 180 days of storage when evaluated by panel of judges on a nine point hedonic scale.

Salt as sanitizer agent is preferred to vinegar as it is cheaper (Rheinlander, 2006). Salt solution is a better sanitizer when compared with potable water at an appropriate concentration and two minutes contact time (Amoah *et al.,* 2007). It has been observed that efficacy of sanitization improved with increasing temperature and increasing concentration; however, high concentration (35 ppm) has a deteriorating effect on the appearance of some crops such as lettuce and it greatly reduced the shelf life. Amoah *et al.* (2007) also observed that increasing the salt concentration from 7 to 35 ppm improved its efficacy from 1.4 to 2.1 log units at a contact time of two minutes.

Reddy (2010) reported that dipping of rajagira leaves (*Amaranthus paniculatus*) in brine (2%) could not extend the shelf life when packed in 100 gauge polypropylene pouches beyond two days and reduced the moisture significantly with a maximum decaying of 7.43 and 10.12 % on first and second day of storage.

2.1.2. Sodium hypochlorite

The most common forms of free chlorine include liquid chlorine and hypochlorites. Sodium hypochlorite increases water pH to above 7.5 which restricts the repeated applications of sodium hypochlorite to recirculating water as it may damage the sensitive produce (Suslow, 1997).

Nascimento *et al.* (2003) reported that washing lettuce samples with chlorinated water for 15 minutes reduced the total mesophilic aerobic bacteria and total coliform counts by 2.63 and 1.91 log, respectively. Baur *et al*. (2004) examined the effect of chlorinated, ozonated and tap water in different washing procedures on sensory and microbiological quality of shredded iceberg lettuce at 4°C and results revealed that, trimmed lettuce heads prewashed in chlorinated water (200 mg/l) exhibited highest shelf life up to seven days, followed by five days in prewashed with 100 mg/l chlorinated water with better control of browning.

About 1 log and 2 log reductions in total mesophilic aerobic bacteria counts following 100 ppm free chlorine (pH 8.7) treatments of lettuce samples for one minute at 4 °C and at 50 °C, respectively was reported by Delaquis *et al.* (2004). Rodgers *et al.* (2004) reported that treatment with 100 ppm chlorine for five minutes reduced *L. monocytogenes* and *E. coli* to non-detectable levels on whole apples, whole lettuce, strawberries, and cantaloupe.

Balla and Farkas (2006) observed chlorine as more effective chemical additive in reducing pathogenic or naturally occurring microorganisms. Sodium hypochlorite fulfils many requirements as the ideal disinfectant and further more it has an excellent cleaning action (Fukuzaki, 2006). Varghese (2006) revealed that surface sanitization with 30 ppm sodium hypochlorite as the most effective sanitizing agent for enhancing shelf life of cut vegetables. Lee and Baek (2008) reported that sodium hypochlorite (NaOCl) is most widely used sanitizer in the fresh-cut industry.

Allende *et al*., (2009) confirmed that sodium hypochlorite inactivated a maximum of 1–1.3 log cfu g^{-1} background microflora present in fresh-cut products. Amith (2012) standardized the effective concentration of 30 ppm, 60 ppm and 90

ppm sodium hypochlorite as the surface sanitizer for minimally processed pomegranate, mango, papaya and pineapple respectively. The lettuce treated with 200 ppm sodium hypochlorite for 15 minutes yielded an approximate reduction of 2 $\log_{10}$ of the total mesophilic aerobic bacteria and coliform counts (Oliveira *et al.,* 2012). Bachelli *et al*. (2013) observed that sodium hypochlorite showed lower values for the count of mesophilic microorganisms in minimally processed lettuce at last day of storage compared to other treatments. Chandran (2013) reported that surface sanitization of fresh-cut vegetables *viz.* cabbage, beans, carrot and beetroot with 30 ppm sodium hypochlorite was effective in reducing the microbial population.

2.1.3. Ozone

Ozone is one of the most powerful oxidizing agents which is applied in gaseous or aqueous form for sanitation purposes. Bazarova (1982) reported that treatment of apples with ozone resulted in lower weight loss and spoilage. A study by Sarig *et al.* (1996) showed that grapes exposed for 20 minutes to ozone (8 mg/l) had considerably reduced the decay of grapes and increased the shelf life during cold storage. Ozone is a highly effective sanitizer at concentrations of 0.5 to 2 ppm and is reported to have 1.5 times oxidizing potential than chlorine and 3,000 times the potential of hypochlorous acid (HOCl) (Suslow, 1998).

Heard (2000) reported that use of sanitizers such as chlorine, peroxy acetic acid, hydrogen peroxide, acidified sodium chloride or ozone can provide an additional reduction in the initial population of microorganisms on the surface of fresh produce. Onions treated with ozone were reported to greatly decrease the mould and bacterial counts without any change in chemical composition and sensory quality during storage (Song *et al.,* 2000).

Ozone is the natural substance in the atmosphere and one of the most potent sanitizers against a wide spectrum of microorganisms (Khadre *et al*., 2001). In 2001, Food and Drug Administration (FDA) approved 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 minimally processed fruits and vegetables (FDA, 2001).

A study was conducted by Garcia *et al.* (2003) to determine the effectiveness of ozone (2.5, 5.0, and 7.5 ppm) on microbiological attributes of shredded lettuce and reported a 0.6 to 0.8 log reduction in aerobic plate count after a 10 minute treatment. Treating fresh fruits and vegetables with ozone has been found to destroy microorganisms by the progressive oxidation of vital cellular components and increases the shelf life of produce (Guzel- Seydim *et al*., 2004). The use of ozonated water has been applied to fresh-cut vegetables for sanitation purposes which reduced microbial populations and extended the shelf life (Beltran *et al.,* 2005). It is a relatively safe fruit and vegetable sanitizer as O_3 is highly unstable in water, spontaneously decomposes into non toxic compounds, such as oxygen and does not leave any toxic residues (Rivera, 2005).

Treatment with ozone appears to have a beneficial effect in extending the storage life of fresh produce such as cucumber, apples, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and by oxidation of ethylene (Kim, 2007). Olmez and Akbas (2009) reported that application of 2 ppm ozonated water treatment for two minutes was the optimum processing conditions for ozone disinfection of green leaf lettuce, in terms of reducing microbial load and maintaining sensory quality during cold storage. In food industry, application of ozone has received a commercial interest due to its effectiveness to extend the shelf life of fresh or fresh-cut products by inhibiting the growth of microorganisms (Sothornvit and Kiatchanapaibul, 2009).

Ozone effectiveness against microorganisms depends on the amount applied, effectiveness of ozone delivery method, type of material, the target microorganisms and physiological state of bacteria cells at the time of treatment (Das and Kim, 2010). Nature and composition of vegetable surfaces and the type and load of microbial contaminants also affect bacteria attachment and overall performances of ozone treatment (Kim and Hung, 2012). Kim (2012) reported that shredded carrot washed

with 2 ppm ozonated water for 20 minutes maintained quality by inhibiting off-odour and high overall quality score. Ong *et al*. (2012) reported that fungal decay of fresh and fresh-cut produce was prevented by application of ozone. It is reported that 3 ppm O_3 is more effective than 3 ppm chlorine dioxide in inactivating bacteria, moulds and yeasts on fruits and vegetables (Chen *et al*., 2013).

Treating tomato slices with 0.4 mg/l ozonated water for 3 minutes achieved the best firmness retention and microbial quality, keeping the glucose and fructose levels upto 10 days at 5˚C (Aguayo *et al.*, 2014). Yeoh *et al.* (2014) revealed that microbial populations on fresh-cut papaya can be reduced without depleting its major antioxidants when subjected to 9.2 ± 0.2 µl/l gaseous ozone for 20 minutes. Ozone is also reported to be efficient in reducing pesticide residues from the fresh produce (Glowacz *et al*., 2015).

2.1.4. Washing with water

Washing vegetables with water is the most used decontamination method at house hold level, 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. Tap water washing of lettuce leaves was reported to reduce the indigenous microflora by approximately 1 log CFU/g (Nguyen-The and Carlin, 1994 and Kim *et al*., 1999).

Gomez *et al.* (2002) showed that washing cauliflower florets in tap water brought about 25 % reduction in bacterial population. 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.

In parsley, maximum reduction was reported when treated with the tap water for total mesophilic aerobic bacteria (0.83 log cfu/g) and total coliform (0.98 log MPN/g) counts (Temiz *et al*., 2011). A study conducted by Ibrahim *et al.* (2012) showed that 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.

Oliveira *et al.* (2012) reported that lettuce leaves washed with potable tap water helped in reducing total mesophilic aerobic microorganisms (0.67 log10 CFU/g) and the coliform population (1.09 log10 CFU/g). Dipping in distilled water was found to be effective in extending the shelf life of rajagira leaves in modified atmosphere packaging (Reddy *et al*., 2013). Feas *et al*. (2014) observed that tap water immersion of ready to eat lettuce was sufficient to reduce aerobic mesophiles under values less than 4 $\log_{10}$ CFU/g.

2.2. EFFECT OF PRETREATMENTS

2.2.1. Effect of benzyl adenine

Metabolic activities *viz.* respiration, transpiration and ethylene production of leafy vegetables are high during post harvest storage period which renders them to be perished in short time. Pretreatment of the produce reduces these metabolic activities which in turn renders a longer shelf life. Guzman (1962) showed that benzyl adenine delays senescence and maintains high quality of escarole held at 4˚C.

Benzyl adenine retards the onset of senescence in certain vegetable crops. Guzman (1962) found that 5.0 and 10.0 ppm of the benzyl adenine for celery and 2.5 ppm for escarole and chicory kept these vegetables with best appearance. Benzyl-adenine applied to leafy vegetables before or after harvesting reduced the rate of respiration during storage and thus maintained their freshness (Wittwer and Dedolph, 1962). Segall *et al*. (1963) confirmed that benzyl adenine retarded the development of senescent tissue and delayed the formation of sites for the entrance and multiplication of many of the weak parasites associated with postharvest decay. Tsujita and Andrew (1967) reported that a concentration of 30 ppm BA was more effective in maintaining the chlorophyll content of cabbage during storage.

Rushing (1990) showed that cytokinin application on broccoli lowered the rate of respiration compared to control which resulted in extension of shelf life. Exogenous application of cytokinins to plant tissues result in delayed senescence,

maintenance of chloroplast activity, decline chlorophyll degradation, production of protein and nucleic acid synthesis and mobilization of nutrients into cytokinin treated area (Clarke *et al*., 1994 and Wingler *et al.*, 1998). Skutnik (1998) showed that benzyl adenine was effective in extending the longevity of *Hosta* leaves conditioned or soaked in this growth regulator and also holds for shoots of *Asparagus densiflorus* 'Sprengerii'.

Costa *et al.* (2005) reported that treatment of broccoli heads with 6-benzylaminopurine (BAP) (100 ppm) can retard senescence related changes. Influence of cytokinin *viz.* BAP in combination with other methods on broccoli heads and asparagus spears were studied and found positive result in postharvest green color retention (Costa *et al*., 2005 and Yuan *et al*., 2010).

The application of benzyladenine (BA) and other cytokines effectively delayed ripening of apples and other fruits (Greene *et al*., 2006). According to Jing *et al*. (2007) greengrocery treated first with 20 mg/l BA followed by nano packaging prolonged the storage life to five days. Similarly, favourable effect of benzyl adenine in extending postharvest longevity of leaves and maintaining the quality expressed as the index of leaf greenness was recorded in *Arum italicum* (Janowska and Schroeter-Zakrzewska 2008). In a study by Rabiza-Swider and Skutnik (2008), the conditioning of leaves of *Hosta* 'Crispula' and 'Undulata mediovariegata' in gibberellic acid and benzyl adenine retarded the degradation of soluble proteins, especially after the use of benzyl adenine.

Bhardwaj *et al*. (2010) found that increase in physiological loss in weight and rotting was low in fruits treated with 20% neem leaf extract and 100 ppm benzyladenine as compared to control and other treatments. Application of benzyladenine at the concentration of 50–150 mg/dm^3 was found to have an influence on higher index of leaf greenness (Janowska and Smigielska, 2010). A combined application of BA and GA_3 inhibit chlorophyll and protein degradation (Janowska and Stanecka, 2011). Siddiqui *et al.*, (2011a) found that a single treatment with 10 ppm of 6-benzylaminopurine (BAP) for 10 minutes could be used commercially to

extend postharvest life of fresh-cut broccoli florets and maintained a better nutritional value (higher vitamin and protein contents) and appearance (greenness or fresh-like state). Janowska *et al.* (2013) reported that benzyladenine applied at concentration of 25, 50 and 75 mg/dm^3 significantly influenced postharvest longevity and leaf quality of *Limonium latifolium* in comparison with the leaves from the control group.

2.2.2. Effect of treatment on cut stem end

Waxing is a common method to decrease moisture loss from fruits and vegetables (Kester and Fennema, 1986). Surface coating has been used as preservation technique for fruits as well as vegetables for decades (Baldwin *et al.*, 1995). Effectiveness of the coating depends on thickness, concentration, and type of coating (Amarante *et al*., 2001). Waxes are effective in blocking the migration of moisture and among waxes it was reported candelilla wax as the most resistant one in comparison with carnauba wax and beeswax (Bosquez-Molina and Vernon- Carter, 2005).

Waxes serve as physical barriers to spoilage microorganisms by preventing their entry and increase the shelf life of fruit and vegetables (Min and Krochta, 2005). Yadav *et al.* (2009) tested the efficacy of different pretreatments in extending shelf life of tomatoes and revealed that physiological loss in weight was less in samples dipped in paraffin wax (37%). Patel *et al*. (2013) concluded from study that coating treatment with 2.0 per cent carnauba wax or 2.0 per cent carnauba wax + BA 25 ppm enhanced the quality of pointed gourd for longer period under ambient conditions. Bahnasawy and Khater (2014) observed that shelf life of cucumber increased from 173.4 to 231.6 h when the wax solution concentration increased from 0 to 100 % and concluded that shelf life increased with increasing wax concentration in solution.

Combination of 12% wax coating and storage at 5°C was reported to be the best treatment for maintaining the quality and extending the shelf-life of tangerine citrus var. Siam Banjar (Hassan *et al.,* 2014). Main objective of the application of surface coating is to reduce water evaporation and thereby slow rate of weight loss, which

ultimately extend the shelf life of the fruits (Hassan *et al.,* 2014). De Leon-Zapata *et al.* (2015) reported that application of candelilla wax with fermented extract of tarbush resulted in a positive effect in reducing the weight loss, maintaining the water activity, improving firmness, quality and shelf life of apples (var. Golden delicious) for eight weeks in marketing conditions.

For long distance transportation of cut flowers wet cotton swab or tube containing water or water with preservatives is commonly practiced. In anthurium (Hettiarachchi and Balas, 2005) and orchids (Hegde, 1999; De *et al.,* 2014) it was reported that cut stem end dipped in water or wrapping with wet cotton swab increased the vase life.

2.3. PREPACKAGING AND STORAGE

Packaging minimizes the postharvest losses of vegetables by reducing physical injury during transit and handling (Gast, 1991). Packaging have the potential to reduce moisture loss, restrict entry of oxygen, lower respiration, retard ethylene production, seal flavor volatiles, and retard discoloration (Ahvenainen, 1996; Luo *et al*., 2004 and Ares *et al*., 2008). Nainar *et al*. (1997) reported that physiological weight loss of a produce decreased with increasing thickness of plastic bags.

O'Hare *et al*. (2001) reported that plastic film packaging of pak choi effectively reduced moisture loss and wilting and was considerably more effective than manual misting or treating leaves with anti-transpirant chemical. Thomson *et al.* (2001) found that modified atmospheric packaging provided storage benefits for chinese garlic chives, spearmint, hot mint, thai basil, snake beans, perilla, hot chillies, pak choi, baby pak choi, kangkong, and kai choi. Das (2004) concluded that modified atmospheric packaging is an attempt to retard physiological processes and also minimize microbial infections in order to maintain optimum quality and extend the shelf life.

Modified atmosphere packaging has shown to prolong the quality of leafy vegetables, through a right balance between product respiration and film oxygen transmission rate (Kim *et al.,* 2004). Jiang and Pearce (2005) found packaging to be extremely effective in retarding yellowing of leafy brassicas used in the fresh-cut vegetable industry. Roshita *et al*. (2005) reported that polypropylene extended the shelf life of minimally processed shredded cabbage upto three weeks with minimum colour change, reduction in weight loss and deterioration in sensory properties. Saito and Rai (2005) studied qualitative changes in radish sprouts under modified atmosphere packaging in micro perforated films and revealed that shelf life of radish sprouts could be extended upto six days when packed in micro perforated oriented polypropylene film packages with a gaseous atmosphere of 9-15 % O_2 and 8-11 % CO_2.

Packaging is reported to protect the nutritive quality along with better appeal (John, 2008) and increases shelf life by creating a modified atmosphere with an increase in concentration of carbon dioxide in the package (Assumi *et al*., 2009). Packaging also decreases the rate of microbial growth (Rodriguez-Aguilera and Oliveira, 2009 and Sandhya, 2010). It creates a barrier between environment and food, in addition to ease of transport, handling and marketing (Reddy, 2010).

Polyethylene bags acted as barriers for loss of moisture resulting in build up of high relative humidity in the vicinity of vegetables and thereby retarded moisture loss through transpiration which in turn reduced the physiological loss in weight of vegetables (Koraddi and Devendrappa, 2011). Suraweera *et al*. (2011) reported that polyethylene film packaging, combined with low temperature storage could be recommended as an appropriate method for extending the postharvest quality of *Amaranthus tricolor* leaves.

Selection of suitable packaging film is of crucial importance to maintain quality and to assure a longer shelf life of the packaged produce (Dulal *et al*., 2012). Reddy *et al*. (2013) found that when rajagira leaves with tender stem were packed in 100 gauge polypropylene bags with vents increased the shelf life upto four days with

86.32 per cent moisture retention, 1.27 % physiological loss in weight, 16.98 and 14.52% yellowing and decaying respectively.

Temperature is a critical factor in maintaining product quality during storage. Low temperature storage has been shown to slow down evapo-transpiration and hence ensures a longer shelf life of the vegetables (Kays, 1991). Ooraikul and Stiles (1991) observed that use of polyethylene pouches reduced the loss of weight, chlorophyll and ascorbic acid of spinach and green bean kept at 10ºC and 20ºC. Refrigerated storage can enhance the shelf life of quality of leafy vegetables (Sankat and Maharaj, 1996). According to Lopresti and Tomkins (1997) longest satisfactory storage of dill (9 - 12 days), parsley and chives (14 - 21 days) was obtained in non-perforated bags at 2°C. Favell (1998) found that leafy vegetables can be easily preserved by storing in polypropylene packages, at temperatures close to 0°C.

Lee and Kader (2000) observed that leafy vegetables held at 6˚C lost 10 % of their ascorbic acid content in six days, while those held at room temperature lost 20% in only two days. Gomez and Artes (2005) reported that celery sticks prepackaged with polypropylene and stored at 4 °C exhibited the best quality after 15 days. During the cold storage, the packaging reduced moisture loss and minimized the degradation of chlorophyll in all fresh green leafy vegetables (Souzan and Abd el-aal, 2007). Jany *et al*. (2008) revealed that cabbage, cauliflower, country bean, tomato and pea can be stored in room temperature for eight days, at refrigerated (4^{o}C) temperature for 12 days and at freezing temperature (-18^{o}C) for 90 days. Onyango (2010) reported that after four days of storage, vegetables held at ambient temperature had lost nearly 10 per cent while those kept in refrigerator lost only 5 % of initial moisture content.

Leafy vegetables with large surface area to volume ratios are particularly vulnerable to loss of water during storage which will increase with storage temperature and vapour pressure deficit (Onyango, 2010). The rate of respiration generally increases as the storage temperature increases (Koraddi and Devendrappa, 2011). It was observed by Zenoozian (2011) that active modified atmosphere

packaging of vegetables showed least weight loss because of restricted respiration rates of vegetables in active modified atmosphere and also carbohydrate resources were consumed slightly in this condition. For vegetables, storage at 5°C in a modified atmospheric package is recommended since the vegetables maintained their quality attributes after storage (Nyaura *et al.,* 2014). Refrigeration temperature used in storage of ready to eat vegetables extended the shelf life by slowing down the growth rate of microorganisms (Feas *et al*., 2014).

A study was conducted by Jiang and Pearce (2005) investigating on the use of fully cling wrap, semi-cling wrap packed shoots (two-thirds of leaves exposed) and non-packaged under supermarket conditions (ambient 28°C) and results revealed that semi-cling wrap packed pak choy performed best whereas fully packaged pak choy had less water loss and tended to have more rots than the other two treatments.

Barth *et al.* (1993) reported that modified atmosphere packaging of broccoli maintained ascorbic acid, chlorophyll and retained moisture compared to broccoli stored in air. Favell (1998) observed greater losses of ascorbic acid with only 10 % retention in spinach within three days of storage at 20^{o}C, and 20 % retention when stored under refrigeration (4^{o}C) for seven days. Decrease in vitamin C was observed over 10 days storage period in all vegetables irrespective of packaging combinations (Hussein *et al.,* 2000). Kuan (2003) reported that levels of soluble oxalates in soya beans remained constant throughout six months of storage at -20^{o}C, 4^{o}C and 25^{o}C.

Catherwood *et al.* (2007) observed a small increase in soluble oxalates during storage which is related to an overall loss in moisture content of vegetables. In lettuce, higher storage temperature of 10°C hastened chlorophyll and carotenoid loss compared with low temperature (4°C) (Ferrante and Maggiore, 2007). Prabhu and Barrett (2009) reported ascorbic acid retention between 28 and 69 % of their initial content in two african leafy vegetables (*Cassia tora* and *Corchorus tridens*) when stored at 4^{o}C for 14 days.

Onyango (2010) observed that soluble oxalate content of fresh samples was 3.4% which increased to 3.5% after storage for four days in the refrigerator (4±1˚C)

and to 3.7% when stored at ambient temperatures (20±3°C). Parsley and dill showed highest retention of chlorophyll content at 5°C and lowest chlorophyll content at 25°C (Zenoozian, 2011). Kramchote *et al.* (2012) reported that cabbage stored at 4°C and 10°C effectively delayed leaf yellowing and maintained leaf chlorophyll content, reduced weight loss, respiration rate and ethylene production and head firmness was maintained. Drumstick leaves prepackaged in 350 gauge thick LDPE was found to be the best in maintaining the colour ,vitamin C, beta carotene during storage and microbial load i.e. fungi, bacteria was less at the end of storage period (Kumar *et al.*, 2013).

Materials and methods

3. MATERIALS AND METHODS

The present investigation on "Postharvest handling for extending shelf life of amaranthus *(Amaranthus tricolor* L.)" was undertaken at Department of Processing Technology, College of Agriculture, Vellayani, during the period of 2013-2015, with the objective to extend the shelf life of amaranthus (*var*. Arun) with minimum nutritional loss through postharvest handling practices.

The study was conducted in three parts

3.1. Evaluation of sanitizing treatments
3.2. Effect of pretreatments
3.3. Prepackaging 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 Keralam (VFPCK) at Pappanchani, Vellayani during morning hours. Amaranthus of uniform size without any visual defects was harvested 30 days after sowing.

Harvested amaranthus stem of 25-30 cm long with leaves (after removing root portion) were taken and fully immersed in following sanitizing agents for five minutes. For each treatment 200 g samples were taken with five replications.

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

After the treatments excess water was drained and samples were stored at room temperature (30±2˚C) for visual and physiological studies and observations were taken at an hourly interval till the end of shelf life. Amaranthus with cumulative physiological loss in weight of more than 25 % and relative water content less than 65 % with lower score of visual observations and overall acceptability was considered as the end of shelf life.

Effectiveness of sanitizing agents for surface decontamination was evaluated microbiologically before and after the treatment.

3.1.1. Enumeration of total microbial load

The quantitative assay of the microflora in pre and post treated samples was carried out by serial dilution spread plate technique. NA (nutrient agar) and RB (Rose Bengal Agar) medium were used for the enumeration of bacterial and fungal population respectively.

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 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 was 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

$$\text{No. of colony forming units (CFU per gram of the sample)} = \frac{\text{Total number of colony formed} \times \text{dilution factor}}{\text{Aliquot plated}}$$

The data were analyzed statistically using Completely Randomized Design.

3.1.2. Physiological parameters

Physiological parameters of sanitized amaranthus were recorded periodically till the end of shelf life.

3.1.2.1. Physiological loss in weight (PLW)

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

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

3.1.2.2. Relative water content (RWC)

Relative water content was estimated in per cent according to the method proposed by Smart and Bingham (1974).

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 upto one hour. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 85˚C. RWC was calculated in per cent by the formula

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

3.1.3. Visual parameters

Visual parameters like colour, texture, appearance, leaf wilting and defoliation/decay of sanitised amaranthus were taken initially and at an interval of two hours till the end of shelf life by conducting a sensory evaluation performed by a

Plate 1. Sanitisation of amaranthus

Plate 2. Moist cotton plugging of cut stem end

Plate 3. Cut stem end dipped in water

30 member semi-trained panel. A score card proposed by Swaminathan (1995) was used for assessing the sensory qualities with a 5 point hedonic scale with following scores.

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

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

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

3.2. EFFECT OF PRETREATMENTS

Amaranthus after harvesting was surface sanitized using the best treatment selected from the Part I experiment (3.1) and was pretreated by dipping in following pretreatment solutions (prepared in distilled water) along with control (distilled water) for five minutes.

R_1 : Benzyl Adenine (10 ppm)

R_2 : Benzyl Adenine (20 ppm)

R_3 : Control (with distilled water)

The pretreated amaranthus of 200 g weight were stored at room temperature (30 ± 2 ˚C) with following conditions in three replications.

C_1 : Cut stem end dipped in water

C_2 : Moist cotton plugging

C_3 : Waxing of the cut stem end

C_4 : Control

For the treatment C_1, 500 ml beaker containing 250 ml tap water was taken and cut stem end of differently pretreated samples of 200 g each with three replication

was dipped in water. In the case of C_2, moist cotton containing 90 ± 2 % of water was taken, plugged on the cut stem end of 200 g bunch (together as a single plug) and the plugged end was covered with LDPE 100 gauge in order to prevent leakage of water. For C_3, cut stem end of 200 g amaranthus was dipped in 50 % bee wax and air dried. All the treated samples were stored at room temperature.

The following visual and physiological parameters of the treated samples were recorded daily till the end of shelf life.

3.2.1. Physiological parameters

The following physiological parameters were recorded for the pretreated amaranthus daily till the end of shelf life.

3.2.1.1. Physiological loss in weight (PLW)

Physiological loss in weight was calculated as described in 3.1.2.1.

3.2.1.2. Relative water content (RWC)

Relative water content was calculated as described in 3.1.2.2.

3.2.2. Visual parameters

Visual parameters like colour, texture, appearance, leaf wilting and defoliation of pretreated amaranthus were evaluated daily by a semi trained panel of 30 members using a 5 point hedonic scale as described in 3.1.3.

3.3. PREPACKAGING AND STORAGE

Amaranthus (*var*. Arun) after harvesting was surface sanitized and pretreated with the best treatments from first (3.1) and second part (3.2) of the experiment respectively and prepackaged (200 g) using following different packages and stored at two different conditions viz. room temperature and refrigerated conditions for storage studies in three replications.

(A)

(B)

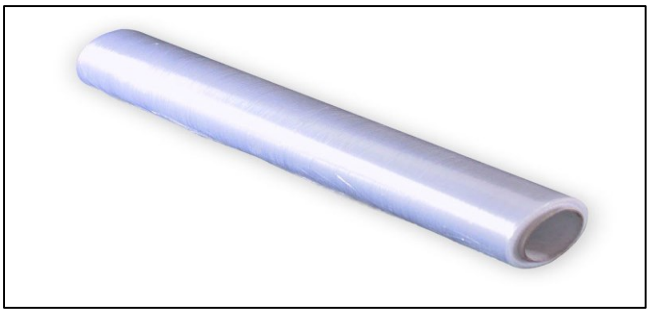
(C)

(D)

Plate 5. Prepackaged amaranthus (A) Macro ventilated PP (100 gauge) without absorbent paper (B) Macro ventilated PP (100 gauge) with absorbent paper (C) Cling film wrapping (D) Sleeve wrapping with LDPE

(A)

(B)



(C)

Plate 4. (A) Macro ventilated LDPE (150 gauge) cover (B) Macro ventilated PP (100 gauge) cover (C) Cling film

P_1: Macro ventilated LDPE cover (150 gauge) with absorbent paper

P_2: Macro ventilated LDPE cover (150 gauge) without absorbent paper

P_3: Macro ventilated PP cover (100 gauge) with absorbent paper

P_4: Macro ventilated PP cover (100 gauge) without absorbent paper

P_5: Sleeve wrap with LDPE

P_6: Cling film (3/4 covering)

P_7: Without prepackaging

Polyethylene and polypropylene covers of size 48×36 cm with 5 per cent ventilation were used in treatments from P_1 to P_4 and in treatments P_1 and P_3 absorbent paper was placed at one side inside each package. The treated amaranthus were packaged in respective polybags and sealed using sealing machine. In treatment P_5, LDPE sheets of 150 gauge thickness were made into sleeve shape and used. In treatment P_6, three-fourth portion of treated amaranthus were wrapped from cut stem end towards the terminal portion with cling film.

All prepackaged amaranthus (200 g) in three replications were stored under two different conditions.

S_1: Room temperature (30±2˚C)

S_2: Refrigerated condition (10±2˚C)

The following visual, physiological, nutritional and microbial parameters were recorded.

3.3.1. Physiological parameters

The following physiological parameters were recorded daily till the end of shelf life.

3.3.1.1. Physiological loss in weight (PLW)

Physiological loss in weight was calculated as described in 3.1.2.1.

3.3.1.2. Relative water content (RWC)

Relative water content was calculated as described in 3.1.2.2.

3.3.2. Nutritional parameters

The following nutritional parameters were recorded initially (pretreated amaranthus before storage) and at the end of shelf life.

3.3.2.1. Vitamin C

Vitamin C content was estimated by 2, 6-dichloro phenol indophenol (DCPIP) dye method (Sadasivam and Manickam, 1992) and expressed as mg/100g. Working standard solution (5 ml) was pipetted out into a 100 ml conical flask. Oxalic acid 4% was added to it and titrated against the dye (V_1 ml). End point was noted on appearance of pink colour which persisted for a few minutes. The sample (1 g) was weighed and ground in a mortar and pestle in 15 ml of 4 % oxalic acid. The homogenate was filtered through a double layered cheese cloth. The filtrate was made upto a known volume and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and made upto 25 ml using oxalic acid. 5 ml aliquot was pipette into a conical flask to which 10 ml of 4 % oxalic acid was added. This was titrated against 2, 6 – dichlorophenol indophenol (DCPIP) solution, until the appearance of pink colour (V_2 ml). The amount of ascorbic acid was calculated as follows:

Ascorbic acid (mg/100g) = (0.5 mg / V_1 ml) × (V_2/ 5 ml) × (100/ weight of sample).

3.3.2.2. β-carotene

β-carotene was estimated according to the method proposed by Srivastava and Kumar (1998). Five gram of fresh sample was weighed and homogenized with 10-15 ml acetone and few crystals of anhydrous sodium sulphite, in a mortar with pestle. The homogenate was filtered and the supernatant was decanted into a beaker. This was repeated twice and transferred the pooled supernatant to a separating funnel. Petroleum ether (10 ml) was added and mixed thoroughly. Two layers were separated on keeping the separating funnel undisturbed for some time. The lower layer was discarded and upper layer was collected in a 100 ml volumetric flask. The volume was made up to 100 ml with petroleum ether and the optical density was recorded at 452 nm using petroleum ether as blank.

$$\text{Amount of β-carotene} = \frac{(\text{OD of sample} \times 13.9 \times 10^4 \times 100)}{(\text{Weight of sample} \times 560 \times 1000)}$$

3.3.2.3. Chlorophyll

Chlorophyll content was estimated using spectrophotometer (Sadasivam and Manickam, 1992). A weighed quantity of sample (1 g) was taken and cut into small bits and ground it with addition of 20 ml of 80% acetone thoroughly in mortar and pestle. Centrifuged at 5000 rpm for five minutes and transferred the supernatant to a 100 ml volumetric flask. Ground the residue with 20 ml of 80% acetone, centrifuged and transferred the supernatant to the same volumetric flask. Repeated this procedure until the residue became colourless. Made up the volume to 100 ml with 80% acetone. Read the absorbance of solution at 645 nm and 663 nm against the solvent (80% acetone) blank.

$$\text{Chl a (mg/g)} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V/1000 \times 1/\text{ Fresh weight}$$

$$\text{Chl b (mg/g)} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times V/1000 \times 1/\text{ Fresh weight}$$

$$\text{Total Chl (a + b)} = (8.02 \times A_{663} + 20.2 \times A_{645}) \times V/1000 \times 1/\text{ Fresh weight}$$

3.3.2.4. Oxalates

Oxalates content was performed using by titration method (Day and Underwood, 1986). One gram of dried powdered sample was weighed and taken in 100 ml conical flask and 75 ml of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 ml of the filtrate was titrated while hot against KMnO4 solution (0.05 M) to the end point. The oxalate content was calculated as percent of dry weight.

3.3.2.5. Calcium

The calcium content of samples was determined using flame photometric method (Alexander, 1963). 0.3 g of oven dried sample was digested in sulphuric acid (H_2SO_4) and made up to a known volume with distill water. Diluted sample was read using flame photometer.

$$\text{Ca (\%)} = \frac{100 \times \text{Reading}}{\text{Sample weight} \times 10{,}000}$$

3.3.2.6. Iron

The iron content was estimated using Atomic Absorption Spectrophotometer (AS). 0.3g of oven dried sample was digested in sulphuric acid (H_2SO_4) and made up to a known volume with distilled water. Diluted sample was read using Atomic Absorption Spectrophotometer (AS).

$$\text{Fe (\%)} = \frac{\text{Reading} \times 100}{\text{Sample weight} \times 10{,}000}$$

3.3.3. Enumeration of total microbial load

Microbial load on prepackaged samples was calculated initially (before storage) and at the end of shelf life, as described in 3.1.1.

3.3.4. Visual parameters

Visual parameters like colour, texture, appearance, leaf wilting and defoliation of packaged amaranthus were evaluated daily by 30 member semi trained panel using a 5 point hedonic scale as described in 3.1.3.

3.4. Statistical analysis

The data generated from experiments were statistically analyzed using Completely Randomized Design (CRD). In visual parameters, different preferences as indicated by scores were analyzed to get the mean rank values using Kruskall – Wallis test.

Results

4. RESULTS

The experimental data collected from the investigation on "Postharvest handling for extending shelf life of amaranthus *(Amaranthus tricolor* L.)" were analysed and the results are presented in this chapter under following heads.

4.1. Evaluation of sanitizing treatments

4.2. Effect of pretreatments

4.3. Prepackaging and storage

4.1. EVALUATION OF SANITIZING TREATMENTS

Effectiveness of sanitizing agents, brine (T_1), sodium hypochlorite (T_2), ozonised water (T_3) and tap water (T_4) for surface decontamination was evaluated after dipping harvested amaranthus in sanitizing agents for five minutes and subjected for analysis of surface microbial count, physiological and physical parameters.

4.1.1. Bacterial population

Microbial count before and after sanitization treatment is depicted in Table 1. Before sanitization, different treatments did not differ significantly in bacterial population which ranged from 24.84 to 25.58 $\times 10^5$ cfug^{-1}. The sanitizing treatments significantly differed from each other in reducing microbial population. Amaranthus treated with 2 ppm ozonised water (T_3) had least bacterial populations (3.24×10^5 cfug^{-1}) with highest reduction percentage (85.68) followed by 30 ppm sodium hypochlorite (T_2) (8.84×10^5 cfug^{-1}) with a reduction percentage of 64.26. Highest bacterial population was observed in amaranthus treated with 2% brine (T_1) (15.51×10^5 cfug^{-1}) with lowest reduction percentage (39.39) which was on par with tap water (T_4) (14.17 $\times 10^5$ cfug^{-1}) having reduction percentage of 42.91%.

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

Treatment	Bacterial population $\times 10^5$ cfug^{-1}			Fungal population	
	Before treatment	After treatment	Reduction %	Before treatment	After treatment
T_1	25.58	15.51[a]	39.39[c] (6.26[c])	TLTC	TLTC
T_2	24.84	8.84[b]	64.26[b] (8.02[b])	TLTC	TLTC
T_3	24.84	3.24[c]	85.68[a] (9.24[a])	TLTC	TLTC
T_4	24.84	14.17[a]	42.91[c] (6.54[c])	TLTC	TLTC
CD (0.05)	NS	4.549	6.914 (0.444)		

Figure in parentheses is square root transformed values

TLTC- Too Less to Count

Figures in a column followed by same letter do not differ significantly (p = 0.05)

4.1.2. Fungal population

Amaranthus treated with different sanitizing agents were evaluated for fungal count. It was found that fungal population was too less to count in amaranthus before and after the sanitization treatments.

4.1.3. Physiological parameters

Effects of different sanitizing agents on physiological parameters of amaranthus stored under room temperature were analyzed periodically and are described as below.

4.1.3.1. Physiological loss in weight (PLW)

Physiological loss in weight (%) of sanitized amaranthus is given in Table 2. Amaranthus treated with 2 ppm ozonised water (T_3) recorded the lowest physiological loss in weight of 6.88 % followed by 30 ppm sodium hypochlorite (T_2) of 8.23 % after 2 h of storage. Highest physiological loss in weight (12.55) was recorded by 2% brine (T_1).

After 3 h of storage, 2 ppm ozonised water (T_3) recorded the lowest cumulative weight loss of 11.17 % followed by 30 ppm sodium hypochlorite (T_2) with 21.36 % loss and 2% brine (T_1) had the highest physiological weight loss of 28.95 %.

Same trend was observed after 4 h of storage also with minimum weight loss of 25.63 % for 2 ppm ozonised water (T_3) which was followed by 30 ppm sodium hypochlorite (T_2) (27.53). Highest weight loss of 31.04 % was observed in 2% brine (T_1) which was more than that of tap water (T_4) (29.12).

4.1.3.2. Relative water content

Relative water content of amaranthus leaves before treatment was found non-significant difference between the treatments and values ranged between 82.69 and 83.22 percentage (Table 3.). After the sanitization treatments, relative water content of amaranthus leaves in treatments T_2 (30 ppm sodium hypochloride), T_3 (2 ppm

Table 2. Effect of sanitizing treatments on physiological loss in weight, %

Treatment	PLW after storage		
	2 h	3 h	4 h
T_1	12.55[a]	28.95[a]	31.04[a]
T_2	8.23[c]	21.36[c]	27.53[c]
T_3	6.88[d]	11.17[d]	25.63[d]
T_4	10.07[b]	22.53[b]	29.12[b]
CD (0.05)	1.285	0.253	0.021

Table 3. Effect of sanitizing treatments on relative water content, %

Relative water content (RWC)					
Treatment	Before sanitization	After sanitization	After storage		
			2 h	3 h	4 h
T_1	83.12	82.03[b]	74.34[d]	60.24[d]	53.64[d]
T_2	83.22	86.22[a]	77.88[c]	65.11[b]	58.43[b]
T_3	82.69	87.41[a]	82.70[a]	75.92[a]	63.11[a]
T_4	82.93	87.28[a]	80.58[b]	64.38[c]	55.23[c]
CD (0.05)	NS	2.930	1.831	0.219	0.028

ozonised water) and T_4 (tap water) did not show any significant difference between each other whereas in treatment T_1 (2% brine) relative water content decreased to 82.03 %.

Highest relative water content after 2 h of storage was recorded by T_3 (2 ppm ozonised water) (82.70) followed by T_2 (30 ppm sodium hypochlorite) (77.88). The treatment T_1 (2% brine) recorded the lowest water content (74.34 %) after 2 h of storage.

Similarly, after 3 h of storage, 2 ppm ozonised water (T_3) maintained the highest relative water content of 75.92% which was followed by T_2 (30 ppm sodium hypochlorite) (65.11) and the lowest water content was observed in amaranthus treated with 2% brine (T_1) (60.24).

The treatment with 2 ppm ozonised water (T_3) had the highest (63.11) water content after 4 h of storage followed by T_2 (58.43) and 2 % brine (T_1) recorded the lowest relative water content of 53.64 %.

4.1.4. Visual parameters

Visual parameters of treated amaranthus viz., colour, texture, leaf wilting, defoliation and overall acceptability were ranked and mean score for each attribute at 2,3 and 4 hours of storage are presented in Tables 4, 5 and 6 respectively. The scores for visual parameters were statistically analyzed using Kruskall - Wallis test and results revealed that significant differences of visual parameter between treatments stored under room temperature at 2, 3 and 4 hours of storage.

Amaranthus treated with 2 ppm ozonised water (T_3) recorded the highest mean score for colour (4.00), texture (4.25) and overall acceptability (3.95) and lowest mean score for defoliation (1.00) and leaf wilting (1.1). It was followed by T_2 (30 ppm sodium hypochlorite) with a mean score for colour (3.55), texture (4.00), overall acceptability (3.35), defoliation (1.00) and leaf wilting (1.15). Minimum mean score was obtained for T_1 (2% brine) in colour (3.25), texture (3.6) and overall

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

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

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

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

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

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

acceptability (3.00) and highest for leaf wilting (2.1) and defoliation (1.45) after 2 h of storage.

After 3 h of storage, amaranthus dipped in 2 ppm ozonised water (T_3) recorded the highest mean score for colour (3.55), texture (3.3) and overall acceptability (3.5) and lowest mean score for leaf wilting (2.00) and defoliation (2.05) which was followed by 30 ppm sodium hypochlorite (T_2) treated amaranthus with a mean score of 2.30, 2.80, 2.90, 2.25 and 2.55 for colour, texture, overall acceptability, leaf wilting and defoliation respectively. Lowest mean score was observed for T_1 (2% brine) for colour (1.8), texture (2.00) and overall acceptability (2.2) and highest for leaf wilting (3.6) and defoliation (3.05).

After 4 h of storage, amaranthus dipped in 2 ppm ozonised water (T_3) showed highest mean score for colour (2.4), texture (2.75) and overall acceptability (2.00) and lowest for leaf wilting (2.3) and defoliation (2.65) which was followed by 30 ppm sodium hypochlorite (T_2). Minimum mean score was obtained by amaranthus dipped in 2% brine (T_1) for colour (1.85), texture (1.55) and overall acceptability (1.6) and maximum for leaf wilting (3.7) and defoliation (3.45).

Based on the efficiency of sanitizers in reducing the microbial count and superiority in physiological and physical parameters, 2 ppm ozonised water (T_3) was selected as the best sanitizer for further studies.

4.2. EFFECT OF PRETREATMENTS

Amaranthus sanitized using 2 ppm ozonised water was subjected to different pretreatments and was stored under different conditions. Physiological and physical parameters were analyzed daily till the end of shelf life.

4.2.1. Physiological parameters

Effect of different pretreatments and conditions on physiological parameters of amaranthus viz. physiological loss in weight and relative water content was recorded daily.

Table 7. Effect of pretreatments on physiological loss in weight (%) after 24 h of storage

Conditions	**Physiological loss in weight (%)**			
	Pretreatments			**Mean**
	R_1	R_2	R_3	
C_1	12.22^{h}	14.96^{g}	16.87^{f}	14.68^{c}
C_2	12.01^{h}	14.93^{g}	17.01^{f}	14.65^{c}
C_3	20.85^{e}	20.81^{e}	22.47^{d}	21.38^{b}
C_4	25.85^{b}	24.14^{c}	28.32^{a}	26.1^{a}
Mean	17.74^{c}	18.71^{b}	21.16^{a}	
CD (0.05)	Pretreatment (R) - 0.793	Conditions (C) -0.919	Interaction (R×C) - 1.587	

Table 8. Effect of pretreatments on physiological loss in weight (%) after 48 h of storage

Conditions	**Physiological loss in weight (%)**			
	Pretreatments			**Mean**
	R_1	R_2	R_3	
C_1	21.41^{c}	22.11^{b}	25.26^{a}	22.93^{a}
C_2	21.35^{c}	22.27^{b}	25.23^{a}	22.95^{a}
Mean	21.38^{c}	22.18^{b}	25.25^{a}	
CD (0.05)	Pretreatment (R) -0.121	Conditions (C) -0.097	Interaction (R×C)- 0.177	

4.2.1.1. Physiological loss in weight (PLW)

Significant difference in physiological loss in weight was observed between different pretreatments and conditions of storage (Table 7.). Amaranthus treated with 10 ppm BA (R_1) showed least PLW of 17.74 % after 24 hours of storage followed by 20 ppm BA (R_2) (18.71). Highest weight loss was recorded by distilled water (R_3) (21.16). Under different conditions of storage, minimum PLW (14.65) was recorded by amaranthus plugged with moist cotton (C_2) which was on par (14.68) with C_1 (cut stem end dipped in water) and maximum weight loss (26.1 %) was recorded by control (C_4) after 24 h of storage. When interaction effect was studied, sample treated with 10 ppm BA + moist cotton plugging (R_1C_2) showed the least (12.01) weight loss which was on par with R_1C_1 (10 ppm BA + cut stem end dipped in water) (12.22) and highest physiological loss in weight was recorded by control (R_3C_4) (28.32 %) after 24 h of storage.

Among different pretreatments 10 ppm BA (R_1) showed lowest cumulative PLW of 21.38 percent after 48 h of storage followed by 20 ppm BA (R_2) (22.18) (Table 8.). Highest loss in weight was observed in amaranthus treated with distilled water (R_3) (25.25). In case of conditions, C_1 (cut stem end dipped in water) had lowest PLW (22.93) which was on par with moist cotton plugging (C_2) (22.95). Observations for C_3 and C_4 were not recorded after 48 h of storage as it was unacceptable due to wilting. In interaction effects, 10 ppm BA + moist cotton plugging (R_1C_2) recorded minimum PLW (21.35) which was on par (21.41) with R_1C_1 (10 ppm BA + cut stem end dipped in water). Maximum PLW of 25.26 % was recorded in R_3C_1 (distilled water + cut stem end dipped in water) which was on par with R_3C_2 (distilled water + moist cotton plugging) (25.23).

4.2.1.2. Relative water content (RWC)

Among pretreated amaranthus, maximum relative water content (69.43) was observed in 10 ppm BA (R_1) treated sample which showed no significant difference

Table 9. Effect of pretreatments on relative water content (%) after 24 h of storage

Conditions	**Relative water content (%)**			
	Pretreatments			**Mean**
	R_1	R_2	R_3	
C_1	77.85^{a}	75.96^{ab}	74.87^{ab}	76.23^{a}
C_2	77.75^{a}	76.23^{ab}	73.3^{b}	75.76^{a}
C_3	67.19^{c}	66.58^{c}	64.14^{c}	65.97^{b}
C_4	54.9^{d}	55.95^{d}	53.64^{d}	54.83^{c}
Mean	69.43^{a}	68.69^{a}	66.49^{b}	
CD (0.05)	Pretreatment - 2.121 (R)	Conditions - 2.441 (C)	Interaction - 4.243 (R×C)	

Table 10. Effect of pretreatments on relative water content (%) after 48 h of storage

Conditions	**Relative water content (%)**			
	Pretreatments			**Mean**
	R_1	R_2	R_3	
C_1	70.45^{a}	67.15^{ab}	64.49^{bc}	67.36^{a}
C_2	70.22^{a}	67.78^{ab}	62.59^{c}	66.86^{a}
Mean	70.33^{a}	67.47^{b}	63.54^{c}	
CD (0.05)	Pretreatment – 2.710 (R)	Conditions – 2.215 (C)	Interaction - 3.830 (R×C)	

from 20 ppm BA (R_2) (68.69). The minimum water content (66.49) was noticed in R_3 (distilled water) after 24 h of storage (Table 9.). With respect to conditions, highest relative water content (76.23) was recorded by amaranthus with cut stem end dipped in water (C_1) which did not show any significant difference (75.76) with moist cotton plugging (C_2) after 24 h of storage. Relative water content was observed to be the lowest (54.83) in control (C_4) treatment. In case of interaction effects, 10 ppm BA + cut stem end dipped in water (R_1C_1) showed the highest (77.85) relative water content which was on par with 10 ppm BA + moist cotton plugging (R_1C_2) having 77.75 % of water content. Lowest RWC (53.64) was observed in samples treated with distilled water + control (R_3C_4) which was non-significant with R_1C_4 (54.9) and R_2C_4 (55.95).

After 48 h of storage, significant difference was observed between the pretreatments (Table 10.). Amaranthus treated with 10 ppm BA (R_1) recorded the highest RWC of 70.33 % followed by 20 ppm BA (R_2) (67.47). Lowest relative water content was recorded by distilled water (R_3) (63.54). Amaranthus stem waxed at cut end (C_3) and untreated sample (C_4) wilted and became unacceptable hence, observation were not recorded. Amaranthus with cut stem end dipped in water (C_1) had maximum RWC of 67.36% which was on par with C_2 (moist cotton plugging) with 66.86% water content. In case of interaction effects, R_1C_1 (10 ppm BA + cut stem end dipped in water) had the highest relative water content of 70.45% which was on par with R_1C_2 (10 ppm BA + moist cotton plugging) with RWC of 70.22%. Lowest relative water content (62.59) was observed in R_3C_2 (distilled water + moist cotton plugging) treated samples.

4.2.2. Visual parameters

Amaranthus treated with different pretreatments and conditions showed significant difference in visual quality parameters like colour, texture, leaf wilting, defoliation and overall acceptability (Table 11.).

Table 11. Effect of pretreatments on visual parameters after 24 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
R_1C_1	188.25	3.75	207.10	4.30	48.50	1.00	76.50	1.00	212.30	4.60
R_1C_2	176.67	3.65	197.50	4.00	48.50	1.00	76.50	1.00	193.10	4.20
R_1C_3	103.68	2.65	92.60	2.30	172.80	3.30	96.45	1.15	76.00	1.85
R_1C_4	101.70	2.45	66.40	1.90	186.33	3.55	143.00	1.50	64.00	1.65
R_2C_1	173.45	3.60	188.50	3.85	57.65	1.15	76.50	1.00	185.75	4.05
R_2C_2	164.98	3.55	176.50	3.65	60.70	1.20	87.65	1.05	183.50	4.00
R_2C_3	73.05	2.05	72.00	2.00	159.45	3.30	103.10	1.20	85.00	2.00
R_2C_4	47.26	1.60	46.80	1.55	191.58	3.65	183.10	1.70	52.00	1.45
R_3C_1	161.47	3.45	167.50	3.50	72.90	1.40	87.65	1.05	165.65	3.65
R_3C_2	151.33	3.35	137.50	3.00	75.95	1.45	96.45	1.15	142.70	3.20
R_3C_3	43.30	1.65	46.80	1.55	178.50	3.60	123.05	1.35	49.00	1.40
R_3C_4	39.10	1.55	46.80	1.55	189.80	3.70	189.55	1.85	37.00	1.20
CV. (0.05)	19.675									

Table 12. Effect of pretreatments on visual parameters after 48 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
R_1C_1	81.00	3.00	85.00	3.00	49.50	2.35	55.00	1.10	83.00	2.95
R_1C_2	78.14	3.05	81.75	3.05	40.50	2.20	52.00	1.05	75.59	2.95
R_2C_1	72.15	2.85	50.50	2.40	55.50	2.45	61.00	1.20	77.00	2.85
R_2C_2	72.15	2.85	76.38	2.85	52.50	2.40	52.00	1.05	77.00	2.85
R_3C_1	30.85	2.15	41.88	2.25	76.50	2.80	70.00	1.35	29.00	2.05
R_3C_2	30.85	2.15	27.50	2.00	88.50	3.00	73.00	1.40	26.00	2.00
CV. (0.05)	11.070									

Amaranthus pretreated with 10 ppm BA with cut stem end dipped in water (R_1C_1) had maximum mean score for colour (3.75), texture (4.3) and overall acceptability (4.6) and lowest score for leaf wilting (1.00) and defoliation (1.00) after 24 h of storage. Minimum mean score was observed in R_3C_4 (distilled water + control) for colour (1.55), texture (1.55) and overall acceptability (1.2) and maximum score for leaf wilting (3.7) and defoliation (1.85).

After 48 h of storage, significant difference was observed in visual parameters of pretreated amaranthus with different conditions (Table 12.). Amaranthus pretreated with 10 ppm BA with moist cotton plugging (R_1C_2) showed highest mean score for colour (3.05) and texture (3.05) and for overall acceptability $R_1C_1$and R_1C_2 scored highest mean score (2.95). In case of leaf wilting, R_1C_2 (10ppm BA + moist cotton plugging) scored lowest mean score (2.2) and for defoliation R_1C_2 and R_2C_2 scored the minimum mean score (1.05). Lowest mean score for colour (2.15), texture (2.00) and overall acceptability (2.00) was observed in R_3C_2 (distilled water + moist cotton plugging) with highest mean score for leaf wilting (3.00) and defoliation (1.4).

Results pertaining to physiological parameters like PLW, RWC, and visual parameters revealed that the pretreatment with 10 ppm BA and storage with moist cotton plugging of stem end significantly increased the shelf life. Hence this treatment R_1C_2 was taken for the further studies.

4.3. PREPACKAGING AND STORAGE

Amaranthus sanitized with ozonised water 2 ppm and pretreated with 10 ppm BA with moist cotton plugging of stem end were prepackaged in different packaging

materials and stored under room temperature and refrigerated conditions. Physiological and physical parameters were recorded daily till the end of shelf life.

4.3.1. Physiological parameters

Effect of prepackaging and storage conditions on physiological parameters of harvested amaranthus was studied and results are described below.

4.3.1.1. Physiological loss in weight (PLW)

Physiological loss in weight in different packages after 24 h of storage at room temperature and refrigerated conditions are given in Table 13. Prepackaging treatments and storage conditions significantly differed in physiological loss in weight. Treated amaranthus prepackaged in macro ventilated LDPE (150 gauge) with absorbent paper (P_1) showed the minimum physiological weight loss (4.93) which was followed by macro ventilated PP (100 gauge) with (P_3) and without absorbent paper (P_4) recording a weight loss of 5.90 and 5.79 % respectively after 24 h of storage. Highest PLW (9.64 %) was observed in treatment P_7 (without prepackaging).

Under different storage conditions, refrigerated condition (S_2) recorded minimum PLW of 4.44% whereas room temperature (S_1) recorded maximum weight loss of 9.91% after 24 h of storage. When interaction effects were studied P_4S_2 (Macro ventilated PP (100 gauge) without absorbent paper + refrigerated storage) showed minimum PLW (2.24) and maximum weight loss (13.2) was observed in P_7S_1 (without prepackaging + room temperature) after 24 h of storage.

After 48 h of storage, P_1 showed the lowest cumulative physiological loss in weight (10.14) followed by P_4 (10.77) and highest PLW of 17.10 % was noticed in P_7 (Table 14). With respect to storage conditions, refrigerated storage (S_2) recorded the least PLW (10.32) and room temperature storage (S_1) recorded highest cumulative weight loss of 16.21 %. Treated amaranthus packaged in macro ventilated LDPE (150 gauge) without absorbent paper stored under refrigerated condition (P_2S_2) had lowest PLW (7.71) which exhibited no significant difference with P_4S_2 (macro ventilated PP (100 gauge) without absorbent paper + refrigerated storage) and P_1S_2 (macro ventilated LDPE (150 gauge) with absorbent paper + refrigerated storage) with a weight loss of 8.04 and 9.16 % respectively. Highest PLW (22.1%) was observed in

Table 13. Effect of prepackaging and storage conditions on physiological loss in weight (%) after 24 h of storage

Prepackaging treatments	**Physiological loss in weight (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	5.71^{def}	4.15^{fg}	4.93^{d}
P_2	9.71^{c}	2.64^{gh}	6.17^{c}
P_3	7.01^{d}	4.80^{efg}	5.90^{cd}
P_4	9.35^{c}	2.24^{h}	5.79^{cd}
P_5	11.42^{b}	5.42^{def}	8.42^{b}
P_6	12.97^{ab}	5.79^{de}	9.38^{ab}
P_7	13.2^{a}	6.08^{de}	9.64^{a}
Mean	9.91^{a}	4.44^{b}	
CD (0.05)	Prepackaging - 1.158 (P)	Storage - 0.613 (S)	Interaction - 1.639 (P×S)

Table 14. Effect of prepackaging and storage conditions on physiological loss in weight (%) after 48 h of storage

Prepackaging treatments	**Physiological loss in weight (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	11.12^{ef}	9.16^{g}	10.14^{d}
P_2	15.43^{c}	7.71^{g}	11.57^{c}
P_3	12.73^{de}	10.96^{f}	11.84^{c}
P_4	13.49^{d}	8.04^{g}	10.77^{cd}
P_5	17.62^{b}	12.01^{def}	14.81^{b}
P_6	20.97^{a}	12.21^{def}	16.59^{a}
P_7	22.1^{a}	12.08^{def}	17.10^{a}
Mean	16.21^{a}	10.32^{b}	
CD (0.05)	Prepackaging – 1.230 (P)	Storage – 0.654 (S)	Interaction – 1.734 (P×S)

treated amaranthus without prepackaging stored under room temperature (P_7S_1) which showed no significant difference (20.97) with P_6S_1 (cling film + room temperature).

Among prepackaged amaranthus, highest PLW (21.58) was observed in treatment without prepackaging (P_7) after 72 h of storage (Table 15.). Macro ventilated LDPE (150 gauge) with absorbent paper (P_1) recorded lowest physiological loss in weight of 12.96 % followed by P_3 (15.23) which was on par with P_4 (15.69) and P_2 (16.06). Under different storage conditions, refrigerated storage (S_2) showed the lowest weight loss (12.96) and highest weight loss (21.42) was recorded by room temperature condition (S_1). When interaction effects were studied, P_2S_2 (macro ventilated LDPE (150 gauge) without absorbent paper + refrigerated storage) recorded minimum weight loss (10.17 %) with no significant difference for P_4S_2 (macro ventilated PP cover (100 gauge) without absorbent paper + refrigerated storage) with a physiological weight loss of 10.24 % after 72 h of storage.

All the treatment stored under room temperature was spoiled by 96 h of storage (Table 16.). Amaranthus prepackaged and stored under refrigerated conditions, P_4 (macro ventilated PP (100 gauge) without absorbent paper) had lowest physiological loss in weight of 13.61 % followed by P_2 (macro ventilated LDPE (150 gauge) without absorbent paper) and P_1 (macro ventilated LDPE (150 gauge) with absorbent paper) with physiological loss in weight of 14.43 and 14.59 %, respectively. Maximum weight loss (17.78) was recorded in P_7 (without prepackaging) which showed no significant difference from P_6 (cling film) and P_5 (sleeve wrap with LDPE).

After 120 h of storage, except P_2 and P_4, other treatments were not determined due to wilting and spoilage (Table 16.). No significant difference was observed between treatments. The treatment P_4 (macro ventilated PP (100 gauge) without absorbent paper) and P_2 (macro ventilated LDPE (150 gauge) without absorbent paper) recorded a weight loss of 17.29 and 17.63 percent respectively.

Table 15. Effect of prepackaging and storage conditions on physiological loss in weight (%) after 72 h of storage

Prepackaging treatments	**Physiological loss in weight (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	13.21^{h}	12.71^{h}	12.96^{d}
P_2	21.95^{cd}	10.17^{i}	16.06^{c}
P_3	17.40^{e}	13.06^{h}	15.23^{c}
P_4	21.14^{d}	10.24^{i}	15.69^{c}
P_5	23.66^{bc}	15.03^{fg}	19.35^{b}
P_6	25.07^{b}	13.86^{gh}	19.47^{b}
P_7	27.54^{a}	15.61^{f}	21.58^{a}
Mean	21.42^{a}	12.96^{b}	
CD (0.05)	Prepackaging – 1.227 (P)	Storage – 0.652 (S)	Interaction – 1.735 (P×S)

Table 16. Effect of prepackaging and storage conditions on physiological loss in weight, %

Treatments	**PLW after refrigerated storage**	
	96 h	**120 h**
P_1S_2	14.59^{bc}	ND
P_2S_2	14.43^{bc}	17.63
P_3S_2	15.34^{b}	ND
P_4S_2	13.61^{c}	17.29
P_5S_2	17.38^{a}	ND
P_6S_2	17.58^{a}	ND
P_7S_2	17.78^{a}	ND
CD (0.05)	0.989	NS

ND: Not determined due to spoilage and wilting; NS: non significant

4.3.1.2. Relative water content (RWC)

Relative water content of amaranthus prepackaged in macro ventilated PP cover (100 gauge) without absorbent paper (P_4) showed highest water content of 84.85 % after 24 h of storage (Table 17.). It was followed by P_3 (Macro ventilated PP cover (100 gauge) with absorbent paper) and P_2 (Macro ventilated LDPE cover (150 gauge) without absorbent paper) with a relative water content of 84.08 and 83.74 % respectively. Lowest relative water content (79.12) was observed in P_7 (without prepackaging) which was on par with P_5 (sleeve wrap with LDPE) (79.83) and P6 (cling film) (80.42). Under different storage conditions, refrigerated storage (S_2) recorded the highest water content (83.96) and room temperature (S_1) recorded the lowest water content of 80.34 %. Amaranthus prepackaged in macro ventilated PP (100 gauge) without absorbent paper stored under refrigerated storage (P_4S_2) had highest relative water content of 85.87 % which showed no significant difference with P_2S_2 (macro ventilated LDPE (150 gauge) without absorbent paper + refrigerated storage) (85.79). Lowest relative water content (76.95) was noticed in P_7S_1 (without prepackaging + room temperature) which was on par with P_6S_1 (cling film + room temperature) and P_5S_1 (sleeve wrap with LDPE + room temperature) with a water content of 77.7 and 78.29 % after 24 hours of storage.

After 48 h of storage, P_4 (macro ventilated PP (100 gauge) without absorbent paper) recorded highest relative water content (82.49) followed by P_2 (macro ventilated LDPE (150 gauge) without absorbent paper) (80.42) (Table 18.). Minimum water content (69.75) was observed in P_7 (without prepackaging). With respect to storage conditions, refrigerated storage (S_2) had highest relative water content (78.40) and treated amaranthus stored at room temperature (S_1) had relative water content of 73.77 % only. Among the interaction effects, P_4S_2 (macro ventilated PP (100 gauge) without absorbent paper + refrigerated storage) showed maximum relative water content of 84.42 % followed by P_2S_2 (83.94 %). Lowest relative water content (68.93) was observed in P_6S_1 (cling film + room temperature) which showed no significant difference with P_7S_1 (without prepackaging + room temperature) (68.98),

Table 17. Effect of prepackaging and storage conditions on relative water content (%) after 24 h of storage

Prepackaging treatments	**Relative water content (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	81.42^{e}	84.55^{abc}	82.98^{b}
P_2	81.68^{e}	85.79^{a}	83.74^{ab}
P_3	82.48^{de}	85.67^{ab}	84.08^{ab}
P_4	83.84^{bcd}	85.87^{a}	84.85^{a}
P_5	78.29^{f}	81.38^{e}	79.83^{c}
P_6	77.7^{f}	83.13^{cde}	80.42^{c}
P_7	76.95^{f}	81.29^{e}	79.12^{c}
Mean	80.34^{b}	83.96^{a}	
CD (0.05)	Prepackaging – 1.341 (P)	Storage – 0.726 (S)	Interaction – 1.905 (P×S)

Table 18. Effect of prepackaging and storage conditions on relative water content (%) after 48 h of storage

Prepackaging treatments	**Relative water content (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	74.79^{def}	81.92^{bc}	78.35^{c}
P_2	76.92^{d}	83.94^{ab}	80.42^{b}
P_3	75.69^{de}	80.72^{c}	78.21^{c}
P_4	80.55^{c}	84.42^{a}	82.49^{a}
P_5	70.56^{g}	74.24^{ef}	72.39^{d}
P_6	68.93^{g}	73.03^{f}	70.98^{de}
P_7	68.98^{g}	70.53^{g}	69.75^{e}
Mean	73.77^{b}	78.40^{a}	
CD (0.05)	Prepackaging – 1.644 (P)	Storage – 0.879 (S)	Interaction – 2.327 (P×S)

P_5S_1 (sleeve wrap with LDPE + room temperature) (70.56) and P_7S_2 (without prepackaging + refrigerated storage) (70.53) after 48 h of storage.

Treated amaranthus prepackaged in macro ventilated PP (100 gauge) without absorbent paper (P_4) recorded highest relative water content (73.43) which was followed by P_2 (macro ventilated LDPE (150 gauge) without absorbent paper) and P_1 (macro ventilated LDPE (150 gauge) with absorbent paper) with water content of 72.79 and 71.62 %, respectively after 72 h of storage (Table 19.). Among different storage conditions refrigerated storage (S_2) recorded highest relative water content of 73.55 % and treated amaranthus stored at room temperature showed 65.43 % of relative water content. Treated amaranthus stored under refrigerated condition prepackaged in macro ventilated PP (100 gauge) without absorbent paper (P_4S_2) recorded maximum relative water content (77.02) which showed no significant difference with P_2S_2 (macro ventilated LDPE (150 gauge) without absorbent paper + refrigerated storage) (76.95) and P_1S_2 (macro ventilated LDPE (150 gauge) with absorbent paper + refrigerated storage) (75.67). Minimum relative water content (56.48 %) was observed in amaranthus stored at room temperature without prepackaging (P_7S_1) after 72 h of storage.

At 96 h of storage, prepackaged amaranthus stored at room temperature was damaged due to wilting and spoilage hence observations were not recorded (Table 20.). Under refrigerated storage, P_2S_2 (macro ventilated LDPE (150 gauge) without absorbent paper) recorded highest relative water content (74.25) which recorded no significant difference with P_4S_2 (macro ventilated PP (100 gauge) without absorbent) (74.23). Lowest relative water content was observed in P_6S_2 (64.12) which showed no significant difference with P_7S_2 (64.50) and P_5S_2 (65.98).

Amaranthus stored under refrigerated condition and prepackaged with macro ventilated PP (100 gauge) without absorbent paper (P_4S_2) (67.91) and macro ventilated LDPE (150 gauge) without absorbent paper (P_2S_2) (63.93) showed no significant difference at 120 h of storage. Observation of other treatments were not recorded due to spoilage and wilting (Table 20.).

Table 19. Effect of prepackaging and storage conditions on relative water content (%) after 72 h of storage

Prepackaging treatments	**Relative water content (%)**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	67.56^{e}	75.67^{a}	71.62^{ab}
P_2	68.62^{de}	76.95^{a}	72.79^{ab}
P_3	68.41^{e}	73.95^{ab}	71.17^{b}
P_4	69.83^{cde}	77.02^{a}	73.43^{a}
P_5	64.37^{f}	71.86^{bc}	68.12^{c}
P_6	62.75^{f}	71.60^{bcd}	67.17^{c}
P_7	56.48^{g}	67.86^{e}	62.18^{d}
Mean	65.43^{b}	73.55^{a}	
CD (0.05)	Prepackaging – 2.194 (P)	Storage – 1.175 (S)	Interaction – 3.106 (P×S)

Table 20. Effect of prepackaging and storage conditions on relative water content, %

Prepackaging treatments	**RWC after refrigerated storage**	
	96 h	**120 h**
P_1S_2	71.99^{ab}	ND
P_2S_2	74.25^{a}	63.93
P_3S_2	69.66^{b}	ND
P_4S_2	74.23^{a}	67.91
P_5S_2	65.98^{c}	ND
P_6S_2	64.12^{c}	ND
P_7S_2	64.5^{c}	ND
CD (0.05)	2.861	NS

ND: Not determined due to storage and wilting; NS: Non Significant

4.3.2. Nutritional parameters

Effect of prepackaging and storage on nutritional parameters viz. ascorbic acid, β-carotene, chlorophyll, oxalates, calcium and iron were recorded initially for the pretreated amaranthus before storage and at the end of shelf life.

4.3.2.1. Ascorbic acid

Initial ascorbic acid content ranged from 18.55 mg/100g to 19.05 mg/100g in all prepackaging treatments. At the end of shelf life ascorbic acid content of prepackaged amaranthus showed significant difference (Table 21.). Highest ascorbic acid (7.39 mg/100g) was noticed in amaranthus prepackaged in macro ventilated PP (100 gauge) without absorbent (P_4) followed by P_2 (macro ventilated LDPE (150 gauge) without absorbent paper) (6.65). Lowest ascorbic acid of 4.02 mg/100g was recorded by P_7 (without prepackaging). Among storage conditions, refrigerated storage retained highest ascorbic acid of 7.59 mg/100g whereas amaranthus stored at room temperature retained 3.26 mg/100g only.

When interaction effects were studied, P_4S_2 (macro ventilated PP (100 gauge) without absorbent + refrigerated storage) showed highest ascorbic acid retention (10.78) which was followed by P_2S_2 (macro ventilated LDPE (150 gauge) without absorbent paper) (9.27). Lowest ascorbic acid was retained by P_7S_1 (without prepackaging + room temperature) (2.51) and P_1S_1 (macro ventilated LDPE (150 gauge) with absorbent paper + room temperature) (2.51).

4.3.2.2. β-carotene

Initial β-carotene content ranged from 6.25 to 6.26 µg/100g in all prepackaging treatments with no significant difference. β-carotene content of different prepackaging were significantly differed at the end of shelf life (Table 22.). Macro ventilated PP cover (100 gauge) without absorbent paper (P_4) had highest β-carotene content of 3.71 µg/100g followed by P_2 (macro ventilated LDPE cover (150 gauge) without absorbent paper) (3.14). Least β-carotene content of 1.77 µg/100g was

Table 21. Effect of prepackaging and storage conditions on ascorbic acid content, mg/100g

Prepackaging treatments	**Before storage**	**Ascorbic acid**		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	18.55	2.51^{i}	7.77^{c}	5.14^{c}
P_2	18.65	4.01^{g}	9.27^{b}	6.65^{b}
P_3	18.80	3.26^{h}	7.02^{d}	5.14^{c}
P_4	19.05	4.01^{g}	10.78^{a}	7.39^{a}
P_5	18.97	3.26^{h}	6.27^{e}	4.76^{d}
P_6	18.92	3.26^{h}	6.52^{e}	4.89^{cd}
P_7	18.55	2.51^{i}	5.51^{f}	4.02^{e}
Mean	NS	3.26^{b}	7.59^{a}	
CD (0.05)		Prepackaging – 0.270 (P)	Storage – 0.140 (S)	Interaction – 0.389 (P×S)

recorded by P_1 (macro ventilated LDPE cover (150 gauge) with absorbent paper). Under different storage conditions, refrigerated storage (S_2) recorded highest (4.16) retention of β-carotene and room temperature storage (S_1) had 0.80 µg/100g β-carotene only.

Amaranthus prepackaged in macro ventilated LDPE cover (150 gauge) without absorbent paper under refrigerated storage (P_2S_2) had highest (5.66 µg/100g) β-carotene content followed by P_4S_2 (macro ventilated PP cover (100 gauge) without absorbent paper + refrigerated storage) (5.34) at the end of shelf life. Minimum β-carotene content (0.47) was observed in P_1S_1 (macro ventilated LDPE cover (150 gauge) with absorbent paper + room temperature).

4.3.2.3. Chlorophyll

Total chlorophyll content of all prepackaged amaranthus before storage ranged from 5.31 to 5.34 mg g^{-1}. Amaranthus prepackaged in macro ventilated PP cover (100 gauge) without absorbent paper (P_4) recorded highest total chlorophyll content of 3.48 mg g^{-1} followed by macro ventilated LDPE cover (150 gauge) without absorbent paper (P_2) (2.98) (Table 23.). Lowest (1.56) total chlorophyll content was observed in P_7 (without prepackaging) which showed no significant difference with P_5 (Sleeve wrap with LDPE) (1.69) at end of shelf life. Under different storage conditions, amaranthus kept in refrigerated storage (S_2) had highest total chlorophyll content of 2.76 mg/g and amaranthus stored at room temperature (S_1) recorded the lowest total chlorophyll content of 1.75 mg g^{-1}.

At the end of shelf life P_4S_2 (macro ventilated PP cover (100 gauge) without absorbent paper + refrigerated storage) recorded maximum total chlorophyll retention of 4.42 mg g^{-1} which was on par with P_2S_2 (macro ventilated LDPE cover (150 gauge) without absorbent paper + refrigerated storage). Lowest total chlorophyll (1.19 mg g^{-1}) was observed in P_7S_1 (without prepackaging + room temperature).

Table 22. Effect of prepackaging and storage conditions on β-carotene content, µg/100g

Prepackaging treatments	Before storage	β-carotene content		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	6.25	0.47^{l}	3.08^{g}	1.77^{g}
P_2	6.26	0.60^{jk}	5.66^{a}	3.14^{b}
P_3	6.25	0.61^{j}	3.27^{e}	1.94^{e}
P_4	6.26	2.09^{h}	5.34^{b}	3.71^{a}
P_5	6.25	0.59^{k}	4.36^{c}	2.48^{c}
P_6	6.25	0.59^{k}	4.28^{d}	2.44^{d}
P_7	6.25	0.63^{i}	3.17^{f}	1.89^{f}
Mean	NS	0.80^{b}	4.16^{a}	
CD (0.05)		Prepackaging - 0.010 (P)	Storage - 0.008 (S)	Interaction- 0.016 (P×S)

Table 23. Effect of prepackaging and storage conditions on total chlorophyll content, mgg^{-1}

Prepackaging treatments	Before storage	Total chlorophyll		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	5.33	1.53^{ef}	2.21^{bcd}	1.87^{cd}
P_2	5.31	1.83^{de}	4.13^{a}	2.98^{b}
P_3	5.31	1.51^{ef}	2.58^{b}	2.04^{c}
P_4	5.34	2.54^{b}	4.42^{a}	3.48^{a}
P_5	5.34	1.78^{de}	1.62^{ef}	1.69^{d}
P_6	5.33	1.91^{cde}	2.38^{bc}	2.15^{c}
P_7	5.32	1.19^{f}	1.93^{cde}	1.56^{d}
Mean	NS	1.75^{b}	2.76^{a}	
CD (0.05)		Prepackaging – 0.339 (P)	Storage – 0.175 (S)	Interaction – 0.473 (P×S)

4.3.2.4. Calcium

Before storage pretreated amaranthus had calcium content ranging from 6.36 to 6.45 % and was noted to be non significant. At the end of shelf life, highest (5.79) calcium content was seen in treated amaranthus prepackaged in macro ventilated PP (100 gauge) without absorbent paper (P_4) which was on par with P_3 (macro ventilated PP (100 gauge) with absorbent paper) with a calcium content of 5.72% (Table 24.). Lowest (4.02) calcium content was recorded in P_6 (cling film). Among storage conditions, no significant difference was observed in calcium content. Refrigerated storage (S_2) had highest calcium content of 5.15% on par with room temperature storage (S_1) of 4.42%.

With respect to interaction effects, maximum (5.99) calcium content was observed in P_4S_2 (macro ventilated PP (100 gauge) without absorbent paper + refrigerated storage) followed by P_2S_2 (5.91) on par with P_3S_2 (5.84). Minimum (3.46) calcium content was recorded by P_1S_1 (macro ventilated LDPE (150 gauge) with absorbent paper + room temperature) at the end of shelf life.

4.3.2.5. Iron

Pretreated amaranthus before storage showed non-significance in initial iron content which ranged from 0.16 to 0.17 %. Among prepackaged amaranthus, highest (0.09) iron content was noted in P_5 (sleeve wrap with LDPE) which differed non significantly with P_4 (macro ventilated PP (100 gauge) without absorbent paper), P_2 (macro ventilated LDPE (150 gauge) without absorbent paper), P_6 (cling film) and P_7 (without prepackaging), all having iron content of 0.08% (Table 25.). Minimum iron content was observed in P_1 (macro ventilated LDPE (150 gauge) with absorbent paper) (0.06) which was on par with P_3 (macro ventilated PP (100 gauge) with absorbent paper) (0.06). At the end of shelf life amaranthus stored under refrigerated conditions showed maximum iron content of 0.08 % than that at room temperature with 0.07 %.

Table 24. Effect of prepackaging and storage conditions on calcium content, %

Prepackaging treatments	**Before storage**	**Calcium**		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	6.36	3.46^{c}	5.04^{abc}	4.25^{ab}
P_2	6.45	3.75^{bc}	5.91^{ab}	4.83^{ab}
P_3	6.45	5.60^{abc}	5.84^{ab}	5.72^{a}
P_4	6.45	5.58^{abc}	5.99^{a}	5.79^{a}
P_5	6.36	4.22^{abc}	4.92^{abc}	4.57^{ab}
P_6	6.42	3.69^{bc}	4.35^{abc}	4.02^{b}
P_7	6.45	4.67^{abc}	4.00^{abc}	4.34^{ab}
Mean	NS	4.42^{a}	5.15^{a}	
CD (0.05)		Prepackaging – 1.574 (P)	Storage – 0.846 (S)	Interaction – 2.224 (P×S)

Table 25. Effect of prepackaging and storage conditions on iron content, %

Prepackaging treatments	**Before storage**	**Iron**		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	0.16	0.05^{d}	0.07^{bc}	0.06^{b}
P_2	0.16	0.08^{ab}	0.08^{ab}	0.08^{a}
P_3	0.17	0.05^{d}	0.06^{cd}	0.06^{b}
P_4	0.17	0.07^{bc}	0.09^{a}	0.08^{a}
P_5	0.17	0.08^{ab}	0.09^{a}	0.09^{a}
P_6	0.16	0.07^{bc}	0.09^{a}	0.08^{a}
P_7	0.16	0.06^{cd}	0.09^{a}	0.08^{a}
Mean	NS	0.07^{b}	0.08^{a}	
CD (0.05)		Prepackaging – 0.011 (P)	Storage – 0.005 (S)	Interaction – 0.012 (P×S)

When interaction effects were studied, highest iron content of 0.09 % was recorded by P_4S_2 (macro ventilated PP cover (100 gauge) without absorbent paper + refrigerated storage), P_5S_2 (Sleeve wrap with LDPE + refrigerated storage), P_6S_2 (Cling film + refrigerated storage) and P_7S_2 (without prepackaging + refrigerated storage). Minimum iron content was recorded by P_1S_1 (macro ventilated LDPE (150 gauge) with absorbent paper + room temperature) (0.05) and P_3S_1 (Macro ventilated PP (100 gauge) with absorbent paper + room temperature) (0.05) at the end of shelf life.

4.3.2.6. Oxalates

Before storage, fresh pretreated amaranthus had oxalate content ranged from 1.70 to 1.72 %. Amaranthus without prepackaging (P_7) and sleeve wrap with LDPE (P_5) showed lowest oxalate content of 1.41 % which was followed by P_6 (cling film) (1.42 %) (Table 26.). Highest oxalate content was observed in P_1 (macro ventilated LDPE (150 gauge) with absorbent paper) (1.60 %) and P_3 (macro ventilated PP (100 gauge) with absorbent paper) (1.60 %). Among storage conditions, refrigerated condition (1.33 %) recorded lowest oxalate content and maximum was recorded by room temperature storage (1.71%) at the end of shelf life.

When interaction was studied amaranthus without prepackaging stored under refrigerated condition recorded lowest (P_7S_2) oxalate content of 1.07 % followed by P_5S_2 (sleeve wrap with LDPE + refrigerated storage) and P_6S_2 (cling film + refrigerated storage). Maximum oxalate content (1.75 %) was observed in P_7S_1 (without prepackaging + room temperature) and P_6S_1 (cling film + room temperature).

4.3.3. Microbial population

Different prepackaging showed significant difference in microbial population at the end of shelf life (Table 27.). Amaranthus prepackaged in P_4S_2 (macro ventilated PP cover (100 gauge) without absorbent paper + refrigerated storage) recorded a

Table 26. Effect of prepackaging and storage conditions on oxalate content, %

Prepackaging treatments	**Before storage**	**Oxalate content**		
		Storage conditions		
		S_1	S_2	**Mean**
P_1	1.70	1.69^{c}	1.51^{f}	1.60^{a}
P_2	1.72	1.68^{d}	1.50^{fg}	1.59^{b}
P_3	1.71	1.70^{c}	1.50^{fg}	1.60^{a}
P_4	1.71	1.68^{d}	1.49^{g}	1.59^{b}
P_5	1.72	1.72^{b}	1.10^{h}	1.41^{d}
P_6	1.70	1.74^{a}	1.09^{h}	1.42^{c}
P_7	1.72	1.75^{a}	1.07^{i}	1.41^{d}
Mean	NS	1.71^{a}	1.33^{b}	
CD (0.05)		Prepackaging – 0.007 (P)	Storage – 0.002 (S)	Interaction – 0.018 (P×S)

Table 27. Effect of prepackaging and storage conditions on bacterial population of amaranthus

Prepackaging treatments	**Bacterial population $\times 10^7$ cfug^{-1}**		
	Storage conditions		
	S_1	S_2	**Mean**
P_1	7.15^{f}	7.71^{d}	7.43^{c}
P_2	6.41^{g}	5.95^{h}	6.18^{d}
P_3	19.00^{a}	8.66^{b}	13.83^{a}
P_4	2.98^{j}	1.11^{m}	2.04^{f}
P_5	3.48^{i}	1.37^{l}	2.43^{e}
P_6	8.26^{c}	7.50^{e}	7.88^{b}
P_7	1.53^{k}	0.12^{n}	0.83^{g}
Mean	6.97^{a}	4.64^{b}	
CD (0.05)	Prepackaging – 0.006 (P)	Storage – 0.001 (S)	Interaction – 0.018 (P×S)

microbial population of 1.11×10^7 cfug^{-1} after 120 h of storage followed by P_5S_2 (sleeve wrap + refrigerated storage). Highest microbial population of 19×10^7 cfug^{-1} was observed in P_3S_1 (macro ventilated PP cover (100 gauge) with absorbent paper + room temperature). Refrigerated storage conditions recorded the lowest microbial population (4.64×10^7 cfug^{-1}) when compared with room temperature storage.

4.3.4. Visual parameters

Effects of different prepackaging and storage conditions on visual parameters of treated amaranthus were statistically analyzed using Kruskall-Wallis test and found that treatments differed significantly.

After 24 h of storage, treated amaranthus prepackaged in macro ventilated PP (100 gauge) with (P_3S_2) and without absorbent paper (P_4S_2) and macro ventilated LDPE (150 gauge) with (P_1S_2) and without absorbent paper (P_2S_2) stored under refrigerated condition showed highest mean score for colour (5.00) (Table 28.). Maximum mean score for texture (4.95) was recorded by P_1S_2 and P_4S_2. Highest mean score for overall acceptability (5.00) was scored by P_3S_2 and P_4S_2. Lowest mean score for colour (4.00), texture (3.75) and overall acceptability (3.9) was noticed in P_7S_1 (without prepackaging + room temperature). With respect to leaf wilting lowest mean score (1.00) was recorded by all prepackaged amaranthus stored under refrigerated condition and highest mean score (1.4) was noticed in P_5S_1, P_6S_1 and P_7S_1. Minimum mean score for defoliation (1.00) was observed in P_1S_2, P_2S_2 and P_4S_2 and maximum mean score (1.45) was obtained by P_5S_1 and P_7S_1. In case of decay lowest mean score (1.00) was recorded by all treatments except P_1S_1, P_3S_1 and P_6S_1. Highest mean score for decay (1.25) was observed in $P_1S_1$and P_3S_1.

Maximum score for colour (5.00) was obtained by amaranthus prepackaged in P_2S_1, P_2S_2, P_4S_1 and P_4S_2 after 48 h of storage (Table 29.). In case of texture (5.00) and overall acceptability (5.00) highest mean score was recorded by P_2S_2 and P_4S_2. Lowest mean score was observed in P_6S_1 and P_7S_1 for colour (4.00) and P_7S_1 for texture (2.3) and overall acceptability (2.75). With respect to leaf wilting (1.00)

Table 28. Effect of prepackaging and storage conditions on visual parameters after 24 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Decay		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
P_1S_1	121.50	4.60	118.48	4.45	138.50	1.10	132.50	1.15	168.50	1.25	149.13	4.75
P_1S_2	177.50	5.00	186.23	4.95	124.50	1.00	111.50	1.00	133.50	1.00	162.88	4.85
P_2S_1	121.50	4.60	159.13	4.75	138.50	1.10	132.50	1.15	133.50	1.00	156.00	4.80
P_2S_2	177.50	5.00	179.45	4.90	124.50	1.00	111.50	1.00	133.50	1.00	176.63	4.95
P_3S_1	121.50	4.60	118.48	4.45	138.50	1.10	146.50	1.25	168.50	1.25	140.10	4.65
P_3S_2	177.50	5.00	179.45	4.90	124.50	1.00	146.50	1.25	133.50	1.00	183.50	5.00
P_4S_1	170.50	4.95	165.90	4.80	138.50	1.10	132.50	1.15	133.50	1.00	156.00	4.80
P_4S_2	177.50	5.00	186.23	4.95	124.50	1.00	111.50	1.00	133.50	1.00	183.50	5.00
P_5S_1	93.50	4.40	71.05	4.10	180.50	1.40	174.50	1.45	133.50	1.00	107.87	4.45
P_5S_2	163.50	4.90	165.90	4.80	124.50	1.00	139.50	1.20	133.50	1.00	142.25	4.70
P_6S_1	114.50	4.55	60.55	3.90	180.50	1.40	167.50	1.40	161.50	1.20	107.87	4.45
P_6S_2	163.50	4.90	165.90	4.80	124.50	1.00	139.50	1.20	133.50	1.00	142.25	4.70
P_7S_1	37.50	4.00	44.38	3.75	180.50	1.40	174.50	1.45	133.50	1.00	51.15	3.90
P_7S_2	149.50	4.80	165.90	4.80	124.50	1.00	146.50	1.25	133.50	1.00	107.88	4.45
CV. (0.05)	22.362											

Table 29.Effect of prepackaging and storage conditions on visual parameters after 48 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Decay		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
P_1S_1	108.50	4.45	84.60	3.70	103.50	1.25	193.90	1.60	249.25	2.50	74.13	3.75
P_1S_2	157.50	4.80	189.10	4.80	109.40	1.30	110.50	1.20	143.33	1.35	175.75	4.75
P_2S_1	185.50	5.00	140.95	4.35	168.40	1.80	221.70	1.00	162.75	1.50	175.75	4.75
P_2S_2	185.50	5.00	210.50	5.00	103.50	1.25	110.50	1.40	110.95	1.10	204.50	5.00
P_3S_1	108.50	4.45	122.05	4.05	138.90	1.55	110.50	1.80	249.25	2.50	74.13	3.75
P_3S_2	157.50	4.80	183.75	4.75	103.50	1.25	110.50	1.60	130.38	1.30	175.75	4.75
P_4S_1	185.50	5.00	140.95	4.35	133.00	1.50	110.50	1.00	126.08	1.25	175.75	4.75
P_4S_2	185.50	5.00	210.50	5.00	74.00	1.00	138.30	1.20	110.95	1.10	204.50	5.00
P_5S_1	150.50	4.75	40.50	3.00	235.80	2.75	166.10	1.00	143.33	1.35	89.50	4.00
P_5S_2	164.50	4.85	189.10	4.80	103.50	1.25	193.90	1.00	98.00	1.00	187.25	4.85
P_6S_1	45.50	4.00	59.40	3.30	253.00	3.00	127.40	1.00	143.33	1.35	28.00	3.00
P_6S_2	164.50	4.85	199.80	4.90	103.50	1.25	110.50	1.30	110.95	1.10	193.00	4.90
P_7S_1	45.50	4.00	17.40	2.30	233.50	3.00	152.20	1.00	98.00	1.0	21.75	2.75
P_7S_2	122.50	4.55	178.40	4.70	103.50	1.25	110.50	1.00	98.00	1.00	187.25	4.85
CV. (0.05)	22.362											

lowest mean score was scored by P_4S_2 and for defoliation P_2S_1, P_4S_1, P_5S_1, P_5S_2, P_6S_1, P_7S_1 and P_7S_2 scored lowest mean score (1.00). Highest mean score (3.00) was noticed in P_6S_1 and P_7S_1 for leaf wilting and P_3S_1 (1.8) for defoliation. Minimum mean score for decay (1.00) was recorded by P_5S_2, P_7S_1 and P_7S_2 and maximum in P_1S_1 (2.50) and P_3S_1 (2.50).

After 72 h of storage amaranthus prepackaged in different prepackages stored under room temperature and refrigerated condition showed significant difference (Table 30.). Highest mean score was recorded by P_2S_2 (3.65) for colour and P_4S_2 for texture (3.85) and overall acceptability (3.4). Lowest mean score was observed in P_7S_1 for colour (2.15), texture (1.15) and overall acceptability (1.05). In case of leaf wilting, lowest mean score was recorded by P_4S_2 (1.15) and highest by P_7S_1 (4.15). Minimum mean score for defoliaton was noticed in P_6S_2 (1.55) and maximum in P_3S_1 (3.05). Mean score for decay was observed lowest in P_7S_2 (1.05). Highest mean score for decay (3.25) was obtained by P_1S_1.

After 96 h of storage, maximum mean score for colour (3.00) was recorded by P_2S_2 and P_4S_2 and rest of the prepackaging treatments scored the lowest mean score (2.35) (Table 31.). For texture (3.05) and overall acceptability (3.05) P_4S_2 recorded the highest mean score. Minimum mean score was obtained by P_1S_2 and P_7S_2 for texture (1.3) and P_6S_2 as well as P_7S_2 scored lowest mean score (1.6) for overall acceptability. Lowest mean score for leaf wilting (1.85) was observed in P_2S_2 and P_4S_2 whereas in case of defoliation lowest mean score was recorded by P_2S_2 (1.6). Highest mean score for leaf wilting (3.75) was recorded by P_6S_2 and P_7S_2 and maximum mean score for defoliation was noticed in P_1S_2 (3.45). Amaranthus without prepackaging and stored under refrigerated condition (P_7S_2) showed minimum mean score for decay (1.35) and maximum mean score (4.1) was recorded by P_3S_2.

Prepackaged amaranthus except P_2S_2 and P_4S_2 were spoiled after 120 h of storage (Table 32.). When treatments P_2S_2 and P_4S_2 were statistically analyzed using Kruskall- Wallis test no significant difference was observed between the treatments.

Table 30. Effect of prepackaging and storage conditions on visual parameters after 72 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Decay		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
P_1S_1	114.00	2.75	86.88	1.85	172.32	2.80	229.18	3.00	247.63	3.25	71.85	1.90
P_1S_2	157.85	3.15	194.23	3.00	59.45	1.25	104.60	1.80	226.68	2.85	190.98	3.10
P_2S_1	113.80	2.75	81.90	1.80	194.40	3.20	185.23	2.60	81.30	1.20	124.78	2.35
P_2S_2	212.35	3.65	225.10	3.60	52.80	1.20	90.80	1.65	81.30	1.20	199.90	3.35
P_3S_1	97.50	2.60	70.95	1.65	176.85	2.85	213.85	3.05	243.78	3.15	121.95	2.30
P_3S_2	141.45	3.00	192.45	3.00	64.42	1.35	103.78	1.80	214.05	2.70	185.65	3.05
P_4S_1	124.75	2.85	94.18	1.95	199.15	3.15	207.70	2.70	101.48	1.45	141.08	2.55
P_4S_2	196.00	3.50	248.08	3.85	49.95	1.15	100.00	1.75	71.15	1.10	216.80	3.40
P_5S_1	103.00	2.65	70.95	1.65	203.25	3.25	128.80	2.00	142.20	1.80	57.73	1.65
P_5S_2	185.10	3.40	191.55	3.05	110.75	1.75	113.55	1.85	137.13	1.75	181.55	3.00
P_6S_1	103.00	2.65	78.25	1.75	233.18	3.85	134.75	2.15	156.13	1.95	71.85	1.90
P_6S_2	185.10	3.40	200.63	3.10	87.30	1.70	85.95	1.55	126.98	1.65	190.98	3.10
P_7S_1	48.00	2.15	34.45	1.15	247.43	4.15	172.92	2.60	71.15	1.10	23.83	1.05
P_7S_2	185.10	3.40	197.43	3.05	99.65	1.85	90.55	1.60	66.08	1.05	188.10	3.05
CV. (0.05)	22.362											

Table 31. Effect of prepackaging and storage conditions on visual parameters after 96 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Decay		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
P_1S_2	58.15	2.35	42.45	1.30	66.95	2.85	115.10	3.45	115.98	4.00	52.40	1.70
P_2S_2	99.75	3.00	118.85	3.00	33.25	1.85	42.98	1.60	45.05	1.70	112.80	3.00
P_3S_2	58.15	2.35	39.87	1.25	66.95	2.85	108.57	3.35	118.35	4.10	62.77	1.90
P_4S_2	103.00	3.00	118.62	3.05	33.25	1.85	51.35	1.75	42.90	1.65	118.72	3.05
P_5S_2	58.15	2.35	65.62	1.75	83.60	3.30	62.10	2.00	61.20	2.10	52.40	1.70
P_6S_2	58.15	2.35	65.62	1.75	104.75	3.75	66.65	2.15	79.50	2.55	47.20	1.60
P_7S_2	58.15	2.35	42.45	1.30	104.75	3.75	46.75	1.65	30.53	1.35	47.20	1.60
CV. (0.05)	12.592											

Table 32. Effect of prepackaging and storage conditions on visual parameters after 120 h of storage

Treatments	Colour		Texture		Leaf wilting		Defoliation		Decay		Overall acceptability	
	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score	Mean rank	Mean score
P_2S_2	20.00	2.65	20.35	2.70	21.40	2.30	20.97	2.85	20.07	3.30	20.00	2.50
P_4S_2	21.00	2.70	20.65	2.70	19.60	2.20	20.02	2.80	20.92	3.05	21.00	2.55
CV. (0.05)	3.841											

4.3.5. Cost of the best treatment

Cost incurred for the sanitization of 200 g amaranthus with 2 ppm ozonised water followed by 10 ppm BA treatment and moist cotton plugging of cut stem end prepackaged in macro ventilated PP (100 gauge) cover without absorbent paper was calculated as Rs. 7.75/- which helped in extending the shelf life of amaranthus upto 120 h in refrigerated conditions. The details of cost is given in Appendix II.

Discussion

5. DISCUSSION

The results obtained from the investigation on "Postharvest handling for extending shelf life of amaranthus *(Amaranthus tricolor* L.)" are discussed in this chapter under following headings.

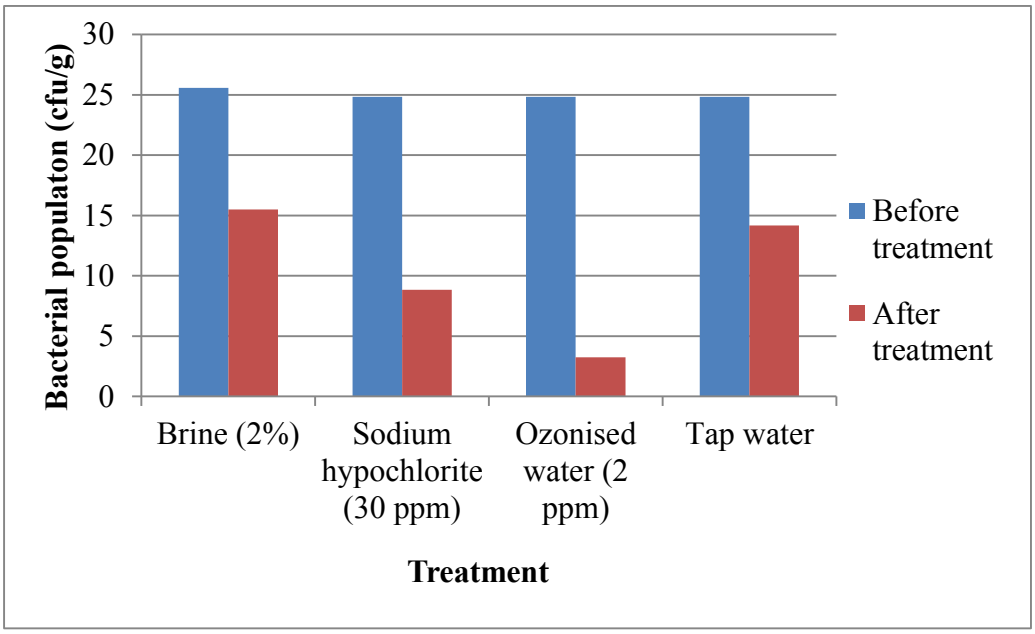
5.1. Evaluation of sanitizing treatments
5.2. Effect of pretreatments
5.3. Prepackaging and storage

5.1. EVALUATION OF SANITIZING TREATMENTS

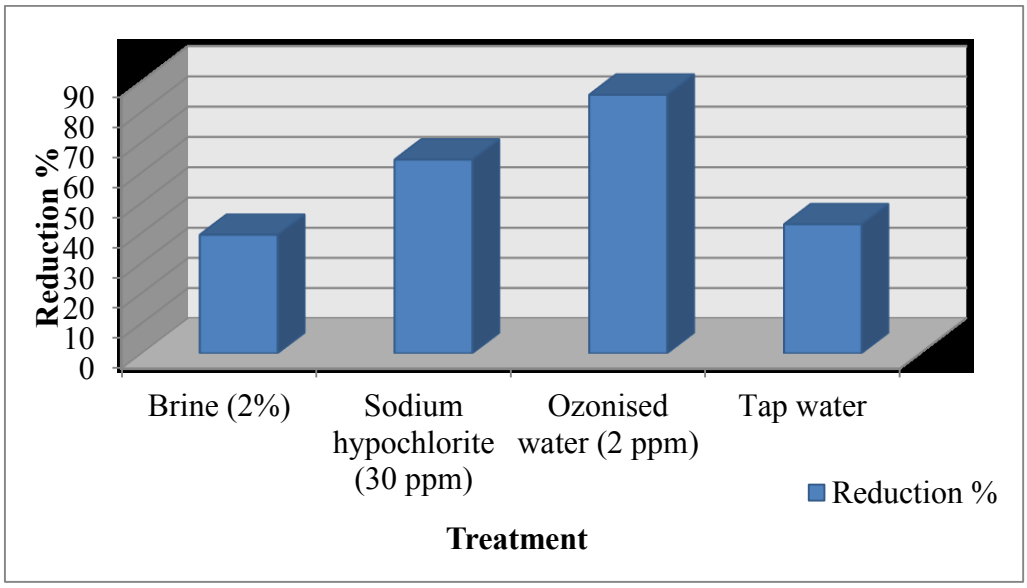
Raw and minimally processed vegetables are essential part of people's diet all around the world. However, in recent years fresh produce consumption has reported the outbreaks of food borne disease which increased the need of sanitization in the chain of postharvest handling. Numerous cleaning compounds and processes have been developed to remove and destroy bacteria, viruses, and parasites from vegetables. Washing with sanitizers is an important step in reducing the microbial population and quality deterioration (Kim, 2012). Objectives of vegetable sanitization are reduction or elimination of microorganisms from vegetables and guarantee it for consumption (Bachelli *et al.*, 2013). In this study, amaranthus was surface sanitized with 2% brine, 30 ppm sodium hypochlorite, 2 ppm ozonised water and tap water and results showed difference in their efficiency of sanitization as well as extending shelf life.

5.1.1. Enumeration of total microbial count

Surface sanitization of amaranthus (var. Arun) with 2 ppm ozonised water was found highly effective with 85.68% reduction in microbial population followed by 30 ppm sodium hypochlorite with a reduction percentage of 64.26 (Fig.1.b.). Similar result was reported by Olmez and Akbas (2009) that application of 2 ppm ozonated



(A)



(B)

Fig 1. Effect of sanitization on bacterial population: (A) Bacterial population, cfug^{-1} (B) Reduction percentage of bacterial population, %

water treatment for two minutes was the optimum processing conditions for disinfection of green leaf lettuce, in terms of reducing the microbial load during cold storage. Ibrahim *et al.* (2012) reported that in turnip greens microbial population was reduced to 1.43 log $cfug^{-1}$ when treated with 5 ppm ozone for five minutes. Chen *et al.* (2013) opined that 3 ppm ozone is found more effective in inactivating bacteria, moulds and yeasts on vegetables. Nath *et al.* (2014) observed that total bacterial counts reduction is greater than 90 % in Chinese cabbage treated with ozonated water (2.3 mg/l) for 60 minutes.

The sanitizing efficiency of ozone may be due to destruction of microorganism by disruption or disintegration of cell envelope and internal cellular proteins by progressive oxidation causing rapid cell death (Komanapall and Lau, 1996; Kim, 2012).

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

Surface sanitization of amaranthus with 2% brine had highest bacterial population (15.51×10^5 $cfug^{-1}$) as compared to other treatments (Fig.1. A.). Amaranthus treatment with 2 % brine showed lowest reduction percentage of 39.39% which was even lower than treatment with tap water (42.91%). This result is supported by the findings of Reddy (2010) in which brine (2%) dipping resulted in maximum decaying of rajagira leaves (*Amaranthus paniculatus*).

As salt acts as osmotic agent, draws water from the tissues leading to condition of moisture on inner surface of the film (Anon., 1999).

5.1.2. Physiological parameters

Physiological weight loss is a phenomenon of weight loss from produce during storage which in turn is related with shelf life of vegetables whether whole or fresh-cut products. It could be noted that there is a relation between physiological loss in weight, relative water content and shelf life or freshness of leafy vegetable. Increase in weight loss decreases the water content and in turn reduces the freshness or shelf life of leafy vegetables.

Amaranthus treated with 2 ppm ozonised water was found to have lowest physiological loss in weight of 6.88, 11.17 and 25.63 % at 2, 3 and 4 hours of storage at room temperature (Fig. 2.). This result was in agreement with findings of Nadas *et al*. (2003) that fruits treated with ozonised water showed less weight loss than non-treated samples after storage. Zhang *et al.* (2005) reported that fresh-cut celery treatment with ozonated water has showed to be effective to reduce the population of microorganisms and retard physiological metabolism. Sothornvit and Kiatchanapaibul (2009) reported fresh or fresh-cut vegetables extended the shelf life by inhibiting the growth of microorganisms after sanitization by ozone.

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

Amaranthus treated with 2% brine were unable to sustain edibility. Lowest relative water content (53.64 %) with highest weight loss (31.04 %) was observed in 2% brine treated amaranthus during storage. Amoah *et al.* (2007) found that high concentration of brine treatment greatly reduced the quality of the lettuce leaves. Reddy (2010) reported that brine (2%) used as dipping solution reduced the moisture significantly and could not extend the shelf life of *rajagira* leaves with or without stem.

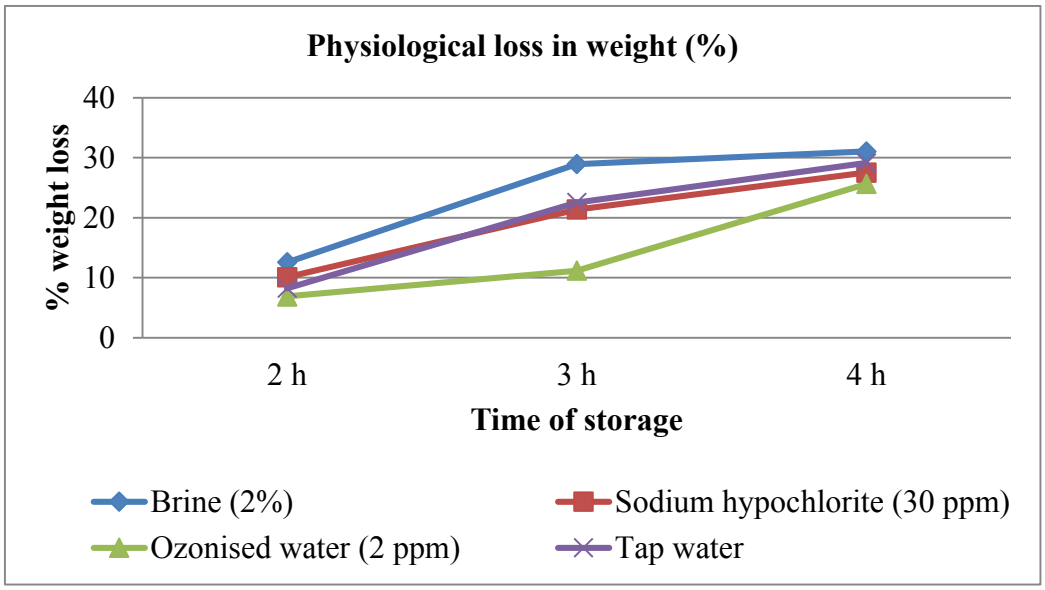


Fig 2. Effect of sanitization on physiological loss in weight of amaranthus

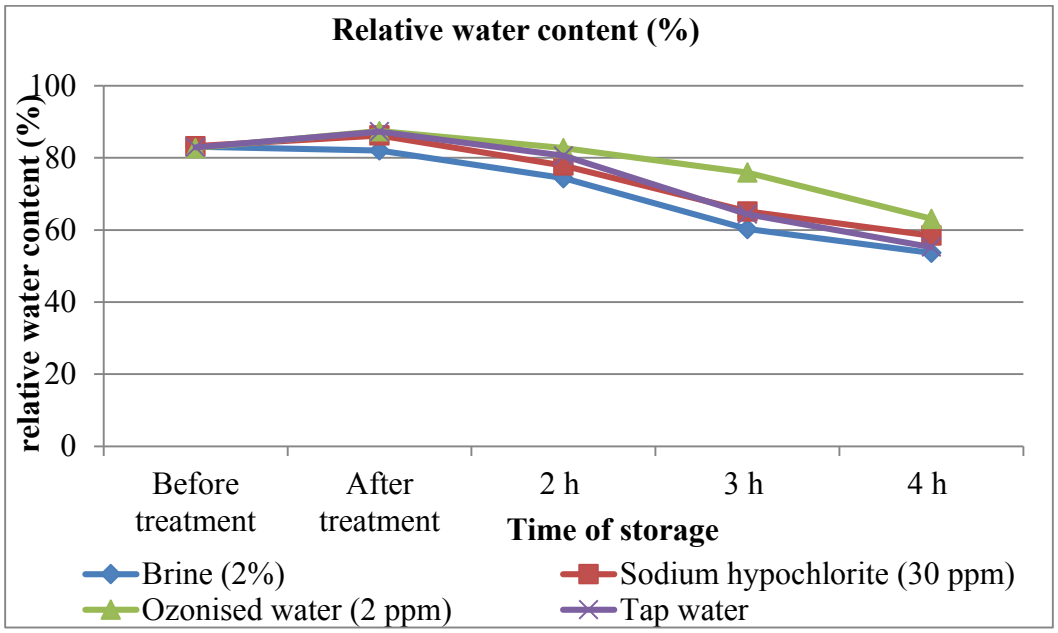


Fig 3. Effect of sanitization on relative water content of amaranthus

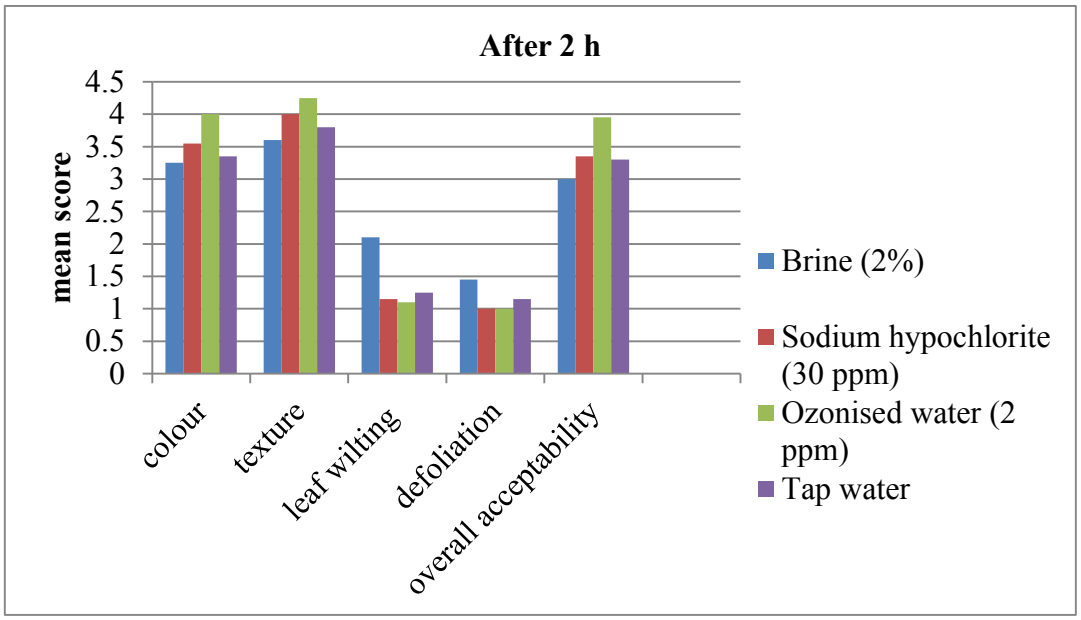
5.1.3. Visual parameters

Amaranthus is highly perishable in nature which leads to loss of freshness during storage. In the study, amaranthus treated with 2 ppm ozonised water exhibited maximum freshness during storage with highest mean score for colour (2.40), texture (2.75) and overall acceptability (2.00). Low leaf wilting (2.30) and defoliation (2.65) were also observed for the same treatment (Fig.4.). Zhang *et al.* (2005) revealed that the best preservation effect was found to be ozonated water treatment by which the microbial population was lowered and nutritional and sensory quality of fresh-cut celery were maintained good for 9 days of storage at 4ºC. Olmez and Akbas (2009) also reported similar results with application of 2 ppm ozonated water treatment for 2 minutes was the optimum processing conditions for disinfection of green leaf lettuce, in terms of reducing the microbial load and maintaining the sensory quality during cold storage.

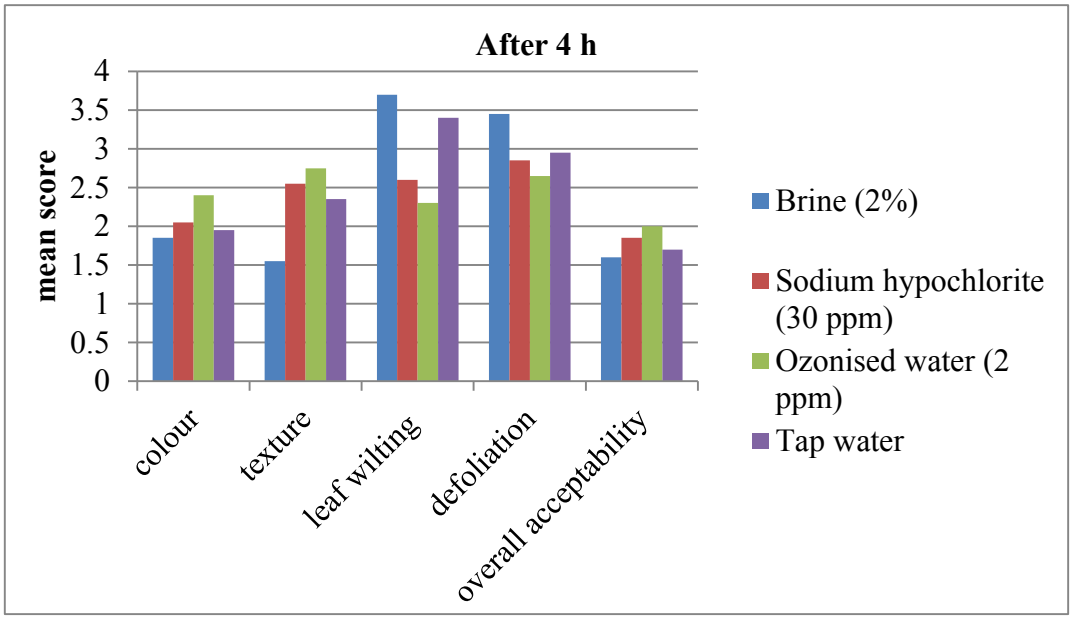
Freshness of a produce is related to the shelf life of a produce. Amaranthus treated with ozonised water (2 ppm) had the longest shelf life upto 4 hours when compared to other treatments. Beltran *et al.* (2005) reported that the use of ozonated water applied to fresh-cut vegetables for sanitation purposes reduced the microbial populations and extended the shelf life. Ozone treatment is found effective in inhibiting the growth of microorganisms and extends the shelf life of fresh or fresh-cut products (Sothornvit and Kiatchanapaibul, 2009).

Amaranthus treated with brine showed the highest wilting (3.70) with lowest mean score for texture (1.55), colour (1.85) and overall acceptability (1.60) during storage. This could be due to the dehydrating nature of salt which renders to loss of moisture during storage which results in loss of freshness and turgidity of produce (Anon., 1999).

Based on the efficiency of sanitizers in reducing the microbial count and superiority in physiological and physical parameters, 2 ppm ozone (T_3) was selected as best sanitizer for further studies.



(A)



(B)

Fig.4. Effect of sanitization on visual parameters: (A) After 2 h (B) After 4 h

5.2. EFFECT OF PRETREATMENTS

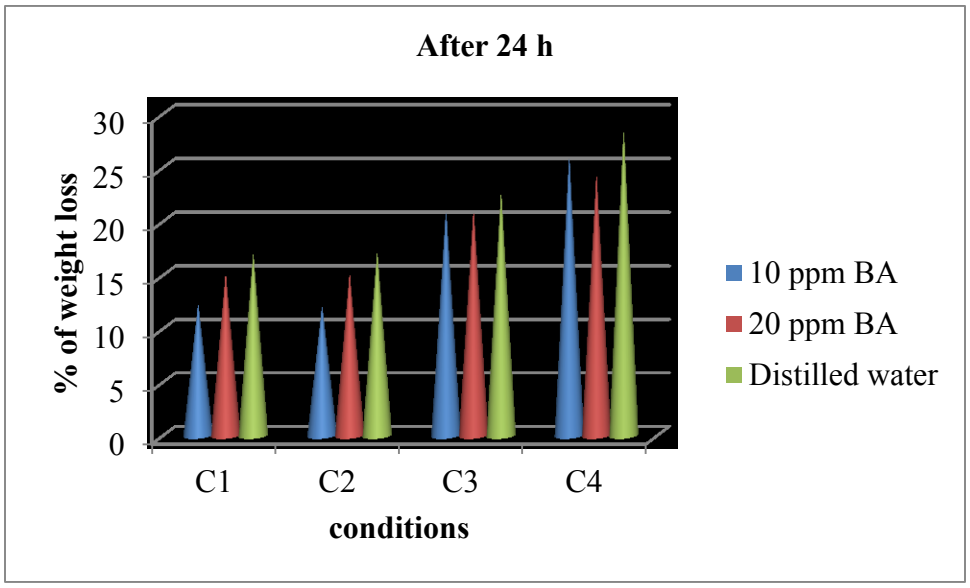
Leafy vegetables have high respiration rate which lead to shorter shelf life. Disinfectants or dipping in chemical solutions, chemical or bio-preservatives, refrigeration, reduction of water activity and additives are used to maintain quality and thereby to extend shelf life of vegetables. In this study, different pretreatments with benzyl adenine (10 ppm, 20 ppm) and distilled water and storage conditions (cut stem dipped in water, moist cotton plugging of cut stem end and waxing of stem end) were studied to extend the shelf life of amaranthus.

5.2.1. Physiological parameters

Amaranthus treated with benzyl adenine had significantly improved the shelf life as compared to distilled water. The results showed that 10 ppm benzyl adenine (BA) was more effective in maintaining the freshness with low physiological weight loss (21.38%) and high relative water content (70.33%) after 48 h of storage (Fig.5.). This was in accordance with Siddiqui *et al.* (2011a) who reported that fresh-cut broccoli florets treated with 10 ppm BA for 10 minutes reduced physiological loss in weight and maintained green color; significantly reduced loss of organoleptic quality and extended postharvest life upto nine days.

The shelf life of 10 ppm BA pretreated amaranthus stored with cut stem dipped in water or moist cotton plugging had shelf life upto 48 h. Increased longevity of vegetable with benzyladenine (BA) treatment before and after harvesting may be a consequence of the decrease in respiration (Dedolph *et al.,* 1961; Dedolph *et al.,* 1962 and Wittwer and Dedolph, 1962) via inhibition of glycolytic kinases (Tuli *et al*., 1964) and thus maintained their freshness. Emongor *et al.* (2000) and Mutui *et al.* (2001) observed that cytokinin treatment delayed leaf senescence and improved the keeping quality of many cut flowers.

When storage conditions were studied, it was observed that cut stem dipped in water or moist cotton plugging of cut stem end had lowest physiological loss in weight (22.93 and 22.95 %) with high retention of water content (67.36 and 66.86 %)



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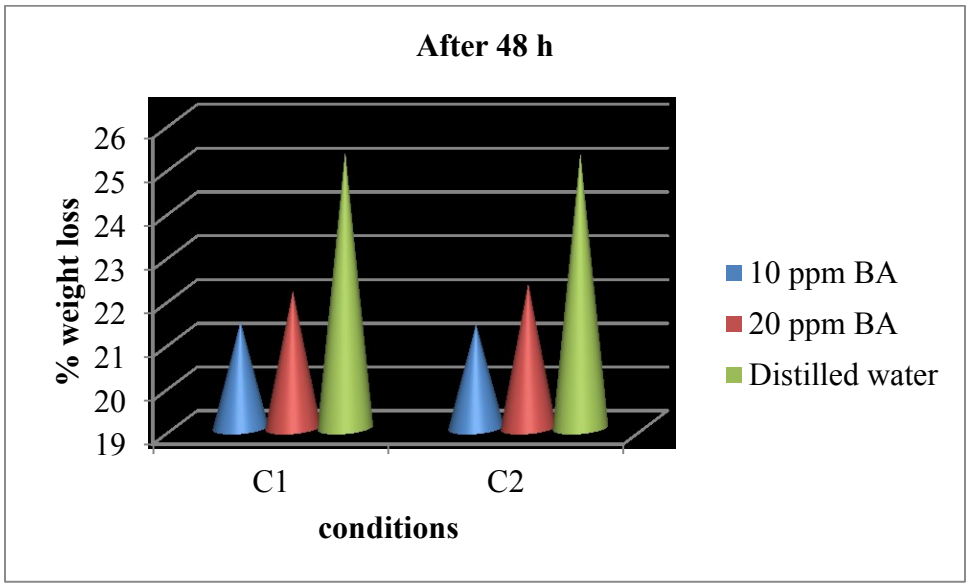
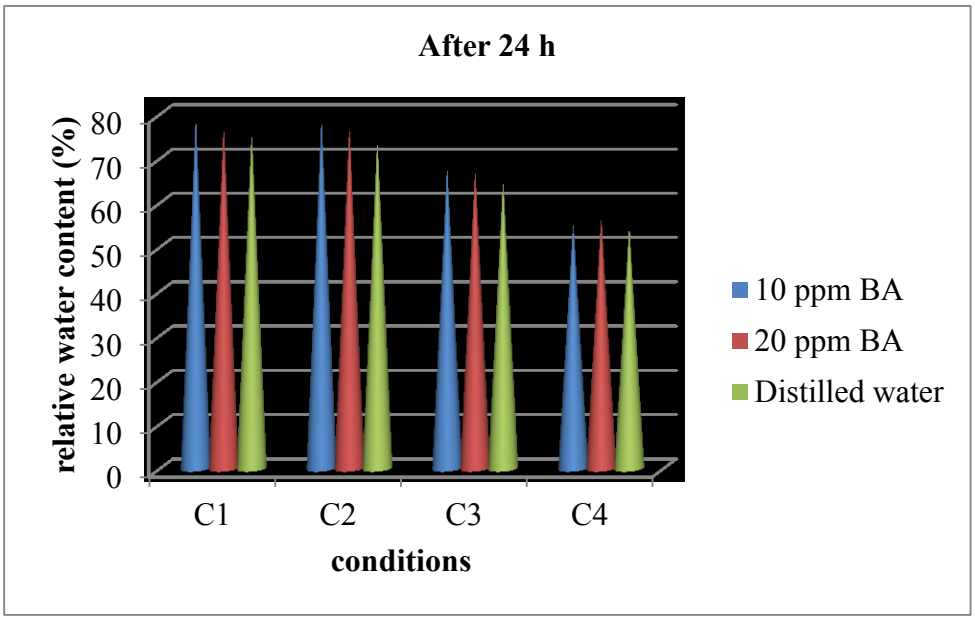
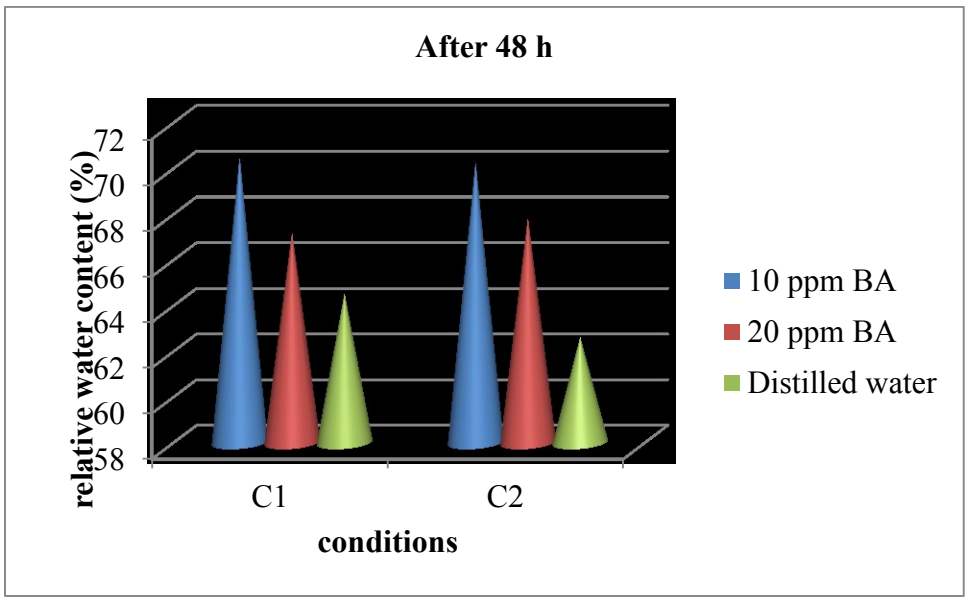


(B)

Fig 5. Effect of pretreatment on physiological loss in weight (%) during storage: (A) After 24 h (B) After 48 h



(A)



(B)

Fig 6. Effect of pretreatment on relative water content (%) during storage: (A) After 24 h (B) After 48 h

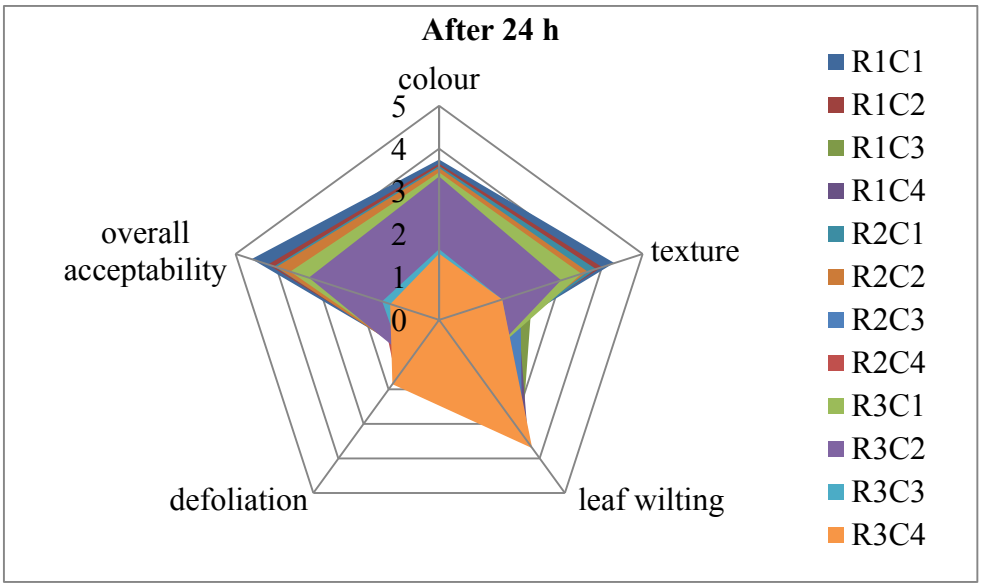
during storage. Similarly in anthurium (Hettiarachchi and Balas, 2005) and orchids (Hegde, 1999; De *et al.,* 2014) it was reported that cut stem end dipped in water or wrapping with wet cotton swab increased the vase life. For long distance transportation of cut flowers preference in the use of wet cotton swab or tube containing water or water with preservatives were reported.

During storage, decrease in relative water content was observed with increase in weight loss irrespective of pretreatments and conditions (Fig. 5. and Fig 6.). Amaranthus pretreated with BA and stored with cut stem dipped under water or moist cotton plugging of cut stem end extended the shelf life and retained the freshness upto 48 h of storage at room temperature. Lowest physiological loss in weight was observed in amaranthus pretreated with 10 ppm benzyl adenine with cut stem dipped in water (21.41%) or moist cotton plugging of cut stem end (21.35%). Similarly, the same treatments retained the highest relative water content of 70.45 and 70.22 % during the storage period. This was in agreement with Asil and Karimi (2010) who reported that BA treatment of cut flowers dipped in water increased water uptake therefore retarded weight loss in comparison with control treatment and extended the vase life.

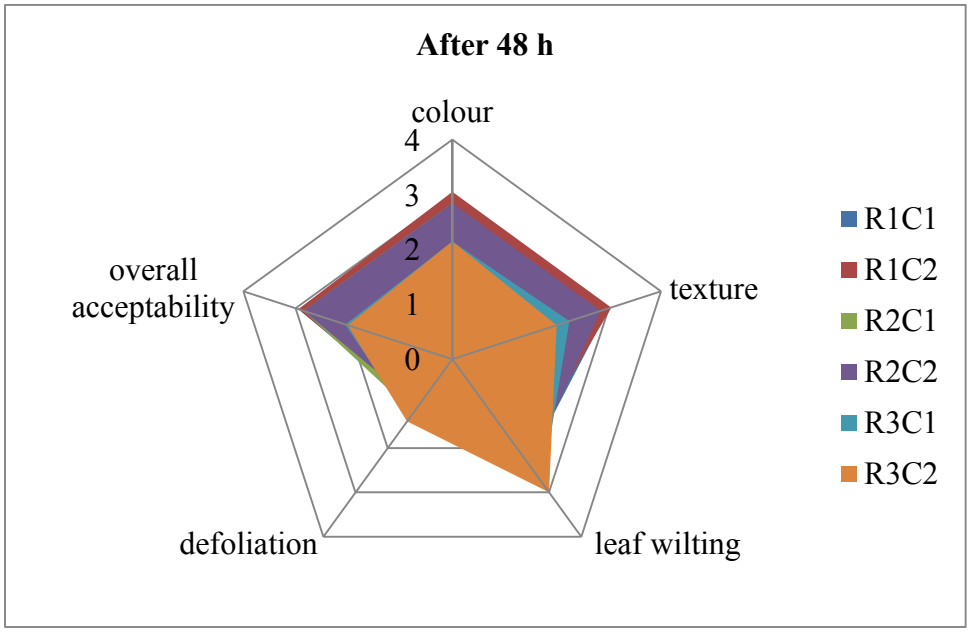
5.2.2. Visual parameters

Moisture content is important for fresh leafy vegetables to remain well hydrated and to maintain sensory attributes *viz.,* texture and appearance over period of storage (Souzan and Abd-el-aal, 2007). Similarly in the study it was noted that the treatment which retained highest moisture content had scored highest mean score for colour, texture and overall acceptability with low leaf wilting and defoliation.

Amaranthus pretreated with 10 ppm benzyl adenine and stored with cut stem end dipped in water or with moist cotton plugging of cut stem end recorded highest mean score for visual parameters during storage (Fig. 7.). This was in agreement with the findings of Siddiqui *et al.* (2011b) that cabbage treated with 15 ppm BA could be used as a safe and effective method to maintain organoleptic quality, delay in



(A)



(B)

Fig 7. Star diagram depicting the effect of pretreatment on visual parameters during storage: (A) After 24 h (B) After 48 h

senescence, maintenance of chloroplast activity, decline in chlorophyll degradation, the production of protein, nucleic acid synthesis and mobilization of nutrients into the benzyl adenine treated area.

Pretreated amaranthus with 10 ppm BA and stored with cut stem end dipped in water or with moist cotton plugging was equally effective. Hence based on the convenience in packaging and transportation 10 ppm BA treatment with moist cotton plugging was selected for further study.

5.3. PREPACKAGING AND STORAGE

Leafy vegetables are highly perishable with an active metabolism such as respiration and transpiration during post harvest period. Because of large surface area to volume ratios of leafy vegetables make it vulnerable to loss of water during storage and increased with storage temperature and vapour pressure deficit (Onyango, 2010). 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 (Reddy, 2010).

In the present study, amaranthus surface sanitized with 2 ppm ozonised water, pretreated with 10 ppm benzyl adenine and moist cotton plugging of cut stem end were prepackaged and stored under room temperature (30±2˚C) and under refrigerated storage condition (10±2˚C). The physiological, physical, nutritional parameters and microbial population were recorded.

5.3.1. Physiological parameters

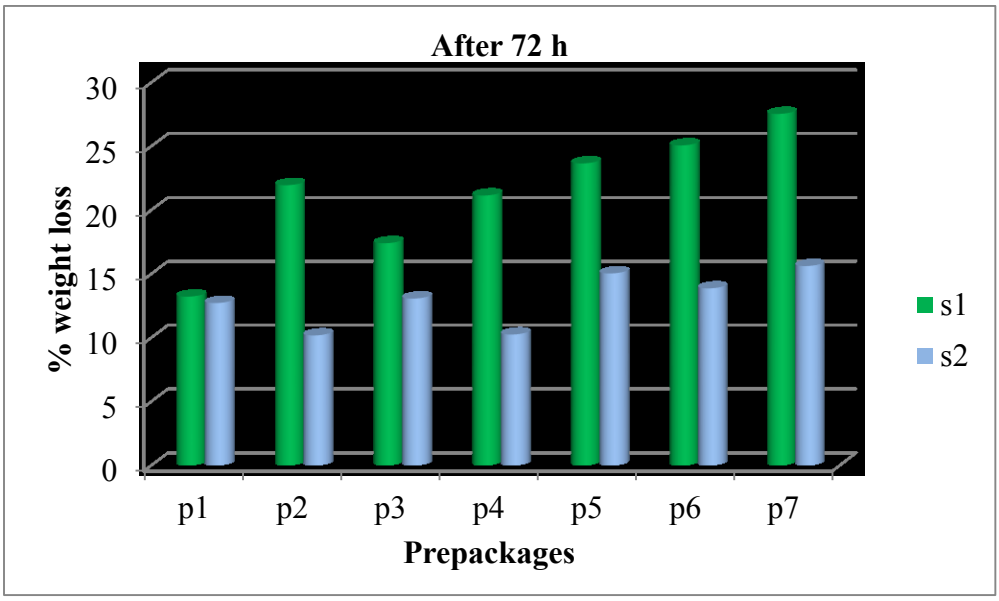
Amaranthus in different prepackages stored under refrigerated conditions showed a significant retention of water content and in turn less physiological loss in weight. Loss of relative water content is one of the major problems in amaranthus which finally results in visible wilting and reduction in produce quality (Jacxsens *et al.*, 2002). The loss of water is a natural process of catabolism which is attributed to

the respiration and other senescence related metabolic process (Souzan and Abd-el-aal, 2007). This can be overcome by using appropriate packaging material and storage condition. The permeability of the packaging material and the temperature during storage are two very important parameters to control senescence of leafy vegetables (Lokke *et al.,* 2010).

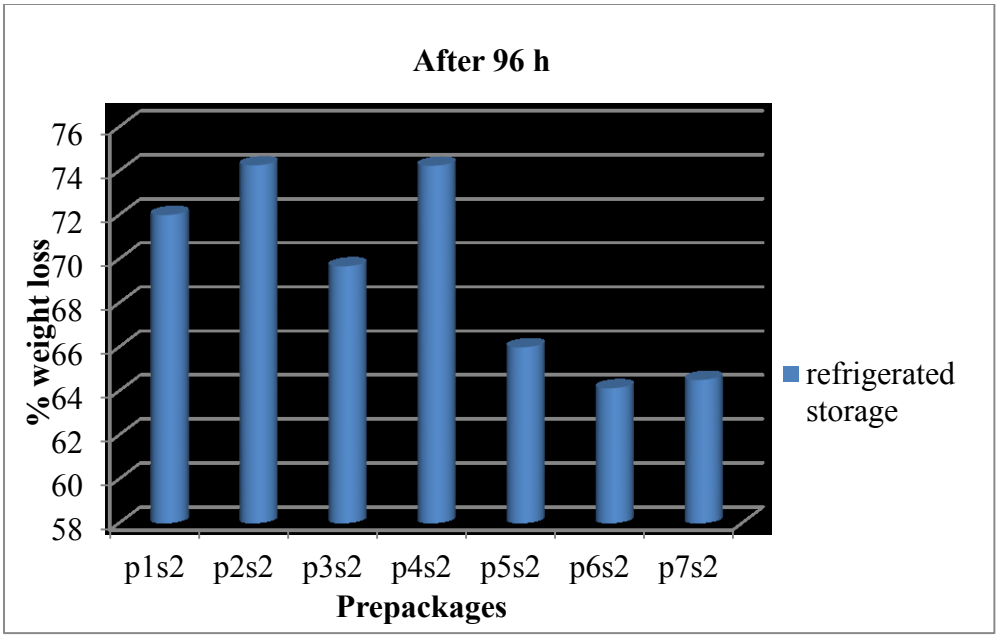
When amaranthus were prepackaged in different packages, 100 gauge macro ventilated PP and 150 gauge LDPE without absorbent paper were capable of retaining the quality in terms of moisture retention with low physiological loss in weight during storage (Fig. 8. and Fig.9.). Similarly Aharoni *et al.,* (1989) and Cantwell and Reid (1993) found that perforated bags reduced water and quality loss of herbs such as watercress, dill, mint and thyme. Packaging has the potential to reduce moisture loss, restrict the entrance of oxygen, lower respiration and retard discoloration (Ahvenainen, 1996). Packaging also helps to increase shelf life by creating a modified atmosphere with an increase in concentration of carbon dioxide in the package (Assumi *et al*., 2009).The result is supported by Reddy *et al.* (2013) who reported that *rajagira* leaves with tender stem packed in 100 gauge polypropylene with vents extended the shelf life upto four days with reduced moisture loss, diminished physiological loss in weight, low yellowing and decaying percent.

Prepackaging of treated amaranthus with cling film or sleeve wrapping with LDPE also showed a higher retention of physiological and visual qualities compared to control samples. This is in agreement with the findings of Jiang and Pearce (2005) who had reported that cling film wrapping (two-thirds of leaves exposed) of pak choy performed best.

Amaranthus prepackaged in macro ventilated PP or LDPE with absorbent paper showed low physiological loss in weight. It might be due to the absorption of transpired water by the absorbent paper which was placed inside the packaging which in turn increased the weight of prepackages containing amaranthus. These treatments showed low relative water retention and was found to be visually inferior and had a

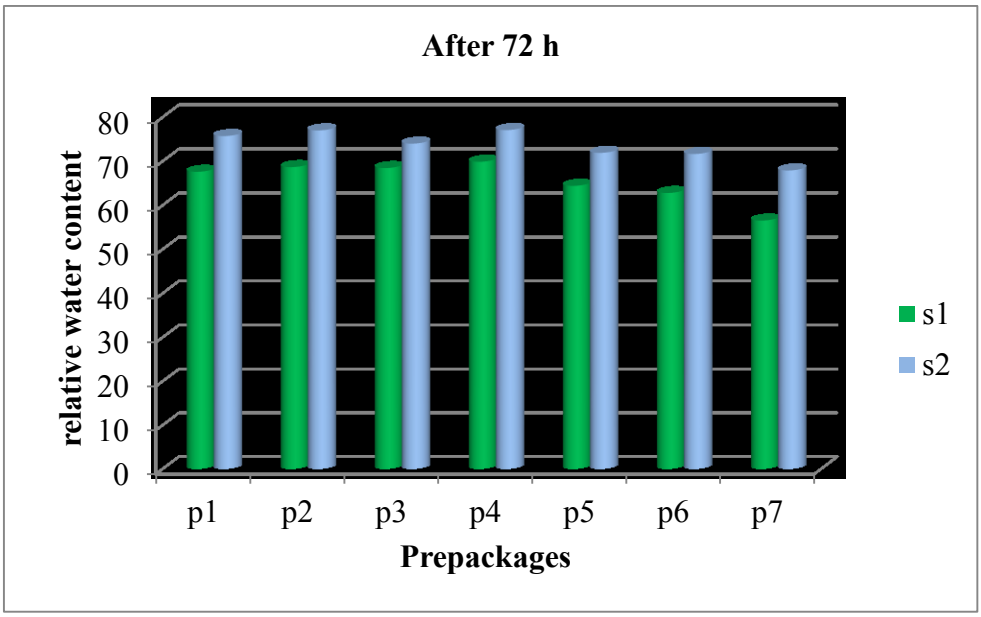


(A)

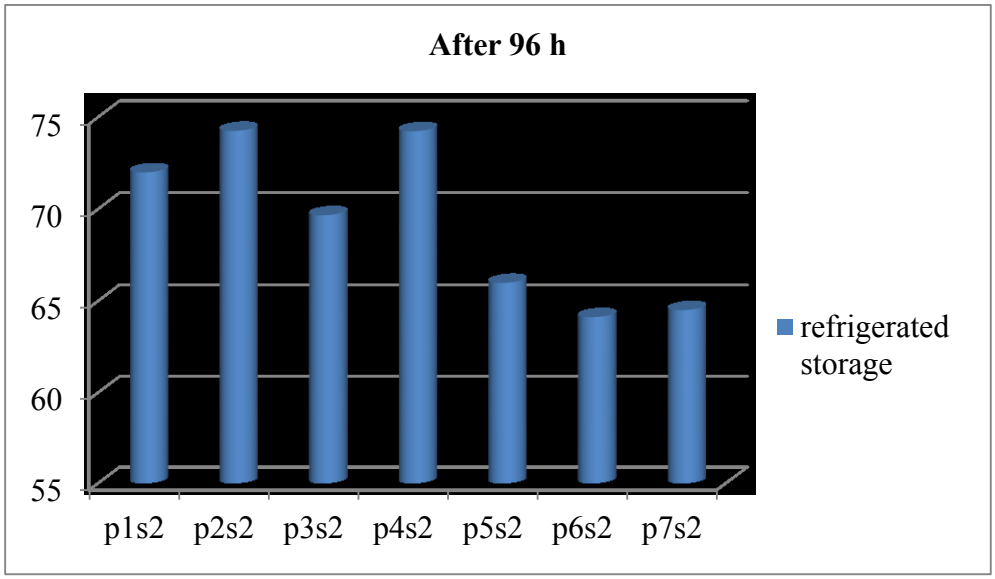


(B)

Fig 8. Effect of prepackaging and storage on physiological loss in weight, %: (A) After 72 h (B) After 96 h



(A)



(B)

Fig 9. Effect of prepackaging and storage on relative water content, % (A) After 72 h (B) After 96 h

shorter shelf life compared to macro ventilated PP or LDPE without absorbent paper in both storage temperatures.

Unpackaged amaranthus showed decrease in freshness and water content due to rapid transpiration and wilting. This was in agreement with the findings of Souzan and Abd-el-aal (2007).

Storage temperature also showed a significant influence in maintaining quality viz. physiological weight loss and relative water content of the treated amaranthus. Refrigerated storage (10 ± 2 ˚C) was found to be the best for differently prepackaged amaranthus as compared to room temperature storage. Amaranthus prepackaged and stored under refrigerated condition recorded the lowest weight loss (12.96 %) and highest relative water content (73.55 %) after 72 h of storage. The slight loss of water content during refrigeration storage was observed which was due to the respiration and others a senescence-related metabolic process (Prabhu and Barrett, 2009).

Low temperature storage slows down the evapo-transpiration and hence ensures a longer shelf life of the vegetables (Kays, 1991). The result was in conformity with the findings of Moreira *et al.,* (2006) who observed an increase in weight loss of lettuce leaves with the increase in storage temperatures after 170 h of storage. Onyango (2010) reported that at the end of four days of storage the vegetables held at 20ºC had lost 10 % of the initial moisture content. Koraddi and Devendrappa (2011) also reported a similar result in vegetables viz. fenugreek, coriander, tomato, chilli, french bean, lady's finger, cucumber and carrot.

Amaranthus prepackaged in macro ventilated PP without absorbent paper stored under refrigerated condition showed the lowest weight loss (17.29 %) with highest relative water content (67.91 %) after 120 h of storage. This was in accordance with Rivera *et al.* (2006) who observed that changes in moisture content of green leafy vegetables were minimized by using polyethylene bags and cold storage.

5.3.2. Nutritional parameters

Amaranthus prepackaged and stored at room temperature and refrigerated condition showed a significant difference in retention of ascorbic acid, β-carotene, total chlorophyll, calcium and iron. 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); Lee and Kader (2000); Kays and Paull (2004) and Onyango (2010).

The ascorbic acid content of vegetables is influenced by various factors such as climatic conditions, cultural practices, maturity at harvest, harvesting method and postharvest handling conditions (Lee and Kader, 2000). Ascorbic acid content of fresh pretreated amaranthus before storage ranged from 18.55 to 19.05 mg/100 g.

Differently prepackaged amaranthus showed a decrease in ascorbic acid at the end of shelf life (Fig.10.a.). Among prepackages, macro ventilated PP without absorbent paper showed the highest retention of ascorbic acid (7.39 mg/100 g) followed by macro ventilated LDPE without absorbent paper (6.65 mg/100 g) at end of shelf life. This was in agreement with Ooraikul and Stiles (1991) who reported that ascorbic acid of green beans and spinach was preserved by packaging and storage at 10ºC.

Reduction of ascorbic acid content was observed during storage which could be attributed to oxidation of ascorbic acid into dehydro-ascorbic acid by the enzyme ascorbic acid oxidase (Pal *et al.*, 2002 ; Nyaura *et al*., 2014) and the process is influenced by temperature, biological deterioration factors (senescence and microbial activity) (Moreira *et al*., 2006). Refrigerated condition showed highest retention of ascorbic acid (4.16 mg/100 g) at the end of shelf life after 120 h of storage as compared to room temperature stored amaranthus. Souzan and Abd-el-aal (2007) showed a similar result when vegetables were stored at cold temperature with the retention of 80 per cent ascorbic acid after eight days.

When combined effect of prepackaging and storage temperature were considered a reduction of ascorbic acid from initial was observed. Similarly Rai *et al.*

(2009) reported a sudden drop of ascorbic acid in storage of shredded cabbage at 15°C packed in polypropylene. Amaranthus prepackaged in macro ventilated PP without absorbent paper stored under refrigerated condition retained the highest ascorbic acid after 120 h of storage.

Loss of β-carotene was observed in the prepackaged amaranthus during storage. Before storage the β-carotene content of fresh pretreated amaranthus ranged from 6.25-6.26 µg/100g but at the end of shelf life slight reduction was observed. Degradation of β-carotene in stored amaranthus in modified atmospheric package is usually due to oxidation and also enzymatic degradation (Gareth *et al*, 1998). Prepackaged amaranthus in macro ventilated PP without absorbent paper had the highest β-carotene (3.71 µg/100g) at the end of shelf life (Fig.10.b.). Amaranthus prepackaged and stored under refrigerated condition (4.16 µg/100g) showed the highest retention when compared to the room temperature (0.80 µg/100g). Nyaura *et al*. (2014) reported that rapid loss of ascorbic acid and β-carotene of the amaranth leaves stored at room temperature can be attributed to the catalysis by light. Amaranthus prepackaged in macro ventilated LDPE without absorbent paper stored under refrigerated condition showed highest retention of β-carotene (5.66 µg/100g) followed by macro ventilated PP without absorbent paper stored under refrigerated condition (5.34 µg/100g).

Total chlorophyll content was found to be decreased during storage (Fig.10.c.). Baardseth and Von Elbe (1989) reported a similar result that chlorophyll of spinach leaves were degraded during storage. Total chlorophyll content before storage ranged from 5.31 to 5.34 mg g^{-1}. Prepackaged amaranthus in macro ventilated PP without absorbent paper had highest total chlorophyll content when compared to other treatments. During storage, refrigerated storage retained the highest total chlorophyll content compared to room temperature. Vina and Chaves (2003) reported that decrease in chlorophyll content during storage is temperature dependent. When the combined effect of storage temperature and prepackaging were considered, it was observed that prepackaging of amaranthus in macro ventilated PP cover without

absorbent paper (4.42 mg g^{-1}) and macro ventilated LDPE without absorbent paper (4.13 mg g^{-1}) stored under refrigerated condition had retained highest total chlorophyll content at the end of shelf life. This result was in accordance with Souzan and Abd-el-aal (2007) who reported decrease in chlorophyll content of leafy vegetables during storage. Gokmen *et al.* (2005) reported that enzymatic action leads to degradation of chlorophylls.

Slight reduction in calcium content was seen at the end of shelf life when compared to the fresh pretreated amaranthus which ranged from 6.36 to 6.45 %. Amaranthus prepackaged in macro ventilated PP with or without absorbent paper showed similar and highest calcium content of 5.79 % and 5.72 % respectively after 96 and 120 h of storage. During storage it was found that refrigerated storage retained more calcium than at room temperature but a slight decrease was observed from initial content which may be due to small losses of ashes by transpiration phenomena during refrigeration (Oulai *et al*., 2015). Prepackaging of amaranthus with macro ventilated PP without absorbent paper stored under refrigerated condition retained the highest calcium content (5.99 %) at the end of shelf life at 120 h of storage (Fig.10.d.).

In the case of iron content also slight reduction was observed during storage (Fig.10.e.). Iron content of fresh pretreated amaranthus was found to range from 0.16 to 0.17 %. Amaranthus stored under refrigerated condition retained the highest iron content (0.08 %) with a slight reduction from initial content. Similar result was reported by Oulai *et al*. (2015) in refrigerated stored leafy vegetable. Different prepackages were found to have less effect on iron retention. Prepackaging of amaranthus in macro ventilated PP without absorbent paper, sleeve wrap with LDPE and cling film wrap stored under refrigerated condition retained more iron content at the end of shelf life.

Before storage oxalate content of pretreated amaranthus ranged from 1.70 to 1.72 %. Oxalate content was reported to be decreased with age of plant (Onyango,2010). Oxalate content of prepackaged amaranthus stored at room temperature was

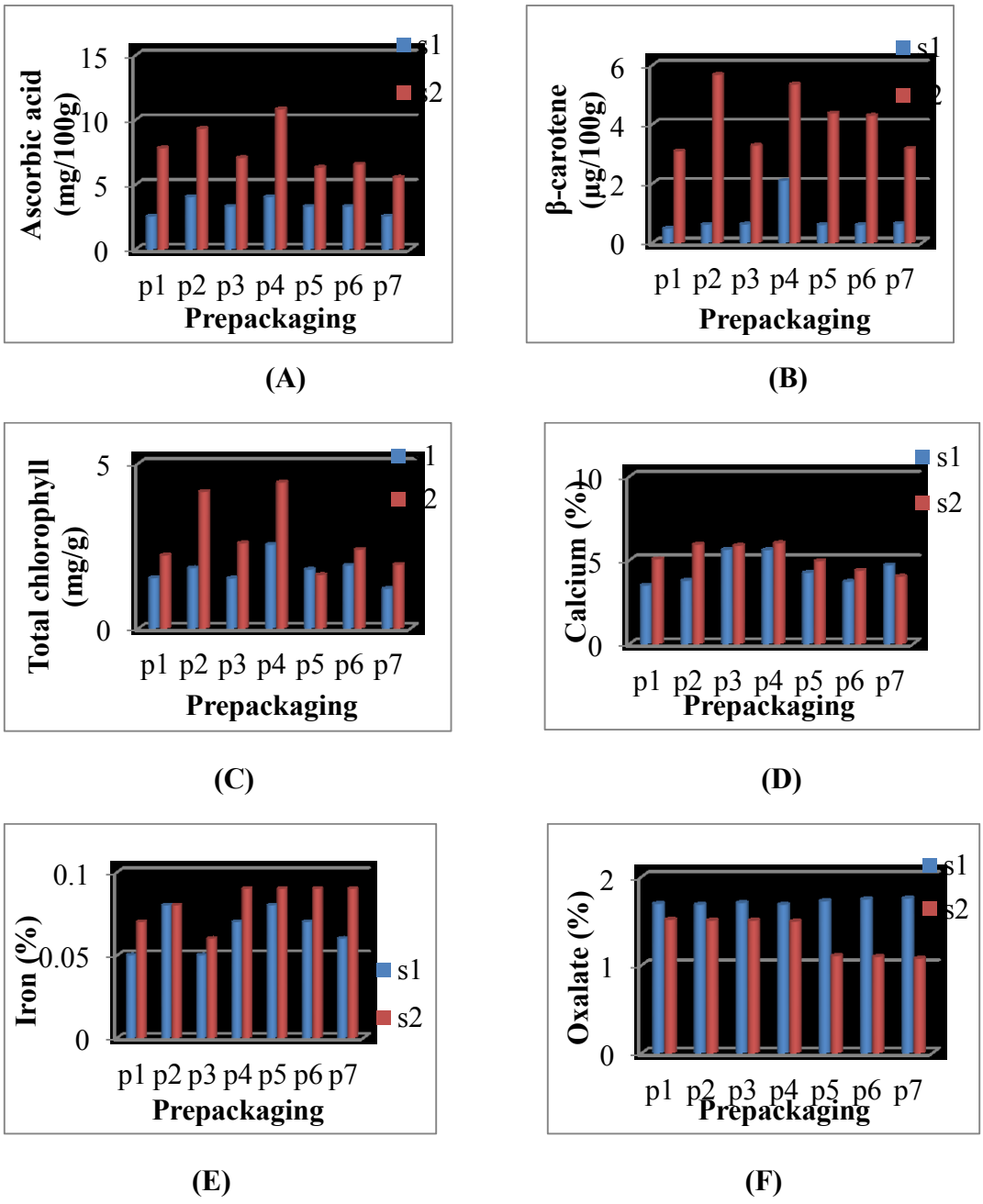


Fig 10. Effect of prepackaging and storage on nutritional parameters of amaranthus (A) Ascorbic acid, mg/100g (B) β-carotene, µg/100g (C) Total chlorophyll, mg g^{-1} (D) Calcium (%) (E) Iron, % (F) Oxalate, %

observed to be slightly increased from initial content. This could be due to the activity of the enzyme ascorbase, which converts ascorbic acid to oxalate under warm conditions (Cleveland and Soleri, 1991). This result was in agreement with Onyango (2010) who observed a similar increase in oxalate content of amaranthus. At refrigerated condition, slight decrease in oxalate content was observed. Ogbadoyi *et al.* (2006) reported that high moisture content in the tissue at freezing temperature result in formation of ice crystals capable of lacerating the cell membranes and cell leakage leading to considerable loss of oxalate from its cellular compartment. Amaranthus without prepackaging stored under refrigerated condition showed the lowest oxalate content compared to other treatments (Fig.10.f.).

5.3.3. Microbial load

Microbial population was observed to be higher in all prepackaged amaranthus than without prepackaging during storage. This might 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 (pH 5.8-6.0) and can end up as the cause of deterioration and growth of microorganisms (King *et al.*, 1991; Ahvenainen, 1996; Brackett, 1999).

Amaranthus without prepackaging showed lower microbial population of 0.83 $\times 10^7$ $cfug^{-1}$ followed by macro ventilated PP without absorbent paper (2.04 $\times 10^7$ $cfug^{-1}$) (Fig. 11.). Reddy *et al*. (2013) observed that rajagira leaves prepackaged in polypropylene 100 gauge with vents had less decaying percentage. It was observed that amaranthus prepackaged in macro ventilated 150 gauge LDPE without absorbent paper had higher bacterial population compared to macro ventilated PP (100 gauge) without absorbent paper. Higher the gauge, more thicker the package which leads to accumulation of transpired or respired moisture resulting in more decaying (Reddy, 2010).

When amaranthus was prepackaged in macro ventilated PP and LDPE with absorbent paper, highest microbial population was noticed compared to other

treatments at the end of shelf life. This might be due to absorption of transpired water by the absorbent paper which increased the moisture content in the package and lead to a favorable condition for microbial growth.

Under refrigerated storage condition microbial population (4.64×10^7 $cfug^{-1}$) was found less as compared to room temperature (6.97×10^7 $cfug^{-1}$). Bacts and Tamplin (2002) reported that low temperature storage inhibits the microbial growth on fresh-cut produce. When combined effect was studied amaranthus without prepackaging (0.12×10^7 $cfug^{-1}$) and macro ventilated PP without absorbent paper stored under refrigerated condition (1.11×10^7 $cfug^{-1}$) showed the lowest microbial count.

5.3.4. Visual parameters

Amaranthus prepackaged and stored under refrigerated condition maintained high score for visual parameters during storage when compared to room temperature (Fig. 12.). Superior visual parameters viz. colour (2.70), texture (2.70) and overall acceptability (2.55) were maintained by macro ventilated PP cover without absorbent paper stored under refrigerated condition with lowest leaf wilting (2.20), defoliation (2.80) and decay (3.05) after 120 h of storage. Jiang and Pearce (2005) found modified atmosphere packaging to be extremely effective in retarding yellowing of leafy brassicas. Suraweera *et al.* (2011) reported that polyethylene film-packaging significantly extended the shelf life of leaves both at room temperature and 10°C. Nyaura *et al.* (2014) observed that different storage temperatures affected the visual appearance and shelf life of amaranthus.

Control samples stored under room temperature recorded the highest score for wilting and lowest score for other visual parameters throughout the storage period which could be due to the direct exposure of amaranthus to external environment. Onyango (2010) reported that higher the storage temperature higher will be the transpiration rate and transit losses (rotting and wilting) leading to a shorter postharvest life of vegetables.

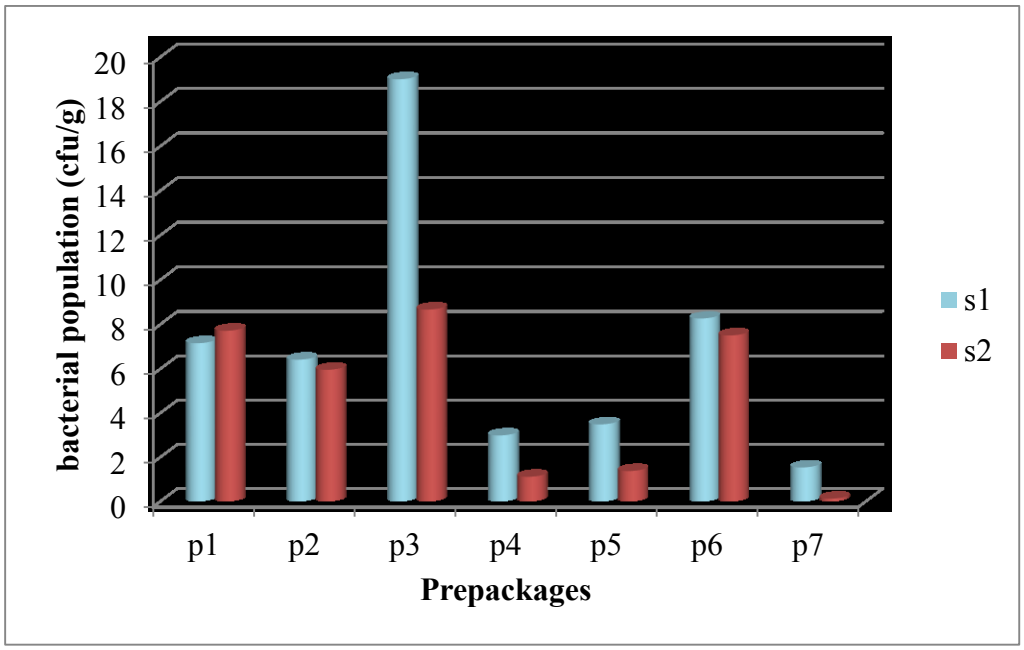


Fig 11. Effect of prepackaging and storage on bacterial population (cfug^{-1})

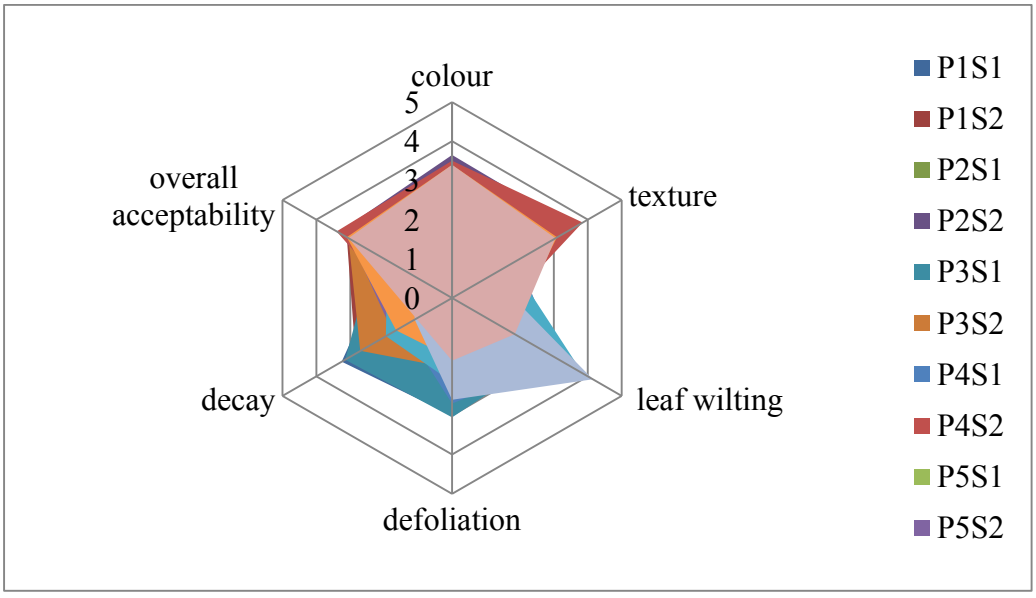


Fig 12. Star diagram depicting the effect of prepackaging and storage on visual parameters

Summary

6. SUMMARY

The study entitled "Postharvest handling for extending shelf life of amaranthus *(Amaranthus tricolor* L.)" was conducted at Department of Processing Technology, College of Agriculture, Vellayani, during 2013-15, with the objective to extend the shelf life of amaranthus (var. Arun) with minimum nutritional loss through postharvest handling practices. Major findings are summarized below.

Amaranthus was surface sanitized with different sanitizing agents and the study revealed that surface sanitization of amaranthus with 2 ppm ozonised water had lowest microbial population (3.24×10^5 $cfug^{-1}$) with highest reduction percentage of 85.68. Surface sanitization of amaranthus with 30 ppm sodium hypochlorite also reduced the microbial population than brine and tap water.

Physiological quality of surface sanitized amaranthus were analyzed and found that amaranthus treated with 2 ppm ozonised water recorded lowest physiological loss in weight of 6.88, 11.17 and 25.63 % at 2, 3 and 4 h of storage at room temperature respectively. Relative water content was also found to be highest in 2 ppm ozonised water treated amaranthus during 2, 3, and 4 h of storage at room temperature. Lowest relative water content (53.64 %) with highest weight loss (31.04 %) was observed in 2 % brine treated amaranthus during storage.

Amaranthus treated with 2 ppm ozonised water recorded highest mean score for colour (2.40), texture (2.75) and overall acceptability (2.00) with minimum mean score for leaf wilting (2.30) and defoliation (2.65) after 4 h of storage at room temperature. Increase in weight loss decreases the water content and in turn reduces the freshness or shelf life of leafy vegetables. Highest mean score for leaf wilting (3.70) and defoliation (3.45) with lowest mean score for texture (1.55), colour (1.85) and overall acceptability (1.60) during storage was recorded by amaranthus treated with 2 % brine. Based on the effectiveness of sanitizing agents in reducing microbial population and maintaining the quality of amaranthus, 2 ppm ozonised water was selected as the best sanitizer.

Effect of different pretreatments on physiological and visual parameters were studied. Results showed that 10 ppm benzyl adenine (BA) was more effective in maintaining the freshness with low physiological weight loss (21.38%) and retained high relative water content (70.33%) after 48 h of storage at room temperature. When storage conditions were studied, it was observed that cut stem end dipped in water and moist cotton plugging of cut stem end did not show significant difference in physiological loss in weight (22.93 %) and relative water content (67.36 %) during storage.

Amaranthus pretreated with benzyl adenine and stored with cut stem dipped under water or moist cotton plugging of cut stem end extended the shelf life and retained the freshness upto 48 h of storage at room temperature. Lowest physiological loss in weight was observed in amaranthus pretreated with 10 ppm benzyl adenine with cut stem dipped in water (21.41%) and moist cotton plugging of cut stem end (21.35 %). Similarly, the same treatments retained the highest relative water content of 70.45 and 70.22 % respectively during the storage period.

The treatment which retained highest moisture content scored highest mean score for colour, texture and overall acceptability with low leaf wilting and defoliation. Amaranthus pretreated with 10 ppm benzyl adenine and stored with cut stem end dipped in water or with moist cotton plugging of cut stem end recorded highest mean score for visual parameters upto 48 h of storage at room temperature. Based on the results and convenience for packaging and transportation, 10 ppm benzyl adenine treatment with moist cotton plugging of cut stem end was selected as the best pretreatment for amaranthus.

Amaranthus surface sanitized with 2 ppm ozonised water for five minutes followed by pretreatment with 10 ppm benzyl adenine for five minutes and moist cotton plugging of cut stem end were prepackaged and stored under room temperature (30±2˚C) and refrigerated storage condition (10±2˚C) and physiological, visual and nutritional parameters and microbial population were recorded.

When amaranthus were prepackaged in different packages, macro ventilated PP 100 gauge without absorbent paper and LDPE 150 gauge without absorbent paper was capable of retaining the quality in terms of low physiological loss in weight and high relative water content during storage. Refrigerated storage (10 ± 2 ˚C) was found to be the best for differently prepackaged amaranthus as compared to room temperature storage. Amaranthus prepackaged in macro ventilated PP without absorbent paper stored under refrigerated condition showed the lowest weight loss (17.29 %) with highest relative water content (67.91 %) after 120 h of storage.

Amaranthus prepackaged and stored at room temperature and refrigerated condition showed a significant difference in retention of nutritional parameters. Ascorbic acid content of fresh pretreated amaranthus before storage ranged from 18.55 to 19.05 mg/100 g. Among prepackages macro ventilated PP without absorbent paper showed the highest retention of ascorbic acid (7.39 mg/100 g) followed by macro ventilated LDPE without absorbent paper (6.65 mg/100 g) at the end of shelf life. In case of storage conditions, refrigerated condition showed highest retention of ascorbic acid (4.16 mg/100 g) at the end of shelf life after 120 h of storage as compared to room temperature (3.26 mg/100g). Amaranthus prepackaged in macro ventilated PP without absorbent paper stored under refrigerated condition retained the highest ascorbic acid (10.78 mg/100g) at the end of shelf life of 120 h of storage.

Before storage, the β-carotene content of fresh pretreated amaranthus ranged from 6.25-6.26 µg/100g but at the end of shelf life slight reduction was observed. Prepackaged amaranthus in macro ventilated PP without absorbent paper had the highest β-carotene (3.71 µg/100g) at the end of shelf life of 120 h at refrigerated condition. When storage condition was studied amaranthus stored under refrigerated condition showed the highest retention (4.16 µg/100g) when compared to the room temperature (0.80 µg/100g). Amaranthus prepackaged in macro ventilated LDPE without absorbent paper stored under refrigerated condition showed highest retention of β-carotene (5.66 µg/100g) followed by macro ventilated PP without absorbent paper stored under refrigerated condition (5.34 µg/100g).

Total chlorophyll content of amaranthus before storage ranged from 5.31 to 5.34 mg g^{-1}. Prepackaged amaranthus in macro ventilated PP without absorbent paper (3.48 mg g^{-1}) had highest total chlorophyll content when compared to other treatments. Among storage methods refrigerated storage retained the highest total chlorophyll content (2.76 mg g^{-1}). When the combined effect of storage temperature and prepackaging were considered, it was observed that prepackaging of amaranthus in macro ventilated PP cover without absorbent paper (4.42 mg g^{-1}) and macro ventilated LDPE without absorbent paper (4.13 mg g^{-1}) stored under refrigerated condition had retained highest total chlorophyll content at the end of shelf life.

In case of calcium retention, slight reduction was observed after 96 h of room temperature storage and 120 h of refrigerated storage when compared to the fresh pretreated amaranthus. Calcium content before storage ranged from 6.36 to 6.45 %. Amaranthus prepackaged in macro ventilated PP with or without absorbent paper showed highest calcium content after 96 and 120 h of storage. During storage it was found that refrigerated storage retained more calcium (5.15 %) than room temperature. Prepackaging of amaranthus with macro ventilated PP without absorbent paper stored under refrigerated condition retained the highest calcium content of 5.99% at 120 h of storage.

Iron content of fresh pretreated amaranthus ranged from 0.16 to 0.17 %. Amaranthus stored under refrigerated condition retained the highest iron content (0.08%) with a slight reduction from initial content. Prepackaging of amaranthus in macro ventilated PP without absorbent paper, sleeve wrap with LDPE and cling film wrap stored under refrigerated condition retained more iron content at the end of shelf life.

Before storage, oxalate content of pretreated amaranthus ranged from 1.70 to 1.72 %. Oxalate content of prepackaged amaranthus stored at room temperature (1.71 %) slightly increased from initial content. Amaranthus without prepackaging stored under refrigerated condition showed the lowest oxalate content (1.07 %) compared to other treatments.

When microbial population was determined it was observed that amaranthus prepackaged in macro ventilated PP without absorbent paper had lower microbial population (2.04×10^5 $cfug^{-1}$) than other prepackaged amaranthus. Under refrigerated storage condition microbial population (4.64×10^5 $cfug^{-1}$) was found to be less as compared to room temperature (6.97×10^5 $cfug^{-1}$). When combined effect was studied, macro ventilated PP without absorbent paper stored under refrigerated condition (1.11×10^5 $cfug^{-1}$) showed the lowest microbial count.

Superior visual quality viz. colour, texture and overall acceptability was maintained by macro ventilated PP cover without absorbent paper stored under refrigerated condition with lowest leaf wilting, defoliation and decay after 120 h of storage. Control samples stored under room temperature recorded the highest score for wilting and lowest score for other visual parameters. Based on the superiority of prepackaging and storage condition in retaining quality parameters with less microbial population, macro ventilated PP (100 gauge) without absorbent paper under refrigerated condition was selected as the best prepackaging and storage condition.

Surface sanitization of amaranthus with 2 ppm ozonised water followed by dipping in 10 ppm Benzyl Adenine with moist cotton plugging of cut stem end and prepackaging in macro ventilated polypropylene (100 gauge) without absorbent paper stored under refrigerated condition extended the shelf life of amaranthus (*var*. Arun) upto 120 h with minimum nutritional loss.

Future line of work

The present study revealed that postharvest handling practices could increase the shelf life of amaranthus with minimum nutritional loss. The technology developed can be commercialized after validating in the current market system as future line of work which will be helpful in reducing postharvest losses and better returns to farmers.

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Appendices

APPENDIX I

Date: **COLLEGE OF AGRICULTURE, VELLAYANI**

Dept. of Processing Technology

Score card for visual parameters

Particulars	T1	T2	T3	T4
Appearance				
Colour				
Texture				
Leaf wilting				
Defoliation				

Score :

Excellent-5

Very good-4

Good-3

Fair-2

Poor-1

Name:

Signature:

Appendix II

Cost incurred for sanitized and pretreated amaranthus (200 g) prepackaged in macro ventilated PP cover

Items	Market Price (Rs)	Quantity	Cost (Rs)
Ozonised water	0.75/ppm	2 ppm	1.50
Benzyl adenine	520/g	5 mg	2.60
Cotton	150/kg	15 g	2.25
PP cover	180/kg	1 no.	0.65
Miscellaneous	-	-	0.75
Total			7.75

Abstract

POSTHARVEST HANDLING FOR EXTENDING SHELF LIFE OF AMARANTHUS *(Amaranthus tricolor* L.)

by

GEOGY MARIAM GEORGE

(2013-12-108)

ABSTRACT

Submitted in partial fulfilment of the

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MASTER OF SCIENCE IN HORTICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PROCESSING TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM- 695 522

KERALA, INDIA

2015

ABSTRACT

The study entitled "Postharvest handling for extending shelf life of amaranthus *(Amaranthus tricolor* L.)" was conducted at Department of Processing Technology, College of Agriculture, Vellayani, during 2013-15, with the objective to extend the shelf life of amaranthus (var. Arun) with minimum nutritional loss through postharvest handling practices.

Amaranthus (var. Arun) raised as per Kerala Agricultural University packages of practices was harvested 30 days after sowing and 25-30 cm long stem with leaves after removing root portion were taken for the study.

Effectiveness of sanitizing agents for surface decontamination of harvested amaranthus was evaluated by analyzing microbial, physiological and visual parameters. The results revealed that amaranthus sanitized with 2 ppm ozonised water had highest microbial reduction (85.68 %), lowest physiological loss in weight (25.63 %), highest relative water content (63.11 %) and score for visual parameters which was followed by those treated with 30 ppm sodium hypochlorite.

When amaranthus sanitized with 2 ppm ozonised water was subjected to different pretreatments and conditions, it was observed that dipping in 10 ppm Benzyl Adenine with moist cotton plugging of cut stem end had lowest physiological loss in weight (21.35 %), highest relative water content (70.22 %) and acceptability which maintained a shelf life up to 48 h of storage at room temperature (30±2˚C).

Amaranthus surface sanitized with 2 ppm ozonised water and pretreated with 10 ppm Benzyl Adenine with moist cotton plugging of cut stem end was packaged in different materials and stored at room temperature (30±2˚C) and refrigerated conditions (10±2˚C) and analysed for physiological, nutritional and visual qualities. The results revealed that prepackaging of sanitized and pretreated amaranthus in 100 gauge macro ventilated polypropylene without absorbent paper had shelf life up to 72 h with highest retention of freshness and nutritional qualities when stored at room temperature and up to 120 h in refrigerated storage. Amaranthus had lowest

physiological loss in weight (17.29 %) and highest relative water content (67.91 %) at 120 h of refrigerated storage.

Nutritional parameters of sanitized and pretreated amaranthus were analyzed and recorded 18.55 to 19.05 mg/100g of ascorbic acid, 5.31 to 5.34 mg g^{-1} of total chlorophyll, 6.25 to 6.26 µg/100g of β-carotene, 6.36 to 6.45 % of calcium and 0.16 to 0.17 % of iron before storage. At the end of shelf life of 120 h of refrigerated storage, amaranthus prepackaged in macro ventilated PP (100 gauge) without absorbent paper recorded maximum retention of ascorbic acid (10.78 mg/100g), total chlorophyll (4.42 mg g^{-1}), β-carotene (5.34 µg/100g), calcium (5.99%) and iron (0.09%) content with lowest microbial population.

Surface sanitization of harvested amaranthus (var. Arun) with 2 ppm ozonised water and dipping in 10 ppm Benzyl Adenine, each for five minutes, followed by moist cotton plugging of cut stem end and prepackaging in macro ventilated polypropylene (100 gauge) without absorbent paper could extend shelf life up to 120 h when stored under refrigerated condition.