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Effect of chemical treatment, storage and packaging on physico-chemical properties of sunflower microgreens

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Abstract
Objective of this investigation was to study effects of chemical treatments [ethanol vapour (EV), acetaldehyde vapour (AV), citric acid (CA), ascorbic acid (AA), citric acid + ethanol (CA+E) and citric acid + ascorbic acid (CA+AA), water as control] and packaging materials (polystyrene and LDPE) on postharvest quality sunflower microgreens for 16 days (sampling at 4th day) stored at 10±1 °C. Total soluble solids first increased with storage (4.5 on 0th day and 5.7 on 8th day) and then decreased (5.1 on 16th day) whereas acidity followed a continuous increase with storage time (0.040% on 0th day to 0.072 on 16th day). Both fresh weight and dry weight also increased with storage period. Fresh weight and dry weight were10.03g and 8.61g on 0th day and 10.47g and 12.46 on 16th day respectively.

Keywords: Sunflower microgreens, acidity, tss, citric acid, ascorbic acid

1. Introduction
Microgreens are new class of speciality greens that are harvested at the cotyledonary leaf stage (appearance of true leaves) sans roots and sees coats. They are often called as vegetable confetti owing to their varied and distinct colours and flavours and are consumed in raw form as salads or cooked and used as garnish (Aggrawal and Aggrawal, 2013) [2]. Microgreens contain 5-10 times higher concentration of nutrients like ascorbic acid, phenols, chlorophyll and carotenoids, vitamins etc., than their mature counterparts (Xiao *et al*., 2012; Kou *et al*., 2013) [15, 8]. Owing to the high concentration of nutrients microgreens contain, they can be classified as functional foods (Sharma *et al*., 2012) [13].

Microgreens are easy to grow and harvest in semi-urban and urban settings and are being offered as fresh salads straight out of the pot in various restaurant chains in India and across the world. They don't need much input in terms of fertilizers, nutrients, water, soil and space because of their short life span. Sunflower seeds are consumed raw, sprouted, roasted, salted or made into flour. Protein make up 24-30% of seed weight containing 8 essential amino acids and have high vitamin concentration (Balasaraswathi and Sadasivam, 1997) [3]. Sunflower microgreens are rich in fibre, protein, total phenols and have high total antioxidant activity (Pajak *et al*., 2014) [10].

Chemicals like citric acid, ascorbic acid, acetaldehyde, ethanol vapour or their combinations fall under GRAS (generally regarded as safe) and have been reported to extend the shelf life of minimally processes fruits and vegetables (Goyal *et al*., 2014; Siddiqui *et al*., 2015) [6, 14]. Organic acids like ascorbic acid and citric acids have been showed to inhibit the enzymatic activity in fresh produce and retain its colour and texture in case of green celery (Gomez and Artes, 2004) [5] and peeled oranges (Pao and Patracek, 1997) [11]. Acetaldehyde and ethanol have been shown to slow down and hasten the ripening process in various fruits and vegetables depending concentration and variety and can be used to extend the shelf life of fresh produce (Pesis, 2005) [12]. MAP (modified atmosphere packaging) is used to extend the shelf life of fresh produce by reducing respiration leading to less substrate depletion. MAP can thus inhibit the chemical and enzymatic mechanisms associated with fresh produce decay (Kader *et al*., 1989) [7]. However, research on posh-harvest shelf life of microgreens with external treatments is scare and there aren't any studies available determine the post-harvest quality of sunflower microgreens. The scope of the present is to determine the effects of individual GRAS chemicals and their combinations, MAP and packaging on the shelf life of sunflower microgreens.

2. Materials and Methods

For growing sunflower microgreens, Sunflower seeds were procured from Department of Oilseeds Technology, CCS HAU, Hisar. Sunflower seeds were soaked for 24h in water and distributed over wet vermiculite kept in shade house at 25 ± 5 °C. The crop was kept moist and no fertilizer was applied. Microgreens were harvested after 7 days of growth, washed with tap water and subjected to the following treatments with distilled water as control.

The following treatments with distilled water as control.

Treatment	Concentration and duration
Ethanol vapour treatment (EV)	100% for 3.5 minutes
Acetaldehyde vapour treatment (AV)	100% for 4 minutes
Citric acid spray treatment (CA)	0.5% w/v
Ascorbic acid spray treatment (AA)	0.5% w/v
Citric acid + Ethanol spray treatment (CA+E)	0.5% w/v + 40% v/v resp.
Citric acid + Ascorbic acid spray treatment (CA+AA)	0.25% w/v each

The treated microgreens were packed either in 0.05% perforated LDPE bags and polystyrene trays wrapped with cling films with 6 pin hole size perforations in it. Filter paper soaked with water was placed in packages maintain high humidity. Packs were stored for 16 days at low temperature (10±1 °C) maintained in B.O.D. incubator having three replicates. Sampling was done at every 4th day.

2.1 Observations recorded

2.1.1 Average fresh weight and dry weight

The fresh weight of 25 microgreens was recorded to get obtain average fresh weight. For dry weight, 25 microgreens were weighed and cut; cut pieces were packed into previously weighed packs of paper that were allowed to dry in hot air oven at 55±2 °C for two days and then for 1 day at 110° C. The weight of the dried samples was recorded and the dry weight was calculated. The reduced weight was noted down and the procedure was repeated until constant weight was observed.

$$\text{Dry weight (\% of fresh weight)} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

2.1.2 Total Soluble Solids

One g of sunflower microgreens was lightly macerated with 1 ml of water and squeezed by hand through a muslin cloth. The juice extracted was immediately used for determination of TSS by using hand refractometer of 32% range. After adjusting the dilution factor, the values were expressed in %.

2.1.3 Acidity

Acidity was estimated by titrating the extract against 0.01 N sodium hydroxide, as per the method described by AOAC (1995).

$$\text{Acidity (\%)} = \frac{\text{Titre} \times \text{Normality of alkali} \times 0.6404 \times \text{Volume made up}}{\text{Volume of aliquot} \times \text{Weight of sample}}$$

2.2 Statistical Analysis

The OPStat software developed by CCS HAU, Hisar was used for statistical analysis of variance (ANOVA). Means were separated by critical difference (CD) at the 5% level of significance. For experiment three factorial CRD was used for analysis.

3. Results and Discussion

3.1 Average fresh weight and dry weight

The data on fresh weight and dry weight of sunflower microgreens under various treatments and packaging during storage are presented in Table 3 and 4 respectively. There was a progressive increase in fresh weight of microgreens with increasing storage period. The average fresh weight at 0th day was 10.04 g, which increased to 10.47 g/25 microgreens at 16th of storage whereas dry weight increased from 8.61 g/25 microgreens on 0th day to 12.46 g/25 microgreens on 16th day. The increase in fresh weight can be attributed to uptake of moisture and slight increase in the size of true leaves of microgreens as reported by Median *et al*. (2015) in minimally processes baby spinach leaves stored under varying humidity levels at 7 °C. The increase in dry weight on per cent fresh weight basis could be due to loss of moisture, as there is no fresh synthesis of dry matter in microgreens. There was no significant effect of packaging material on fresh weight of microgreens, however, the microgreens packed polystyrene trays maintained higher dry weight than LDPE bags. The differences in weight loss can be attributed to different permeabilities of the packaging material to moisture and gasses as reported by Chitravathi *et al*. (2015) [4]. The fresh weight was not significantly affected with respect to control by various treatments, except acetaldehyde treatment showing decreased fresh weight which could be due to its lethal effect.

3.2 Total soluble solids (TSS)

TSS increased slightly with time (from 4.5 on 0th day to 5.7 on 8th day) and then decreased at later storage periods (5.1 on 16th day) (Table 1). TSS was slightly decreased with respect to control by various treatments, except acetaldehyde vapour treatment. Microgreen packed in LDPE bags showed a slight higher TSS than the ones packed in polystyrene trays. This increase can be attributed to conversion of insoluble reserved metabolites to soluble one during the initial stages of growth and at later stages, a decrease been observed due to utilisation of soluble metabolites for growth. The differences in the TSS of microgreens packed in different packaging materials could be due to differential utilisation of metabolites in by respiration getting influenced by the permeabilities of the packaging material to gasses. Goyal and Siddiqui (2014) [6] also observed that TSS of mung bean sprouts at room temperature increased during the first 24 h and then decreased at 48h. Balasaraswathi and Sadasivam (1997) [3] conducted a study to analyse the nutrient content of sunflower as it germinated for 5 days. Reducing sugars that contribute to TSS were found to increase from 0.50% to 4.40% from day 1 to day 5.

3.3 Acidity

The data on acidity (%) of sunflower microgreens under various treatments and packaging during storage is presented in Table 2. There was a progressive increase in acidity of microgreens with storage period with 0.040% on 0th day to 0.071% on 16th day. The acidity was significantly decreased with respect to control by various treatments, maximum being for acetaldehyde treatment. There was no significant difference observed in acidity of microgreens packed either in LDPE bags or polystyrene trays. McGill *et al*. (2006) [9] also reported an increase in oxalic acid content of spinach leaves stored under CA and MA storage. The increasing acidity maybe attributed to the biochemical conversion of fatty acids to acids with time.

Table 1: Effect of various treatments and packaging on average fresh weight (g/25 microgreens) of microgreens during storage

Treatments	Period of storage (days)														
	0			4			8			12			16		
	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean
Control	10.00	10.02	10.01	10.12	10.04	10.08	10.23	10.14	10.18	10.31	10.14	10.23	10.64	10.43	10.54
EV	10.03	10.04	10.03	10.15	10.07	10.11	10.25	10.15	10.20	10.31	10.42	10.37	10.53	10.64	10.58
AV	10.03	10.03	10.03	10.11	10.05	10.08	9.90	10.13	10.01	(9.90)*	10.45	10.17	(9.90)*	10.46	10.18
CA	10.07	10.04	10.06	10.18	10.08	10.13	10.24	10.22	10.23	10.35	10.45	10.40	10.65	10.44	10.55
AA	10.05	10.05	10.05	10.17	10.08	10.12	10.26	10.21	10.24	10.34	10.45	10.39	10.65	10.44	10.54
CA+E	10.02	10.04	10.03	10.14	10.08	10.11	10.23	10.22	10.23	10.35	10.44	10.40	10.65	10.24	10.45
CA+AA	10.03	10.02	10.03	10.17	10.07	10.12	10.27	10.21	10.24	10.35	10.45	10.40	10.53	10.44	10.49
Mean			10.03			10.11			10.19			10.34			10.47

Treatments	Packaging		Overall mean
	LDPE	PS	
Control	10.26	10.15	10.21
EV	10.25	10.26	10.26
AV	9.97	10.22	10.09
CA	10.30	10.25	10.27
AA	10.29	10.25	10.27
CA+E	10.28	10.21	10.24
CA+AA	10.27	10.24	10.25
Mean	10.23	10.23	

CD at 5%	Treatment (T) = 0.057	Packaging (P) = NS	Storage (S) = 0.024	SxT = NS	SxP = 0.033	TxP = 0.039	SxTxP = 0.086

* Treatment was terminated due to spoilage of the microgreens. Values in the parenthesis are assumed values equivalent to the values at the last day before termination of the treatment. The values have been taken for the purpose of ANOVA only.
LDPE: Low density polyethylene, **PS**: Polystyrene

Table 2: Effect of various treatments and packaging methods on dry weight (% fresh weight) of microgreens during storage

Treatments	Period of storage (days)														
	0			4			8			12			16		
	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean
Control	10.06	10.03	10.04	10.72	10.91	10.82	11.01	11.39	11.20	12.26	12.39	12.32	12.82	13.38	13.10
EV	9.03	9.02	9.02	10.10	10.27	10.18	10.71	11.60	11.16	12.27	11.76	12.02	12.92	12.19	12.55
AV	8.59	8.64	8.61	9.64	10.81	10.22	10.79	11.22	11.01	(10.79)*	11.74	11.26	(10.79)*	13.05	11.92
CA	7.99	8.01	8.00	8.90	9.00	8.95	11.08	11.51	11.30	11.55	12.20	11.87	11.51	12.34	11.93
AA	8.09	8.11	8.10	8.81	9.98	9.39	10.87	11.32	11.09	11.45	12.97	12.21	11.51	12.14	11.83
CA+E	8.23	8.22	8.22	8.62	9.27	8.95	11.16	11.40	11.28	12.40	11.74	12.07	12.46	13.56	13.01
CA+AA	8.22	8.33	8.28	8.61	9.21	8.91	10.84	11.50	11.17	12.54	12.03	12.28	12.50	13.35	12.92
Mean			8.61			9.63			11.17			12.01			12.46

Treatments	Packaging Material		Overall Mean
	LDPE	PS	
Control	11.37	11.62	11.50
EV	11.01	10.97	10.99
AV	10.12	11.09	10.61
CA	10.21	10.61	10.41
AA	10.15	10.90	10.52
CA+E	10.57	10.84	10.71
CA+AA	10.54	10.88	10.71
Mean	10.57	10.99	

Packaging (P) = 0.063	Storage (S) = 0.100	SxT = 0.265	SxP = 0.142	TxP = 0.168	SxTxP = 0.375

* Treatment was terminated due to spoilage of the microgreens. Values in the parenthesis are assumed values equivalent to the values at the last day before termination of the treatment. The values have been taken for the purpose of ANOVA only.
LDPE: Low density polyethylene, PS: Polystyrene

Table 3: Effect of various treatments and packaging on total soluble solids (%) of microgreens during storage

Treatments	Period of storage (days)														
	0			4			8			12			16		
	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean
Control	4.5	4.5	4.5	4.6	4.5	4.5	5.8	5.6	5.7	5.4	5.3	5.3	5.2	5.2	5.2
Ethanol vapour	4.4	4.4	4.4	4.5	4.4	4.5	5.4	5.5	5.5	5.0	5.0	5.0	4.8	4.9	4.8
Acetaldehyde vapour	4.5	4.5	4.5	4.6	4.4	4.5	7.2	5.5	6.3	(7.2)*	5.1	6.1	(7.2)*	4.9	6.0
Citric acid	4.6	4.6	4.6	4.7	4.6	4.6	5.6	5.7	5.6	5.1	5.2	5.1	4.9	5.0	4.9
Ascorbic acid	4.5	4.5	4.5	4.5	4.5	4.5	5.5	5.4	5.5	5.1	5.0	5.0	5.0	4.9	4.9
Citric acid + ethanol	4.4	4.4	4.4	4.5	4.5	4.5	5.4	5.6	5.5	4.9	5.1	5.0	4.7	4.9	4.8
Citric acid + ascorbic acid	4.4	4.4	4.4	4.5	4.4	4.5	5.6	5.4	5.6	5.1	5.1	5.1	4.9	4.9	4.9
Mean			4.5			4.5			5.7			5.3			5.1

Treatments	Packaging		Overall mean
	LDPE	PS	
Control	5.1	5.0	5.0
Ethanol vapour	4.8	4.9	4.9
Acetaldehyde vapour	6.1	4.9	5.5
Citric acid	5.0	5.0	5.0
Ascorbic acid	4.9	4.9	4.9
Citric acid + ethanol	4.8	4.9	4.8
Citric acid + ascorbic acid	4.9	4.9	4.9
Mean	5.1	4.9	

CD at 5%	Treatment (T) = 0.11	Packaging (P) = 0.06	Storage (S) = 0.10	SxT = 0.26	SxP = 0.14	TxP = 0.16	SxTxP = 0.36

* Treatment was terminated due to spoilage of the microgreens. Values in the parenthesis are assumed values equivalent to the values at the last day before termination of the treatment. The values have been taken for the purpose of ANOVA only.
LDPE: Low density polyethylene, PS: Polystyrene

Table 4: Effect of various treatments and packaging on acidity (%) of microgreen during storage

Treatments	Period of storage (days)														
	0			4			8			12			16		
	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean	LDPE	PS	Mean
Control	0.033	0.033	0.033	0.063	0.049	0.056	0.069	0.067	0.068	0.073	0.060	0.067	0.085	0.070	0.078
Ethanol vapour	0.048	0.048	0.048	0.070	0.051	0.061	0.078	0.057	0.068	0.078	0.053	0.066	0.082	0.063	0.073
Acetaldehyde vapour	0.038	0.038	0.038	0.058	0.050	0.054	0.063	0.055	0.059	(0.063)*	0.059	0.061	(0.063)*	0.068	0.066
Citric acid	0.035	0.035	0.035	0.050	0.054	0.052	0.057	0.057	0.057	0.056	0.067	0.062	0.059	0.082	0.071
Ascorbic acid	0.042	0.042	0.042	0.063	0.053	0.058	0.084	0.053	0.069	0.082	0.070	0.076	0.087	0.065	0.076
Citric acid + ethanol	0.043	0.043	0.043	0.069	0.050	0.060	0.067	0.051	0.059	0.068	0.073	0.071	0.074	0.074	0.074
Citric acid + ascorbic acid	0.040	0.040	0.040	0.043	0.036	0.040	0.063	0.048	0.056	0.065	0.070	0.068	0.069	0.072	0.071
Mean			0.040			0.054			0.062			0.067			0.072

Treatments	Packaging		Overall mean
	LDPE	PS	
Control	0.065	0.085	0.075
Ethanol vapour	0.071	0.055	0.063
Acetaldehyde vapour	0.057	0.054	0.055
Citric acid	0.052	0.059	0.055
Ascorbic acid	0.072	0.057	0.064
Citric acid + ethanol	0.064	0.058	0.061
Citric acid + ascorbic acid	0.056	0.053	0.055
Mean	0.062	0.060	

CD at 5%:	Treatment (T) = 0.001	Packaging (P) = NS	Storage (S) = NS	SxT = 0.001	SxP = 0.001	TxP = 0.001	SxTxP = 0.002

LDPE: Low density polyethylene, PS: Polystyrene

4. Conclusion
On the basis of the results obtained in the present investigation, it can be concluded that the sunflower microgreens that are ready for harvest by 7th day of sowing at 25 ± 5 °C, having desirable configuration of length 6.13-6.23 cm and fresh weight 10.04-10.47 g/25 microgreens, remained acceptable upto 12 days when stored at low temperature of 10 °C. The shelf life and nutritional quality was better when microgreens were packed in polystyrene trays as compared to LDPE bags. The various chemical vapour or spray treatments to microgreens were not influencing significantly the shelf life, except the acetaldehyde treatment that adversely affected the shelf life. Future body of work can include application of other biochemical like phytohormones, natural antimicrobials and pre-harvest sprays to enhance the shelf life and nutritional quality.

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