Influence of packaging and storage conditions on biochemical quality and enzymatic activity in relation to shelf life enhancement of fresh basil leaf

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Abstract Biochemical changes in response to storage conditions and packaging were investigated in basil leaves of variety Genovese in order to find out suitable conditions for their storage. Fresh basil leaves placed in open trays or packed in lowdensity polyethylene (LDPE) bags were stored at 5, 12 °C and under ambient conditions (12.2–21.0 °C) for 12 days. Chlorophyll content, total phenols, total flavonoids and antioxidant activity of basil leaves decreased with simultaneous increase in electrolyte leakage and TBA reactive compounds during whole storage period. Packaging of leaves and storage at 5 °C slowed down the changes in these quality parameters in comparison to those kept in open trays and stored at 12 °C and ambient temperature respectively. Activities of enzymes catechol oxidase and lipoxygenase which increased during storage were slowed down by packaging and decrease in storage temperature. Irrespective of storage conditions and packaging, activities of antioxidative enzymes viz. catalase, peroxidase and superoxide dismutase increased during storage. Rapid increase in activities of antioxidative enzymes was observed LDPE packed leaves than those held in open trays and in leaves stored at 5 °C as compared to 12 °C and ambient temperature. Basil leaves packed in LDPE and stored at 5 °C showed the slowest changes in biochemical parameters and the highest activities of antioxidative enzymes and could be stored for 12 days in comparison to 6 days in open trays at ambient temperature.

Keywords Antioxidative enzymes · Basil leaves · Packaging · Quality parameters · Shelf life · Storage conditions

Introduction

Basil (*Ocimum basilicum*) is a culinary herb prominently featured in Italian and South East Asian countries. Basil is related to tropical regions of Asia particularly India and Iran, but now it is widely available throughout the world. Basil, being an aromatic herb, is used extensively in food to add a distinctive aroma and flavor as well as for its antimicrobial, anti-inflammatory, antioxidant, antiviral or medicinal properties (Boateng 2013). Fresh or dried leaves of basil can be used as a spice. Basil contains a wide range of essential oils rich in phenolic compounds (Phippen and Simon 2000) and other natural products including polyphenols such as flavonoids and anthocyanins (Phippen and Simon 1998) which contribute to its strong antioxidant activity.

Basil leaves constantly deteriorate in quality during transportation and storage depending on the environmental conditions. Several biochemical processes occur during the storage or leaf senescence such as degradation of proteins, chlorophyll, nucleic acid and membrane integrity (Buchanan-Wollaston 1997) with the involvement of several classes of enzymes. Change in colour of leaves due to chlorophyll degradation is the most visible symptom. Browning of leaves during storage might be due to the activity of enzyme peroxidase (POD) or catechol oxidase (Wongsheree et al. 2009). POD catalyzes browning reactions through the generation of hydrogen peroxide. Free phenolics or quinines are used as substrates in POD catalysed browning reactions. Catechol oxidase can

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enhance POD-mediated browning or act independently. Catechol oxidase also uses phenolic compounds as a substrate and its activity has been correlated with chilling injury symptoms in some leafy vegetables (Ose et al. 1995). Maintenance of membrane integrity during storage has been considered important which changes with the change in temperature. Loss of membrane integrity during storage has been related to lipid peroxidation and the accumulation of free radicals (Wills et al. 2007). A part of lipid degradation of membrane might be due to the activity of enzyme lipoxxygenase (Maalekuu et al. 2006). The production of free radicals during membrane degradation is considered to be the prime cause of oxidative damage during senescence. Plant cells are protected against the effects of these free radicals by a complex antioxidant system that involves antioxidants and antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and POD. Specific responses has been observed for different antioxidative enzymes in relation to senescence and ripening processes (Jimenez et al. 2003) that can take place during storage. Phytochemical reactions during storage under different environmental conditions may change the level of antioxidants and antioxidative enzymes. Therefore, quantification of these reactions is critical in understanding their role in shelf life enhancement of basil leaves.

The deterioration of basil leaf following harvest is influenced by many factors such as storage conditions and packaging material. The quality of most of the fresh culinary herbs is best maintained at low temperature (Cantwell and Reid 2002). However, chilling injury can occur when basil is exposed to near freezing point temperature. Sensitivity to chilling temperature has been observed higher in lemon basil and holy basil than in sweet basil in which the leaf darkening occurred within 24 h during storage at 4 °C (Wongsheree et al. 2009). Packaging of fresh produce in polyethylene bags has become a common practice in now a-days. Packaging changes the composition of air to provide an optimum atmosphere for maintaining the quality of produce. A modified atmosphere created by packaging extends the storage life of produce by reducing respiration rate, improving moisture retention and through inhibition of physiological deterioration (Mangaraj et al. 2009). So far, little work has been done to extend the storage life of fresh basil leaves. In order to formulate storage conditions for enhancement of shelf life of basil leaves, it is essential to understand the biochemical basis of quality maintenance of leaves during storage under different conditions. So, the present study was undertaken to ascertain the effect of packaging and storage conditions on biochemical quality parameters in relation to activities of various enzymes of fresh basil leaves for revealing targeted strategies for increasing shelf life of basil leaves.

Materials and methods

Collection and storage of basil leaves

The present investigation was carried out on basil variety i.e. Genovese obtained from Field Fresh Pvt. Ltd. Ludhiana. Fresh basil leaves were cleaned manually and removed from stalk. Cleaned fresh basil leaves were placed in open trays or packed in LDPE (Low density polyethylene) bags (20 g in each bag) and were stored at 5 °C (90–95% humidity), 12 °C (90–95% humidity) and ambient (12.2–21.0 °C, 37–98% humidity) temperature. Ten bags of each treatment were taken on alternative days, mixed and analyzed for quality parameters and enzymatic activities in triplicates.

Physiological and biochemical quality parameters

Physiological loss in weight was periodically calculated as percentage of difference between initial weight and final weight of tested basil leaves divided by their initial weight. Ascorbic acid was estimated by the standard method of AOAC (1984). Basil leaves (0.5 g) were crushed in pestle and mortar using 10 ml of metaphosphoric acid-acetic acid solution and was filtered. Filtrate was titrated against dye 2, 6 dichloroindophenol till the appearance of pink colour and volume of dye used to oxidize ascorbic acid in sample was noted. Ascorbic acid content was determined by titrating the standard ascorbic acid (0.2 mg/ml) with dye and was expressed as mg/100 g of fresh basil leaves. For estimation of chlorophyll, basil leaves (0.5 g) were crushed in pestle and mortar and chlorophyll was extracted five times using 10 ml of acetone. The extract was covered with aluminium foil to prevent photo-bleaching. Combined mixture was finally placed on shaker at 140 rpm for 30 min and then centrifuged at 12,000 rpm for 15 min. Final volume of supernatant was made to 100 ml by adding acetone. Chlorophyll content was determined by taking absorbance at 644.8 and 663 nm (Sozzi et al. 1998).

Phenolic compounds

For extraction of phenolic compounds, basil leaves (1 g) were crushed and refluxed with 5 ml of 80% methanol for 1 h. The refluxed sample was filtered through Whatman filter paper No. 1 and the volume was made 10 ml by washing with hot 80% methanol. The methanol extract, thus prepared was used for estimation of phenolic compounds. For estimation of total phenols, methanolic extract (0.5 ml) was evaporated to dryness and the residue was dissolved in 6.5 ml of distilled water. To this, 0.5 ml of Folin's reagent was added and shaken thoroughly. After

5 min, 1 ml of saturated solution of Na_2CO_3 was added and the reaction mixture was incubated for 1 h at room temperature. The absorbance of blue color was read at 760 nm against a blank. The concentration of total phenol was determined from standard curve prepared simultaneously using gallic acid (10–50 μg) (Singleton et al. 1999). Flavonoids from the methanolic extract were estimated by the method of Balabaa et al. (1974). Methanolic extract (3 ml) was evaporated to dryness. The residue left was dissolved in 10 ml of 0.1 M methanolic solution of aluminium chloride. Intensity of yellow color so developed was read at 420 nm against blank. Concentration of total soluble flavonoids was determined from standard curve prepared simultaneously using rutin (40–200 μg).

Ion leakage and thiobarbituric acid (TBA)-reactive compounds

Basil leaves (1.0 g) were put in 20 ml of distilled water and were kept overnight at 25 °C. Conductivity of the water was measured with the conductivity meter. After this, the samples were boiled at 95 °C for 1 h and conductivity was measured again. The ratio of reading before boiling and after boiling indicated the ion leakage (Bailey et al. 1996). TBA-reactive compounds were determined according to Hodges et al. (1999) with some modifications. Basil leaves (2 g) were homogenized in 4 ml of distilled water. To this homogenate, 4 ml of 10% TCA was added and solution was filtered through filter paper. To 4 ml of filtrate, 1 ml of 0.06 M thiobarbituric acid was added and the solution was heated for 10 min at 100 °C. After cooling, absorbance was read at 532 nm. The content of malondialdehyde was calculated using 1.56 as extinction coefficient.

Total antioxidant activity

The method of Dasgupta and De (2006) was used for the estimation of total antioxidant activity. To 1 g of crushed basil leaves, 10 ml of distilled water was added. Sample was boiled for 1 h in boiling water bath and left overnight. To 2 ml of above extract, 1 ml of TAA reagent (0.6 M H_2SO_4 , 28 mM sodium phosphate and 4 mM ammonium molybdate, mixed in equal amounts before use) was added and mixture was incubated at 95 °C for 90 min. After cooling, intensity of blue color was read at 695 nm. The total antioxidant activity was measured as ascorbic acid equivalent and was determined from standard curve prepared simultaneously using ascorbic acid (40–200 μg).

Estimation of activities of CAT, SOD and POD

Extraction and assay of CAT and SOD

Basil leaves (0.5 g) were homogenized in 5 ml of cold (4 °C) extraction buffer (0.1 M sodium phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone and 1 mM EDTA) using pre chilled pestle and mortar. The mixture was then centrifuged at 8000 rpm for 15 min at 4 °C. Supernatant was collected and analyzed for activities of enzymes CAT and SOD. For assaying CAT enzyme, 0.1 ml enzyme extract was added to 1.9 ml of chilled 0.1 M sodium phosphate buffer (pH 7.0). Reaction was started by adding 1 ml of H_2O_2 (8.25 $\mu\text{l/ml}$) to the reaction mixture. The rate of decrease in absorbance at 240 nm was measured at 10 s interval for 3 min. Enzyme activity was expressed as $\mu\text{moles of H}_2\text{O}_2$ decomposed/min/g fresh weight using 0.0394 as extinction coefficient (Rao et al. 1997). For estimation of SOD, 3 ml of reaction mixture contained 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM nitroblue tetrazolium (NBT), 0.1 ml of 1 mM EDTA, 0.1 ml of 0.1 M Na_2CO_3 , 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 0.95 ml distilled water. The enzyme extract (0.1 ml) was added and reaction was started by adding 0.1 ml of 2 μM riboflavin. The test tubes were then placed under normal sunlight till the development of blue color and the reaction was stopped by keeping the tubes in dark. The intensity of color developed was read at 560 nm. The activity was expressed as the amount of enzyme required for the 50% inhibition of photochemical reduction of NBT (Xing et al. 2008).

Extraction and assay of POD

For extraction of enzyme POD, basil leaves (0.2 g) were homogenized in 2 ml of cold (4 °C) extraction buffer (0.1 M Tris–HCl buffer containing 1 mM EDTA, 1% (w/v) polyvinylpyrrolidone and 10 μM β -mercaptoethanol) using pre chilled pestle and mortar. The mixture was then centrifuged at 8000 rpm for 15 min at 4 °C and supernatant was analyzed for enzyme activity. For assay of enzyme POD, enzyme extract (0.1 ml) was added to 3 ml of chilled guaiacol (111 $\mu\text{l}/100$ ml of 0.1 M sodium phosphate buffer pH 6.5). The reaction was started by adding 0.1 ml of H_2O_2 (82.5 $\mu\text{l/ml}$) and the decrease in absorbance (470 nm) was measured at 30 s interval for 3 min (Hemeda and Klein 1990). The activity was expressed as units/min/g fresh weight. The unit of enzyme was expressed as change in absorbance by 1.0 under standard conditions.

Lipoxygenase and catechol oxidase

Basil leaves (1 g) were homogenized in 10 ml of cold (4 °C) extraction buffer (0.1 M sodium phosphate buffer of pH 6.8 containing 1.5% polyvinylpolypyrrolidone) using pre chilled pestle and mortar. The mixture was then centrifuged at 8000 rpm for 15 min at 4 °C and supernatant was analysed for activities of lipoxygenase and catechol oxidase. For estimation of lipoxygenase, the assay was performed at 25 °C by adding 50 µl of crude enzyme extract to 100 µl of substrate reagent (10 µl of linoleic acid substrate, 4 ml of water, 1 ml of 0.1 N NaOH and 5 µl of Tween 20) and 1.85 ml of buffer (100 mM phosphate buffer, pH 6.8) and absorbance was observed at 234 nm up to 3 min at 30 s interval. One unit of activity was defined as amount of the enzyme that showed an increase in absorbance of 0.1 per min at 234 nm (Sovrano et al. 2006). The method described by Dan et al. (1999) was followed for the estimation of catechol oxidase. The reaction mixture contained 1.95 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 10 mM caffeic acid substrate and 0.05 ml of crude enzyme extract. The activity was determined by monitoring the increase in absorbance of the mixture at 420 nm after adding the enzyme extract. One unit of enzyme activity was defined as the amount of the enzyme that showed an increase in absorbance of 0.001 per min at 420 nm.

Statistical analysis

All the experiments were performed in triplicate and data were expressed as mean $\pm$ standard error. The data were subjected to two-way analysis of variance (ANOVA) to determine the significance of differences. Critical difference at 5% level was determined by using completely randomized design in CPCS software.

Results

Physiological and biochemical quality parameters

Physiological loss in weight of basil leaves increased significantly ($p \leq 0.05$) during storage under all conditions. However, loss was quite lower at 5 °C as compared to basil leaves stored at 12 °C or under ambient conditions. LDPE packaging was helpful in declining the weight loss significantly ($p \leq 0.05$) as compared to basil leaves placed in open trays under all three storage conditions (Table 1). On 6th day of storage, loss in weight under ambient temperature, 12 and 5 °C was 24.10, 14.21 and 10.28% in leaves placed in open trays and 3.05, 2.23 and 1.60% in LDPE bags respectively. On 12th day of storage, leaves in open

trays at 5 °C showed a weight loss of 74.37% and were almost dry. However, LDPE packaging resulted in weight loss of 4.36 and 6.44% at 5 and 12 °C, respectively on 12th day of storage. Leaves kept in open trays at ambient temperature could not be stored beyond 6th day because of deterioration whereas in LDPE packaging, storage life was up to 8th day at same temperature. Irrespective of storage conditions, a decline in total chlorophyll content of leaves was observed with increase in storage period. This decrease was significant ($p \leq 0.05$) even after 2nd day of storage in all the samples except for samples packed in LDPE bags and stored at 5 °C where a significant ($p \leq 0.05$) difference in chlorophyll content was found after 4th day of storage. However, LDPE packed leaves retained more chlorophyll as compared to leaves placed in open trays and also chlorophyll degradation was slowest at 5 °C followed by 12 °C. At storage temperature of 5 °C, chlorophyll retention was 75% in leaves stored in LDPE bags and 58.95% in leaves placed in open trays on 6th day of storage, which reduced to 71.91% in LDPE packaging and 42.28% in samples in open trays at 12 °C. However, retention in chlorophyll was lowest at ambient temperature where leaves stored in LDPE packaging and open trays retained 54.32 and 41.35% of chlorophyll content respectively on 6th day of storage (Table 1). A significant ($p \leq 0.05$) decline in ascorbic acid content of leaves was found with increase in storage period under all storage conditions. Ascorbic acid retention was higher in leaves kept in LDPE bags as compared to open trays under all storage conditions. Low temperature storage was found to reduce the rate of ascorbic acid loss significantly ($p \leq 0.05$). On 6th day of storage, ascorbic acid loss was highest in leaves stored under ambient conditions i.e. 83.65% in LDPE packaging and 87.43% in open trays, whereas this loss at 12 °C was 75.97 and 79.89% in LDPE packaging and open trays respectively during same period of storage. The loss of ascorbic acid was lowest in leaves stored at 5 °C i.e. 65.90% in LDPE packaging and 70.74% in open trays on 6th day of storage (Table 2).

Total antioxidant activity comprises of lipophilic antioxidant activity and hydrophilic antioxidant activity. The total antioxidant activity also decreased throughout the storage period and the decrease was significantly ($p \leq 0.05$) lower in packed leaves than in leaves placed in open trays and also at 5 °C followed by leaves stored at 12 °C. Among all storage conditions, basil leaves stored under ambient temperature and in open trays displayed highest decrease in antioxidant activity. At 5 °C, the retention of antioxidant activity on 6th day of storage was 82.88 and 76.22% in LDPE packed and open trays leaves respectively, whereas at 12 °C, this retention was 58.58 and 57.66% in samples kept in LDPE bags and open trays respectively. Retention of antioxidant activity was lowest

Table 1 Effect of storage conditions and packaging on the weight loss (%) and chlorophyll content (mg/g) of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of storage (S) →	0	2	4	6	8	10	12	CD ($p \leq 0.05$)
<i>Weight loss</i>										
5 °C	LDPE		0.00 ± 0.00	0.40 ± 0.01	0.80 ± 0.05	1.60 ± 0.10	2.80 ± 0.07	3.60 ± 0.17	4.36 ± 0.09	T-0.74
	Open		0.00 ± 0.00	0.60 ± 0.01	1.86 ± 0.05	10.28 ± 0.70	28.20 ± 0.67	33.46 ± 1.72	74.37 ± 1.94	P-0.60
12 °C	LDPE		0.00 ± 0.00	0.82 ± 0.01	1.63 ± 0.10	2.23 ± 0.12	3.82 ± 0.11	4.84 ± 0.09	6.44 ± 0.12	S-0.85
	Open		0.00 ± 0.00	1.88 ± 0.08	4.42 ± 0.13	14.21 ± 1.25	54.13 ± 1.18	66.31 ± 1.34	n.d.	TP-1.04
Ambient	LDPE		0.00 ± 0.00	1.41 ± 0.06	2.55 ± 0.11	3.05 ± 1.44	4.57 ± 1.25	n.d.	n.d.	TS-1.48
	Open		0.00 ± 0.00	8.21 ± 1.26	16.56 ± 0.11	24.10 ± 1.15	n.d.	n.d.	n.d.	PS-1.21
<i>Chlorophyll</i>										
5 °C	LDPE		3.24 ± 0.02	2.90 ± 0.06	2.65 ± 0.03	2.43 ± 0.04	2.24 ± 0.07	1.94 ± 0.06	1.24 ± 0.05	T-NS
	Open		3.24 ± 0.02	2.64 ± 0.02	2.34 ± 0.06	1.91 ± 0.03	1.47 ± 0.06	1.22 ± 0.06	0.86 ± 0.07	P-0.26
12 °C	LDPE		3.24 ± 0.02	2.82 ± 0.08	2.60 ± 0.06	2.33 ± 0.03	1.68 ± 0.07	1.40 ± 0.04	1.06 ± 0.02	S-0.37
	Open		3.24 ± 0.02	2.23 ± 0.03	2.13 ± 0.08	1.37 ± 0.07	1.24 ± 0.12	1.04 ± 0.01	n.d.	TP-NS
Ambient	LDPE		3.24 ± 0.02	2.78 ± 0.03	2.27 ± 0.06	1.76 ± 0.07	1.45 ± 0.06	n.d.	n.d.	TS-NS
	Open		3.24 ± 0.02	2.13 ± 0.04	1.95 ± 0.03	1.34 ± 0.05	n.d.	n.d.	n.d.	PS-NS
TPS-2.09										

Values are mean ± SE of three determinations

n.d. values not determined due to deterioration of basil leaves, *CD* values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

Table 2 Effect of storage conditions and packaging on ascorbic acid content (mg/100 g) and antioxidant activity (μg ascorbic acid equivalent/g) of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of storage (S) →	0	2	4	6	8	10	12	CD ($p \leq 0.05$)
<i>Ascorbic acid</i>										
5 °C	LDPE		76.61 ± 1.18	44.29 ± 1.15	33.17 ± 0.57	26.13 ± 0.56	25.47 ± 1.14	19.40 ± 0.57	16.29 ± 0.96	T-1.68
	Open		76.61 ± 1.18	37.54 ± 1.30	30.19 ± 0.68	22.42 ± 0.84	19.12 ± 0.76	17.21 ± 0.41	11.91 ± 0.52	P-1.37
12 °C	LDPE		76.61 ± 1.18	31.21 ± 1.42	31.51 ± 0.45	18.41 ± 0.18	15.27 ± 1.75	11.39 ± 1.64	8.52 ± 1.10	S-1.94
	Open		76.61 ± 1.18	27.16 ± 1.74	21.18 ± 1.16	15.41 ± 1.16	10.50 ± 0.49	9.78 ± 1.57	nd.	TP-2.38
Ambient	LDPE		76.61 ± 1.18	25.38 ± 1.22	13.32 ± 0.06	12.53 ± 0.22	11.42 ± 0.16	nd.	nd.	TS-3.37
	Open		76.61 ± 1.18	16.72 ± 0.46	13.34 ± 1.14	9.63 ± 0.84	nd.	nd.	nd.	PS-2.75
<i>Antioxidant activity</i>										
5 °C	LDPE		309.14 ± 1.15	295.43 ± 1.01	262.28 ± 1.21	256.22 ± 1.15	192.35 ± 1.13	183.18 ± 1.12	111.62 ± 1.75	T-1.17
	Open		309.14 ± 1.15	283.40 ± 1.11	245.00 ± 1.15	235.633 ± 0.86	140.38 ± 1.25	126.23 ± 1.15	105.56 ± 1.09	P-0.96
12 °C	LDPE		309.14 ± 1.15	289.37 ± 1.04	260.15 ± 1.44	181.21 ± 1.42	138.42 ± 1.15	104.45 ± 1.06	98.40 ± 1.17	S-1.36
	Open		309.14 ± 1.15	283.23 ± 1.73	228.38 ± 0.49	178.28 ± 1.27	106.83 ± 1.09	101.23 ± 1.14	nd.	TP-1.66
Ambient	LDPE		309.14 ± 1.15	276.14 ± 1.15	196.10 ± 1.15	110.36 ± 0.94	104.60 ± 1.14	nd.	nd.	TS-2.35
	Open		309.14 ± 1.15	238.55 ± 1.22	132.57 ± 1.23	99.52 ± 1.21	nd.	nd.	nd.	PS-1.92
TPS-3.3										

Values are mean ± S.E. of three determinations

n.d. values not determined due to deterioration of basil leaves, CD values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

in samples stored under ambient conditions i.e. 35.69 and 32.19% in leaves packaged in LDPE bags and kept in open trays respectively (Table 2). Total phenols and flavonoids decreased significantly ($p \leq 0.05$) with progression of storage period. This decrease was significantly ($p \leq 0.05$) rapid under ambient conditions as compared to leaves kept at 12 and 5 °C. The phenol and flavonoids content remained highest in samples packed in LDPE bags stored at 5 °C during whole storage period (Table 3). Electrolyte leakage and TBA reactive compounds of leaves increased significantly ($p \leq 0.05$) with increase in storage period but leaves at ambient conditions showed earlier rise and higher electrolyte leakage as compared to those stored at 12 and 5 °C. Slower increase in electrolyte leakage and TBA reactive compound was found in packed leaves than those kept in open trays (Table 4).

Activities of enzymes

The activities of enzymes catechol oxidase and POD increased significantly ($p \leq 0.05$) with increase in storage period, resulting in onset of browning (Tables 5, 6). During the whole storage period, basil leaves packed in LDPE bags showed significantly ($p \leq 0.05$) lower increase in activity of catechol oxidase and more increase in activity of POD than basil leaves kept in open trays at all storage conditions. Significant ($p \leq 0.05$) difference in activities of catechol oxidase and POD were found in leaves stored under different storage temperatures. Samples stored at 5 °C showed lowest enhancement in activity of catechol oxidase followed by samples at 12° C and ambient temperature, whereas reverse trend was found for POD activity where enhancement in activity was highest at 5 °C. Increase in POD activity was highest during first 2 days of storage. Lipoxygenase activity in leaves also increased significantly ($p \leq 0.05$) with increase in storage period (Table 5). However, the increase was significantly ($p \leq 0.05$) lower at 5 °C storage temperature and in LDPE packaging as compared to storage at other temperatures and in leaves kept in open trays. Activity of enzyme CAT was lower than activity of POD during whole storage period under all storage conditions. A significant ($p \leq 0.05$) enhancement in activities of enzymes CAT and SOD was also found with advancement in storage period under all experimental conditions (Table 6). The increase in activity was significantly ($p \leq 0.05$) rapid and was for a longer storage period in LDPE packed samples at 5 °C as compared to other storage conditions.

Discussion

Weight loss of fresh basil leaves could be due to transpiration and respiration. Transpiration, the greatest contributor to reduction in weight is a mechanism by which water is lost due to difference in vapor pressure in atmosphere and test sample. Respiration causes weight reduction because with a loss of every carbon atom, a molecule of water is produced. LDPE packaging was helpful in declining the rate of weight loss in basil leaves as compared to storage in open trays. This might be due to the fact that an atmosphere of low oxygen and high carbon dioxide created by packaging reduced the respiration rate thus reducing weight loss and extended the storage life of produce through inhibition of physiological deterioration (Mangaraj et al. 2009). Reduced rate of transpiration and respiration at low temperature might also be reason of lower loss in weight at 5 °C. Loss in chlorophyll during storage might be due to increased activity of both Mg-dechelataase and POD as indicated by Chairat et al. (2013) while working on Chinese kale. Lipid peroxidation during storage as indicated by increase in TBA reactive compounds could result in degradation of chlorophyll as implied by Imamura and Shimizu (1974). Ascorbic acid is required in diet in greater amounts as compared to other vitamins combined (FAO/WHO 1974). Ascorbic acid is fairly labile and its retention is often monitored when evaluating post harvest storage effects on nutritional quality in fruits and vegetables. The decomposition of ascorbic acid during storage was attributed to oxidation processes that could occur because of the presence of catalyst and oxidizing enzymes (Lee and Kader 2000). Christakou et al. (2005) also observed that increasing storage period caused a decrease of ascorbic acid in fruits and vegetables. Low temperature storage was found to reduce the rate of ascorbic acid loss as compared to high temperature storage. Similar results have also been reported in tomatoes by Znidarcic et al. (2010).

Phenolic compounds are the secondary metabolites of plants, characterized by having at least one aromatic ring with one or more hydroxyl group. Phenols and flavonoids are the major phenolic contents in basil leaves. Enzyme catechol oxidase and POD could be responsible for browning reactions during storage (Wongsheree et al. 2009) and total free phenolics are the substrates of most browning and blackening reactions. The increase in activity of catechol oxidase probably resulted in decrease in the level of phenols and flavonoids in basil leaves. Slower increase in activity of catechol oxidase at 5 °C resulted in slower decrease in free phenolics. Higher increase in POD activity and slower decrease in free phenolics under LDPE packaging indicated that POD might not be responsible for

Table 3 Effect of storage conditions and packaging on phenol (mg/100 g) and flavonoid (mg/100 g) content of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of Storage (S) →	0	2	4	6	8	10	12	CD ($p \leq 0.05$)
<i>Phenols</i>										
5 °C	LDPE		292.70 ± 1.18	290.97 ± 2.60	260.12 ± 2.50	205.68 ± 2.90	179.53 ± 4.02	141.19 ± 4.69	117.92 ± 2.87	T-2.69
	Open		292.70 ± 1.18	286.40 ± 2.35	237.18 ± 4.38	194.21 ± 2.57	132.39 ± 2.39	112.13 ± 3.91	92.24 ± 4.87	P-2.19
12 °C	LDPE		292.70 ± 1.18	289.35 ± 1.03	246.95 ± 4.62	200.53 ± 2.03	149.32 ± 5.24	124.58 ± 2.89	104.54 ± 2.57	S-3.11
	Open		292.70 ± 1.18	279.75 ± 1.33	216.98 ± 3.37	149.32 ± 5.24	124.58 ± 2.89	102.54 ± 3.57	n.d.	TP-3.80
Ambient	LDPE		292.70 ± 1.18	281.27 ± 2.31	234.16 ± 3.94	156.95 ± 2.79	104.72 ± 3.00	n.d.	n.d.	TS-5.38
	Open		292.70 ± 1.18	280.15 ± 2.89	200.53 ± 2.03	106.95 ± 1.66	n.d.	n.d.	n.d.	PS-4.39
<i>Flavonoids</i>										
5 °C	LDPE		309.20 ± 1.48	295.37 ± 2.39	253.58 ± 1.79	246.77 ± 5.93	241.44 ± 0.58	202.68 ± 1.30	126.53 ± 0.81	T-2.35
	Open		309.20 ± 1.48	291.75 ± 3.18	212.127 ± 1.1	173.43 ± 0.63	155.21 ± 0.5	133.8 ± 1.51	116.94 ± 0.80	P-1.92
12 °C	LDPE		309.20 ± 1.48	295.85 ± 1.97	229.28 ± 1.21	206.51 ± 2.74	185.88 ± 2.19	150.99 ± 2.47	109.26 ± 1.16	S-2.71
	Open		309.20 ± 1.48	243.70 ± 1.18	186.51 ± 0.55	169.57 ± 1.64	135.20 ± 1.20	109.72 ± 2.63	n.d.	TP-3.32
Ambient	LDPE		309.20 ± 1.48	281.35 ± 2.97	226.75 ± 3.6	201.41 ± 1.24	86.00 ± 0.77	n.d.	n.d.	TS-4.70
	Open		309.20 ± 1.48	230.35 ± 2.15	173.77 ± 3.08	154.8 ± 2.71	n.d.	n.d.	n.d.	PS-3.84
TPS-6.65										

Values are mean ± S.E. of three determinations

n.d values not determined due to deterioration of basil leaves, CD values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

Table 4 Effect of storage conditions and packaging material on electrolyte leakage (%) and TBA reactive compounds (nmol/g) of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of Storage (S) →	0	2	4	6	8	10	12	CD ($p \leq 0.05$)
<i>Electrolyte leakage</i>										
5 °C	LDPE		12.16 ± 1.15	15.19 ± 1.69	23.64 ± 1.44	31.83 ± 1.76	40.18 ± 1.15	50.53 ± 3.17	62.79 ± 0.72	T-1.81
	Open		12.16 ± 1.15	20.76 ± 1.80	32.59 ± 1.99	41.81 ± 1.45	53.48 ± 1.45	66.58 ± 1.66	75.63 ± 1.27	P-1.48
12 °C	LDPE		12.16 ± 1.15	19.89 ± 1.75	36.86 ± 2.02	47.26 ± 1.73	57.96 ± 2.61	68.90 ± 1.51	80.48 ± 2.60	S-2.09
	Open		12.16 ± 1.15	24.10 ± 2.31	39.01 ± 2.52	57.81 ± 3.17	68.81 ± 2.60	79.26 ± 2.41	n.d.	TP-2.57
<i>Ambient</i>	LDPE		12.16 ± 1.15	27.15 ± 1.73	44.93 ± 2.42	66.28 ± 2.31	74.60 ± 1.94	n.d.	n.d.	TS-3.63
	Open		12.16 ± 1.15	42.28 ± 1.23	74.98 ± 1.89	92.30 ± 1.28	n.d.	n.d.	n.d.	PS-2.96
<i>TBA reactive compound</i>										
5 °C	LDPE		0.53 ± 0.05	0.94 ± 0.16	1.55 ± 0.21	3.52 ± 0.23	5.32 ± 0.09	7.18 ± 0.32	8.48 ± 0.19	T-NS
	Open		0.53 ± 0.05	2.21 ± 0.30	3.34 ± 0.13	4.52 ± 0.06	5.66 ± 0.32	7.91 ± 0.39	9.36 ± 0.06	P-0.522
12 °C	LDPE		0.53 ± 0.05	1.35 ± 0.05	1.77 ± 0.08	4.30 ± 0.22	5.54 ± 0.08	7.83 ± 0.05	9.48 ± 0.08	S-0.73
	Open		0.53 ± 0.05	2.35 ± 0.04	4.25 ± 0.06	5.35 ± 0.09	6.62 ± 0.29	8.46 ± 0.35	n.d.	TP-NS
<i>Ambient</i>	LDPE		0.53 ± 0.05	1.42 ± 0.20	2.49 ± 0.32	4.66 ± 0.03	7.36 ± 0.32	n.d.	n.d.	TS-NS
	Open		0.53 ± 0.05	2.45 ± 0.12	4.27 ± 0.06	6.39 ± 0.21	n.d.	n.d.	n.d.	PS-NS
TPS-NS										

Values are mean ± S.E. of three determinations

n.d values not determined due to deterioration of basil leaves, CD values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

Table 5 Effect of storage conditions and packaging material on catechol oxidase (change in O.D. $\text{min}^{-1} \text{g}^{-1}$ FW) and lipoxxygenase (change in O.D. $\text{min}^{-1} \text{g}^{-1}$ FW) activities of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of Storage (S) →	0	2	4	6	8	10	12	CD ($p \leq 0.05$)
<i>Catechol-oxidase activity</i>										
5 °C	LDPE		11.4 ± 1.21	136.5 ± 4.04	213.8 ± 3.35	233.9 ± 2.14	254.6 ± 2.70	270.9 ± 4.36	287.5 ± 1.4	T-2.75
	Open		11.4 ± 1.21	167.2 ± 1.16	221.1 ± 4.27	238.0 ± 3.98	261.3 ± 4.24	273.5 ± 2.82	296.6 ± 1.86	P-2.27
12 °C	LDPE		11.4 ± 1.21	203.6 ± 1.32	228.1 ± 4.60	252.5 ± 3.83	262.9 ± 2.07	276.5 ± 3.72	297.7 ± 4.79	S-3.17
	Open		11.4 ± 1.21	206.4 ± 2.81	237.9 ± 2.72	263.0 ± 3.33	283.5 ± 3.78	295.2 ± 2.94	n.d.	TP-3.89
Ambient	LDPE		11.4 ± 1.21	207.3 ± 2.76	235.3 ± 1.63	257.6 ± 1.36	274.0 ± 3.02	nd	n.d.	TS-5.50
	Open		11.4 ± 1.21	209.0 ± 1.54	245.9 ± 4.92	268.4 ± 0.61	n.d.	n.d.	n.d.	PS-4.49
<i>Lipoxxygenase activity</i>										
5 °C	LDPE		31.6 ± 0.99	36.0 ± 1.70	44.5 ± 1.46	53.2 ± 1.46	72.3 ± 1.64	150.8 ± 0.85	155.1 ± 0.68	T-1.78
	Open		31.6 ± 0.99	48.8 ± 1.76	67.8 ± 1.45	89.1 ± 1.73	142.8 ± 2.45	151.8 ± 1.17	177.7 ± 0.60	P-1.45
12 °C	LDPE		31.6 ± 0.99	49.4 ± 0.53	69.9 ± 1.25	105.4 ± 0.57	144.7 ± 2.90	160.0 ± 3.00	299.3 ± 0.61	S-2.05
	Open		31.6 ± 0.99	55.6 ± 1.71	72.3 ± 1.78	133.0 ± 3.56	152.4 ± 1.30	163.3 ± 2.74	n.d.	TP-2.51
Ambient	LDPE		31.6 ± 0.99	58.5 ± 2.10	72.7 ± 4.78	143.5 ± 0.62	162.8 ± 1.96	n.d.	n.d.	TS-3.56
	Open		31.6 ± 0.99	73.5 ± 1.16	130.0 ± 1.54	172.8 ± 1.26	n.d.	n.d.	n.d.	PS-2.90
TPS-5.03										

Values are mean ± S.E. of three determinations

n.d. values not determined due to deterioration of basil leaves, CD values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

Table 6 Effect of storage conditions and packaging on POD ($\mu\text{moles of H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{g}^{-1}$ FW), CAT (units $\text{min}^{-1} \text{g}^{-1}$ FW) and SOD (units $\text{min}^{-1} \text{g}^{-1}$ FW) activities of fresh basil leaves

Storage conditions (T)	Packaging (P) ↓	Days of Storage (S) →	0	2	4	6	8	10	CD ($p \leq 0.05$)
<i>POD activity</i>									
5 °C	LDPE		0.815 ± 0.13	12.86 ± 0.86	17.11 ± 0.89	17.84 ± 0.64	19.63 ± 0.51	21.63 ± 0.68	22.68 ± 0.63 T-1.18
	Open		0.815 ± 0.13	3.52 ± 0.85	3.57 ± 0.75	4.23 ± 0.34	5.07 ± 0.35	9.28 ± 0.43	10.86 ± 0.58 P-0.96
12 °C	LDPE		0.815 ± 0.13	11.17 ± 0.65	12.75 ± 0.63	14.33 ± 0.48	17.54 ± 0.95	18.52 ± 0.98	19.1 ± 0.45 S-1.36
	Open		0.815 ± 0.13	2.37 ± 0.93	2.78 ± 0.65	2.98 ± 0.25	5.23 ± 0.48	7.13 ± 0.54	n.d. TP-NS
Ambient	LDPE		0.815 ± 0.13	9.47 ± 0.54	12.48 ± 0.43	13.2 ± 0.76	16.5 ± 0.68	n.d.	n.d. TS-NS
	Open		0.815 ± 0.13	1.54 ± 0.58	2.59 ± 0.67	2.55 ± 0.42	n.d.	n.d.	n.d. PS-1.93
<i>CAT activity</i>									
5 °C	LDPE		0.28 ± 0.09	3.21 ± 0.54	5.48 ± 0.56	9.145 ± 0.42	14.31 ± 0.58	16.46 ± 0.54	18.46 ± 0.16 T-0.66
	Open		0.28 ± 0.09	3.11 ± 0.58	4.98 ± 0.32	6.93 ± 0.69	9.51 ± 0.08	11.15 ± 0.48	15.63 ± 0.35 P-0.54
12 °C	LDPE		0.28 ± 0.09	2.39 ± 0.65	4.32 ± 0.42	5.52 ± 0.64	6.17 ± 0.51	6.62 ± 0.34	9.52 ± 0.54 S-0.76
	Open		0.28 ± 0.09	0.84 ± 0.06	2.77 ± 0.42	4.30 ± 0.72	4.11 ± 0.54	4.35 ± 0.60	n.d. TP-NS
Ambient	LDPE		0.28 ± 0.09	0.79 ± 0.12	1.55 ± 0.13	3.37 ± 0.60	2.68 ± 0.38	n.d.	n.d. TS-1.32
	Open		0.28 ± 0.09	0.60 ± 0.09	1.19 ± 0.18	1.31 ± 0.44	n.d.	n.d.	n.d. PS-NS
<i>SOD activity</i>									
5 °C	LDPE		LDPE	901 ± 1.06	1526 ± 2.96	1544 ± 2.60	1584 ± 4.02	1617 ± 3.15	1622 ± 1.87 T-2.66
	Open		Open	901 ± 1.06	1073 ± 2.64	1405 ± 3.67	1417 ± 6.07	1435 ± 3.11	1457 ± 3.57 P-2.17
12 °C	LDPE		LDPE	901 ± 1.06	1476 ± 1.65	1507 ± 2.32	1522 ± 2.66	1536 ± 3.39	1538 ± 2.05 S-3.08
	Open		Open	901 ± 1.06	982 ± 3.03	1216 ± 2.23	1270 ± 1.60	1404 ± 2.82	1418 ± 1.81 TP-3.77
Ambient	LDPE		LDPE	901 ± 1.06	981 ± 1.82	1389 ± 4.05	1429 ± 1.48	1433 ± 3.21	n.d. TS-5.33
	Open		Open	901 ± 1.06	939 ± 1.65	957 ± 2.91	973 ± 1.79	n.d.	n.d. PS-4.35
TPS-7.55									

Values are mean ± S.E. of three determinations

n.d. values not determined due to deterioration of basil leaves, CD values of T, P and S represent critical difference between mean values at different storage conditions, packaging and days of storage respectively

browning reactions and chlorophyll degradation rather it might have role as an antioxidative enzyme. These results were at variance with the results of Wongsheree et al. (2009) who showed inverse relation between catechol oxidase with leaf browning in basil. However, our results are in accordance with the results of Promyou et al. (2008) where catechol oxidase seemed to be a limiting factor. The increase in POD activity might reflect greater rate of lignin biosynthesis and greater healing rate adopted by leaves in an attempt to compensate for damage and protect tissue from pathogen attack, as suggested by Howard and Griffin (1993). Decrease in content of phenols, flavonoids and ascorbic acid during storage might be responsible for decrease in antioxidant activity since they are the major non-enzymatic antioxidants. Increase in electrolyte leakage might be due to the loss of membrane integrity with senescence during storage consequently leading to leakage of ions. Marangoni et al. (1995) correlated ion leakage to changes in membrane lipids; particularly to the loss of linolenic acid, a fatty acid prone to oxidation. Lipoxigenase oxidizes the double bonds between carbon atoms, thus increases the degree of unsaturation of fatty acids (Lee et al. 2005). Increase in TBA reactive compounds and increase in electrolyte leakage was found with parallel increase in lipoxigenase activity during storage. Leaves packed in LDPE bags and stored at 5 °C showed slower increase in electrolyte leakage and activity of lipoxigenase than basil leaves kept in open trays and higher temperatures, thus lowering the loss of membrane integrity to a greater extent, so that basil leaves could be stored for 12 days under these conditions as compared to 6 days in open trays and 8 days in LDPE packaging under ambient conditions.

Both POD and CAT enzymes use hydrogen peroxide as common substrate. POD reduces hydrogen peroxide to water, due to its higher affinity to hydrogen peroxide as compared to CAT (Kawakami et al. 2002). During storage, the increase in activities of POD and CAT were higher and were for longer storage period at 5 °C as compared to ambient conditions, thus protecting the basil leaf from damaging effect of reactive oxygen species, therefore, prolonging the shelf life of basil leaves at 5 °C. Higher increase in CAT under 5 °C could have resulted in lower increase in electrolyte leakage and TBA reactive compounds under these conditions. A correlation between decrease in CAT activity and increased membrane leakage as well as TBA reactive compounds has been reported by Bailey et al. (1996). Increase in CAT activity during storage was also reported by Xing et al. (2008). The SOD enzyme is believed to function in the elimination of reactive oxygen species produced during ripening that rapidly attacks all macromolecules in fruits, causing serious damage in cellular components and metabolic dysfunction

(Matheson et al. 1975). The increase in activity of SOD enzyme during storage was found parallel with increase in activity of CAT and POD as these enzymes are the key components of cellular antioxidant systems. SOD catalyzed the dismutation of superoxide anions to produce hydrogen peroxide which was then removed by CAT and POD. These enzymes are reported to extend food freshness by protecting the integrity of membranes and are also related with stress tolerance gene encoding the enzyme reported to up regulate post harvest (Xing et al. 2008). The results of present study also indicated the possible involvement of antioxidative enzymes viz POD, SOD and CAT in delaying the senescence process in basil leaves.

Conclusion

LDPE packaging of basil leaves and storage at 5 °C slowed down the changes in biochemical parameters and increase in catechol oxidase and lipoxigenase as compared to basil leaves kept in open trays and stored at 12 °C or ambient temperature respectively. Maximum enhancement in activities of antioxidative enzymes viz. POD, CAT and SOD was also found in leaves stored under these conditions. In conclusion, among all the storage conditions studied, LDPE packaging and 5 °C storage temperature maintained the quality of basil leaves for longer period probably by reducing the oxidative stress to larger extent due to enhanced activities of antioxidative enzymes and thus can be used to extend the shelf life of basil leaves.

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Compliance with ethical standards

Conflict of interest We declare that there is no conflict of interest.

References

- AOAC (1984) Official methods of analysis, 14th edn. Association of Official Analytical Chemists, Washington
- Bailey C, Benama A, Corbineau F, Come D (1996) Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. *Physiol Plant* 97:104–110. <https://doi.org/10.1111/j.1399-3054.1996.tb00485.x>
- Balabaa SI, Zaki AY, Elshamy AM (1974) Total flavonoids and rutin content of different organs of *Sophora japonica* L. *J Assoc Off Anal Chem* 57:752–755
- Boateng LD (2013) Effect of drying methods on nutrient quality of basil (*Ocimum viride*) leaves cultivated in Ghana. *Int Food Res J* 20(4):1569–1573

- Buchanan-Wollaston V (1997) The molecular biology of leaf senescence. *J Exp Bot* 48:181–199. <https://doi.org/10.1093/jxb/48.2.181>
- Cantwell MI, Reid MS (2002) Post harvest handling systems: fresh herbs. *Postharvest technol for hort crops*. University of California Press, California, pp 327–331
- Chairat B, Nutthachai P, Varit S (2013) Effects of UV-C treatment on chlorophyll degradation, antioxidant enzyme activities and senescence in chinese kale (*Brassica oleracea* var. *alloglabra*). *Int Food Res J* 20:623–628
- Christakou EC, Arvanitoyannis IS, Khan EM, Bletsos F (2005) Effect of grafting and modified atmosphere packing (MAP) on melon quality parameters during storage. *J Food Agric Env* 3:204–252
- Dan K, Nagata M, Yamashita I (1999) Changes in phenylalanine ammonia lyase and polyphenol oxidase activities with occurrence of browning in shredded lettuce during storage. *Food Preserv Sci* 25:209–212. <https://doi.org/10.5891/jafps.25.209>
- Dasgupta N, De B (2006) Antioxidant activity of some leafy vegetables of India. A comparative study. *Food Chem* 101:471–474. <https://doi.org/10.1016/j.foodchem.2006.02.003>
- FAO/WHO (1974) Handbook on human nutritional requirements. Monogram series. WHO, Rome, p 35
- Hemeda HM, Klein BP (1990) Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *J Food Sci* 58:184–186. <https://doi.org/10.1111/j.1365-2621.1990.tb06048.x>
- Hodges DM, DeLong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid -reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207:604–611. <https://doi.org/10.1007/s004250050524>
- Howard LR, Griffin LE (1993) Lignin formation and surface discoloration of minimally processed carrot sticks. *J Food Sci* 58:1065–1068. <https://doi.org/10.1111/j.1365-2621.1993.tb06113.x>
- Imamura M, Schimizu S (1974) Metabolism of chlorophyll in higher plants. IV. Relationship between fatty acid oxidation and chlorophyll bleaching in plant extracts. *Plant Cell Physiol* 15:187–190. <https://doi.org/10.1093/oxfordjournals.pcp.a074982>
- Jimenez A, Romojaro F, Gomez JM, Llanos MR, Sevilla F (2003) Antioxidant systems and their relationship in response of pepper fruits to storage at 20 °C. *J Agri Food Chem* 51:6293–6299. <https://doi.org/10.1021/jf030052i>
- Kawakami S, Matsumoto Y, Matsunaga A, Mayama S, Mizuno A (2002) Molecular cloning of ascorbate peroxidase in potato tubers and its response during storage at low temperature. *Plant Sci* 163:829–836. [https://doi.org/10.1016/S0168-9452\(02\)00232-7](https://doi.org/10.1016/S0168-9452(02)00232-7)
- Lee SK, Kader AA (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol Technol* 20:207–220. [https://doi.org/10.1016/S0925-5214\(00\)00133-2](https://doi.org/10.1016/S0925-5214(00)00133-2)
- Lee SH, Ahn SJ, Im YJ, Cho K, Chung GC, Cho BH, Han O (2005) Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleaf gourd and cucumber roots. *Biochem Biophys Res Commun* 330:1194–1198. <https://doi.org/10.1016/j.bbrc.2005.03.098>
- Maalekuu K, Elkind Y, Leikin-Frenkel A, Lurie S, Fallik E (2006) The relationship between water loss, lipid content, membrane integrity and LOX activity in ripe pepper fruit after storage. *Postharvest Biol Technol* 42:248–255. <https://doi.org/10.1016/j.postharvbio.2006.06.012>
- Mangaraj S, Goswami TK, Mahajan PV (2009) Applications of plastic films for modified atmosphere packaging of fruits and vegetables: a review. *Food Eng Rev* 1:133–158. <https://doi.org/10.1007/s12393-009-9007-3>
- Marangoni AG, Smith AK, Stanley DW (1995) A micro structural evolution of chilling injury in mature green tomato fruit. *Can Inst Food Sci Technol J* 22:52–55. [https://doi.org/10.1016/S0315-5463\(89\)1097.1975.tb06647.x](https://doi.org/10.1016/S0315-5463(89)1097.1975.tb06647.x)
- Matheson IB, Etheridge RD, Kratowich NR, Lee J (1975) The quenching of singlet oxygen by amino acid and proteins. *Photobiology* 21:165–171. <https://doi.org/10.1111/j.1751-7030.1-1>
- Ose K, Chachin K, Imahori Y (1995) Browning mechanism of water convolvulus (*Ipomoea aquatica* Forsk.) stored at low temperature. In: Chang YL, Whitaker JR (eds) *Enzymatic browning and its prevention*. ACS Symposium Series 600, Washington, DC, pp. 178–187. <https://doi.org/10.1021/bk-1995-0600.ch014>
- Phippen WB, Simon JE (1998) Anthocyanins in basil (*Ocimum basilicum* L.). *J Agr Food Chem* 46:1734–1738. <https://doi.org/10.1021/jf970887r>
- Phippen WB, Simon JE (2000) Anthocyanin inheritance and instability in purple basil (*Ocimum basilicum* L.). *J Hered* 91:289–296 (PMID:10912675)
- Promyou S, Ketsa S, Van Doorn WG (2008) Hot water treatments delay cold induced banana peel blackening. *Postharvest Biol Technol* 48:132–138. <https://doi.org/10.1016/j.postharvbio.2007.09.006>
- Rao MV, Paliyath G, Ormrod DP, Murr DP, Watkins CB (1997) Influence of salicylic acid on H₂O₂ production, oxidative stress, and H₂O₂-metabolizing enzymes: salicylic acid-mediated oxidative damage requires H₂O₂. *Plant Physiol* 115:137–149. <https://doi.org/10.1104/pp.115.1.137>
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Method Enzymol* 299:152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Sovrano S, Buiatti S, Anese M (2006) Influence of malt browning degree on lipoxygenase activity. *Food Chem* 99:711–717. <https://doi.org/10.1016/j.foodchem.2005.08.048>
- Sozzi GO, Camperi SA, Cascone O, Frascina AA (1998) Galactosidases in tomato fruit ontogeny: decreased galactosidase activities in antisense ACC synthase fruit during ripening and reversal with exogenous ethylene. *Aust J Plant Physiol* 25:237–244. <https://doi.org/10.1071/PP97124>
- Wills RBH, McGlasson WB, Graham D, Joyce DC (2007) *Postharvest, an introduction to physiology and handling of fruit, vegetables and ornamentals*, 5th edn. University of New South Wales Press Ltd, Sydney
- Wongsheree T, Ketsa S, Doorn WGV (2009) The relationship between chilling injury and membrane damage in lemon basil (*Ocimum* × *Citriodorum*) leaves. *Postharvest Biol Technol* 51:91–96
- Xing Z, Wang Z, Tan Q (2008) Effect of different packaging films on postharvest quality and selected enzyme activities of *Hypsizygus marmoreus* mushrooms. *J Agric Food Chem* 56:11838–11844. <https://doi.org/10.1021/jf8024387>
- Znidarcic D, Ban D, Oplanic M, Karic L, Pozrl T (2010) Influence of post harvest temperatures on physiochemical quality of tomatoes (*Lycopersicon esculentum* Mill.). *J Food Agric Environ* 8:21–25