Effectsof hexanal on shelf life of strawberry (*Fragaria* x *ananassa*Duch.)

ABSTRACT

We evaluated the role of 'Enhanced Freshness Formulation' (EFF) having hexanal as the main component on shelf life enhancement of strawberry (*Fragaria* x *ananassa*) cv. Sweet Charlie. After crop harvest, a laboratory experiment was done at ambient temperature (about22-30°C) with 5-treatments (dipping of freshly harvested berries in hexanal of varying strengths)*viz.*, 1 % hexanal for 2.5 min (V_1), 1 % hexanal for 5 min (V_2), 2 % hexanal for 2.5 min (V_3) and 2 % hexanal for 5 min (V_4). Undipped berries were considered as control treatment (V_5).The study revealed that dipping the berries in 2 % hexanal for 2.5 min proved most effective to prolong the shelf life of strawberry. Maximum shelf life (5.90 days) and fruit firmness (5.88 N mm^{-1}) were obtained in the most efficacious treatment, thereby extending the marketing period.

Keywords: Enhanced Freshness Formulation**,** Hexanal, Shelflife, Storage, Strawberry

INTRODUCTION

Allelochemicals are released from plant tissue in a variety of ways including emission of volatile substances from living plant parts, exudation from roots, or leaching from above ground parts by rain, dew, fog, etc. (30).A product called Enhanced Freshness Formulation (a hexanal formulation) has the potential to preserve quality, reduce postharvest losses and assure longevity of fresh produce without compromising on fruit quality, environmental and human health (19).Hexanal is produced in plant tissues through a lipoxygenase pathway as a naturally occurring volatile with generally-recognized-as-safe status(27,30,35). When the plants are wounded or cut, there is a grassy odour which is chemically referred as "hexanal" (10). Hexanal slows down the ripening process and treated fruits maintains the marketable quality for longer periods due to the retention by the membrane (16). Hexanal is antifungal against *Alternaria alternata*, *Botrytis cinerea* and *Penicillium expansum*. Apart from the antimicrobial activity of hexanal, it enhances the sensory quality of ripe fruit (1,11,36). Pre and postharvest applications of hexanal in several fruits, vegetables, fresh cut produce and flowers enhances their shelf-life (23,32).Synthetic fungicides used to delay spoilage, ripening and senescence of fruits are environmental concerns regarding food safety for the consumers (17). The use of new technologies (hexanal treatment) retains fruit quality, thereby reducing the postharvest losses and extending their shelf life.

Strawberries are perishable and therefore needs very careful postharvest handling primarily due to their susceptibility to mechanical damage, physiological degradation, water loss and microbial deterioration. Economic loss of fruit quality due to short shelf life begins with the loss of membrane integrity that largely plays the leading role in the senescence process (24,29). The susceptibility of strawberries to microbial decay is due to their soft skin, which is easily ruptured facilitating entry of most organisms to attack and multiply (42).In recent years advances has been made in post-harvest storage technology with the onset of Phospholipase D (PLD) inhibiting technology, the important enzyme that initiates membrane phospholipid degradation in the cell membrane during ripening (20). Phospholipase D is a key enzyme involved in membrane deterioration that occurs during fruit ripening and senescence.The quality of highly perishable horticultural produce is reduced by excessive degradation of membranes. Hexanal, an inhibitor of phospholipase D has been successfully showing significance in enhancing storage life and fruit quality attributes by preserving membrane integrity. Hexanal, a C6 aldehyde, is a naturally occurring compound (12,44) and is responsible for downregulating the expression of PLD enzyme which degrades the phospholipid bilayer (9,21,22).

Enhancing shelf life of fruits and vegetables is a major challenge. In modern agriculture, farmers use synthetic chemicals, whose indiscriminate usage has created numerous problems such as environment and health issues. Increased awareness about these harmful effects has led to the development of alternate ecofriendlyshelf life enhancement strategies. Enhanced Freshness Formulation based on hexanal enhances shelf life of many fruit crops. However, studies regarding activity of hexanal for enhancing shelf life in strawberry are rare. Therefore, this study was done to assess the effects of hexanal basedEnhanced Freshness Formulation on shelf life of strawberry.

Figure1. Strawberry populationFigure 2. Strawberry plant at fruiting stage

MATERIALS AND METHODS

Fruits were collected from strawberry plant at fruiting stage during February,2020 and 2021 from farmer’s field atDhankhuloi, Jorhat district India. Dhankhuloi is located at a latitude 26°48′ 7.3476''N and 94°3′ 23.7996''E longitude having an altitude of 84 m above mean sea level with annual rainfall of 1879.62 mm. Maximum and minimum temperatures during February are 25.56 °C and 11.19 °C, respectively.

The fruits were harvested,when more than 75 % fruit attained colour from strawberry plants grown in open under plastic mulched condition.The shelf life assessment experimentwas done in completely randomized designwith 4-replications(Fig 5). Strawberry fruits were subjected to hexanal based Enhanced Freshness Formulation (EFF) for shelf life treatments on the same day of harvest and stored at ambient temperature (22-30°C).Five treatments consisted of dipping of harvested fruits (V_1-dipped in 1 %hexanal for 2.5 min, V_2-1 %hexanal for 5 min, V_3- 2 %hexanal for 2.5 min , V_4- 2 %hexanal for 5 min) and Control (V_5 - fruits without dipping).Fruit firmness (N mm^{-1})was determined by using a digital Fruit Penetrometer (Model: DFP-001, Parisa Technology). The shelf life(days) was estimated by observing the number of days the fruits remained in sound condition from harvesting without spoiling to optimum eating stage at room temperature(28). Physiological loss in weight (%) of fruit was calculated as loss of weight in grams to the initial weight and expressed in percentage (13). The loss due to pathogenic infection, shrivelling and other deformity till fruits became unmarketable was considered as decay loss (%) and was determined visually during the course of the experiment (4,25). Total anthocyanins (mg $100g^{-1}$) were determined according to the pH differential method on 2^{nd} day of harvest (45).

Statistical analysis

All the data were subjected to the statistical analysis of variance using completely randomised design as described by (26) at 5 % level of significance with the help of a windows-based computer package OPSTAT (34) and SPSS software.

RESULTS AND DISCUSSION

Fruit firmness (N mm^{-1})

Pooled data of both the years recorded highest fruit firmness (5.88 N mm^{-1}) in $V_3$and minimum fruit firmness (0.44 N mm^{-1}) was observed in V_5(Table 1 and Fig 3).Fruit firmness is a major quality attribute that often dictates the shelf life of fruits. Genetic background, growing conditions and fruit constitution at the time of testing (degree of ripeness, size, post-harvest handling, internal temperature,method for fruit firmness testing etc.) affect fruit firmness. Further, as the days of storage progressed, the pulp firmness decreased, which might be due to continued ripening of fruits during storage, wherein, insoluble pectic substances of the cell wall are degraded into soluble pectin, resulting in pulp softening (8,35).Higher firmness was observed in hexanal treated samples compared to control which might be due to the delayed ripening and reduced activity of enzyme phospholipase D. Hexanal application on fruits influenced the firmness positively for a longer period of time, primarily because hexanal is a potent inhibitor of enzymes that disturb the membrane integrity. Hexanal application at optimum concentration as dip maintained the best firmness, primarily because of damaging effect at higher concentration. Furthermore, hexanal down regulates the genes responsible for enzymes involved in ethylene biosynthesis, preserves the cell membrane integrity after harvest and resulting in better pulp firmness. The activity of phospholipase D is also stimulated by calcium and low pH. Thus, if the action of phospholipase D is inhibited, then the rest of enzymes are unable to act on the intermediates. This would prevent the accumulation of neutral lipids and the destabilization of the membranes. Similar views were expressed by (2,3,6,9,38,41)in their studies.

Shelf life (days)

In the pooled analysis of data of two years showed that V_3 recorded highest shelf life (5.90 days) and shortest shelf life (2.37 days) inV_5(Table 1 and Fig 4).Maximum shelf life was observed in fruits treated with hexanal of optimum concentration and duration(2% for 2.5 min). The fruits treated with hexanal showed a naturaldelayed ripening process. Delayed softening of fruits extends shelf life of fresh produces. High concentrations and short duration of exposure resulted in more changesthan lower concentration treatment for long time exposure time. This might be due to fact that lower concentration were not enough to inhibit action of PLD enzyme. A simple inhibition of membrane deterioration by hexanal may enhance the shelf life by preserving membrane structure. Hexanal enhances the shelf life of fruits by maintaining fruit firmness of the treated fruits. Similar results were reported for apple, cherry, peach, plum, guava and vegetables by (2,5,33,39,41).

Table 1. Effects of hexanal treatment on fruit firmness (N mm^{-1}), shelf life (days) and anthocyanin content (mg 100g^{-1})

Treatment	Fruit firmness (N mm^{-1})			Shelf life (days)			Anthocvanin content (mg 100g^{-1}) on 2nd DAH		
	2019-20	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**
V_1 (2.5 min 1% hexanal)	5.05^{c}	5.09c	5.07^{c}	4.90^{b}	4.95^{b}	4.92^{b}	51.11^{c}	50.88^{c}	51.00^{c}
V_2 (5.0 min 1% hexanal)	5.30^{b}	5.34^{b}	5.32^{b}	5.00^{b}	5.05^{b}	5.02^{b}	57.42^{b}	56.77^{b}	57.10^{b}
V_3 (2.5 min 2% hexanal)	5.85^{a}	5.91^{a}	**5.88^{a}**	5.80^{a}	6.00^{a}	**5.90^{a}**	63.13^{a}	62.85^{a}	**62.99^{a}**
V_4 (2.5 min 2% hexanal)	5.36^{b}	5.42^{b}	5.39^{b}	5.10^{b}	5.15^{b}	5.12^{b}	47.40^{d}	45.34^{d}	46.37^{d}
V_5(Control, Fruits not dipped)	0.42^{d}	0.46^{d}	0.44^{d}	2.25^{c}	2.50^{c}	2.37^{c}	45.75^{d}	44.15^{d}	44.95^{e}
SEd(±)	0.10	0.11	0.07	0.21	0.19	0.14	0.82	0.62	0.52
CD(P=0.05)	0.21	0.23	0.15	0.44	0.40	0.29	1.74	1.32	1.05

Physiological loss in weight (%)

The physiological loss in weight increased with the increasing period of storage after harvest irrespective of the treatments. Data regarding the physiological loss in weight (%) as influenced by the treatments are presented in Table 2. In the combined analysis of data of both years, on 6th day of harvest, the fruits under V_3 exhibited minimum physiological loss in weight (27.86 %) whereas maximum physiological loss in weight (44.89 %) was recorded under V_5.Hexanal treated strawberry fruits showed minimum physiological loss in weight than control. Physiological loss in weight of stored produce occurs due to direct water losses vis-à-vis higher respiration rate. Strawberries are subjected to rapid water loss causing them to shrivel and deteriorate (13).The reduced weight losses due to hexanal application might be ascribed to phospholipase D inhibition and reduction in phospholipids degradation as well as downstream oxidative processes and preserved membrane integrity. Post harvest application of hexanal might have effectively maintained the cell structures which lead to reduction of respiration rate and turgor loss that contributed to reduced weight loss in treated fruits. Hexanal treatments could have reduced the catabolic processes and quality losses by maintaining the membrane integrity and cell structure. Similar effects of hexanal application on physiological weight loss wereobserved by (6,7,9,15,23,41).

Table2. Effects of hexanal treatment on physiological loss in weight (%)

Treatment	**Days after harvest**

	2nd day			4th day			6th day		
	2019-20	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**
V_1 (2.5 min 1% hexanal)	5.76^{b}	6.42^{b}	6.09^{bc}	16.70^{b}	16.19^{b}	16.45^{b}	36.25^{b}	37.11^{b}	36.68^{b}
V_2 (5.0 min 1% hexanal)	5.87^{b}	7.30^{b}	6.58^{b}	16.30^{b}	15.75^{b}	16.03^{b}	36.36^{b}	37.45^{b}	36.91^{b}
V_3 (2.5 min 2% hexanal)	4.15^{b}	5.29^{b}	4.72^{c}	13.45^{c}	14.98^{b}	14.22^{c}	26.96^{c}	28.76^{c}	**27.86^{c}**
V_4 (2.5 min 2% hexanal)	5.32^{b}	6.08^{b}	5.70^{bc}	14.67^{bc}	16.57^{b}	15.62^{bc}	34.15^{b}	34.97^{b}	34.56^{b}
V_5(Control, Fruits not dipped)	9.25^{a}	9.81^{a}	9.53^{a}	20.88^{a}	20.12^{a}	20.50^{a}	43.84^{a}	45.94^{a}	44.89^{a}
SEd(±)	0.79	1.06	0.66	1.19	0.75	0.70	2.12	1.56	1.31
CD(P=0.05)	1.68	2.26	1.31	2.53	1.60	1.43	4.52	3.33	2.69

Decay loss (%)

The decay loss increased in all the treatments with increasing day of storage after harvest (Table3). The combined analysis of the data of both the years, on 6th day of harvest, the fruits under V_3 exhibited minimum decay loss (59.03 %) whereas maximum decay loss (74.73 %) was recorded under V_5.Hexanal is highly volatile and possess antifungal properties against *Alternaria alternata*, *Botrytis cinerea*and*Penicilliumexpansum*. Infection by pathogens may occur during the growing season, at harvest time or during handling and transport. Inoculum load carried by the fruits from growing field and sanitation during storage handling plays an important role to minimize and maximize contamination by decay pathogens. Moreover, fruit composition and fruit maturity status could also affect the decay severity. Higher decay loss could be due to higher presence of disease causing inoculums due to favourable environmental conditions. The ability to control previously established infections in the postharvest environment is of crucial importance.Hexanal has the ability to penetrate tissues to control latent or established infections. Hexanal along with other constituents of EFF(Enhanced Freshness Formulation) such as antioxidants may help maintain the membrane structure, as well as provide protection from the oxidative damage. In our study, strawberry fruits treated with hexanal resulted in a significant decrease in fungal decay upto some extent confirming the previous reports about its antifungal effect. Fruits without dip in the hexanal based EFF and stored in the container developed latent fungal growth which resulted in the fruit decay. However dipping in hexanal based EFF for longer duration might have increased the chance of fungal infection due to the increase in surface moisture that favoured fungal infection. The lower concentration and longer exposure times of hexanal treatment might have resulted in less effect on percentage decay. The results of the study have conformity with previous studies of (9,14,37,40,43,46).

Table3. Effects of hexanal treatment on decay loss (%)

Treatment	Days after harvest								
	2nd day			4th day			6th day		
	2019-20	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**	**2019-20**	**2020-21**	**Pooled**
V_1(2.5 min 1% hexanal)	15.47^{bc}	18.49^{b}	16.98^{bc}	32.14^{b}	35.41^{b}	33.78^{b}	65.62^{b}	64.58^{b}	65.10^{b}
V_2(5.0 min 1% hexanal)	14.87^{bc}	17.79^{b}	16.33^{bc}	30.62^{b}	34.37^{b}	32.50^{b}	63.33^{b}	62.91^{b}	63.12^{b}
V_3(2.5 min 2% hexanal)	13.98^{c}	16.78^{b}	15.38^{c}	28.24^{b}	29.11^{c}	28.68^{c}	59.16^{b}	58.89^{c}	**59.03^{c}**
V_4(2.5 min 2% hexanal)	17.49^{b}	18.80^{b}	18.15^{b}	30.95^{b}	35.41^{b}	33.18^{b}	63.54^{b}	62.29^{bc}	62.91^{b}
V_5(Control, Fruits not dipped)	23.75^{a}	25.17^{a}	24.46^{a}	46.61^{a}	51.39^{a}	50.00^{a}	75.35^{a}	74.10^{a}	74.73^{a}
SEd(±)	1.27	2.06	1.21	1.77	1.78	1.25	2.88	1.77	1.69
CD(P=0.05)	2.71	4.39	2.47	3.78	3.81	2.57	6.14	3.78	3.45

Superscript by same letter means they are at par

Anthocyanin content (mg/100g)

The pooled analysis of data of two years (Table 1) showed that highest amount of anthocyanin (62.99 mg $100g^{-1}$) was recorded in V_3andminimum (44.95 mg $100g^{-1}$) in V_5. Hexanal application significantly reduced the degradation of anthocyanin which might be ascribed to inhibited activities of cell wall degrading phospholipase D enzyme responsible for senescence of tissue. Hexanal formulation also contained cinnamic acid and it might have induced the synthesis of anthocyanin. Supplemented anthocyanin accumulation in hexanal treated fruits might also be ascribed to increased biosynthesis of volatile and antioxidant compounds which contributed to enhanced anthocyanin synthesis. However, there was difference in results between the concentrations of hexanal and the duration of dip in the experiment. Effectiveness of hexanal was significantly affected by duration of application at optimum dose level as effective concentration of hexanal varies with commodity and variety. The results are in agreement with findings of (15,18,24) and in contrast to the findings of (33) in cherry where no change in anthocyanin content was noticed in stored fruits treated with hexanal containing formulations.

CONCLUSIONS

The application of hexanal dip at appropriate dose established minimum decay loss, physiological loss in weight and maximum level of fruit firmness, shelf life and anthocyanin content. The results reflected maximum inhibition percentage due to hexanal treatment which showed that hexanal had a strong inhibitory effect on microbial growth in strawberry thereby enhanced its shelf life. Shorter duration of exposure of 2.5 min allowed easy and sufficient penetration of treatment solution in strawberry compared to 5 min resulting to its effectiveness which could be due to its thin skin.During postharvest handling, strawberry fruit have high susceptibility to mechanical damage, and thus they need to be handled with care, to avoid damage that can create an entry point for the decay causing fungi. For this reason, strawberry fruit are harvested and often packaged in the field. Dipping of freshly harvested fruits in 2 % hexanal for 2.5 min proved a better option in terms of effective concentration and effective treatment duration respectively for maintaining overall strawberry fruit quality.

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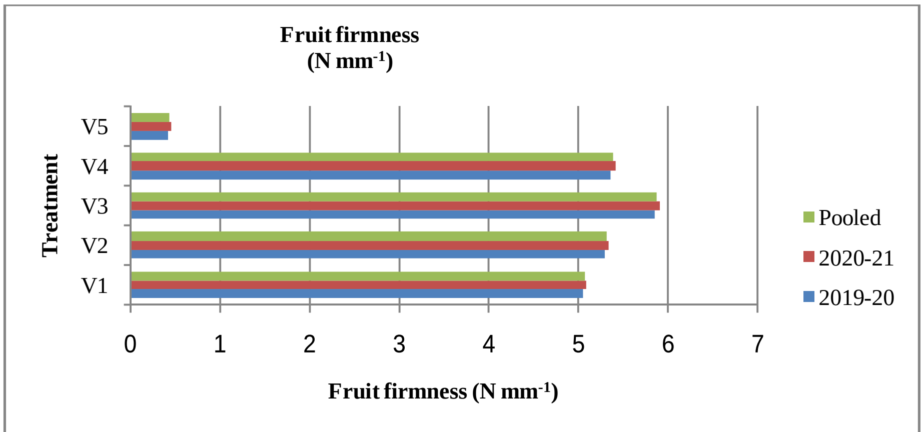

Figure 3. Effects of Hexanal treatment on Fruit firmness (N mm-1)

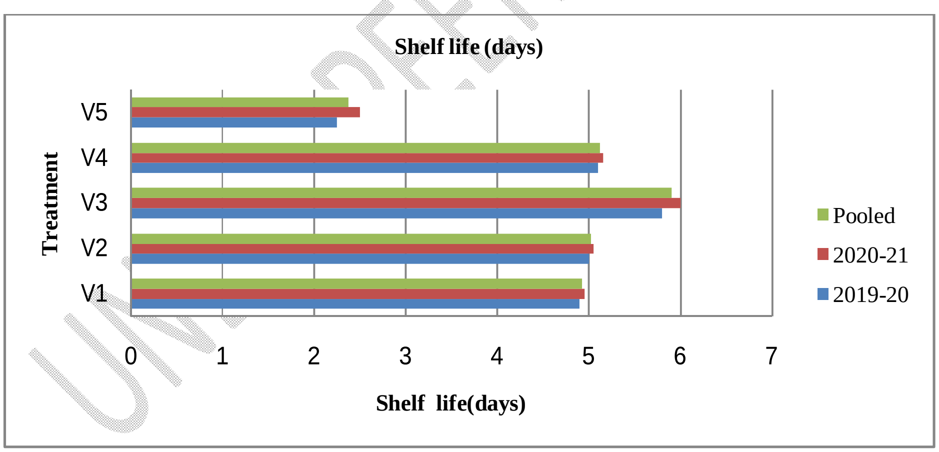

Figure 4. EffectsofHexanaltreatment onShelf life (days)

Figure 5.Post harvest dip of Strawberry in Hexanal