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RESEARCH ARTICLE

Influence of ethrel on spatiotemporal changes and biosynthesis of volatile metabolites of Mango cultivars

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Abstract

Mango, a highly preferred fruit known for its distinctive aroma and flavour, requires proper ripening to enhance its quality and shelf life. The use of calcium carbide for ripening has been banned due to its harmful effects on human health. While ethrel treatment in temperature-controlled chambers offers a safer alternative, it remains unaffordable for small-scale farmers and traders. This study evaluated mango ripening using ethrel in three chambers: silpaulin chamber, zero energy cool chambers and cold chamber. Among these, the zero-energy cool chambers demonstrated the most promising results. Fruits ripened in this chamber exhibited the lowest per cent disease index, indicating superior quality. Additionally, these fruits recorded higher colour values and total carotenoid content than those ripened in the cold chamber. The silpaulin chamber, however, showed increased antioxidant enzyme activity due to a higher respiration rate and disease index. Importantly, mangoes ripened in the zero energy cool chambers had the highest area percentage of volatile metabolites, which are key contributors to aroma and defence mechanisms. This suggests that the zero-energy cool chamber enhances fruit quality while minimizing postharvest losses. Besides being an eco-friendly and cost-effective alternative to traditional cold chambers, the zero-energy cool chamber can serve dual purposes. When not used for ripening, it can be utilized for the postharvest storage of fruits, effectively extending their shelf life. Thus, adopting zero-energy cool chambers offers a sustainable solution for small-scale mango traders and farmers.

Keywords

cost-effective; eco-friendly; ethrel; mango; physico-chemical properties; zero energy cool chamber

Introduction

Mango is Indias' second most consumed fruit crop, producing 20336 thousand MT from 2339 thousand ha (1). Andhra Pradesh ranks first in area with 389670 ha, followed by Uttar Pradesh (279310 ha). Uttar Pradesh ranks first in production with 4807830 MT, followed by Andhra Pradesh (4676060 MT). Mango cultivation occupies 146070 ha in Tamil Nadu, with an annual production of 639640 MT (1). Fruits that ripen after harvesting by producing ethylene, which acts as an autocatalyst, are called Climacteric fruits. As a

climacteric fruit crop, mangoes have a high demand during the season and early sale in the market fetches higher returns. Hence, farmers practice bulk harvesting of fruits with varied fruit maturity. Naturally ripened mangoes of varied maturity take longer to ripen, lacking uniform ripening patterns, poor colour development and higher weight loss. During transportation, these naturally ripened fruits become overripe and become inedible due to frequent handling of fruits (2). Therefore, the fruits are harvested in a mature green stage and force-ripened using artificial ripening agents to overcome these problems (3). Artificial ripening agents promote early fruit ripening with uniform colour development, resulting in aesthetic appearance, improving export potential and minimizing the economic loss caused by the natural ripening of mangoes. Amrapali mangoes treated with 1000 ppm of ethrel resulted in deep yellow fruits with excellent flavour and taste. The lowest Physiological loss in weight of 12.50 % was also observed in fruits treated with 1000 ppm of ethrel (4).

Alphonso mangoes are regarded as the "King of Mango" and the choicest variety because of their aroma, flavour and taste. The fruits are medium to big-sized (200-300 g), oblong to oval. The fruit pulp has a Total Soluble Solids range of 17.2-19.5 ˚B, with 0.2 % - 0.35 % acidity. The fruits have melting, non-fibrous and juicy pulp with excellent export potential and contribute more than 80 % of the export of mango products (5, 6). Mulgoa is a commercial variety of South India. It is extensively cultivated in Tamil Nadu and Karnataka. It is a large-fruited variety with good fruit quality, flavour and sweetness.

Calcium carbide is farmers' and traders' most commonly used artificial ripening agents. It is greyish-black in colour with a garlic odour. When kept along with the fruits, the small pieces of calcium carbide release acetylene gas upon reacting with moisture in the atmosphere. The effect is analogous to ethylene and initiates ripening (7). The fruits ripened using calcium carbide contain traces of arsenic and phosphorus hydride, which are found to be hazardous to human health as they cause vomiting, diarrhoea, burning sensation in chest and abdomen, thirst, weakness, cerebral oedema, seizures and memory loss (8). Thus, the use of this chemical for ripening purposes is illegal in most countries and banned in India under the PFA Act. Hence, an alternate method of using ethylene gas in cold ripening chambers to initiate ripening was practised. It requires high maintenance and is not cost -effective for small traders and farmers. Hence, ethrel (2-choloro) was used by dipping the fruits. As this process was cumbersome and banned by FSSAI because the fruits directly come into contact with the chemical, a new approach was sought.

Considering all these facts, ethrel with sodium hydroxide was used to initiate the ripening process by producing ethylene gas, which triggers the ripening process. Ethrel reacts with NaOH (alkali) and hydrolyses to produce ethylene gas. Exposure time and temperature during ethrel treatment play a vital role in the quality of the fruits. Temperature profoundly impacts the quality and shelf life of fruits (9). When fruits are ripened at ambient temperature, a sharp rise in respiration rate and ethylene production is observed after 3 to 4 days of harvesting (10), thereby limiting its consumer acceptance and postharvest life. The best temperature range was 21˚C to 24˚C and mangoes ripened at a temperature of 27 ˚C and above had mottled skin and strong flavour (10). This ethrel treatment, when given in low-cost chambers made of plastics and silpaulin with high temperatures, results in poor quality and shelf-life fruits. Hence, ethrel treatment should be provided in low temperatures. Using cold ripening chambers for ethrel treatment might not be affordable for farmers and small traders. At this juncture, zero energy cool chambers can be used as an alternate environment-friendly structure to a controlled ripening chamber for the ripening of fruits by ethylene treatment. This would be cost-effective with less maintenance and infrastructure, thus promoting the on-farm ripening of fruits among farmers and small traders. Considering the facts mentioned above, the present study was carried out to examine the effect of temperature during ethrel treatment on the fruit quality of the ripened fruits and whether zero energy cool chambers can be used as an alternative to cold ripening chambers.

Materials and Methods

Treatment details

Two mango cultivars, Alphonso and Mulgoa, were subjected to ethrel treatment at 100 ppm in three different chambers with different temperatures for 24 hrs.

S.No.	Particulars	Treatment Details
1	T_1	Silpaulin chamber (33 ± 1 °C)
2	T_2	Zero energy cool chamber (26 ± 1°C)
3	T_3	Cold chamber (22 ± 1 °C)

Alphonso is one of the choicest varieties and is called as the 'King of mangoes' as it is known for its unique taste, aroma, golden yellow colour flesh and nutritional value. Mulgoa is a commercial variety of South India owing to the high quality of its fruits and is regarded as the mother of coloured mango varieties. Hence, these two commercial varieties were used in the study. Silpaulin chamber of size 0.9 m × 0.9 m × 0.9 m, which can hold a capacity of 80 kg (4 crates of 20 kg each), was used. The framework comprises PVC pipes (12 pipes of 91cm each) attached with L-bends and the silpaulin sheet is mounted on them. The chamber has a vent on one side connected with velcro tape to make the chamber airtight and prevent the escape of ethylene gas. The fruits were treated with ethylene only for 24 hours in a small chamber, ensuring uniform distribution. In the case of commercial usage, where the quantity would be higher, airflow distribution can be provided for uniform treatment.

A zero energy cool chamber of size 0.74 m × 0.59 m × 0.109 m, which can hold a capacity of 80kg (4 crates of 20 kg each), was used. The zero-energy cool chamber is a double-layered structure built with bricks and filled with

sand in between. Watering the sand regularly maintains the moisture, creating a cooling effect inside the chamber. Cold chamber of size 2.2 m × 2.8 m × 2.8 m capable of holding 800 kg (40 crates of 20 kg each). The temperature of the chamber can be controlled, ranging from 5˚C to 40˚C. A forced aircraft circulation system inside the chamber ensures the dissipation of field heat and a uniform supply of ethylene to every fruit.

Harvesting of fruits

The mango cultivars Alphonso and Mulgoa were harvested at a mature green stage from the College Orchard, TNAU, Coimbatore, with a stem length of 1cm, using a mango harvester to prevent the fruits from bruises. The maturity indices include fullness of cheeks, change in fruit colour (dark green to light green), outgrown shoulders and pit formation with ridges at the stem end. The fruits were arranged in crates cushioned with paddy straw to avoid mechanical damage to the fruits during postharvest handling, which may synthesize ethylene production.

Hot water treatment

The harvested fruits are brought to the Analytical Laboratory, Department of Fruit Science, TNAU, washed in fresh water, cleaned and air dried completely. The air-dried fruits are subjected to hot water treatment at 52 °C and 0.1 % Bavistin for 10 min. This treatment was the best treatment for disease control in Kesar mango As the fruit flies' maggots are inside the pulp (11). The latent anthracnose infection is found on the peel or the surface immediately below the peel and hot water treatment along with Bavistin helps to reduce pest and postharvest diseases. After hot water treatment, the fruits are dried thoroughly.

Ethrel treatment

The hot water-treated fruits are treated ethrel in different chambers with different temperatures for 24 hours. The ripening mixture includes ethrel (2-chloroethyl phosphonic acid -39 % active ingredient), sodium hydroxide pellets and water. Ethrel becomes strongly acidic when dissolved in water. Hence, when ethrel is dissolved in solution above pH 5 (alkali medium), ethrel hydrolyses, liberating ethylene gas (12). The crates are placed inside the chamber on bricks to ensure the circulation of ethylene gas beneath the crates. The glass beaker containing the required quantity of ethrel and water is placed inside the chambers. As soon as the sodium hydroxide pellets are added, the chambers are closed airtight to prevent the escape of the ethylene gas. The amount of ethrel, sodium hydroxide and water required to produce 100 ppm of ethylene varies on the chamber size (Fig. 1). The composition of the mixture was determined by the formula in Equation 1 (Table 1).

$$\text{Concentration (ppm)} = \frac{\text{Weight (g)} \times 10^6}{\text{Volume (ml)}} \quad \text{(Eqn. 1)}$$

Table 1. Required quantity of ethrel, water and NaOH to release 100 ppm of ethylene

S.No	Chamber	Ethrel (mL)	Water (mL)	NaOH (g)
1.	Silpaulin chamber	1.3	650	0.26
2.	Zero energy cool chamber	0.9	450	0.18
3.	Cold chamber	31.6	15,800	6.3

A. Silpaulin chamber.

B. Zero energy cool chamber.

C. Cold chamber.

Fig. 1. Ethrel treatment in different ripening chambers of mango cv. Alphonso and Mulgoa.

When enough sodium hydroxide is added to 200 mL of ethrel, 28000 cm3 (28000 mL) of ethylene gas is released. 5 L of water, 10 mL of 39 % ethrel and 2 g of sodium hydroxide were used for the ripening of mango (13). After 24 hours of ethrel treatment, the fruits were taken out and kept under the ambient condition in cardboard boxes cushioned with paddy straw to continue the ripening process. Seven replications with eight fruits per replication were kept for analysis on different days of storage. The boxes were appropriately labelled and subjected to periodical assessment.

Observation recorded

Physical parameters: The physical parameters like colour values, total carotenoids and percent disease index were analyzed. The colour values and total carotenoid content were analyzed regularly from the 0^{th} day after treatment till the 10^{th} day after treatment. The percent disease index for stem end rot and anthracnose was calculated on the 3^{rd} day after the fruit reached full ripeness.

The mango pulps' colour values (L*, a*, b*) were measured using the Lovibond colour measurement tintometer (14). It works on the transmission principle as it accurately measures the reflected colour. The mango pulp was cut into small pieces and placed on a white plate

while the tintometer camera focused on the sample. As the light flashes on the sample, the tintometer measures the colour values of the sample. The L*, a*, b* values represent the following,

L* - Lightness (0 (black) to 100 (white))

a* - Redness/greenness (-a (greenness) to +a (redness))
b* - Yellowness/blueness (-*b* (blueness) to +b (yellowness))

The total carotenoid content was measured by the method suggested previously (15) with a petroleum ether: acetone mixture (3:2) using the formula and indicated in terms of µg/100g in Equation 2.

$$\text{Carotenoid (µg/100g)} = \frac{3.875 \times \text{OD value } V_1 \times \text{Volume made upto} \times 100}{\text{Weight of the sample taken}} \quad \text{(Eqn. 2.)}$$

The per cent disease index was computed by rating the surface area covered by rot and lesions on the fruits with a grade of 0-5 using the formula in Equation. 3(16).

$$\text{Per cent disease index} = \frac{\text{Sum of individual ratings}}{\text{Total number of fruits}} \times \frac{100}{\text{Maximum grade}} \quad \text{(Eqn. 3.)}$$

Grades	Percentage of the area affected.
0	No infection
1	Upto 5 % of fruit surface area covered
2	6 - 10 % of the fruit surface area covered
3	Between 11 and 20 % of the fruit surface area is covered.
4	21 - 50 % of the fruit surface area covered
5	More than 50 % of the fruit surface area is covered.

Antioxidant enzymes: An enzyme extract was prepared by taking 2g of mango pulp and macerated in a prechilled pestle and mortar using 10 mL of phosphate buffer (pH - 7.0). The supernatant obtained was used as an enzyme extract to assess the activity of the antioxidant enzymes catalase and peroxidase.

The catalase activity was estimated using the method based on the absorbance of hydrogen peroxide at 240 nm (17). The reaction mixture of 3 mL was prepared using 1.5 mL of phosphate buffer, 0.5 mL of hydrogen peroxide (775 µL of 30 % H_2O_2 in 100 mL of distilled water), 50 µl enzyme and made up to 3 mL with distilled water. The catalase activity was calculated using the formula in Equation 4 and expressed in terms of the samples' activity/ min/g fresh weight.

$$\text{Catalase (activity/min/g)} = \frac{2.3}{\text{Time (sec)}} \times \log \frac{A1}{A2} \times \frac{10 \times 1000}{50} \times \frac{60 \times 1}{2} \quad \text{(Eqn. 4)}$$

Whereas,

Time- time interval

A1 & A2- Decrease in absorbance of H_2O_2 concentration

To convert µL to g, (10*1000)/50 is given and to convert sec to min, (60x1)/2 is given

The peroxidase activity was estimated using the O-dianidisine method (18). The reaction mixture consists of 3.5 mL phosphate buffer, 0.2 mL enzyme extract, 0.1 mL freshly prepared O-dianidisine (1 mg O-diandisine in 1 mL methanol), 0.2 mL H_2O_2 (0.14 mL of 30 % H_2O_2 in 100 mL of distilled water). Enzyme activity is expressed in terms ΔA/min/g fresh weight of the sample as per Equation 5.

$$\text{Peroxidase (}\Delta\text{A/min / g)} = \frac{\Delta\text{A/min} \times 10 \times 1}{0.2 \times 2} \quad \text{(Eqn. 5)}$$

GC-MS analysis

The GC-MS analysis was carried out on the samples taken on the day of the fruits' complete ripening. The sample for GC-MS analysis was extracted as per the procedure suggested previously (19) with slight modifications. Two grams of fruit sample was weighed and macerated with one gram of sodium sulfite to remove excess moisture and 10 mL ethyl acetate - HPLC grade was added (20) as the best solvent for extracting volatile compounds. The mixture was allowed to stand at room temperature for 1 hour and kept in vortex for 15 min. The extract was centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge at 4˚C. The supernatant was transferred to Eppendorf tubes, closed tightly with cling wrap and used for GC-MS analysis.

The extract was analyzed through GC-7890A and MS -5975C equipped with column DB-5MS 30 m x 250 µm x 0.25 µm and AGILENT made under the following conditions: carrier gas as helium with flow rate at 1 mL per minute and 1µl sample injection split mode injection with a split ratio of 1: 10. The column temperature maintained initially at 50°C which hold for 1 minute at the increasing rate of 10 °C/min, followed by increasing up to 300°C which hold for 4 min, the electron impact energy was 70eV, transfer line temperature was set at 280°C and the ion source temperature was set at 230°C. The chromatogram was obtained for each sample and the compounds were identified by comparing the mass spectra obtained and their retention time with the NIST library.

Statistical analysis

The experiment was laid out in Completely Randomized Design and one-way analysis of variance (ANOVA) was performed to compare the means by least significant difference (LSD) analysis of ANOVA. The significance of the data was determined at $p < 0.05$. Statistical analysis was performed using R software (R version 4.3.1) with suitable packages. Since only two treatments were analyzed on the 10th day, an F-test was performed to check the equality of variance based on which t-test was performed, from which the critical difference and standard error of difference were computed. The GC-MS data were statistically analyzed and a heat map was generated using Metaboanalyst 5.0.

Results

Colour values

The L* value decreased throughout the storage period, regardless of treatments and cultivars and was significantly influenced by different treatments. Mango cv. Alphonso treated in cold chamber (T_3) had the highest L* value (65.63) on day 0, followed by zero energy cool chamber (T_2) (61.43) with the lowest in silpaulin chamber (T_1) (59.34). A similar trend was observed in Mulgoa, with T_3 showing the highest L* value (74.47) followed by T_2 (72.27) and the lowest in T_1 (69.51) (Fig. 2). The a* values increased significantly throughout the storage period regardless of treatments and cultivars. Among the treatments, mango cv. Alphonso and Mulgoa treated in silpaulin chamber (T_1) recorded the highest a* values of 20.44 and 12.89, respectively, followed by zero energy cool chamber (T_2) (12.91 and 6.31, respectively) on the 4th day of storage. The lowest a* values of 9.23 and 5.26 were recorded in Alphonso and Mulgoa fruits treated in the cold chamber on the 4th day of storage (Fig. 3). The increasing trend of b* values was observed in both the cultivars throughout the storage period regardless of treatments. Among the treatments, mango cv. Alphonso and Mulgoa, subjected to ethrel treatment in silpaulin chamber (T_1), registered the highest b* values (45.90 and 45.74, respectively) followed by zero energy cool chamber (T_2) (41.53 and 42.60, respectively) on 0th day of storage. The lowest b* value was recorded in both Alphonso (35.24) and Mulgoa (39.37) treated in the cold chamber (T_3) (Fig. 4).

Total carotenoids

The total carotenoid content increased significantly throughout the storage period, irrespective of the treatments and cultivars. The fruits treated in the cold chamber (lower temperature) recorded a gradual increase in the total carotenoid content (Alphonso - 809.78µg/100g in 0th day to 3567.98 µg/100g in 4th day; Mulgoa - 234.17 µg/100g in 0th day to 1176.38 µg/100g in 4th day) followed by zero energy cool chamber (Alphonso - 1311.95 µg/100g in 0th day to 4859.43 µg/100g in 4th day; Mulgoa - 308.56 µg/100g in 0th day to 2027.68 in 4th day) whereas the rapid

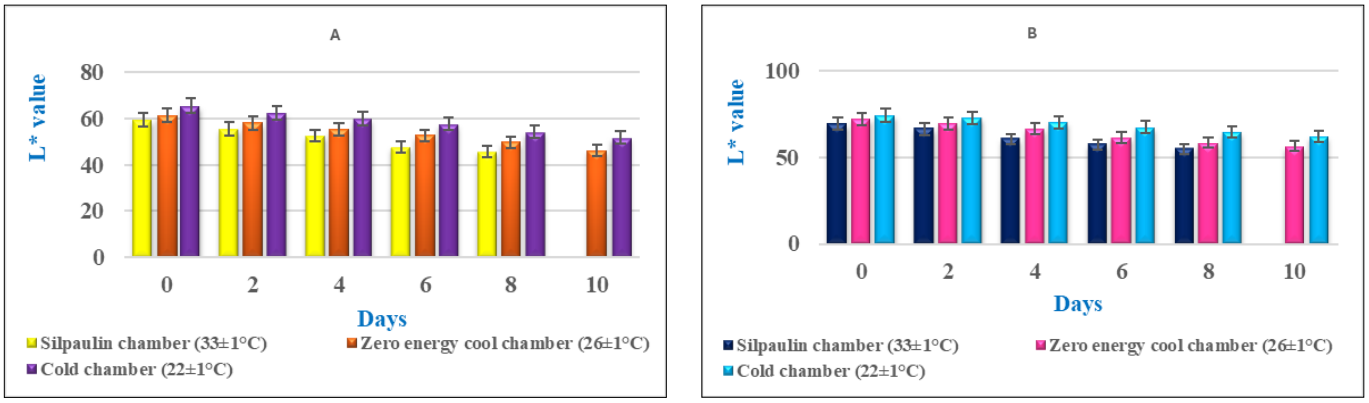

Fig. 2. Effect of temperature during ethrel treatment in different chambers on L* value of mango A. Alphonso; B. Mulgoa.

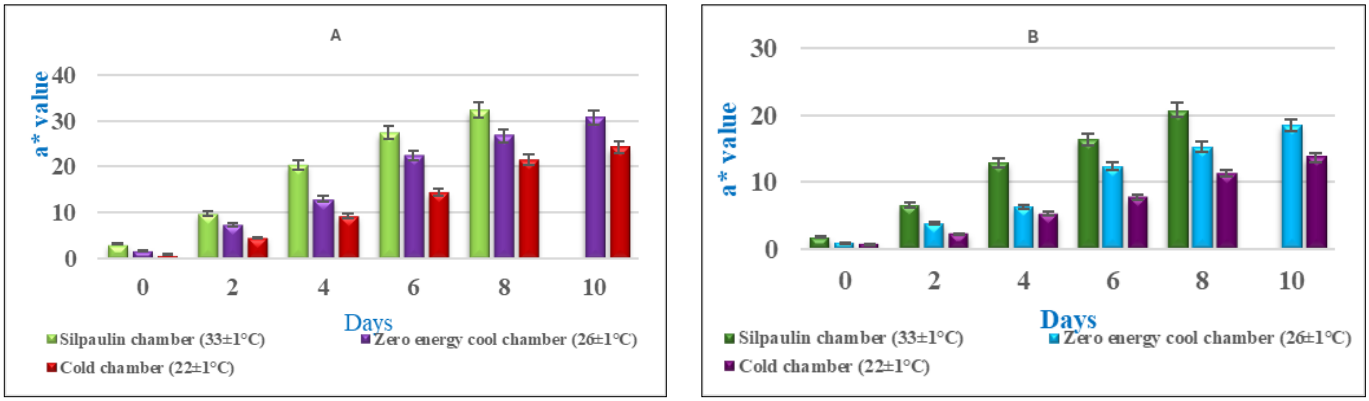

Fig. 3. Effect of temperature during ethrel treatment in different chambers on a* value of mango A. Alphonso; B. Mulgoa.

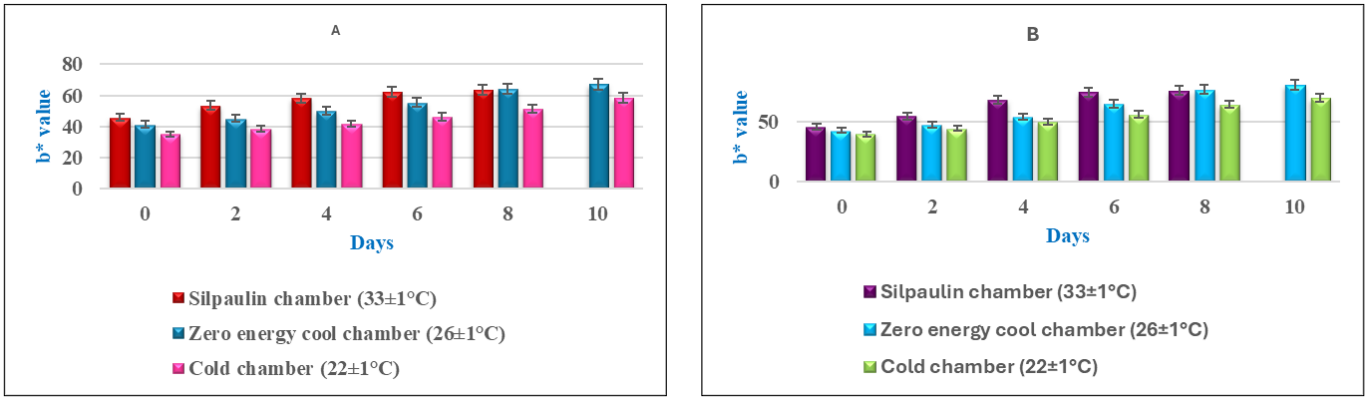

Fig. 4. Effect of temperature during ethrel treatment in different chambers on b* value of mango A. Alphonso; B. Mulgoa.

increase of carotenoid content was seen in fruits treated in silpaulin chamber (higher temperature) (Alphonso - 1947.53 µg/100g in 0th day to 9236.74 µg/100g in 4th day; Mulgoa - 603.34µg/100g in 0th day to 3085.60 in 4th day) in both the cultivars (Table 2).

Percent disease index

The percent disease index of stem end rot varied significantly in mango cv. Alphonso and Mulgoa treated ethrel in different ripening chambers (Table 3). The highest percent disease index was recorded in mango cv. Alphonso subjected to ethrel treatment in silpaulin chamber (T_1) (24.00 %) followed by zero energy cool chamber (T_2) (12.57 %) and the lowest percent disease index was recorded in fruits treated in the cold chamber (T_3) (8.00 %). The mango cv. Mulgoa treated in the silpaulin chamber recorded the highest percent disease index of 9.71 %, which was on par with the fruits treated in the cold chamber (6.86 %). The lowest percent disease index was observed in fruits subjected to ethrel treatment in zero energy cool chambers (4.00 %). The disease index was not only reduced but also delayed at low temperatures. Anthracnose incidence was found only in mango cv. Mulgoa. The percent disease index was significantly higher in fruits treated in the cold chamber (15.43 %), followed by a silpaulin chamber (6.29 %). The lowest PDI was observed in fruits subjected to ethrel treatment in zero-energy cool chambers (4.00 %).

Antioxidant enzyme activity

The catalase activity was found to increase till the day of complete ripening and thereafter showed a decreasing trend. The conversion rate was higher in fruits treated in a silpaulin chamber followed by zero energy cool chambers. A 3.7-fold increase in catalase activity was recorded in fruits treated in a silpaulin chamber, followed by a 2.7-fold increase in fruits treated in a zero energy cool chamber and a 2.2-fold increase in fruits treated in the cold chamber from the 0th day to the 4th day. Similar trends were observed in Mulgoa (Table 3).

Peroxidase activity increased until the fruits were fully ripe, decreasing irrespective of treatments and cultivars. The rate of increase in activity was found to be higher in fruits treated at higher temperatures (silpaulin chamber) (Alphonso -0.22 ΔA/min/g in 0th day to 0.82 ΔA/min/g in 4thday; Mulgoa -0.31 ΔA/min/g in 0thday to 1.02 ΔA/min/g in 4th day) followed by zero energy cool chamber and lower in fruits treated in cold chamber (Table 4).

Volatile compounds identified in mango cv. Alphonso

The volatile compounds were identified by comparing the mass spectra with the retention time (RT). The volatile compounds emitted by the fruits subjected to ethrel treatment in the silpaulin chamber were detected at the retention time from 3.26 to 27.07. A total of 30 volatile compounds were identified out of which five were alkanes (cyclododecane, tetradecane, hexadecane, cyclopentadecane and cycloeicosane), six were ester compounds (acetic acid, butyl ester, 2-ropenoicacid, pentadecylester, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, diethyl Phthalate, 1-Butanol,3-methyl-acetate, Diisooctyl phthalate), two were alkenes(1-Octadecene, cis-1-Chloro-9-octadecene), four ketones (2,5-imethyl-4-methoxy-3(2H)-furanone, cyclopentadecanone, 2-hydroxy, 7,9-Di-tert-butyl-1-oxaspiro [4,5]deca-6,9-diene-2,8-dione, cyclononanone), three were

Table 3. Effect of temperature during ethrel treatment in different ripening chambers on percent disease index (stem end rot and anthracnose) on 3rd day after complete ripening of mango cv. Alphonso and Mulgoa

Treatments	percent disease index (Stem end rot)		percent disease index (Anthracnose)
	Alphonso	Mulgoa	Mulgoa
T_1	24.00 (29.29^a)	9.71 (18.08^a)	6.29 (15.58^b)
T_2	12.57 (20.67^b)	4.00 (10.77^b)	4.00 (10.77^c)
T_3	8.00 (15.98^c)	6.86 (15.03^a)	15.43 (23.01^a)
SE(d)	1.69	1.71	1.92
CD (p=0.05)	3.54	3.60	4.02

Within a column means having different letters indicate a significant difference at p=0.05 according to the LSD test. Figures enclosed in parentheses indicate arcsine transformed values (T1 - Silpaulin chamber (33 ± 1 °C); T2 - Zero energy cool chamber (26 ± 1°C); T3 - Cold chamber (22 ± 1°C))

Table 2. Effect of temperature during ethrel treatment in different ripening chambers on total carotenoids value of mango cv. Alphonso and Mulgoa

Treatments	Alphonso						Mulgoa					
	0th day	2nd day	4th day	6th day	8th day	10th day	0th day	2nd day	4th day	6th day	8th day	10th day
T_1	1947.53 (3.29^a)	5303.10 (3.72a)	9236.74 (3.97^a)	14309.23 (4.15^a)	17232.13 (4.24^a)	NA#	603.34 (2.78^a)	1484.95 (3.17^a)	3085.60 (3.49^a)	4014.03 (3.60^a)	4782.69 (3.68^a)	NA#
T_2	1311.95 (3.12^b)	2544.77 (3.40^b)	4859.43 (3.69^b)	9557.47 (3.98^b)	14112.11 (4.15^b)	16732.86 (4.22^a)	308.56 (2.49^b)	925.68 (2.97^b)	2027.68 (3.31^b)	3049.79 (3.48^b)	3686.19 (3.57^b)	4126.99 (3.62^a)
T_3	809.78 (2.91^c)	2082.77 (3.32^c)	3567.98 (3.56^c)	5689.94 (3.76^c)	8457.64 (3.93^c)	12195.92 (4.08^b)	234.17 (2.37^c)	655.69 (2.82^c)	1176.38 (3.07^c)	1810.04 (3.26^c)	2617.26 (3.42^c)	3115.91 (3.49^b)
SE(d)	0.005	0.016	0.016	0.016	0.016	0.023	0.016	0.007	0.004	0.003	0.003	0.002
CD (p=0.05)	0.013	0.035	0.025	0.038	0.039	0.054	0.042	0.019	0.009	0.006	0.006	0.007

NA# - not analyzed as the fruits were deteriorated. Within a column means having different letters indicate a significant difference at p=0.05 according to the LSD test. Figures enclosed in parentheses indicate log-transformed values (T1 - Silpaulin chamber (33 ± 1°C); T2 - Zero energy cool chamber (26 ± 1 °C); T3 - Cold chamber (22 ± 1°C)

Table 4. Effect of temperature during ethrel treatment in different ripening chambers on catalase of mango cv. Alphonso and Mulgoa

Treatments	Alphonso						Mulgoa					
	0th day	2nd day	4th day	6th day	8th day	10th day	0th day	2nd day	4th day	6th day	8th day	10th day
T_1	1.21^a	2.41^a	4.45^a	5.89^a	4.95^a	NA#	1.61^a	2.92^a	5.02^a	6.33^a	5.53^a	NA#
T_2	0.88^b	1.48^b	2.39^b	3.84^b	4.64^b	4.07^a	1.28^b	1.82^b	2.67^b	4.10^b	5.04^b	4.52^a
T_3	0.69^c	0.92^c	1.55^c	2.15^c	3.41^c	4.17^a	1.04^c	1.22^c	1.85^c	2.41^c	3.82^c	4.62^a
SE(d)	0.01	0.02	0.04	0.06	0.06	NS	0.02	0.03	0.05	0.06	0.07	NS
CD (p=0.05)	0.03	0.05	0.08	0.12	0.14	NS	0.04	0.06	0.11	0.13	0.14	NS

NA# - not analyzed as the fruits were deteriorated. Within a column means having different letters indicate a significant difference at p=0.05 according to the LSD test. (T1 - Silpaulin chamber (33 °C); T2 - Zero energy cool chamber (26 °C); T3 - Cold chamber (22 °C))

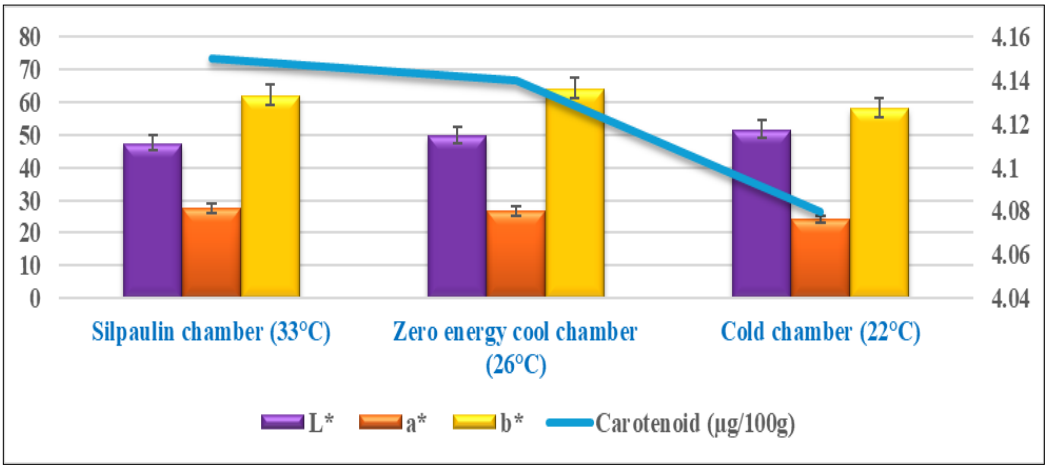

Fig. 5. Relating the findings of L*, a* and b* values with carotenoid content (µg/100g).

aromatics (ethyl benzene, benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl, 1,4-bis(trimethylsilyl)benzene), one nitrile compound (2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)-), one aldehyde (cyclopropane octanal, 2-octyl-), four acids(1,3-dimethyl-1H-pyrazole-5-carboxylic acid, octadecanoic acid, n-hexadecenoic acid, oleic acid) and three other compounds.

The GC-MS analysis of volatile compounds emitted by fruits subjected to ethrel treatment in zero energy cool chambers was detected at the retention time from 3.26 to 26.40. A total of twenty-nine volatile compounds were identified in the ripe fruit. Among them, seven were esters (acetic acid, butylester, 1-Butanol,3-methyl-acetate, 2-Propenoicacid, pentadecylester, sulfurous acid, butyl heptadecyl ester, dichloroacetic acid, heptadecyl ester, Bis(2-ethylhexyl) phthalate, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester), were four ketones (2,5-Dimethyl-4-methoxy-3(2H)-furanone, ethanone,1-(9-anthracenyl), propiophenone, 2-(trimethyl siloxy, 7,9-Di-tert-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione), five were alkanes (dodecane, tetradecane, hexadecane, cyclopentadecane, 2-methyloctacosane), four were alkenes (2-Hexene, (Z)-2-dodecene, 1-Nonadecene, 1-Octadecene), one alcohol (2-Methyl-Z, Z-3,13-octadecadienol), one aldehyde (cis-9-Hexadecenal), three were acids (octadec-9 -enoicacid, octadecanoicacid, n-Hexadecanoicacid), one N-, S compound (2-methyl-6-methylsulfanylpyrazine) and three other compounds.

The GC-MS analysis of volatile compounds emitted by fruits subjected to ethrel treatment in the cold chamber was detected at the retention time from 3.26 to 25.00. Thirty volatile compounds were identified among which, six were esters (aceticacid, butyl ester, 1-Butanol, 3-methyl-, acetate, 2-Propenoicacid, pentadecylester, dichloroacetic acid, heptadecyl ester, Bis(2-ethylhexyl)phthalate, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester), three were alkanes (tetradecane, hexadecane, cycloeicosane), two were alkenes (1-Dodecene, 1-Eicosene), one was terpene (trans-.beta.-Ocimene), one was aromatics (1,2-Bis(trimethylsilyl) benzene), one alcohol (2-Methyl-Z, Z-3,13-octadecadienoic), three ketones (2,5-Dimethyl-4-methoxy-3(2H)-furanone, 2-(6-Chlorohexanoyl) cyclododecanone, 7,9-Di-tert-butyl-1-oxaspiro[4,5] deca-6,9-diene-2,8-dione), four acids (oleic acid, octadecanoic acid, 9,12-Octadecadienoicacid(Z, Z)-, n-Hexadecanoic acid), one sulphur compound (disulfide, di-tert-dodecyl), one aldehyde (cyclopropane octanal,2-octyl-), two pyrans (2H-Pyran, 3,4-dihydro-, Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl) and five other compounds (Fig. 6).

Volatile compounds identified in mango cv. Mulgoa

The volatile compounds were identified by comparing the mass spectra with the retention time (RT). The volatile compounds emitted by the fruits subjected to ethrel treatment in zero energy cool chambers were detected at the retention time from 3.26 to 26.40. Totally 35 volatile compounds were identified out of which six were esters (acetic acid, butyl ester, methoxy acetic acid, 2-tetradecyl ester, 2-Propenoic acid, pentadecyl ester, Diisooctylphthalate, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester, 1-Butanol,3-methyl-, acetate), nine were alkanes (Decane, cyclododecane, tetradecane, pentacosane, Decane, 3,6-dimethyl-, hexadecane, 2,6,11,15-tetramethyl decosane, cycloeicosane, eicosane), four were alkenes (2-Hexene,(E)-, 1-Octadecene, 5-Eicosene (E)-, cis-1-Chloro-9-octadecene), one alcohol (benzyl alcohol), one aromatics (ethyl benzene), six ketones (1,6-dioxacyclododecane-7,12-dione, Ethanone, 1-cyclododecyl, 1-(4-Butoxy-2,6-dimethylphenyl)ethanone, 7,9-Di-tert-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione, 9-Acetylphenanthrene), one aldehyde (9-Octadecenal, (Z)-, cyclopropane octanal,2-octyl-), five acids (n-Hexadecanoic acid, oleic acid, octadecanoic acid, tridecanedioicacid, Octadec-9-enoicacid) and two other compounds.

The GC-MS analysis of mango fruit pulp subjected to ethrel treatment in a cold chamber was detected at a retention time from 3.49 to 26.49. A total of 50 compounds were identified among them, five were esters (aceticacid, butylester, 2-chloro-,octadecyl ester, 1-Butanol,3-methyl-,acetate, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester), thirty were alkanes (octane,4-methyl-, decane, 4-ethyl-, cis-1-hexyl-2-propyl-Cyclopropane, tridecane,1-iodo-, tetradecane, dodecane,4,6-dimethyl, decane,2,3,7-trimethyl-, Heptadecane, Decane,3,7-dimethyl-, tetracasane, pentadecane, eicosane, Heptadecane,8-methyl-, Heneicosane, Cyclopentane,1-butyl-2-propyl-, Hexadecane, Hexacosane, Octadecane,1-iodo-, Dodecane 1,1'-oxybis-, heptacosane, octacosane, heptadecane, nonacosane, octadecane, Heptadecane,2-methyl-, docosane, tetradecane, 2,6,10-trimethyl, hentriacontane, Heneicosane, 3-methyl, dotriacontane), four were alkenes (1-Undecene,4-methyl-, Undecane,4-methyl-, 3-

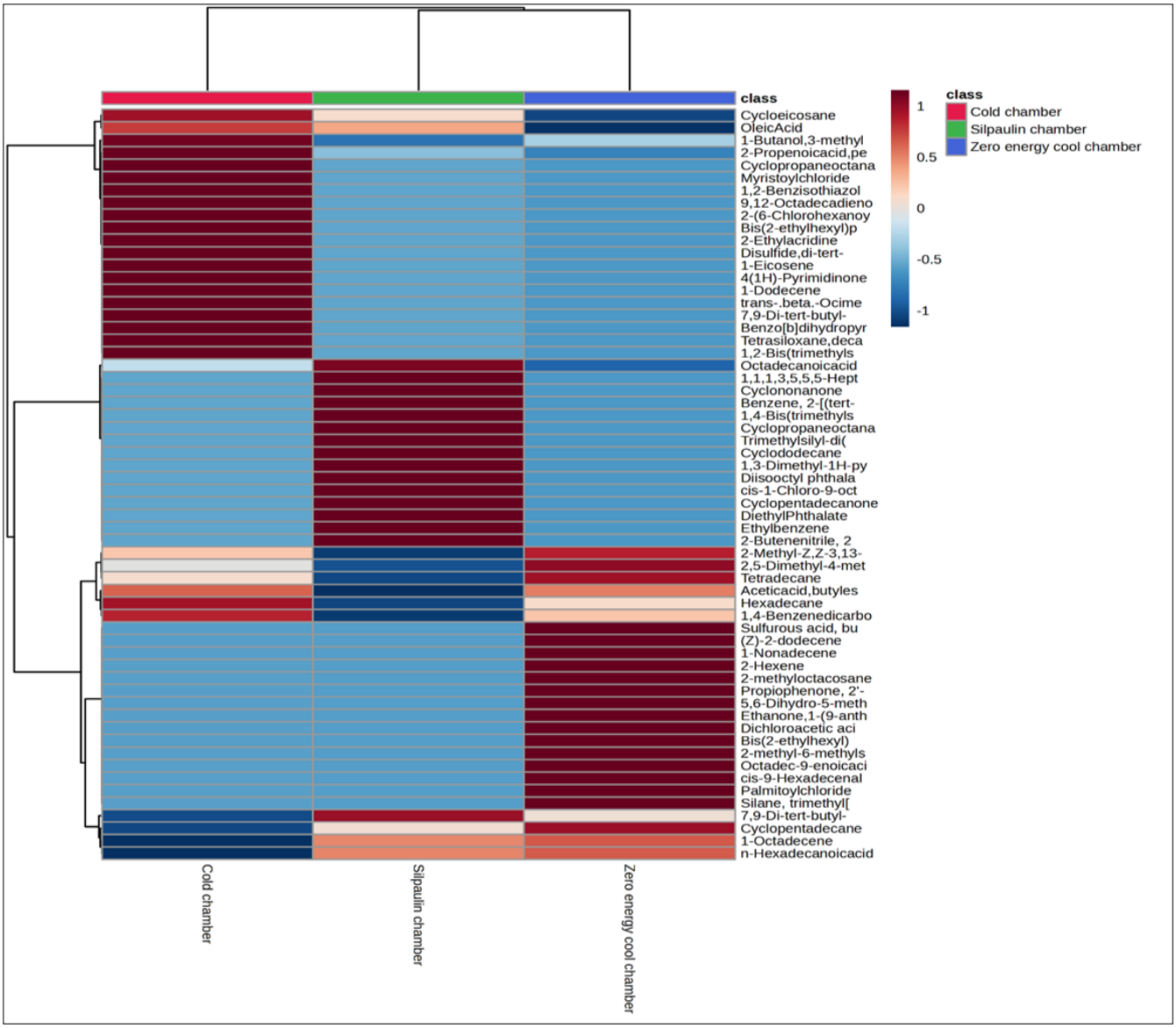

Fig. 6. Heat map of volatile compounds evolved from mango cv. Alphonso subjected to ethrel treatment in different chambers.

Hexene, 2,2,5,5-tetramethyl-, (Z), 2-Tetradecene,(E)-), two aldehydes (benzaldehyde,4-ethyl-, cis-9-Hexadecenal), one ketone ((E)-2-bromobutyloxychalcone), one alkyne (7-Pentadecyne), two acids (oleicacid, n-Hexadecanoicacid) and four other compounds (Fig. 7).

Discussion

The L* value indicates the lightness (luminosity) of the fruit, which decreases as ripening progresses. The pulp colour changes from lighter (white) to darker (yellow), resulting in a reduction of the L* value (21). A higher L* value means the fruits are still unripe, while the lowest value was recorded in fully ripened fruits. The L* value was found to be higher in Alphonso fruits treated in cold chamber (51.84) followed by zero energy cool chamber (49.80), which was on par with fruits treated in the silpaulin chamber (47.60) on the day of complete ripening. The results of the current study correspond to the research findings on mangoes (22). The a* value indicates the greenness and redness of the fruit pulp increased during the ripening of ethrel-treated fruits. The highest a* value was recorded in Alphonso fruits treated at high temperature (silpaulin chamber) (27.41), which was on par with zero energy cool chamber (26.70) and cold chamber (low temperature) (24.20) on the day of complete ripening. Redness was more predominant in Alphonso than in Mulgoa, which may be a cultivar characteristic. On the contrary, results also revealed that the a* value of fruits in higher temperatures increased significantly up to 144 hrs, followed by a non-significant decline. Further, it was stated that the alteration in O_2 and CO_2 concentration might be the reason for changes in colour values in different treatments (23). The mesocarp colour turned yellow during the ripening progression, increasing in b* value during storage. Endogenous ethylene production positively correlated with the b* value (24). The highest b* value was recorded in Alphonso fruits treated in a zero energy cool chamber (64.43), which was on par with fruits treated in the silpaulin chamber (62.29) on the day of complete ripening. This might be due to the higher carotenoid content in the silpaulin chamber (14309.23 µg/100g), which was on par with zero energy cool chambers (14112.11 µg/100g) on the day of complete ripening. The results align with the findings of the previous studies, in which the intensity of the yellow-orange colour increases as the fruit ripens, along with an increase in carotenoid content.

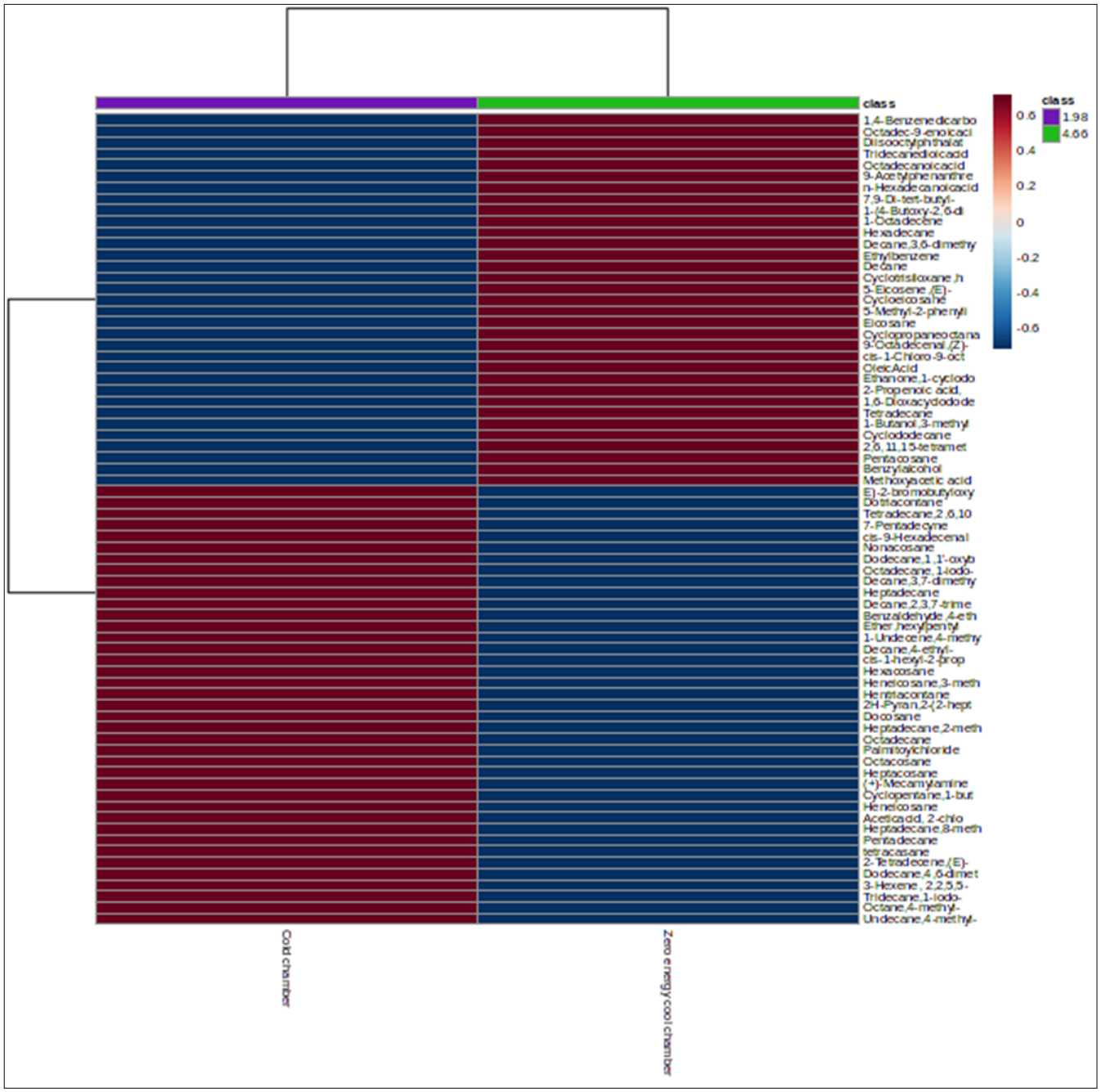

Fig. 7. Heat map of volatile compounds evolved from mango cv. Mulgoa subjected to ethrel treatment in different ripening chambers.

Total carotenoids increased throughout the ripening period in all treatments and cultivars studied. Ethrel treatment enhanced the enzyme activity responsible for carotene synthesis (26). This might be due to the increased synthesis of mevalonic acid and geraniol, precursors for carotenes synthesis (22). The total carotenoid content was higher in Alphonso fruits treated in a silpaulin chamber (14309.23 µg/100g), which was on par with zero energy cool chamber (14112.11 µg/100g) on the day of complete ripening. The increased a* value in the silpaulin chamber (27.41), which was on par with the zero energy cool chamber (26.70), might be attributed to the higher carotenoid content. The results align with the findings of the previous study in Mango (27). The a* and b* values positively correlated with carotenoids, whereas the L* value negatively correlated with total carotenoids (Fig. 5). The results align with the previous studys' findings in mango (28). Total carotenoids increased with an increase in temperature during ripening (29). The total carotenoid content was lower (12195.92 µg/100g) in Alphonso fruits treated in the cold chamber on the day of complete ripening. The present studys' findings are in close conformity with the result that the carotenoid synthesis of Alphonso mango was affected when ripened at low temperatures (10). The total carotenoids in the pulp and its conversion rate were found to increase as the temperature increased. The results are in agreement with those reported in mango var. Tommy Atkins (30).

Anthracnose and stem-end rot are the most common postharvest fungal disease in mango. The percent disease index of stem end rot and anthracnose was less, irrespective of the treatments and cultivars. This might be due to the hot water treatment of fruits combined with 0.1 % Bavistin at 52°C for 10 min. Similar outcomes were reported by previous studies (31, 32). Hot water and bavistin treatment control the spores and latent infections in the fruit surface or the initial few layers beneath the peel (33). The maximum PDI of stem end rot

was observed in fruits treated in a silpaulin chamber in both Alphonso (24.00 %) and Mulgoa (9.71 %) and the minimum was observed in Mulgoa fruits treated in a zero energy cool chamber (4.00 %). Stem end rot is found to develop after harvest under high temperatures and high humidity and increases at a temperature above 28 °C (31, 34). Ethrel treatment involves the formation of fruit abscission zones between the fruit and button tissue due to increased abscission enzymes, resulting in natural openings that allow the pathogen to cause infection (35). The higher ethylene production in the silpaulin chamber due to increased temperature might have caused an increase in the stem end rot index of mango cv. Alphonso and Mulgoa (36). High humidity prevailing in cold chambers (94 %) might be the reason for the rise in anthracnoses' PDI in cold storage. This conforms with a previous study on mangoes (37).

The antioxidant enzymes catalase and peroxidase play a vital role in protecting the cells by scavenging the ROS (Reactive oxygen species). Both catalase and peroxidase are responsible for removing H_2O_2, which is associated with fruit ripening and senescence. The catalase and peroxidase activities increased during ripening (Table 4-5). These results are consistent with those reported previously (38, 39). In contrast, a reduction in catalytic activity was also reported in mangoes (39). The enzyme activity was found to increase the rising temperature. Ethylene treatment has increased the hydrogen peroxidase content (40). The increase in hydrogen peroxidase content was due to increased respiration at higher temperatures, which might have led to increased peroxidase activity to scavenge the H_2O_2 produced (41). Exogenous ethylene application stimulated the catalase and peroxidase activity in Alphonso mango (42). Maximum peroxidase activity was observed in fruits treated at higher temperatures, where maximum activities were found at 30 °C. Fruit ripening is an oxidative process and these antioxidant enzymes play a vital role in protecting the cell wall from oxidative damage by maintaining ROS production below a threshold level (44). The increased antioxidant enzyme activity in fruits treated in a silpaulin chamber might also be due to the increased percent disease index.

Research indicates that 375 volatile compounds from 20 Cuban cultivars were noticed (45). Some compounds in the present study are similar to those in Cuban cultivars (guaiacol, quinones). The differences in compounds detected in fruits treated in different ripening chambers indicate that the temperature during ethrel treatment might have influenced the biosynthetic pathway of the volatile compounds. Mango aroma is said to be formed by a complex mixture of compounds (46). Synthesis of aroma volatile compounds is one of the key features of ripening of fruits. Ethylene is involved in the biosynthesis of furanone and lactones in ripened fruits (47). Research indicates that similar compounds-related observations were also reported by Alphonso Mango (48). Mango fruits ripened at 20°C resulted in better aroma compounds than fruits ripened at various temperatures (49). This temperature promotes the activity of enzymes involved in the biosynthesis of key volatile compounds, such as esters, terpenes and aldehydes, contributing to Alphonso mangoes' characteristic aroma and flavour.

The effect of 20°C on aroma synthesis is not exclusive to Alphonso mangoes, though the specific profile of volatile compounds and their intensities can vary among varieties. The acids and esters are produced by lipid metabolism, which is responsible for flavour development in mango during ripening (50). The ester compounds are higher (seven) in Alphonso fruits subjected to ethylene treatment in zero energy cool chamber compared to fruits subjected to ethrel treatment in a silpaulin chamber (six esters) and cold chamber (six esters). At the same time, six esters were detected in mango cv. Mulgoa was treated in a zero-energy cool chamber, whereas five esters were detected in fruits treated in the cold chamber.

The furan compounds, i.e., 2,5-Dimethyl-4-methoxy -3(2H)-furanone (Mesifuran), are major volatile compounds in Alphonso mango. Mesifuran is the methyl ether of furaneol, which contributes to the odour character of the complex Alphonso blend (30). This compound produces a sherry wine-like note in mango fruits (51). A study (52) reported that mesifuran is said to have a coumarin-like fruity odour. The area percentage of this compound is high in ripened fruits that were subjected to ethrel treatment in a zero energy cool chamber (2.74) followed by a cold chamber (2.22) and silpaulin chamber (2.02). The compound was detected in most of the Alphonso mango cultivars (53) and others (54).

Acetic acid, butyl ester, or n-butyl acetate, is a volatile ester with a strong pear-like aroma (55). Similarly, butyl acetate, a volatile compound with a fruity, sweet and grassy aroma, was reported in Fuji apples (56). Butyl acetate is found to impart characteristic flavour to the fruits, which is found to be higher in fruits treated in zero

Table 5. Effect of temperature during ethrel treatment in different ripening chambers peroxidase (ΔA/min/g) of mango cv. Alphonso and Mulgoa

Treatments	Alphonso						Mulgoa					
	0th day	2nd day	4th day	6th day	8th day	10th day	0th day	2nd day	4th day	6th day	8th day	10th day
T_1	0.22[a]	0.47[a]	0.82[a]	1.02[a]	0.92[a]	NA#	0.31[a]	0.62[a]	1.02[a]	1.23[a]	1.15[a]	NA#
T_2	0.15[b]	0.28[b]	0.42[b]	0.77[b]	0.90[a]	0.83[b]	0.26[b]	0.42b	0.62[b]	0.91[b]	1.11[b]	1.06[b]
T_3	0.12[c]	0.22[c]	0.34[c]	0.47[c]	0.73[b]	0.88[a]	0.21[c]	0.34[c]	0.53[c]	0.71[c]	1.02[c]	1.14[a]
SE(d)	0.003	0.004	0.007	0.01	0.02	0.01	0.004	0.01	0.01	0.01	0.02	0.01
CD (p=0.05)	0.006	0.009	0.015	0.02	0.01	0.03	0.009	0.02	0.02	0.03	0.04	0.03

NA# - not analyzed as the fruits were deteriorated. Within a column means having different letters indicate a significant difference at p=0.05 according to the LSD test. (T1 - Silpaulin chamber (33 °C); T2 - Zero energy cool chamber (26 °C); T3 - Cold chamber (22 °C))

energy cool chamber (4.83 %) followed by a cold chamber (4.41 %) and lower in silpaulin chamber (3.51 %). In Mulgoa, it was found to be higher in fruits treated in zero energy cool chambers (4.66 %) compared to the cold chamber (1.98 %). Oleic acid is a monounsaturated fatty acid. As ripening progresses, the membrane permeability increases in climacteric fruits and the membrane integrity is lost, resulting in lipid degradation, especially lipid peroxidation of unsaturated fatty acid. Unsaturated fatty acids play an important role in fruit senescence. Oleic acid exhibited antifungal properties (57). Mango cv. Alphonso treated in a cold chamber had a 1.3-fold increase in oleic acid compared to fruits treated in the silpaulin chamber. The Mulgoa fruits treated in zero energy cool chambers recorded the highest oleic acid percentage of 11.93 compared to fruits treated in the cold chamber (2.33). The oleic acid and firmness are positively correlated (58). This might be the reason for the increased firmness of Mulgoa fruits treated in zero-energy cool chambers.

Fatty acids such as palmitic acid, palmitoleic acid and lactones determine the flavour quality of mangoes during ripening (59, 60). Hexadecanoic acid, a type of palmitic acid, was found to be higher in mango cv. Alphonso was treated in the zero energy cool chamber, with an area percentage of 12.91 %, followed by fruits treated in the cold chamber (14.12 %) and silpaulin chamber (12.91 %). In mango cv. Mulgoa, hexadecenoic acid was higher in fruits treated in zero energy cool chambers, with an area percentage of 15.32 % compared to those treated in the cold chamber (9.04 %). Apart from aroma, n-hexadecanoic acid is also reported to have antibacterial and antioxidant activity (61). Research indicates hexadecanoic acids' antifungal and antibacterial properties, which might explain the disease index of stem end rot in fruits treated in the zero energy cool chamber and cold chamber (62). Octadecanoic acid, or stearic acid, is a long-chain fatty acid with antioxidant and antibacterial activity (63). It was higher in the zero energy cool chamber (6.58 %) than in the cold chamber (6.25 %) in mango cv. Mulgoa, octadecanoic acid was only present in fruits treated in the zero energy cool chamber with an area percentage of 7.18 %. Octadecanoic acid has also been reported in mango (46), acerola(64),and genipap (46). 1-Nonadecene, which has antioxidant and antimicrobial activity, was found only in fruits treated in zero energy cool chamber with an area percentage of 0.88 %. The increase in the compounds having such antioxidant, antimicrobial and antifungal activity helps prevent postharvest diseases during storage, thereby increasing the fruit quality.

Tetradecane and hexadecane belong to the alkane family, the aroma compound in Algerian date cultivars (65). In Alphonso mango, the compounds tetradecane and hexadecane are higher in fruits treated in zero energy cool chamber (2.71 % and 4.43 %, respectively), followed by cold chamber (2.33 % and 4.31 %, respectively) and lower in fruits treated in silpaulin chamber (2.21 % and 3.62 % respectively). In Mulgoa mango, tetradecane and hexadecane are higher in fruits treated in zero energy cool chambers (2.67 % and 4.64 %, respectively) compared to the cold chamber (1.57 % and 3.42 %, respectively). Tetradecane and hexadecane were two significant components contributing to the aroma of volatile compounds in mango and banana, respectively (66).

1,4-Benzenedicarboxylic acid, bis (2-ethyl hexyl ester), was reported to have antimicrobial activity (67, 68). It is also an essential bioactive constituent of the medicinal plant *Rumex patientia*. The area percentage of 1,4-Benzenedicarboxylic acid, bis (2-ethyl hexyl ester) was found to be highest in fruits (69, 70) treated in cold chamber (5.09 %) followed by zero energy cool chamber (4.70 %) and low in fruits treated in silpaulin chamber (2.74 %). The area percentage of 1,4-Benzenedicarboxylic acid, bis (2-ethyl hexyl ester) showed a 3.6-fold increase in mango cv. Mulgoa is treated in a cool chamber with zero energy compared to fruits in a cold chamber.

Conclusion

The mango cv. Alphonso and Mulgoa, subjected to ethrel treatment at lower temperatures (cold chamber and zero energy cool chamber), showed a reduced percent disease index and a profound effect on the synthesis of volatile metabolites. The area percentage of major volatile compounds responsible for the aroma and antioxidant activities was higher in fruits treated in the zero energy cool chambers, followed by the cold chamber. The colour values and total carotenoid content were also superior in fruits treated in the zero-energy cool chambers compared to the cold chamber. The antioxidant enzyme activity was lower in fruits treated in zero energy cool chambers due to a lower respiration rate and percent disease index. Hence, zero energy cool chamber can be used as an alternative to the cold chamber for ripening mangoes among the farmers and small traders, as it is eco-friendly and cost-effective. When not used for ripening, the zero-energy cool chambers can be used for the postharvest storage of fruits and vegetables to extend their shelf life.

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Authors' contributions

PSK and RR carried out the lab analysis and drafted the manuscript. MKK is involved in analysing the volatile metabolites and reviewing and editing them. MI participated in the design and editing of the study. GR performed the GC-MS analysis. DP and SKA did the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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