



Physio-Chemical Changes during Ethrel Treatment of Mango cv. Alphonso under Different Temperature Regimes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJECC/2023/v13i102618

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/104346>

Original Research Article

Received: 02/06/2023

Accepted: 05/08/2023

Published: 12/08/2023

ABSTRACT

Aim: Mango (*Mangifera indica* L.) is the second most consumed fruit which belongs to the family Anacardiaceae. Ripening in mango (climacteric fruit) is hastened by application of ethrel. The experiment was conducted to study the effect of different temperatures during ethrel treatment on shelf life and quality of mango cv. Alphonso.

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Study Design: The experiment consisted of three treatments and seven replications laid out in Completely randomized design (CRD).

Place and Duration of Study: The study was conducted at Department of Fruit Science, Horticulture College and research Institute, Tamil Nadu Agricultural University, Coimbatore during 2022-2023.

Methodology: Mango cv. Alphonso was subjected to ethrel treatment in three different ripening chambers having different temperatures viz., T₁ – silpaulin chamber (33°C), T₂ – zero energy cool chamber (26°C), T₃ – cold chamber (22°C) for a period of 24 hours. After ethylene treatment, the fruits were stored at ambient temperature to study the ripening behaviour. Shelf life and quality parameters were observed and recorded.

Results: The days taken for ripening was lesser (6 days) and the rate of conversion of quality attributes were higher in fruits subjected to ethrel treatment in silpaulin chamber. The quality attributes like TSS (22°Brix), acidity (0.25 %), TSS : acid ratio (88) were significantly higher in fruits treated in silpaulin chamber on the day of ripening. The physiological loss in weight was found to be significantly lesser in fruits subjected to ethylene treatment in cold chamber (6.56 %) and were on par with fruits treated in zero energy cool chamber (6.71%) whereas the firmness and shelf life were found to be significantly higher in cold chamber treated fruits (2.35 N, 15 days respectively) but firmness was on par with fruits subjected to ethylene treatment in zero energy cool chamber (2.21 N) on ripening. The overall acceptability of fruits in sensory evaluations was significantly higher in fruits treated in zero energy cool chamber (8.3) which was on par with fruits treated in cold chamber (8.1).

Conclusion: The fruits treated in silpaulin chamber had better TSS, acidity and TSS: acid blend. The fruits treated in cold chamber were superior in shelf life and sensory score which was also on par with fruits treated in zero energy cool chamber. Hence zero energy cool chamber can be used as an alternative to cold chamber for ethrel treatment which would be cost effective, environment friendly and can be used by small traders and farmers.

Keywords: *Mango; ethrel; silpaulin chamber; zero energy cool chamber; cold chamber; quality parameters; shelf life.*

1. INTRODUCTION

Mango (*Mangifera indica* Linn) belonging to the family Anacardiaceae is a tropical fruit crop known for its delicious taste, rich flavour and aroma with high nutritional quality. It relishes the status of being the 'National fruit of India' and 'King of fruits' [1]. In India, Andhra Pradesh ranks first in area (3,89,670 ha) and Uttar Pradesh ranks first in production (48,07,830 MT) (Anon, 2021-2022). India is the largest producer of mangoes and is a home for more than 1000 cultivars with unique taste and flavour. Alphonso mangoes are regarded as the 'King of mangoes' as it is the choicest variety with attracting reddish-yellow colour flesh, unique flavour and aroma, excellent sugar-acid blend there by standing first in the export market of India with more than 80% of the share [2].

Mangoes are harvested with different maturity stages in bulk to meet out the demands in the market which results in postharvest loss of 40-60% [3]. The mangoes harvested at different maturity stages do not ripen uniformly and takes a longer time followed by high loss in weight. Therefore artificial ripening of mangoes are

practiced to get uniform ripening, to reduce the ripening time and has great importance in export of mango. Calcium carbide is used by traders and farmers which is banned due to their hazardous effect on human health. Mango is regarded as climacteric fruit where ethylene is produced which act as autocatalyst and declines after ripening [4]. Hence ethrel even at very low concentration (0.01 ppm) can initiate uniform ripening in mango. Ethylene releasing substances such as ethrel (2-Chloroethyl phosphonic acid, C₇H₆ClO₃P) when given at a concentration of 100 ppm is regarded as safe ripening agent by FSSAI and triggers the ripening process. Due to rise in endogenous ethylene level, respiration rate increases and physio-chemical changes such as increase in peel and pulp colour, sugars, carotenoids, fruit pH, volatile compounds; decrease in organic acids, fruit firmness, fruit weight occurs during ripening [5].

Mango fruits are commercially ripened at 20 – 22 °C & 90 – 95 % RH in ripening chamber for 24 – 48 hours. These chambers are high of cost and require infrastructure facility. As an alternate low cost ripening chambers [6] were designed but the

fruits ripened have poor keeping quality. Temperature during ethrel treatment have a remarkable impact on the quality and shelf life of fruits [7]. Fruits ripened at a temperature between 16-25 °C and 90-95% RH have good quality with increased volatile compounds and shelf life. Fruits ripened at ambient temperature have limited postharvest life and consumer acceptance, mottled skin and strong flavour [8]. Mango fruits treated with 150ppm of ethylene for 25 h at 23 ± 2°C resulted in uniform ripening without affecting flavour and taste [9]. Fruits ripened at 24 °C were superior in quality compared to fruits ripened at 18 °C [10]. Cold ripening chamber with controlled temperature is not affordable by small traders and farmers due to its increased cost. In this situation zero energy cool chamber where the temperature is 10-15 °C less than the outside temperature which works on the principle of evaporative cooling [11] can be very well used to get a temperature of 24 – 26 °C without electrical energy. In consideration of the aforementioned facts, this study was made to evaluate the Zero energy cool chamber along with cold chamber and silpaulin chamber to find an alternative to cold ripening chamber with increased fruit quality, shelf life, environment friendly and cost effectiveness at the Department of Fruit Science, Horticultural College and Research Institute, Coimbatore.

2. MATERIALS AND METHODS

Mango fruits cv. Alphonso were harvested from the College orchard, Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore using a mango harvester with a stem length of 1cm to avoid sap burn. The harvested fruits were washed in clean water and then subjected to hot water treatment at 52 °C combined with 0.1% Bavistin for 10 minutes to control the pest (fruit flies) and postharvest disease incidence in mango. The fruits were air dried completely and then proceeded for ethrel treatment.

2.1 Ethrel Treatment

The fruits were arranged in crates and kept at a height of 15 cm above the floor by means of bricks inside different ripening chambers having different temperatures. The study was conducted with three treatments and seven replications (Table 1). Silpaulin chamber supported by PVC pipe (framework) and was provided with velcro tape to make the chamber airtight. Zero energy cool chamber, a double walled chamber made of

bricks, filled with sand in between the two walls. Watering (100 l / day) was done daily for 5 days before treatment to maintain the temperature inside the chamber. Cold chamber equipped with forced air draft circulation system with a temperature range of 5 °C – 40 °C was used. The size of the chambers were as mentioned in Table 2.

Ethrel (2-chloroethyl phosphonic acid – 39% active ingredient) was used as a source of ethylene gas. Ethrel when reacted with NaOH (alkali), hydrolysed into ethylene gas [12]. The amount of ethrel and NaOH taken to release 100 ppm of ethrel was calculated for different ripening chambers of different size (Table 2). The volume of ethylene (cm³) was determined from the formula (Concentration (ppm)= (Weight (g) × 10⁶) / Volume (ml)) by taking concentration as 100 ppm, volume as size of the chamber (l × b × h). According to Kader [13], enough sodium hydroxide when added to 200 ml of ethrel releases 28000 cm³ (28000 ml) of ethylene gas. From this, ethrel needed for the required volume of ethylene gas was calculated. 2g of sodium hydroxide, 10 ml of 39 % ethrel, 5 litre of water were used for ripening of mango [14]. From this the amount of NaOH and water needed were calculated with the known volume of ethrel.

Table 1. Treatment details

Treatments	Details	Temperature (°C)
T ₁	Silpaulin chamber	33
T ₂	Zero energy cool chamber	26
T ₃	Cold chamber	22

2.2 Storage of Fruits

The fruits were subjected to ethrel treatment for a period of 24 hours in different ripening chambers after which they were stored in cardboard boxes cushioned with paper shreds and kept in ambient condition for ripening. The fruits were selected randomly from each treatment and subjected to periodical assessment of physio-chemical parameters on alternate days from zero day after treatment till the day of ripening.

2.3 Physiological Parameters

Green life: The number of days taken by more than 90% of the fruits to get ripened in a treatment is said to be the days taken to ripe. The fruits are observed daily to know the ripening pattern and to calculate the number of days taken to ripe.

Table 2. Amount of ethrel, NaOH and water required to release 100 ppm of ethrel gas in different ripening chambers

S.No	Chamber	Size of chamber (cm)	Ethrel (ml)	Water (ml)	NaOH (g)
1.	Silpaulin chamber	90 × 90 × 90	1.3	650	0.26
2.	Zero energy cool chamber	74 × 59 × 109	0.9	450	0.18
3.	Cold chamber	220 × 280 × 280	31.6	15,800	6.30

Shelf life is the number of days from harvesting of fruits till fruits start to rot [15]. It is inferred through visual appearance and expressed in days.

The physiological loss in weight was computed using the given formula and expressed in terms of percentage [16].

$$\text{Physiological loss in weight (\%)} = \frac{I-F}{F} \times 100$$

I = Initial weight of fruits (gram), F = Final weight of fruits (gram).

Fruit firmness was measured using digital fruit hardness tester (Model: FHT 1122) in three different locations (Proximal, Distal, Middle) of the samples and averaged. The values were recorded and expressed in terms of Newton (N) [17].

2.4 Quality Parameters

Total soluble solids (° Brix) was determined using hand held refractometer with a range of 0-32. The juice was extracted by macerating the pulp in pestle and mortar. Using cotton the juice was squeezed and two drops were placed on the prism and the lid was closed. The values were recorded and expressed as (°Brix) [18]. Titratable acidity (%) was determined as suggested by Ranganna [19] using titration method and values were expressed in percentage. To 5g of fruit sample 30ml of distilled water was added and 5ml of filtrate was taken. Two drops of phenolphthalein indicator was added and titrated against 0.1 N NaOH till pink colour appears and calculated using the formula,

$$\text{Titrateable acidity (\%)} = \frac{(\text{Titre value} \times \text{Normality of alkali} \times \text{Volume made upto} \times \text{Equivalent weight of the acid} \times 100)}{(\text{Volume of sample taken} \times \text{weight of the sample taken} \times 1000)}$$

TSS: acid ratio was determined as ratio between the TSS (° Brix) and Titratable acidity (%) and expressed as value. Organoleptic evaluation was carried out for sensory attributes such as

appearance, colour, flavour, taste and overall acceptability by using a 9-point hedonic scale [20]. This experiment was laid out in Completely randomized design (CRD). The data collected were statistically analysed by computing ANOVA at 5% level of significance using R studio software version 4.3.1 with suitable packages.

3. RESULTS AND DISCUSSION

The various physio-chemical parameters of alphonso fruits subjected to ethrel treatment in different ripening chambers with different temperatures were analysed on alternate days from zero day after treatment till ripening of fruits (0th, 2nd, 4th, 6th, 8th, 10th) and the results are discussed.

3.1 Physiological Parameters

Fruits subjected to ethrel treatment ripe early than untreated fruits [21]. Number of days taken for ripening (Green life) of alphonso mangoes were less for fruits subjected to ethrel treatment in silpaulin chamber (5.9 days) followed by zero energy cool chamber (8 days) and cold chamber (10 days) (Table 3). At high temperature during ethylene treatment, the firmness and acidity tends to decline at a higher rate leading to fast ripening of fruits. The minimum number of days taken to ripe fruits subjected to ethrel treatment in silpaulin chamber might be due to increase in the enzyme activity which catalyses starch hydrolysis and pectin degradation compared to fruits subjected to ethrel treatment in zero energy cool chamber and cold chamber as enzyme activity is positively correlated with temperature [22].

Abdualrahman [23] reported that fruits with low moisture content have increased shelf life. Moisture content on ripening and shelf life of the fruits were found to be negatively correlated with a correlation coefficient of -0.9817 (Fig. 1a). The shelf life (17 days) was found to be significantly higher in the fruits subjected to ethrel treatment in cold chamber (T₃) followed by zero energy cool chamber (15 days). The minimum shelf life was found in fruits subjected to ethrel treatment

in silpaulin chamber (10 days) (Table 3). Slow rate of fruit ripening might have increased the shelf life of fruits subjected to ethrel treatment in cold chamber [24]. The disease severity was also found to be less in fruits subjected to ethrel treatment in cold chamber and zero energy cool chamber than in silpaulin chamber which increased the shelf life of fruits. Fruit firmness was found to be positively correlated with shelf life [25] and hence the fruits subjected to ethrel treatment in cold chamber and zero energy cool chamber was found to have high shelf life.

Table 3. Effect of temperature during ethrel treatment in different ripening chambers on green life and shelf life of mango cv. alphonso

Treatment	Green life (days)	Shelf life (days)
T ₁	6 ^c	10 ^c
T ₂	8 ^b	15 ^b
T ₃	10 ^a	17.1 ^a
SE d	0.42	0.42
CD (p=0.05)	0.87	0.87

**Each value is the mean of seven replications
Within a column means having different letters indicate significance difference at p=0.05 according to LSD test*

Physiological loss in weight was found to increase during ripening in fruits subjected to ethrel treatment which might be due to increase in transpiration and respiration process. Similar results were also obtained by Venkatram and Pandiarajan [26]. The maximum weight loss was observed in fruits subjected to ethrel treatment in silpaulin chamber (8.37%) from the day of

treatment till the day of ripening (6th day). The minimum weight loss was observed on fruits subjected to ethrel treatment in cold chamber (6.56 %) which was also on par with fruits treated in zero energy cool chamber (6.71 %) (Table 4). The elevated physiological loss in weight recorded in silpaulin chamber was due to higher temperature during ethrel treatment that resulted in increased evapo-transpiration [27]. The rate of increase of PLW was lowest in fruits subjected to ethrel treatment in cold chamber followed by zero energy cool chamber due to slow ripening and removal of field heat [28].

Firmness is one of the characteristic change that occurs during the process of ripening leading to the development of soft edible fruits [29]. Firmness decreased with the progression of ripening in fruits subjected to ethrel treatment due to breakdown of insoluble protopectin into soluble pectic substances resulting in dissolution of middle lamella which are catalysed by enzymes like polygalacturonase and cellulase [30]. Fruits subjected to ethrel treatment in cold chamber recorded the highest firmness of 2.35 N on the day of ripening followed by zero energy cool chamber (2.21 N). The firmness was significantly lowest (1.88 N) in fruits treated in silpaulin chamber (Table 4). Positive correlation was found between Titratable acidity and fruit firmness [31] and the correlation coefficient between acidity and firmness on the day of ripening was found to be 0.9518 (Fig. 1b). Maximum loss of firmness observed in fruits subjected to ethrel treatment in silpaulin chamber might be due to increase in the enzymatic activity with increase in temperature.

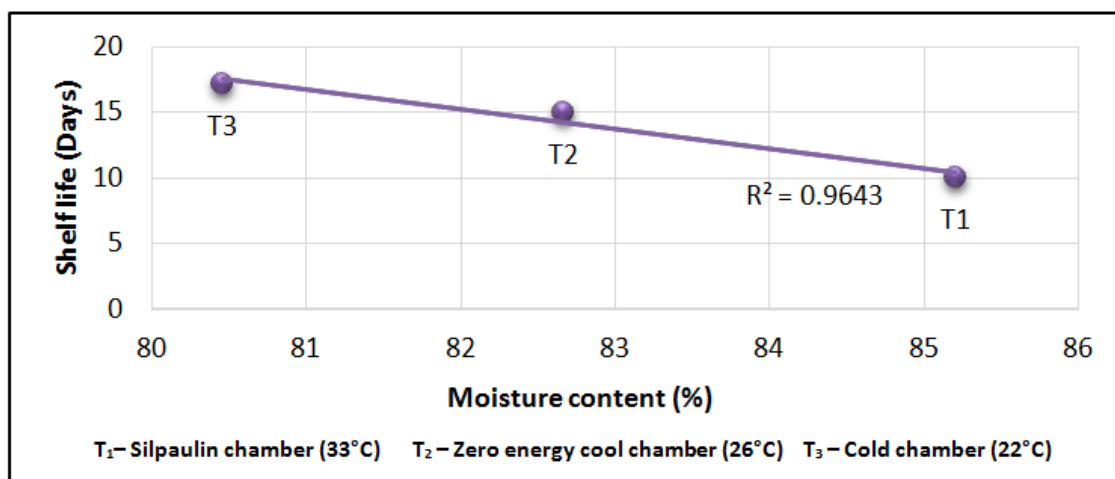


Fig. 1a. Effect of temperature during ethrel treatment in different ripening chambers on correlation between moisture content (%) on ripening and shelf life (days) of mango cv. alphonso

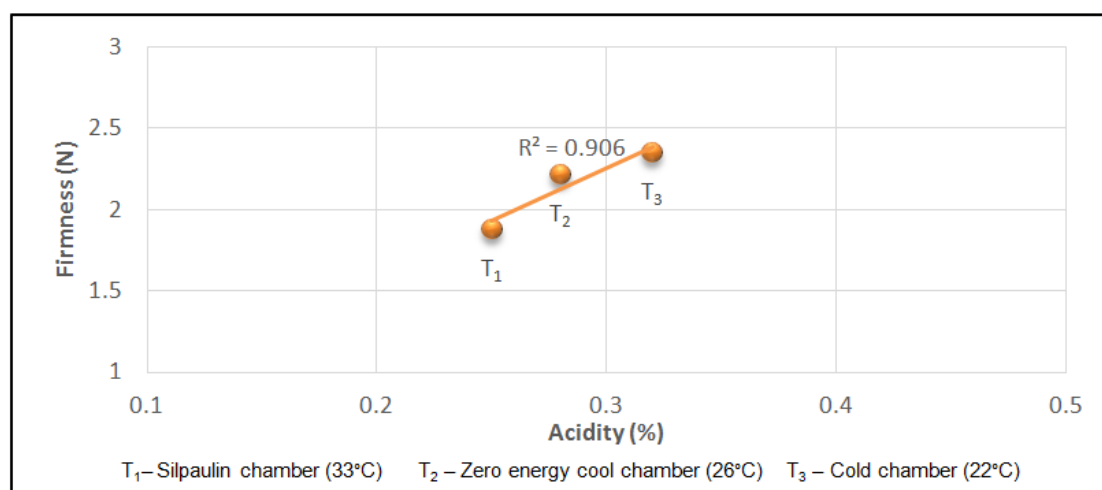


Fig. 1b. Effect of temperature during ethrel treatment in different ripening chambers on correlation between acidity (%) and firmness (N) on ripening of mango cv. alphonso

Table 4. Effect of temperature during ethrel treatment in different ripening chambers on physiological loss in weight (%) and Firmness (N) of mango cv. alphonso

Treatments	Physiological loss in weight (%)					Firmness (N)					
	2 nd day	4 th day	6 th day	8 th day	10 th day	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day
T ₁	1.49 ^a	3.81 ^a	8.37 ^a	10.76 ^a	NA#	14.53 ^b	10.43 ^c	3.21 ^c	1.88 ^c	1.31 ^c	NA#
T ₂	1.06 ^b	2.22 ^b	3.95 ^b	6.71 ^b	8.77	16.92 ^a	12.11 ^b	9.56 ^b	4.13 ^b	2.21 ^b	1.99 ^b
T ₃	0.91 ^c	2.12 ^b	3.08 ^c	4.47 ^c	6.56	17.14 ^a	15.23 ^a	10.4 ^a	7.52 ^a	4.35 ^a	2.35 ^a
SE d	0.06	0.11	0.11	0.15	0.15	0.38	0.21	0.19	0.11	0.14	0.14
CD (p=0.05)	0.12	0.22	0.23	0.32	0.32	0.81	0.44	0.40	0.23	0.29	0.30

NA# - fruits were deteriorated; *Each value is the mean of seven replications

Within a column means having different letters indicate significant difference at p=0.05 according to LSD test

3.2 Quality Parameters

Total soluble solids showed increasing trend during the ripening days which might be due to the postharvest treatment with ethrel which induced fruit ripening [32] and hydrolysis of starch into soluble sugars like glucose and sucrose [33]. Similar findings were reported by Roy et al. [34]. The total soluble solids were found to be significantly higher in fruits subjected to ethrel treatment in silpaulin chamber (22 °Brix) followed by zero energy cool chamber (T₂) (21.2 °Brix) on ripening. The fruits subjected to ethrel treatment in cold chamber recorded lowest TSS value of 20.07 °Brix and were also on par with T₂ (zero energy cool chamber) on ripening (Table 5, Fig. 2a). TSS increased with increase in temperature as the enzyme activity (Total amylase) was positively correlated with temperature [35] and the rate of conversion was found to be dependent on temperature.

Citric acid is the predominantly found organic acid in mango. Acidity has been considered as

one of the important quality parameters and tends to decline linearly by postharvest treatment of ethrel during ripening [36]. This might be due to the utilization of organic acids as substrates in respiration pathways like glycolysis and tricarboxylic acid (TCA) [37]. Ethrel was also found to enhance the conversion of organic acids to sugars [38]. Titratable acidity was found to be lowest in fruits subjected to ethrel treatment in silpaulin chamber (0.25 %) followed by zero energy cool chamber (0.28 %) on the day of ripening. Fruits subjected to ethrel treatment in cold chamber recorded significantly higher titratable acidity of 0.32 % on the day of ripening (Table 5, Fig. 2b). Similar results were reported by Gill et al. [39] in mango. The rate of conversion of acidity was statistically higher in silpaulin chamber compared to zero energy cool chamber and cold chamber as conversion rate increased at high temperatures and gradually decreased at low temperatures [31].

TSS: acid ratio is an indicator of quality fruits. Fruits with high TSS: acid ratio have high quality

with characteristic flavour [40]. TSS: acid ratio increased with progression of ripening. Similar results were reported by Bakshi et al. [41]. Fruits subjected to ethrel treatment in silpaulin chamber recorded the maximum TSS : acid ratio of 88 followed by fruits subjected to ethrel treatment in zero energy cool chamber (75) whereas the fruits subjected to ethrel treatment in cold chamber recorded the minimum TSS: acid ratio of 62.72 on the day of ripening (Table 6, Fig. 2c).

Organoleptic evaluation was carried out on the day of fruit ripening. The overall acceptability was significantly higher in fruits subjected to ethrel

treatment in zero energy cool chamber (T_2) i.e. 8.3 and were on par (8.1) with fruits subjected to ethrel treatment in cold chamber (T_1). The minimum overall acceptability of 7.4 was recorded in fruits subjected to ethrel treatment in silpaulin chamber (Table 7, Fig. 2d). The flavour, colour and appearance of peel was found to be statistically higher in fruits subjected to ethrel treatment in zero energy cool chamber. Similar findings were reported by Gill et al. [31] in mango. Fruits subjected to ethrel treatment in high temperature were good in taste which also supports the findings of Ahmad et al. [42] in banana.

Table 5. Effect of temperature during ethrel treatment in different ripening chambers on TSS ($^{\circ}$ Brix) and titratable acidity (%) of mango cv. alphonso

Treatments	Total soluble solids ($^{\circ}$ Brix)						Titratable acidity (%)					
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day
T_1	12.51 ^a	15.71 ^a	18.80 ^a	22 ^a	25.37 ^a	NA#	1.34 ^c	1.09 ^c	0.73 ^c	0.25 ^c	0.16 ^c	NA#
T_2	10.43 ^b	12.81 ^b	15.63 ^b	18.81 ^b	21.21 ^b	23.11 ^a	1.85 ^b	1.66 ^b	1.35 ^b	0.91 ^b	0.28 ^b	0.21 ^b
T_3	9.62 ^c	11.51 ^c	14.21 ^c	16.7 ^c	18.6 ^c	20.07 ^b	2.02 ^a	1.86 ^a	1.60 ^a	1.27 ^a	0.81 ^a	0.32 ^a
SE d	0.29	0.19	0.20	0.41	0.83	0.87	0.036	0.033	0.020	0.022	0.024	0.02
CD ($p=0.05$)	0.61	0.41	0.42	0.86	1.73	1.90	0.074	0.071	0.040	0.045	0.052	0.04

NA# - fruits were deteriorated; *Each value is the mean of seven replications

Within a column means having different letters indicate significant difference at $p=0.05$ according to LSD test

Table 6. Effect of temperature during ethrel treatment in different ripening chambers on TSS: acid of mango cv. alphonso

Treatments	TSS: Acid					
	0 th day	2 nd day	4 th day	6 th day	8 th day	10 th day
T_1	9.33 (0.97 ^a)	14.42 (1.16 ^a)	25.75 (1.41 ^a)	88.00 (1.94 ^a)	153.1 (2.21 ^a)	NA#
T_2	5.61 (0.74 ^b)	7.72 (0.89 ^b)	11.58 (1.07 ^b)	20.68 (1.31 ^b)	75.00(1.88 ^b)	112.4 (2.05 ^a)
T_3	4.75 (0.68 ^c)	6.20 (0.80 ^c)	8.88 (0.94 ^c)	13.22 (1.12 ^c)	22.98 (1.37 ^c)	62.72 (1.80 ^b)
SE d	0.01	0.017	0.01	0.024	0.056	0.038
CD ($p=0.05$)	0.022	0.029	0.020	0.047	0.116	0.079

NA# - fruits were deteriorated; *Each value is the mean of seven replications

Within a column means having different letters indicate significant difference at $p=0.05$ according to LSD test

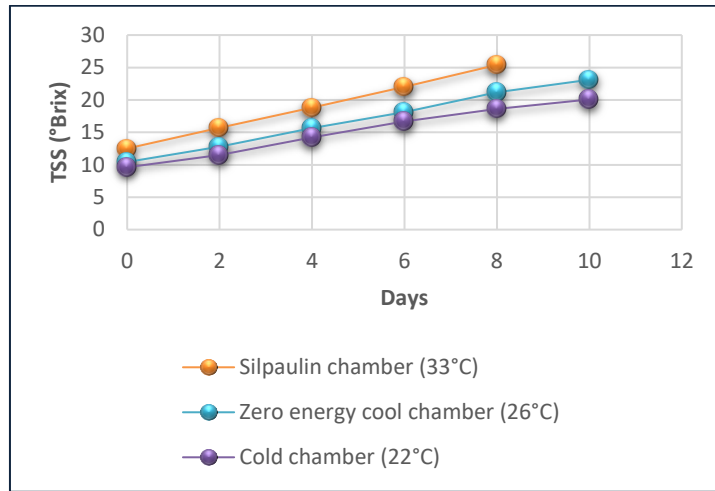
Log-transformed values are enclosed in parentheses

Table 7. Effect of temperature during ethrel treatment in different ripening chambers on organoleptic evaluation (hedonic scale) of mango cv. alphonso

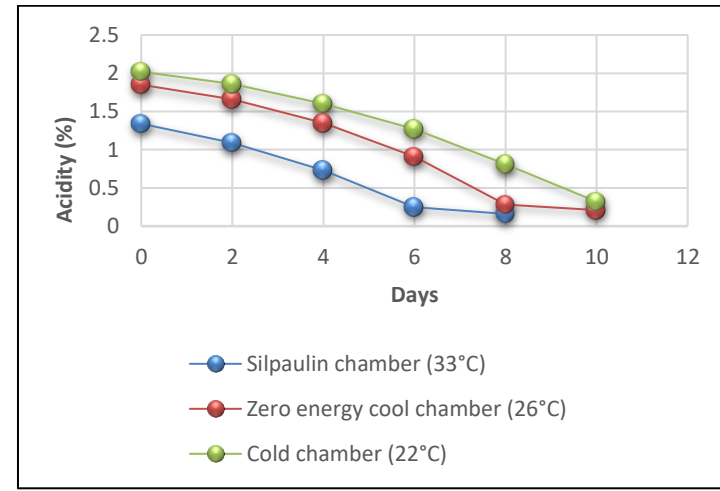
Treatments	Organoleptic evaluation (hedonic scale)					
	Colour and appearance (peel)	Colour and appearance (pulp)	texture	taste	flavour	Overall acceptability
T_1	7.0 ^b	8.4 ^a	7.0 ^b	8.6 ^a	7.6 ^b	7.3 ^b
T_2	8.6 ^a	8.1 ^a	8.3 ^a	8.3 ^a	8.6 ^a	8.3 ^a
T_3	9.0 ^a	7.4 ^b	8.9 ^a	7.6 ^b	8.4 ^a	8.1 ^a
SE d	0.24	0.31	0.36	0.32	0.28	0.39
CD (0.05)	0.51	0.66	0.76	0.69	0.60	0.82

*Each value is the mean of seven replication

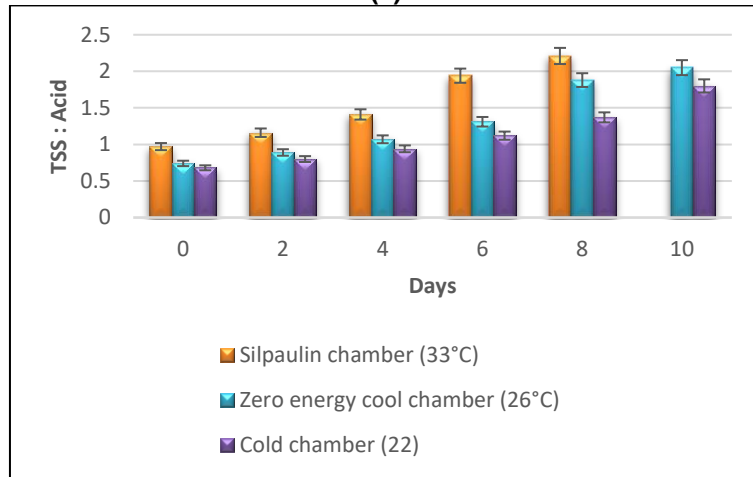
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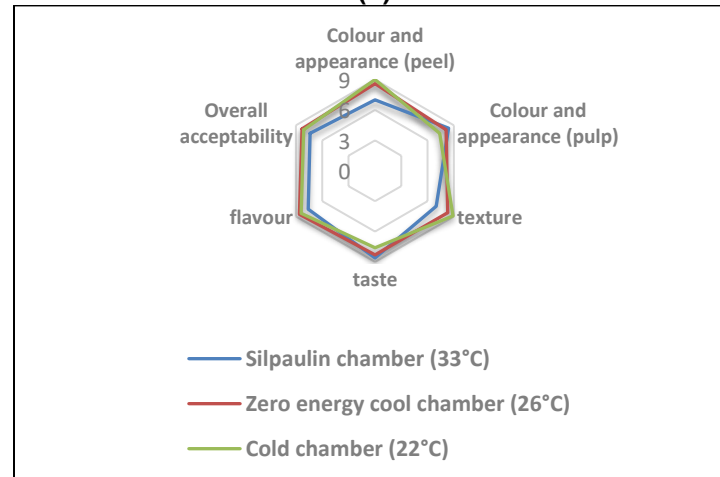
(a)



(b)



(c)



(d)

Fig. 2. Effect of temperature during ethrel treatment in different ripening chambers on quality parameters i.e, total soluble solids (a), titratable acidity (b), TSS: Acid (c) and sensory evaluation (d) of mango cv. alphonso

4. CONCLUSION

From the current investigation, it could be concluded that the temperature prevailing during ethrel treatment plays a vital role in ripening of mango cv. Alphonso. Increase in TSS and TSS: acid ratio was observed in fruits subjected to ethrel treatment in high temperature (Silpaulin chamber – T₁) where in the fruits were poor in shelf life, firmness and sensory qualities. The fruits subjected to ethrel treatment in low temperature (cold chamber – T₃) exhibited superior quality on ripening with extended shelf life. The results were also on par with fruits subjected to ethrel treatment in zero energy cool chamber (T₂). Besides the fruits were less in acidity and high in TSS, sugar- acid ratio in T₂ (zero energy cool chamber) compared to T₃ (cold chamber). Thus zero energy cool chamber can be used as an alternate to cold chamber for ethrel treatment to ripen mango fruits. Additionally, it is more affordable, environment friendly and needs less upkeep than the cold chamber, hence it can be recommended for use by small traders and farmers. The zero energy cool chamber can also be used for postharvest storage of fruits to increase their shelf life when not used for ripening of mangoes.

ACKNOWLEDGEMENT

I am eternally grateful to Tamil Nadu Agricultural University, Coimbatore for providing all facilities during the entire research work. A special mention to Department of Fruit science for providing financial and technical support throughout the research period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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