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Effect of quarantine and fungicidal hot water treatments on quality of mangoes cv. Banganapalli during low temperature storage at 13 °C

Anusree Anand, DV Sudhakar Rao and Deep Lata

Abstract
Quarantine hot water treatment (HWT) is mandatory for the export of mangoes from India to some foreign countries. However, effect of such treatments on the quality of mango during storage at low temperature for long duration has to be studied. In the present investigation, Banganapalli mangoes, which is one of the excellent export varieties in India, were subjected to quarantine HWT (48 °C for 60 min), fungicidal HWT (55 °C for 10 min) and their combination (48 °C for 60 min followed by 55 °C for 10 min) and then stored at 13 °C for 4 weeks. Non-treated fruits were considered as control. At the end of storage, respiration rate and ethylene production rate was higher in the combination HWT than other treatments. This treatment also showed a decreased TSS and ascorbic acid content. Combination of two HWTs resulted in heat injury in those fruits. Quarantine HWT or fungicidal HWT alone maintained quality attributes like firmness, TSS, titratable acidity, ascorbic acid content, total sugars and carotenoid content. Total Phenolics and antioxidant activity were significantly higher in hot water treated fruits than that of control during 4 weeks storage at 13 °C.

Keywords: Quarantine, mango, hot water treatment, quality, cold storage

1. Introduction
India cultivates mango (*Mangifera indica*) on its largest area of 2.31 million hectares and Producing 20.89 million metric tonnes annually [1]. Mango is grown in almost all states of India, where Uttar Pradesh, Andhra Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu are the leading producers.

Mango, being climacteric fruit, ripens after harvest and the shelf life varies between 4 to 8 days at ambient temperature and 2-3 weeks at cold storage of 13 °C, depending upon the varieties [2]. During ripening process, mango reaches its respiration peak on third or fourth day after harvesting, leading to its short shelf life [3]. This short life of mango limits its long distance commercial shipment, both national and international.

Although India has more than 1000 varieties of mangoes, only 3% of these are having prevalence in the trade and export. Lately, there has been increasing popularity and demand for better quality Indian mangoes, even in the international markets. The major export cultivars in India are Alphonso, Banganapalli, Kesar, Dashehari and Totapuri [4]. Banganapalli is grown majorly in Andhra Pradesh, in a town called Banganapalli, from where it derived its name. It is large in size (400g-1kg) with peculiar obliquely oval shape, golden yellow colour when ripe and good for canning [5].

For the commercial export of mangoes from India to countries like USA, Australia, Japan, UK, New Zealand, *etc.*, quarantine measures like irradiation, VHT and hot water treatments (HWT) are mandatory. HWT, being one among these, may affect the shelf life and quality of mangoes when stored/transported under different conditions. Hence there is a need to standardize appropriate storage protocols that help to prolong the storability of mangoes that were subjected to different hot water treatments in order to ease domestic transportation as well as to boost Indian mango export.

2. Materials and Methods
Mango fruits (*Mangifera indica* L. cv. Banganapalli) for the study were procured from the experimental mango orchards of ICAR-IIHR during 2019-20. Fruits, harvested manually were brought to the postharvest laboratory of ICAR-IIHR, where, uniformly matured (mature green) fruits, free from any form of mechanical damages, injury, blemishes or disease were selected

after visual sorting. Then the fruits were thoroughly washed to clean the latex and other dust particles.

2.1 Hot water treatment (HWT)

ICAR-IIHR has designed and developed a rectangular batch type hot water treatment plant with a capacity of 500 kg/batch. It contains a 302 grade stainless steel fabricated water bath of dimensions 2.4x1.2x1.0 m. Spread type 8 electrical heating coils, each of 3 kW capacity, are used to heat water and the hot water is circulated using a 0.5 hp water pump in order to maintain uniform temperature throughout the water bath. Experiments were conducted in this hot water plant with the following temperature and time combinations:

1. 48 °C for 60 min (recommended quarantine HWT for control of fruit fly)
2. 55 °C for 10 min (recommended HWT for control of anthracnose disease)
3. 48 °C for 60 min followed by 55 °C for 10 min (combination HWT)

The temperature was kept 2 °C higher than the proposed temperatures initially, in order to avoid any temperature drop after immersion of fruits. Freshly harvested mature green mango fruits were immersed in the hot water bath after filling in plastic crates of 20 kg capacity. For the combination treatment, fruits were first immersed at 48 °C for 60 min and then taken out, kept in room temperature for about 30-40 min till the water bath reached the temperature of 55 °C.

2.2 Storage

After subjecting to hot water treatments, fruits along with a set of control were stored at 13 ºC ± 1 ºC with relative humidity of 85-90% (walk-in cold room). The fruits were stored after weighing and packing in CFB boxes in 3 replications and 4 kg per replication as sample size. Sampling of fruits for various physical, physiological and biochemical attributes was done at different intervals.

2.3 Respiration and ethylene production rate

Respiration and ethylene production rate of treated and untreated fruits were measured by enclosing them in hermetic containers of known volume and specific time duration. Five fruits from individual treatments were kept as replications. CO_2 gas concentration and ethylene gas at head space was recorded using gas analyzer (Checkmate O_2/CO_2 analyzer, Densensor, Denmark) and ethylene analyzer (Bioconservation, *ppm* ethylene) respectively, by piercing the probe of analyzers into the container through a septum (Silicon-Teflon septum) fixed on its lid. The respiration and ethylene production rate were calculated using formula and expressed as mg CO_2/kg/h and μl C_2H_4/kg/h, respectively [4].

2.4 Physiological loss in weight and fruit firmness

Individual fruits' weight in all treatments (15-30 numbered fruits) were obtained in electronic balance (Model: Sartoris, BSA 320 2S d=0.01g) and cumulative losses in weight were calculated as the percentages of initial weights. Fruit firmness was determined using Instron-Universal testing machine (Model 4201, USA). A probe of 8 mm diameter was used to puncture the fruit surface and the force (expressed in terms of kg/cm^2) with which the fruit pierced was recorded.

2.5 Quality analysis

Five fruits were selected at random from each treatment after cold storage and shifting to room temperature for quality attributes analysis. Mesocarp was extracted and cut into small pieces and stored at -21 °C until different biochemical analysis. The samples were taken out, thawed, grinded in a mixer grinder and then homogenized (specific quantity required for individual parameters) using IKA T25 digital Ultra Turrax® homogenizer before analysis. TSS was measured using hand refractometer calibrated to 25 °C (Erma Inc., Tokyo, Japan). Parameters like acidity (%), ascorbic acid (mg/100 g) and sugars (%) were estimated using standard methods of analysis [6]. 5 g of mango pulp was grinded in pestle and mortar using a solution of petroleum ether and acetone (3:2) along with acid washed sand to extract the carotenoid pigments. The OD values were read in spectrophotometer (T80+ UV/VIS spectrophotometer, PG instruments Ltd. UK) at 452 nm using petroleum ether-acetone solution as blank. Total carotenoid content was then calculated with reference to the standard curve prepared with β-carotene and expressed as μg/100 g pulp. The total phenols content was estimated and expressed as milligram of gallic acid equivalent per 100 g of fresh weight (mg GAE/100 g FW) [7]. Total antioxidants were estimated using FRAP (ferric reducing antioxidant power) assay, and expressed as mg acetic acid equivalent (AAE)/100g FW [8].

2.6 Statistical analysis: The effects of different treatments over the variables were evaluated by two-way analysis of variance at 5% of statistical significance based on a completely randomized design. Software WINDOSTAT 9.3 version was employed to analyze the analysis of variance at 5% significance level and statistical significance of differences between the means ($p \leq 0.05$).

3. Results and Discussion

Respiration rate and ethylene production rate of Banganapalli mangoes were measured up to 4 weeks. At the end of the storage, the combination HWT showed highest respiration as well as ethylene production rate (Fig 1 (a) and (b)). This can be attributed to the increased heat injury in the fruits due to long duration of heat exposure compared to other treatments. Any wounding on the plant cells and tissues causes an increase in respiration rate. Wounding stimulates respiration, ethylene production rate, senescence and ripening of fruits and vegetables. "The respiration rate may gradually increase over time until a maximum value is reached and then decrease to the matter before the wounding or to a higher value" [9]. Damage to cells due to diseases or low temperature breakdown may lead to a wounding response of high ethylene production through stimulation of enzymes in the ethylene biosynthesis pathway [10, 11].

e wounding may be due to mechanical damage or cutting of the product. Wounding plant cells and tissues causes the respiration rate to increase. Wounding stimulates ethylene production rate, respiration, deterioration, senescence and ripening of fruits and vegetables. The respiration rate may gradually increase over time until a maximum value is reached and then decrease to the matter before the wounding or to a higher value. The respiratory rate of apple slices was about 2–3 times that of the whole fruit (Lakakul *et al*., 1999)

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The physiological loss in weight (PLW) of Banganapalli fruits stored at 13 °C was recorded for 4 weeks (Fig 2). PLW showed a gradual increase in all the treatments along with the storage duration. Highest PLW was recorded in the control fruits and the fruits of combination HWT. Fruits that were subjected to HWT showed less weight loss than that of non-treated ones and hence has maintained the marketability of mango fruits [12]. As HWTs reduced the incidence of anthracnose disease and fruit fly attack effectively, weight loss due to such factors were controlled in quarantine HWT and fungicidal HWT. The mango fruits of Kesar subjected to hot water dip treatment at 48 °C for 60 min maintained lower percentage of physiological loss in weight when stored at 13 °C [13]. A temperature higher than 45 °C kills fruit fly eggs and larvae in mangoes [14]. Post harvest hot water dip of mango fruits for 10-15 minutes at about 49–55 °C reduce the development of anthracnose during storage [15]. Mango anthracnose disease incidence was significantly ($p < 0.001$) reduced by hot water treatment as that of control [16]. The time-temperature couple varies according to varieties.

Fruit firmness was recorded after three and four weeks of continuous storage at 13 °C and also recorded after 4 days of shifting to room temperature following 3 weeks of storage at 13 °C (Table 1). Firmness was significantly higher for the hot water treated mango fruits compared to control. Similar reports where, firmness was maintained in hot water treated fruits, were recorded by in mangoes [17], banana [18] and papaya fruits [19]. This could be due to inactivation of texture degrading enzymes. A higher firmness value was recorded in heat stressed strawberry fruits, which was also correlated to the higher cellulose, hemicellulose, neutral sugars and bounded pectins. Genes responsible for pectin disassembly and fruit softening (*FaPG1*, *FaPLB*, *FaPLC*, *FaAra1*, *FaβGal4*) were down-regulated and genes relevant for cell wall reinforcement (*FaPME1* and *FaXTH1*) was up-regulated by heat treatment [20].

Among different HWTs, combination HWT showed lesser TSS, total sugars and TA (table 1 and table 2), while these were maintained in other treatments. Higher hot water temperature might have resulted in an increased ripening which was absent in control fruits. Sugar production in ripening fruits is due to the hydrolysis of starch granules in chloroplast. In combination HWT, there was long duration exposure to higher temperatures which have led to heat injury and also might have hindered the normal metabolic process of starch hydrolysis [21]. TA was decreased during storage and ripening due to increase in respiration and ethylene production. In general, quarantine or fungicidal HWTs alone did not have any negative effect on TA, whereas combination HWT recorded the lowest TA.

Ascorbic acid content was slightly higher in the HW treated fruits than that of control, where, fungicidal HWT being the premier (Table 2). Different studies reported that heat treatments did not affect ascorbic acid content of fruits but slightly maintained compared to control [22, 23]. Also, in Zucchini fruit, hot water dipping and hot water forced convection treatments increased and maintained ascorbic acid content during storage [24]. HWTs did not hinder the ripening process after shifting to ambient temperature and fruits ripened normally. The result is in-line with the study of [25], where hot water treated Sindhri mangoes enhanced the ripening process and had no adverse effect on shelf life.

The total carotenoid content was significantly higher in hot water treated fruits than that of control at the end of storage (Table 2). Application of HWT increased pulp carotenoid contents in Sammar Bahit Chaunsa and Sufaid Chaunsa mango varieties [26]. Similarly, heat treatments significantly maintained carotenoid content in minimally processed Keit mangoes [22] and increased in Sufed Chausa mangoes during storage [23]. Total phenols and antioxidants were significantly higher in the hot water treated fruits than that of non-treated ones (Table 3). Hot air exposure and HWT suppressed reactive oxygen species (ROS) and increased antioxidant capacity of Peach cv. Xiahui [27]. Sammar Bahisht Chaunsa mangoes showed slightly higher total antioxidants and a significantly higher phenolic contents (irrespective of cultivars) after hot water treatment than that of control [26]. Similarly, hot water dipping significantly increased total phenolics and antioxidants capacity of Satsuma mandarin immediately after treatment and maintained during storage [28]. Phenolic contents during low temperature storage of cucumber was enhanced by short hot water dip [29].

Control fruits recorded significantly less (mean value) firmness, total sugars, ascorbic acid, carotenoid content (Table 2), total phenols and antioxidant capacity (Table 3) at the end of the storage, than that of hot water treated fruits. This indicates that, hot water treatment maintained fruit firmness throughout the storage period and has also triggered some ripening process in the fruits which was absent in control fruits. Though low temperature retards ripening related changes [30], hot water treatment maintained the ripening quality after 28 days of storage. Quarantine HWT and fungicidal HWT did not affect the quality of mangoes during and after the storage. When the fruits were shifted to room temperature after three weeks storage at 13 °C, the fruits showed optimum firmness and titratable acidity, better TSS, higher total sugar content, ascorbic acid and total carotenoids than other combinations (table 1 and table 2) indicating proper ripening.

Table 1: Effect of different hot water treatments on firmness (kg/cm^2), TSS (°B) and total sugars (%) during different storage durations at 13 °C

Treatments	Firmness (kg/cm^2)			Mean(T)	TSS (°B)			Mean(T)	Total sugars (%)			Mean(T)
	3weeks	3w+4d	4weeks		3weeks	3w+4d	4weeks		3weeks	3w+4d	4weeks	
T_1	10.84	5.38	3.33	6.51	21.00	21.47	21.13	21.20	14.59	16.22	13.20	14.67
T_2	5.31	3.67	4.93	4.64	20.47	19.47	20.27	20.07	14.35	16.48	13.23	14.69
T_3	4.95	5.93	5.13	5.34	16.07	18.63	19.20	17.97	14.36	16.61	12.57	14.51
T_4	3.60	2.70	2.66	2.99	19.03	21.33	20.73	20.37	13.46	15.08	10.37	12.97
Mean (D)	4.94	3.54	3.21		19.14	20.23	20.33		14.19	16.10	12.34	

	T	D	TxD		T	D	TxD		T	D	TxD	
F test	**	**	**		**	**	**		**	**	**	
S.Em ±	0.29	0.37	0.64		0.27	0.32	0.55		0.13	0.15	0.26	
CD (5%)	0.83	1.08	1.85		0.80	0.92	1.59		0.38	0.44	0.75	

T_1: 48 °C for 60 min 3w+4d: 3 weeks at 13 °C and 4 days at room temperature
T_2: 55 °C for 10 min
T_3: 48 °C for 60 min + 55 °C for 10 min
T_4: Control (without treatment)

Table 2: Effect of different hot water treatments on treatable acidity (%), ascorbic acid (mg/100g) and total carotenoids (μg/100g) during different storage durations at 13 °C

Treatments	Treatable acidity (%)			Mean(T)	Ascorbic acid (mg/100g)			Mean(T)	Total carotenoids(μg/100g)			Mean(T)
	3weeks	3w+4d	4weeks		3weeks	3w+4d	4weeks		3weeks	3w+4d	4weeks	
T_1	0.92	0.64	0.75	0.77	5.67	11.40	4.20	7.09	439.7	993.3	549.6	660.8
T_2	0.77	0.68	0.77	0.74	8.53	10.58	4.80	7.97	453.3	918.6	543.4	638.4
T_3	0.70	0.64	0.64	0.66	6.23	10.60	5.87	7.57	460.3	910.3	571.8	647.5
T_4	0.92	0.70	0.71	0.78	4.53	8.47	4.80	5.93	519.4	658.7	547.7	575.3
Mean (D)	0.83	0.67	0.72		6.24	10.26	4.92		468.2	870.2	553.1	
	T	D	TxD		T	D	TxD		T	D	TxD	
F test	**	**	**		**	**	**		**	**	**	
S.Em ±	0.02	0.02	0.04		0.14	0.17	0.29		14.68	16.95	29.37	
CD (5%)	0.05	0.06	0.11		0.42	0.48	0.84		42.86	49.49	85.71	

T_1: 48 °C for 60 min 3w+4d: 3 weeks at 13 °C and 4 days at room temperature
T_2: 55 °C for 10 min
T_3: 48 °C for 60 min + 55 °C for 10 min
T_4: Control (without treatment)

Table 3: Effect of different hot water treatments on total phenols (mg GAE/100g) and antioxidants (mg AAE/100g) firmness, TSS and total sugars during different storage durations at 13°C

Treatments	Total phenols (mg GAE/100g)			Mean(T)	Total antioxidants (mg AAE/100g)			Mean(T)
	3weeks	3w+4d	4weeks		3weeks	3w+4d	4weeks	
T_1	44.55	41.74	40.12	42.14	22.22	25.29	21.40	22.97
T_2	41.43	40.94	39.79	40.72	21.33	24.01	21.25	22.19
T_3	42.38	39.80	39.64	40.61	21.80	23.38	22.02	22.40
T_4	39.29	37.44	38.14	38.29	21.25	22.87	20.84	21.65
Mean (D)	41.91	39.98	39.42		21.65	23.89	21.38	
	T	D	TxD		T	D	TxD	
F test	**	**	**		**	**	**	
S.Em ±	0.26	0.30	0.52		0.14	0.17	0.29	
CD (5%)	0.75	0.87	1.51		0.42	0.49	0.84	

T_1: 48 °C for 60 min
T_2: 55 °C for 10 min
T_3: 48 °C for 60 min + 55 °C for 10 min 3w+4d: 3 weeks at 13 °C and 4 days at room temperature
T_4: Control (without treatment)

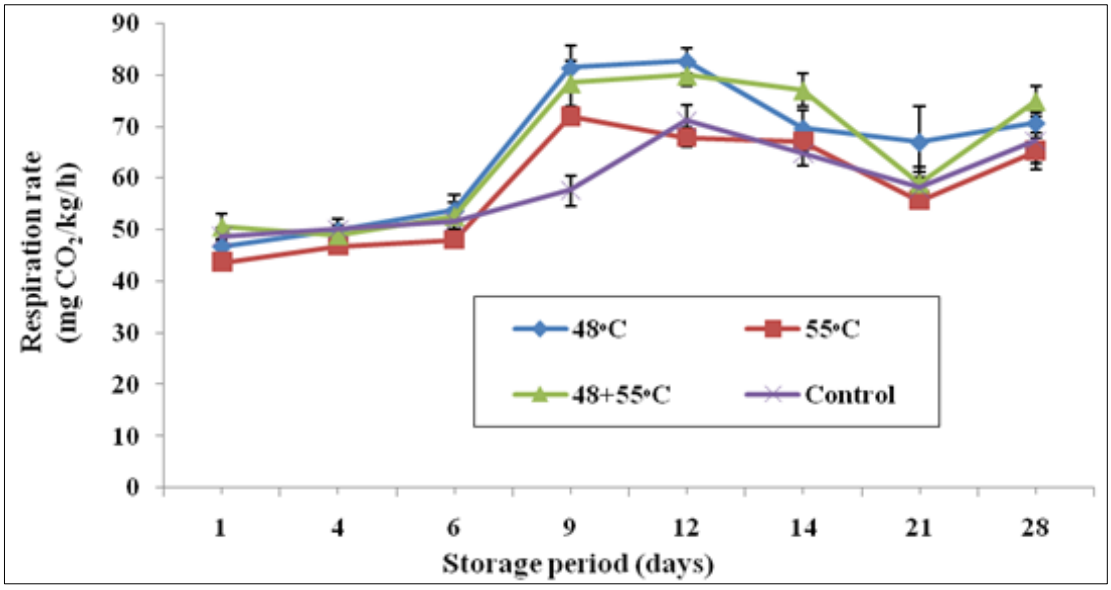

Fig 1 (a): Effect of hot water treatments on respiration rate (mg CO_2/kg/h) of mangoes stored at 13 °C

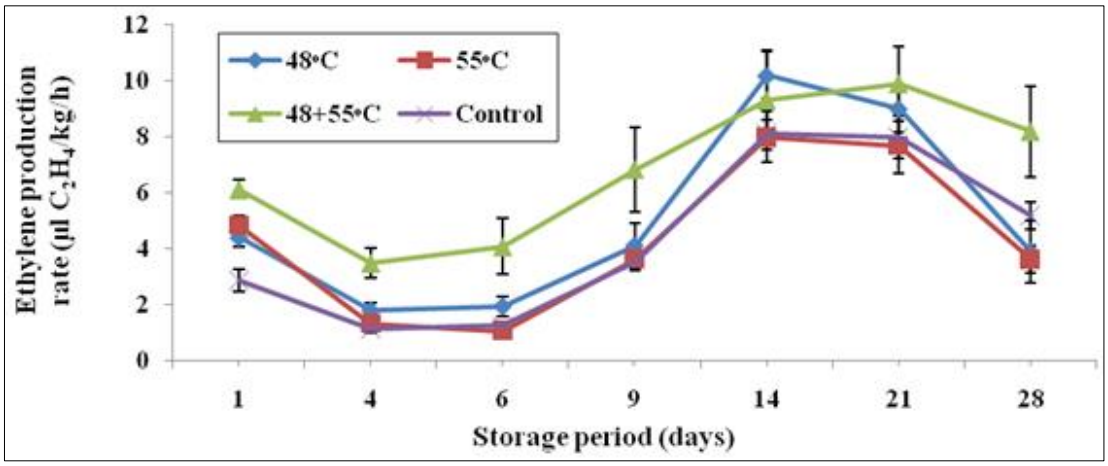

Fig 1(b): Effect of hot water treatments on ethylene production rate (µl C_2H_4/kg/h) of mangoes stored at 13 °C

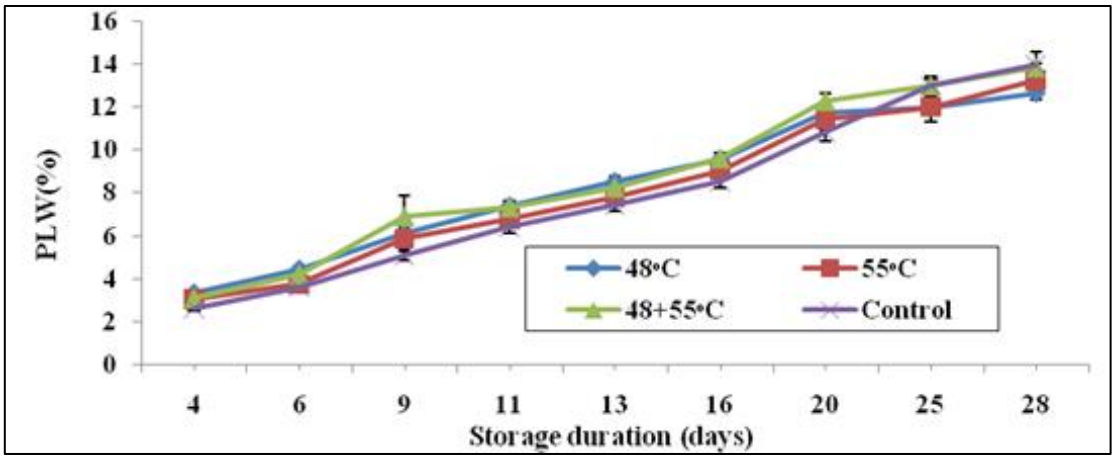

Fig 2: Effect of hot water treatments on physiological loss in weight (%) of mangoes stored at 13 °C

4. Conclusion

Hot water treatment along with chemical fungicide dip has proven to extend the shelf life of mango fruits during overseas shipments [31]. In our study, without the use of any chemical fungicides, HW treated fruits could be stored up to 4 weeks at optimum low temperature. Quality attributes were not adversely affected by quarantine HWT and fungicidal HWT while used alone. The quality was maintained and some parameters like TSS, carotenoids, total antioxidants and phenols were enhanced by HWTs. Hence, the use of HWT, which is an environment friendly technique and leaves no side-effects to the consumers, has to be encouraged among farmers to augment the income from export and reduce capital loss.

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