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Research Article

Physiological and Biochemical Attributes Associated with Spongy Tissue Disorder in *Mangifera Indica* L CV Alphonso during Ripening

Patil Rajvardhan Kiran[1]*, Parth Jadhav[2], Roaf Ahmad Parray[1] and Manish Srivastav[2]

[1]*Division of Agricultural Engineering, ICAR- Indian Agricultural Research Institute, New Delhi, India*

[2]*Division of Fruit and Horticultural Technology, ICAR- Indian Agricultural Research Institute, New Delhi, India*

***Corresponding Author:** Patil Rajvardhan Kiran, Division of Agricultural Engineering, ICAR- Indian Agricultural Research Institute, New Delhi, India.

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Abstract

Spongy tissue is one of the most important malady found in few cultivars of Mangifera indica throughout the world. This causes qualitative and quantitative transformation in affected fruits which results in decline in export potential as well as consumer acceptability. Due to the unclear understanding of the genesis of spongy tissue disorder and the paucity of research, a study was conducted to determine the physiological and biochemical factors that contribute to the occurrence of this disorder in Alphonso mango during ripening under different storage conditions (Ambient and cold storage). The results showed that weight loss (16 and 20.22%) and respiration rate (97.66 and 130.45 ml kg^{-1} h^{-1} CO_2) in healthy and spongy tissue-affected (STA) fruits respectively showed higher metabolic activities in STA fruits in ambient storage. The higher rate of weight loss and respiration in ambient storage as compared to cold storage conditions revealed that accumulated heat in fruits is highly responsible for occurrence of spongy tissue tragedy. A significant difference in carotenoid content (0.836 and 0.407 g kg^{-1}), TSS (18.3 and 12.42 °Brix), and pulp firmness (1.44 and 0.168 N) in healthy and STA fruits confirms absorption of nutrients towards the seed kernel due to initiation of germination. In STA fruits higher respiration rate, weight loss lead to quantitative losses and lower pulp firmness, carotenoid content, total and reducing sugars, TSS results in significant qualitative losses. The fruits in cold storage during ripening showed significant control over spongy tissue disorder as compared to ambient storage by stopping heat accumulation and seed germination during ripening.

Keywords: Spongy tissue; Respiration; Storage; Firmness; Carotenoid

Introduction

Mango is a highly demanded fruit in domestic and international markets due to its mouthwatering taste, delicious flavor, and high nutritional value. Generally, it is known as the king of fruits [1]. It is a rich source of dietary fibers, phenols, antioxidants, essential minerals, and phytochemicals required for a healthy and better lifestyle [2]. India ranked first in area as well as production of various varieties of mango. In 2022, India records mango production up to 21.01 million tons in 21.12 million Ha (www.statista.com). India is considered home to nearly 1600 cultivars out of that Alphonso, Banganpalli, Kesar, and Totapuri are leading and highly exporting cultivars from India [3]. Alphonso is a most famous and highly demanded variety due its attractive appearance, flavors, aroma, and textural properties. The west coastal region of India (*Konkan* region) is well known for ample and authentic production of Alphonso mango [1].

India is one of the important mango exporting country with 7 ranks with 4.64% share in global market in 2022 (www.fao.org). India exported over 49658 MT of mangoes in 2019-20, earning roughly 48.38 million USD from the international market. In 2022–2023, this reduced to 22963 MT, earning 45.72 million USD (Ministry of Commerce, GoI, www.agriexchange.apeda.gov.in). There is huge controversial situation in mango production and export from India. There are many factors responsible for hampering India's mango production and export such as climate change, diseases (Dieback, anthracnose, powdery mildew), pests (stone weevil, fruit

fly), and pesticide residues. Additional physiological disorders such as nutrient deficiency, malformation, soft nose, and internal tissue breakdown such as spongy tissue, jelly seed, and stem end cavity are major quality disintegrators of mango [4,5]. Among all above spongy tissue is highly deleterious disorder found in some of the major cultivars like Alphonso, Tommy Atkins, Keitt, Vanraj, and Haden [5-7].

Cheema and Dani (1934) [8] first described spongy tissue disorder in Alphonso mango as a physiological problem. There was considerable work happened on this disorder but still exact reason and its causes are unanswered. The mesocarp close to the stone displayed yellow-white pulp, a mildly corky character with or without air pockets, and inconsistent fruit ripening in this condition. It occurs close to the centre of the fruit and is only detectable when the fruit is sliced open because there are no visible signs [9]. In past few decades, spongy tissue disorder influenced huge production of Alphonso mango in India which significantly reduces the export potential. About 30 to 40% production of Alphonso mango affected with spongy tissue disorder [2] reported that spongy tissue in mango significantly affects flavor and pulp characters of Alphonso mango resulting in reduced consumer acceptability.

Due to the significant variation in nutrients throughout the fruit and deficiency of Ca content in flesh causes non-typical dispersion of Ca in mesocarp of mango which triggered the breakdown of flesh [5]. In several other studies, causes of internal tissue breakdown reported as orchard management, nutrient, and irrigation management, and climatic conditions also significantly affect [10]. Some factors such as time and season of harvest, effect of mulching, location, high temperature, low fruit transpiration, and biological factors are known to influence internal quality of mango fruit [11,12]. Spongy tissue is mainly associated with stage of maturity, nutritional imbalance, ecological factors, physiological and biochemical attributes [13-15]. Due to spongy disorder fruits quality deteriorated poorly and causing off-flavors in mango fruits. Calcium deficiency and environmental effects are key factors responsible for the internal breakdown of fruits [16]. By observing previous literature, it was found that the etiology of the disorder has been the subject of extensive research over the past five to six decades, but no successful outcomes have yet been noted.

The present study put efforts into determining physiological and biochemical parameters of healthy and spongy tissue affected (STA) Alphonso mango fruits under different storage conditions during ripening. The objective of this study is to understand the association between physicochemical parameters and spongy tissue disorder during storage under different storage conditions in Alphonso mango fruit and to study the significance of each parameter on spongy tissue disorder. By differentiating fruits based on physiochemical parameters this study will be further helpful for postharvest management and non-destructive quality assessment of internal breakdown of mango fruits.

Material and Methods

Fruit material

A Sample of 300 units of Alphonso mango fruit at 75 to 80 % maturity (based on color and specific gravity) were collected from commercial orchards in Ratnagiri (16° 59′ 39′′ N, 73° 37′ 09′′ E) and Sindhudurg 16° 10′ 53′′ N, 73° 44′ 86′′ E) districts of Maharashtra state, India. After harvesting mangoes were immediately kept for pre-cooling. Sorting and grading of mango was done based on weight and color. In order to maintain uniformity mangoes with weight between 225 to 250 g and dark green in color were only selected for further study. The selected mangoes were washed with chlorinated water (3 mg/L) and stored at 15 °C till further treatment.

Sample preparation and storage conditions

During storage duration physicochemical properties mango fruits were evaluated in interval of 5 day. Before physicochemical analysis mango fruits subjected to X-ray imaging for the confirmation of spongy tissue affected fruits non-destructively without cutting shown in figure 1**.** The X-ray imaging were done on first day as well as during 5-day interval. Once confirmed the spongy tissue affected fruits separated from healthy fruits for further analysis. Three fruits from each storage condition and healthy and spongy tissue affected were taken for further destructive physicochemical analysis.

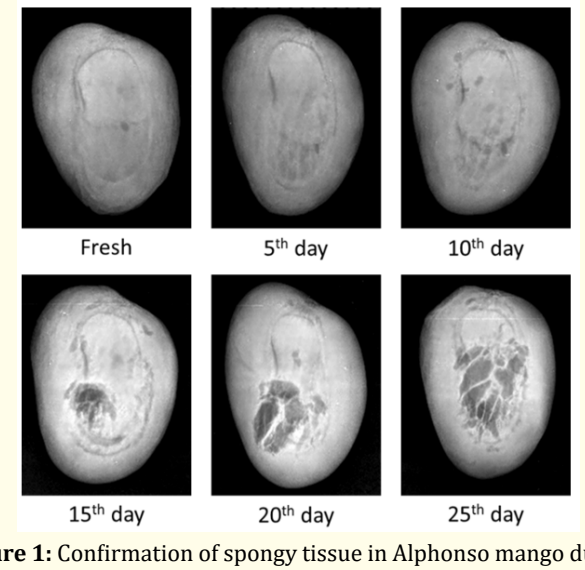


Figure 1: Confirmation of spongy tissue in Alphonso mango during ripening by using X-ray imaging technique.

Quality analysis

The quality of fruits during 25 days storage was assessed in terms of weight loss (PLW), total soluble solids (TSS), firmness, peel and pulp colour, carotenoid content, and respiration rate, postharvest physico-chemical changes. While the samples were being stored, every analysis was done in triplicate at 5 d intervals.

Fruit weight loss (PLW)

Weight of samples was taken with three replicates during storage using a digital weighing balance (Make-Mettler Toledo, Model-ML503, Switzerland, least count- 1 mg). Cumulative weight loss of fruit was determined in 5 d interval throughout the storage duration as difference between initial and final weights of fruit measured with weighing balance of 0.01 g accuracy and compared with initial weight in percentage [17].

Respiration rate

The respiration rate of mango samples was measured by using O_2 and CO_2 analyser (Model: Systech illionis). Samples were kept in a trap box of known volume (300 ml) for one hour for metabolic activities. Then O_2 and CO_2 analyser equipment started 30 min before the actual analysis. Then samples after one hour were analysed for respiration rate measurement with a pre-calibrated O_2 and CO_2 analyser started 30 min before the actual analysis. The concentration of CO_2 was noted down for further calculation [18]. Respiration rate was expressed as (ml kg^{-1} h^{-1} CO_2)

Respiration rate = (CO_2 concentration × Head space Volume × 6.31)/(100×Weight of fruit ×Time of holding----------(2)

Carotenoid content

The carotenoid content of mango sample was determined by the method described by Ranganna, (1986) [19]. For estimation of the carotenoid content of mango pulp 10 g fruit tissue sample was crushed with 5 ml acetone in a motor and pestle and the procedure was repeated till residue became colorless. The extract was transferred to separating funnel followed by addition of 10 ml petroleum ether and 5 ml of 5% sodium sulphate. The mixture was shaken and allowed to stand undisturbed for 5 minutes for phase separation. The top layer was separated and collected into the amber color bottle and making up the volume with 25 ml of petroleum ether. The absorbance value in terms of Optical Density (OD) was taken with the double beam UV-Vis spectrophotometer (Make-Accumax, India, Model- 2201, Wavelength- 195 to 1100 nm) at 452 nm against petroleum ether as a blank. The O.D. values were noted and quantity of total carotenoid measured with standard curve and expressed in g kg^{-1} of fruit tissue.

Total carotenoid content (g kg^{-1}) = (3.87×Final Volume×Optical density×100)/(Weight of sample×1000)----------(3)

Titratable acidity

The titratable acidity was determined by the procedure as reported by AOAC (2000) [20]. One ml of fresh sample was taken in a 100 mL volumetric flask and the volume was made up to 100 mL with distilled water. From this 10 ml was taken in a conical flask and titrated against standard 0.1N sodium hydroxide solution using phenolphthalein as an indicator, until faint pink color persisted for 15 sec. The titratable acidity was calculated and expressed in terms of anhydrous citric acid in percent.

Titratable acidity (%)= ((Normality of NaOH x Titre x 0.64 x 100))/(Volume of juice taken)------(4)

Firmness evaluation

The firmness of mango fruit was measured by using a texture analyser (TA- XT plus connect texture analyser of Stable Micro Systems, Ltd., Surrey, UK) in compression mode with a 2- mm diameter cylindrical probe (SMS-P/2, Stable Micro Systems, Ltd., Surrey, UK). The operating parameters were pre-test speed (2 mm/sec), test speed (0.5 mm/sec), post-test speed (10 mm/sec), trigger force (5.0 g), and distance (15 mm). During the compression test data acquisition rate was set at 200 points/sec as described by Jha., *et al.* (2013) [21].

During firmness measurement pulp firmness were measured separately. In pulp firmness measurement, mango fruit samples were peeled manually without disturbing the pulp texture and then used for compression test. Firmness was measured as the maximum force recorded in a force–time curve obtained during the compression test of mangoes. The firmness of mango was measured in triplicates at three different locations and average values were recorded for the comparison.

Total soluble solids (TSS)

The TSS of mango samples stored under ambient and cold storage conditions was determined by digital hand-held refractometer (Make: ATAGO, Japan, Model: PAL Brix/RI range: 0 to 93% resolution 0.2%). The refractometer was standardized against distilled water (0% TSS). A sample of 10 g mango fruit pulp was crushed using a pestle and mortar and the juice was obtained by filtering the crushed pulp through a muslin cloth. For obtaining TSS, 1 to 2 drops of clear juice were placed on the prism of the refractometer [22].

Total sugars

Total sugars were determined by the volumetric method as reported by Ranganna (1986) [19]. In a 100 mL volumetric flask, 10 mL of juice was taken. In this 2 mL concentrated HCL was added and the flask was kept in the hot water bath at 70-80°C for 30 min.

After cooling the hydrolysate was neutralized by adding a pinch of sodium carbonate till the formation of effervescence stopped. The volume of the neutralized hydrolysate was made to 100 mL with distilled water. The total sugar in the neutralized hydrolysate was determined in the same way as described under reducing sugars.

Total Sugars (%)= ((Factor x volume made up x 100))/((Titre x Wt.of sample taken))----------(5)

Reducing sugars

The reducing sugars were estimated by the volumetric method as reported by Ranganna (1986) [19] as follows. Ten ml of juice was taken in 100 mL volumetric flask. Then 40 mL of distilled water was added and content was neutralized with 1N sodium hydroxide solution (colorless). Two ml lead acetate (45%) was added and kept for 10 min followed by the addition of 10 ml potassium oxalate solution (22%). The volume was made to 100 mL with distilled water and then filtered through filter paper. The filtrate was further used for titration against Fehling's A and B solutions. Five ml of each Fehling's 'A' and 'B' solution were pipetted in 250 ml conical flask and diluted to about 50 ml with distilled water. The mixture was heated to boiling. During boiling, a clarified sample of juice was added carefully through the burette until the brick red color appeared. Finally, 2-3 drops of methylene blue indicator were added and titration was continued until a brick-red precipitate was formed. The reducing sugars content was calculated and expressed in percentage.

Reducing Sugars (%)= ((Factor x Volume madeup x 100))/((Titre x Volume of sample taken))---------(6)

Statistical analysis

The data obtained during the experiment was analysed using Factorial Completely Randomized Design (FCRD) in the pattern for optimizing heat treatment, and storage conditions. For treatment effects data was subjected to ANOVA and means were compared using Tukey HSD test at significant level of 5 percent. All the statistical analysis was performed using SPSS software (Version: SPSS Statistics 20. Ink, IBM Corp., USA). Further, the data was subjected to find out correlation between parameters using Pearson correlation using Python (Google Colab, CA, USA).

Results and Discussion

Weight loss

In present study, an increased trend of weight loss along with storage period was observed and highest weight loss was recorded by spongy tissue-affected (STA) fruit as compared to healthy fruits (Figure 1). Results showed that healthy fruits stored in ambient conditions showed higher weight loss from 3.6 to 16% from 5 to 25 d of storage as compared to healthy fruits stored in cold storage with lowest loss in weight from 2.12 to 8.5% respectively. In case of STA fruits higher weight loss was observed as compared to healthy fruits irrespective of storage conditions. STA fruits stored in ambient conditions recorded highest weight loss of 4.50% at 5th d to 20.22% at 25th d of storage. Nearly about 26.37% more weight loss was observed in STA fruits as compared to healthy ones in ambient condition. Whereas in STA fruits stored in cold storage showed weight loss of 14.43% at 25th d which was 69.76% more than healthy fruits stored in cold storage (Figure 2a).

During ripening of mango fruits continued weight loss was observed due to increased metabolic activities. In this study it was observed that STA fruits had higher and vigorous metabolic activities than healthy fruits. A similar trend was observed by Janave., *et al.* (2007) [23] and reported that ripe spongy fruits attained higher weight loss than ripe non-spongy fruits during 12 days of storage. Major probable reasons for spongy tissue disorder in mango are higher respiration rate, osmotic gradient between mesocarp and seed. and large heat unit accumulation in fruit [24].

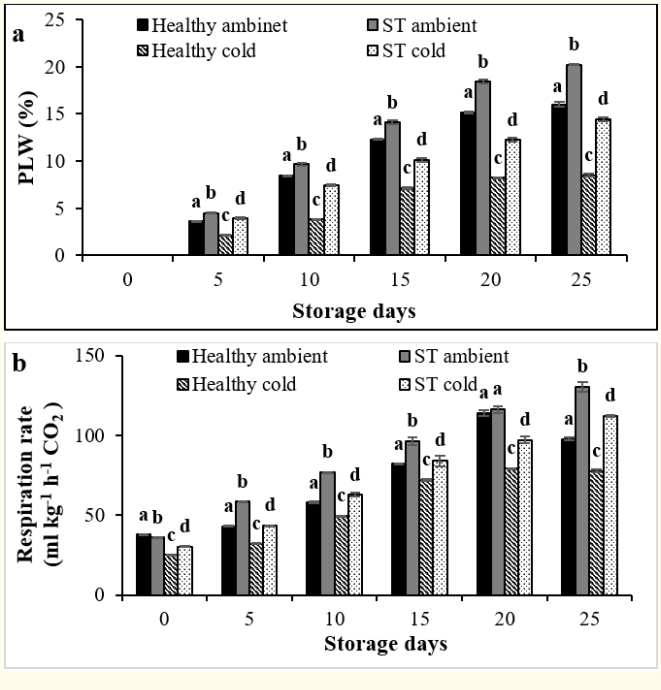


Figure 2: Variation in a) Physiological Loss in Weight (PLW) and b) Respiration rate of healthy and STA Alphonso mango during ripening at different storage conditions.

Respiration rate

Healthy fruits and STA fruits showed nearly similar respiration rates of 36.12 to 37.89 ml kg^{-1} h^{-1} CO_2 at the time of harvesting. In cold storage conditions healthy fruits record low respiration rate of 25.12 ml kg^{-1} h^{-1} CO_2 as compared to STA fruits (30.32 ml kg^{-1} h^{-1} CO_2). After 5th d STA fruits observed drastic increased respiration rate and continue till at the end of storage (58.37 to 130.45 ml kg^{-1} h^{-1} CO_2) in ambient storage (Figure 2b). In healthy fruits peak of respiration rate was reached at 20th d (114.16 ml kg^{-1} h^{-1} CO_2) and then decreased up to 25th d (97.66 ml kg^{-1} h^{-1} CO_2) due to reaching full physiological maturity and low transpiration rate. During storage, STA fruits showed 3.66 times more respiration rate observed at end of storage as compared to initial value. In cold storage conditions higher respiration rate from initial to up to end of storage was observed in STA fruits (30.32 to 112.33 ml kg^{-1} h^{-1} CO_2) as compared to healthy fruits (25.12 to 77.74 ml kg^{-1} h^{-1} CO_2). So, at the end of storage STA fruits showed about 44.5% more respiration rate than healthy fruits. Whereas at 25th d STA fruits stored in ambient storage showed 16% more respiration rate than in cold conditions.

In STA fruits sudden consumption of O_2 were observed due to simultaneous combined respiration of mesocarp and seed during ripening. This resulted into anaerobiosis due higher concentration of CO_2 generation in mesocarp. In present healthy fruits showed increasing trend in respiration but recorded low respiration rate as compared to STA fruits and declined during the last few days of storage. Higher respiration rates in ripe spongy Alphonso fruits were observed due to infection of *staphylococcus* strain which results in formation of air cavity inside the fruit [4]. The STA pulp in contact with seed was observed to have air pockets along with unripened, disintegrated, and white corky pulp and showed higher respiration rate than healthy fruits. Shivashankar., *et al.* (2016) [25] and Rama Krishna., *et al*. (2020) [26] found that jelly seed affected fruit showed higher respiration rate due to viviparous nature of affected fruits. Ravindra and Shivashankar (2004) [27] elaborated that STA Alphonso fruits showed higher respiration rate due to initiation of seed germination, not due to mesocarp tissue. While seed of healthy fruits is in dormant state with firm pulp. It was found that cold storage conditions reduce or stop the germination of seed which results in a significant decline in respiration rate. Vasanthaiah., *et al*. (2008) [11] and Ravindra., *et al*. (2010) [27] reported that STA fruits showed higher respiration rate than healthy fruits leading to elevated temperature throughout the fruit and intern affected the ripening mechanism.

Carotenoid content

The changes in carotenoid content of healthy and STA fruits during storage under different storage conditions presented in Fig. 3a. Initially after harvesting all fruits showed carotenoid content of 0.0045 g/kg irrespective of disorder because fruits were harvested at 70 to 75% maturity. During ripening healthy fruits showed highest carotenoid content of 1.13 g/kg at 20th d of storage and declined to 0.83 g/kg at 25th d due to the senescence (over ripening). But healthy fruits stored in cold storage showed consistently increased trend up to 25th d (0.11 g/kg). While STA fruits showed significantly lower carotenoid content in both storage conditions. STA fruits followed similar trend of increased carotenoid from 0 to 20th d (0.0045 to 0.596 g/kg) then declined to 0.407 g/kg at 25th d. From results its revealed that only 47.34% of carotenoids were biosynthesized in STA fruit compared to healthy Alphonso fruits in ambient conditions. In cold storage condition STA fruits reached carotenoid content up to 0.75 g/kg at 25th d which was less than healthy fruits (1.13 g/kg) but it was significantly higher than STA fruits stored in ambient condition.

The results showed that healthy fruits attained higher carotenoid content than STA fruits. Pulp near to seed seen white, unripe, or blackish clearly showed very less or deflected carotenoid content in that particular area. In fully ripe STA fruits black pulp around the seed showed that carotenoid might be degraded by enzymatic actions involving lipoxygenase mediated oxidation. Janave (2007) [23] reported that Alphonso fruits with spongy tissue showed 2.5-fold less carotenoid content than healthy fruits because of affected tissue unable to synthesize carotenoid. Chytil (1999) [28] reported that during ripening of STA fruits carotenoid is used as antioxidant to deactivate reactive oxygen which results in lower carotenoid content. Oak., *et al*. (2019) [2] reported that due deficiency of antioxidants in STA fruits occurred due to dissipation of reactive oxygen in mesocarp which further deteriorated the physiology of fruit. Similarly, Singh and Swati (2011) [29] and RamaKrishna., *et al.* (2020) [26] also reported that lower β-carotenoid were found in jelly seed affected pulp than healthy pulp. Oak., *et al.* (2022) [24] reported that in STA mesocarp carotene hydroxylase enzyme was 4-fold less as compared to healthy mesocarp. Cold storage conditions significantly lower the rate of metabolic activities in STA fruits. Therefore, showed higher carotenoid content in both STA and healthy fruits as compared to ambient storage. Thus, it is imperative to summarize that spongy disorder significantly affects the biosynthesis of pigments and volatiles during storage.

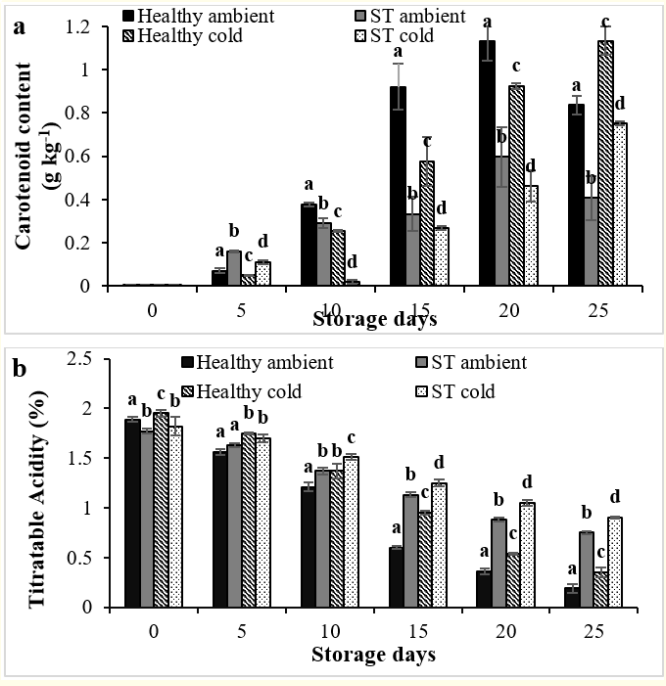


Figure 3: Variation in a) carotenoid content and b) Titratable acidity of healthy and STA Alphonso mango during ripening at different storage conditions.

Titratable acidity

From data presented in Fig. 3b revealed that the acidity of STA fruits was recorded higher than healthy fruits throughout the storage duration irrespective of storage condition. But fruits stored in cold storage showed significantly higher acidity than ambient storage. In healthy fruits initially, acidity was observed in range of 1.89 to 1.95%, and in STA fruits ranged from 1.77 to 1.82%. As ripening begins acidity of healthy fruits declined up to 0.19 and 0.35% at end of storage in ambient and cold storage respectively. Whereas STA fruits retain significantly higher acidity during whole storage duration observed up to 0.75 and 0.9% in ambient and cold storage respectively as compared to healthy fruits.

Reducing titratable acidity during ripening of Alphonso mango was found during study. Palafox Carlos., *et al*. (2012) [22] and Javed., *et al.* (2022) [30] reported reduced acidity due to increased metabolic rate and conversion of organic acids into sugars during ripening. In general, STA fruits showed higher acidity than healthy fruits. Janave and Sharma (2008) [4] and Vasanthaiah., *et al.* (2008) [11] found that because of higher respiration rate in STA Alphonso fruits large amount of CO_2 and heat got accumulated in fruit resulting into higher acidity and anaerobic condition than healthy fruits. Oak., *et al.* (2019) [2] observed that STA mesocarp was tangy and acidic due to higher accumulation of iso-citric acid and amino acids in pulp even after complete ripening. Amrapali, Dashehari, and Langra fruits affected with jelly seed also showed higher titratable acidity than healthy ones [25,26]. It was observed that due to physiological imbalance in STA fruits sweetness and acidity were not maintained resulting in hampered mesocarp taste, decreased acceptability.

Pulp firmness

The pulp firmness of mango decreased during the ripening of fruits. Initially, healthy fruits stored in ambient conditions showed highest firmness at 0th and 5th d (6.78 and 6.03 N) then similarity was observed in healthy fruits stored in both conditions up to 20th d (2.87 and 2.78 N respectively in ambient and cold conditions). But at the end of storage, healthy fruits in cold storage showed higher pulp firmness of 2.12 N as compared to all others (Figure 4). Healthy fruits showed about 76.75 and 66% loss in pulp firmness in ambient and cold conditions respectively. In STA fruits initially pulp firmness of 4.03 and 5 N were observed in both ambient and cold storage and then significant decreased in pulp firmness up to 0.168 and 0.89 N respectively was observed at the end of storage.

During the ripening of Alphonso mango firmness was found to decrease and similar trend was reported by Jha., *et al.* (2013) [21]. In this study it was observed that healthy Alphonso fruits retained high firmness with fleshy, firm pulp as compared to STA fruits. In cold storage samples more firmness was observed than ambient storage in both healthy and STA fruits. Sometimes shifting of water from mesocarp to seed due to the initiation of seed germination results into dried white corky pulp which records hard and strong firmness. Reymond., *et al.* (1998) [14] found that STA fruit pulp showed softer and paste like texture than healthy fruits near to the seed. During ripening of mango water content is reduced due to respiration and metabolic activities and intense activity of softening related firmness was reduced [30]. Oak., *et al.* (2019) [2] reported that fruit firmness is generally maintained by lignification of cellular structure. But in STA fruits concentration of feruloyl glucose was very low as compared to healthy fruits which affects the lignin biosynthesis during ripening. So, uneven and reduced activity of ripening enzymes affects texture of fruits. Increased activities of pectin degrading enzymes during fruit ripening causes pectin degradation and softening of pulp [31,32]. The data revealed that towards the end of storage no significant difference was observed in both types of fruits under ambient and cold storage conditions. It was concluded that there was no significant variation in peel firmness of Alphonso mango with spongy tissue disorder. However, pulp firmness showed higher variation with nature of spongy tissue varying from white hard corky appearance to black soft, air pocket type appearance.

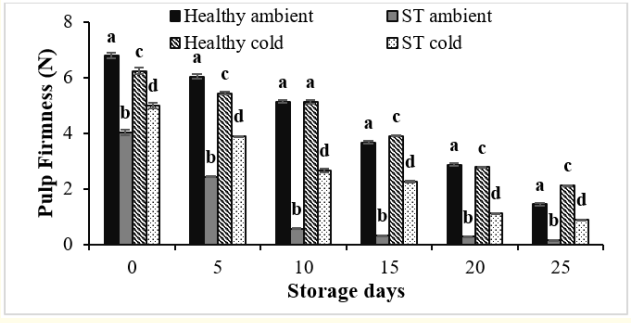


Figure 4: Pulp firmness of Alphonso mango during ripening under different storage conditions.

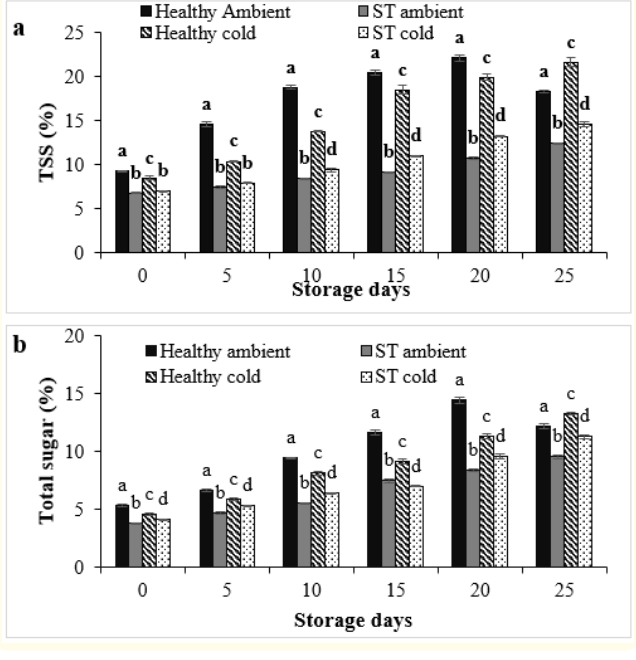
Total Soluble Solids (TSS)

During the ripening of mango, sugar concentration increased 2 to 3 times as compared to raw matured fruits. Initially after harvesting TSS content of healthy fruits varies from 8.5 to 9.3 °Brix, whereas in STA fruits vary from 6.82 to 7.02 °Brix. As ripening is going on in ambient conditions TSS of healthy fruits increased from 9.3 °Brix at 0th d to 22.1 °Brix at 20th d due to conversion of starch into sugars shown in figure 5a. But up to 25th d significant reduction in TSS was observed 18.3 °Brix due to senescence (over-ripening) of fruits. Whereas in cold storage healthy fruits observed gradual and significant increasing trend in TSS from 8.5 to 21.5 °Brix in 25 d storage duration. In case of STA fruits significantly low TSS was observed in ambient and cold storage condition up to 12.42 and 14.61 °Brix respectively at 25th d of storage. At 25th d of storage STA fruits stored in ambient condition showed 31.69% less TSS whereas in cold storage 32.36% less TSS was observed as compared to healthy fruits.

In the present study TSS (°Brix) of STA fruits was found lower than healthy fruits during the whole ripening duration in Alphonso mango. During ripening of mango, starch converts into sugars rapidly in healthy fruits by α-amylase activity resulting a significantly higher increase in TSS compared to STA fruits. After 20th d of storage significant decrease in TSS was observed due to senescence or overripening of fruits in ambient conditions. This was probably because of the reason that during overripening sugar gets converted into organic acids or is used for metabolic activities. In case of STA fruits lower TSS was recorded continuously as compared to healthy fruits due to spongy tissue affected portion of fruits accumulating higher proportion of starch granules than healthy pulp. The TSS of 13.2 and 10.2% were observed in healthy and STA fruits, respectively During ripening of STA fruits delayed or arrested hydrolysis of cell wall as well as degradation of starch might be observed as compared to healthy fruits [2]. When fruit starts ripening large amount of heat is generated which is also responsible for altering carbohydrate metabolism in STA fruits. Shivashankar., *et al*. (2007) [7] reported that healthy fruits showed higher amylase and lipase activity in pulp while STA fruits showed higher amylase and lipase activity in seed due to initiation of germination and decline in pulp which resulted in lower TSS. Incomplete breakdown of starch near to seed resulted into less TSS in mesocarp as compared to healthy pulp [33]. In contrast, Lin., *et al*. (2013) [34] and Rama Krishn., *et al*. (2020) [26] observed higher α-amylase activity in jelly seed-affected fruits than in healthy one resulting in higher TSS in jelly seed-affected pulp compared to healthy pulp. STA fruits showed significantly less TSS than healthy fruits because of absorption of nutrients towards the seed of fruit with white corky pulp remaining behind. But cold storage conditions consistently increased TSS in both fruits without senescence.

Total sugars

Healthy fruits recorded higher total sugar content than STA fruits in both storage conditions. In ambient storage, total sugar increased from 5.32% at 0th d to 14.43% at 20th d but due to senescence slightly decreased up to 12.16% at 25th d of storage shown in figure 5b. In case of cold storage continuous increasing trend was observed in sugar content of healthy fruits records from 4.54 to 13.29% during ripening from initial to 25th d storage respectively. In case of ambient storage STA fruits showed 21.54% less sugar content than healthy fruits whereas in cold storage STA fruits showed 15.19% less sugar content than healthy ones.



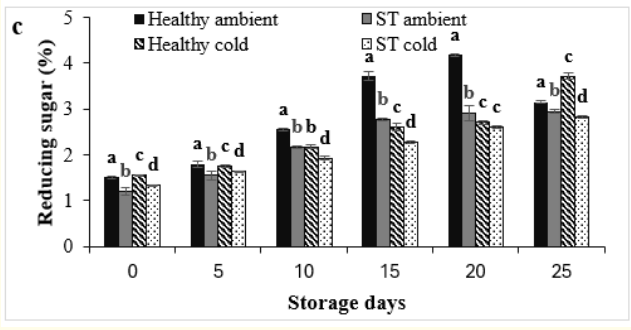


Figure 5: Variation in a) TSS, b) Total sugars, c) Reducing sugars at different days of interval during the ripening of Alphonso mango in ambient and cold storage condition.

Reducing sugars

As data presented in Fig. 5c elucidated that reducing sugar content in healthy fruits was higher than in STA fruits. In case of healthy fruits in ambient storage reducing sugar increased from 1.51 to 4.18% from initial to 20th d storage and decreased to 3.15% up to 25th d storage. In cold storage, it increased from 1.55 to 3.72% throughout the 25-day storage. STA fruits recorded lower reducing sugar content as compared to healthy fruits at 25th d storage 2.95 and 2.82% in ambient and cold storage respectively. So, nearly 6.34 and 2.41% less reducing sugar was observed in STA fruits as compared to healthy fruits in ambient and cold storage respectively.

In present study total and reducing sugar were found higher in healthy pulp than STA pulp during storage. Initiation of germination in seed of mango during ripening records higher respiration rate which created diffusion of CO_2 and hypoxic environment, declined amylase enzyme activity and thus resulted into incomplete starch breakdown. Hence STA fruits showed less total and reducing sugar during ripening [7]. The results highly corroborated with the findings of [13,15,33] for Alphonso and Tommy Atkins mango wherein reduced amylase activity was found responsible for lower sugar content in STA fruits. In contrast Rama Krishna., *et al.* (2020) [26] reported in jelly seed-affected pulp higher values of total and reducing sugar due to breakdown of starch in sugar and it accumulation in jelly seed. Similarly, in Amrapali and Chin Hwang cultivars fruits with jelly seed disorder were found higher total and reducing sugar content than healthy fruits [25,34]. Gill., *et al.* (2017) [35] observed that for higher sugar content in jelly seed affected fruit conversion of ascorbic acid into sugars were responsible. Lower sugar content in both healthy and STA fruits stored under ambient conditions was due to high rate of metabolic activities and respiration rate compared to cold storage.

Correlation analysis of physiological and biochemical parameters

The strength of the correlations between all pairs of parameters was evaluated to investigate the links between physiological and biochemical measures. As shown in figure 6 Strong positive associations existed between PLW and respiration rate, carotenoid content and reducing sugars and total sugars and reducing sugars r = 0.97, r = 0.93 and r = 0.94 respectively. In contrast significant negative correlation observed between carotenoid content and titratable acidity, total sugars and titratable acidity, and reducing sugars and titratable acidity r = 0.95, r = 0.94 and r = 0.93 respectively

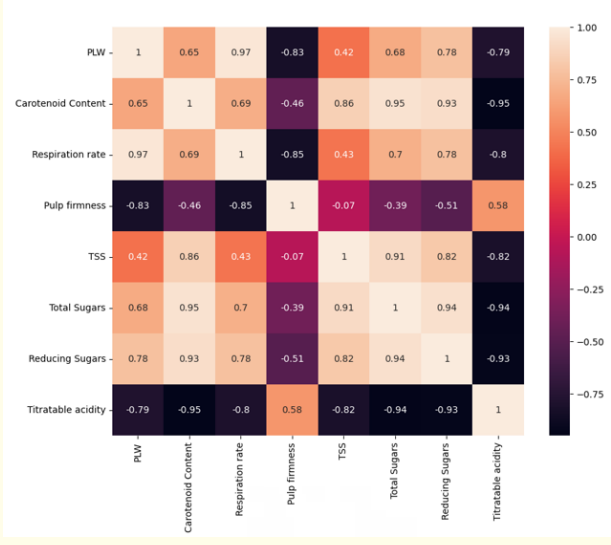


Figure 6: Pearson correlation heat map showing correlations among quality parameters of Alphonso mango.

Conclusion

Based on results of our study it was found that even mango harvested at 70 to 80% maturity also susceptible to spongy tissue disorder. Initially after immediate harvesting spongy tissue not found in mango but it seen after 10 to 15 days of storage. It was confirmed by X-ray imaging of fruits during ripening. During ripening of Alphonso mango fruit causing of spongy tissue disorder starts earlier in ambient storage than in cold storage conditions. So, its concluded that mango stored at low temperature during ripening improves the postharvest quality with higher retention of nutrient contents and delaying cause of spongy tissue up to 20 days after harvesting. Mango stored in cold storage showed proper removal of field heat as well as removal of accumulated heat during ripening it results in slowing the rate of seed germination followed by decrease in meta-

bolic activities. Findings of this study also proves that maintaining cold chain thought the supply chain of Alphonso mango helped to stop spreading of spongy tissue and increase the marketability of mango fruits by retaining high quality.

Author Contribution Statement

Patil Rajvardhan Kiran- conducted the experiments, analysed the samples, analysed the data and drafted the manuscript; Roaf Ahamad Parray- conceptualization, supervised the research, review and edited the manuscript; Pramod Aradwad- review and editing; Indra Mani- conceptualization and original research plan; Arunkumar TV- review and editing; Manish Srivastav- editing the manuscript; All authors have read and approved the final manuscript.

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