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Review Article

EFFECT OF DIFFERENT TREATMENTS TO INDUCE THE FRUIT QUALITY OF DIFFERENT CULTIVARS ON LITCHI

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Abstract: The quality and acceptability of some value added litchi products have been essentially tailored to satisfy the health needs and prevent wastage. Litchi fruit is dipped in SO_2 fumigated with diluted HCl to restore the red colour following SO_2 bleaching; this practice has gained commercial acceptance. The titratable acidity in cold-stored fruit rapidly decreased by 6 hour of shelf time and slowly decreased thereafter at 25°C. Thus, titratable acidity content tended to decline with extended storage duration and ascorbic acid content decreased with self life increased during storage period at low temperature. Anthocyanin and phenolics contents of the fruit decreased during storage but increase fist few day of storage. The pericarp browning, postharvest decay and micro-cracking are the major constraints affecting the commercial quality of litchi during storage, transportation or at the consumer shelf. Commercial SO_2 fumigation and sulphur padding were prevention of pericap browning and micro-cracking in the pericarp. Packaging material like MAP using PVC/PE film wrapping and plastic bags or liners combined with low storage temperature generally provided the best extension of shelf life by reducing weight loss, pericarp browning and maintaining the red colour of fruit. Storage life was found to be the maximum (21 days) when sulphur fumigated fruits were packed in polythene bags and stored at 14°C. In case of fruit stored at 5.5°C showed a statistically superior colour than those at 1°C and remained good for up to 40 day of storage.

Keywords: *Fruit improvement, Anthocyanin, Browning, Minimizing rotting, Sugars*

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Introduction

Litchi (*Litchi chinensis* Sonn.) belongs to the family Sapindaceae, is an evergreen fruit tree native to South China. Sucrose, fructose and glucose are found to be the major sugars in litchi [1,2]. The total sugars in the aril tissues may range from 55.9-61.4% on dry weight basis (Jiang *et al*., 2006), reducing sugars represent more than 70% of the total sugars in the aril (Jiang *et al*., 2006). Due to the presence of significant number of vitamins, minerals and phenolic compounds, litchi fruit is highly cherished for its medicinal value [3].

Postharvest loss of litchi is estimated to be 20 to 30% of the harvested fruit, even as high as 50% prior to consumption, mainly due to the decay caused by infection of microorganisms such as *Peronophythora lithci, Penicillium* spp., *Colletotrichum* spp.[4,5]. Sulphur dioxide controls browning by discoloration of the skin of litchi through the inhibition of PPO [6] and by combining with anthocyanin to form more stable complex [7]. Immediately after sulphur dioxide treatment, litchi fruit may appear as a uniform yellow colour and then turn red again after 1–2 days [8]. Plastic bags reduce moisture loss from stored fruit over a broad temperature range (1-30°C) [9-11].

Harvest maturity

In litchi, maturity can be determined by fruit weight, colour, sugar or total soluble solids (TSS, %), titratable acidity (TA, %), sugar: acid ratio, flavour and days from anthesis. As litchi fruit mature, the concentrations of sugars, principally those of sucrose, glucose and fructose increase [12], while the concentrations of organic acids, predominantly malic acid decrease. Furthermore, pericarp colour was suggested as a picking maturity indicator [13,14], while excess ripeness may be excluded by consideration of acetaldehyde or ethanol contents [15]. In practice, growers' decisions often rely on individual experience in terms of size, pericarp structure, colour, flavour, and taste of the fruit as well as a characteristic time after anthesis [16,17].

For local markets, litchi is ideally harvested when fully red and ripe (Underhill and Wong, 1990), whereas fruit intended for long shipping distances is often picked when the pericarp partly turns red or at 75-80% maturity [18,19]. The TSS/acidity ratio varied from 3.8 at 80 days after blossom to 58.9 at 124 days after blossom respectively. A wide range of TSS/TA ratios from 15 to 65 (based on TA as malic acid and TSS in g hg−1) was recommended for mature fruit of different producing areas (Finger *et al*., 1997; Pesis *et al*., 2002). Underhill and Wong (1990) recommended a TSS: TA ratio of 30-40 while fruit with a TSS: TA ratio of greater than 80:1 is considered to be over-mature [20]. Pesis *et al*. (2001) reported that the peel colour of mature litchi fruits (pink and red pericarp) deteriorated when kept longer on the tree during harvesting season. Dwivedi and Jha (2000) [21] reported that the colour of litchi fruits depends on variety Bedana, Kasha, Muzaffarpur, Purbi, Desi, and Bombai cultivars of litchi which possess light brown, red, reddish, deep pink deep orange to pink light, brown red, light red to deep red carmine red and pinkish brown to red tubercles, respectively.

Fruit composition and physico-chemical changes during postharvest practices

Fruit morphology

The mature pericarp consists of 3 distinct layers and varies slightly in thickness depending on the cultivar. The hard outer epicarp comprises a continuous cuticle, a uniseriate epidermis and subepidermal sclerenchyma. The fleshy middle mesocarp is parenchymatous. The inner endocarp is made up of small, thin-walled and unsuberised epidermal cells [22,23]. The mature litchi pericarp is 1-3 mm thick and consists of three distinct layers. The outermost epicarp has a continuous cuticle 1-3 mm thick, a single epidermal layer and sub-epidermal schlerenchyma. The mesocarp is composed of parenchyma and contains chlorophyll and most of the anthocyanins.

The innermost endocarp is membranous and comprises small, thin-walled, unsuberized epidermal cells [24]. Litchi fruit are conical, heart-shaped or spherical in shape, with a thin pericarp [25]. The pericarp can vary in colour from green-red to full red, depending on fruit maturity and the cultivar. The edible portion of the fruit is a fleshy, semi-translucent white aril. The aril is an extension of the funiculus or seed stalk. It arises from the placenta and surrounds the seed. Fruit size varies between cultivars (Jiang *et al*., 2006).

Fruit Colour

The high post-harvest losses reported of the ripe litchi fruit are due to the rapid colour degradation of the pericarp. Litchi fruit rapidly lose their red colour after harvest. The important causes of colour degradation are blanching or dehydration [26], browning reactions, and active polyphenol oxidase and peroxidase [27] and the existence of other anthocyanin related compounds present in the pericarp [28]. Litchi fruit is dipped in SO_2 fumigated with diluted HCl to restore the red colour following SO_2 bleaching; this practice has gained commercial acceptance (Zauberman *et al*., 1991). The litchi fruit pericarp is initially green but becomes red due to decreased chlorophyll concentration and increased anthocyanin synthesis, which account for the red skin of ripe fruit (Huang, 1995; Underhill and Critchley, 1992). The initial browning that is manifested does not affect eating quality, but brown fruit fetches lower prices than red fruit. Therefore, rapid skin browning is one of the major problems of the litchi industry [29].

Fruit Weight

Badiyala and Awasthi (1991) [30] reported that the average fruit weight ranged between 14.15 to 21.00g with cultivars Muzaffarpur and Rose Scented produced significantly large fruits (21.3g) as compared to all other cultivars while experiment on 9 litchi cultivars. Weight of litchi fruit ranges from 8.27 to 21.1g, the edible portion i.e., pulp weight ranging from 37.30 to 73.6%. Peel weight and stone weight ranging from 11.73 to 28.7% and 3.2 to 22.9%, respectively (Revathy and Narasimham, 1997). 'Huaizhi' fruit weigh about 22g, whereas fruit of other cultivars can weigh over 30g. However, a high proportion of edible aril is more desirable to consumers than a large fruit (Jiang *et al*., 2006).

Sugars

In litchi, total soluble solids (TSS) increase during ripening, reaching 13-20% by harvest (Pauli *et al*., 1984). The total sugars in the aril tissues may range from 55.9-61.4% on dry weight basis (Jiang *et al*., 2006) and the reducing sugars represent more than 70% of the total sugars in the aril (Jiang *et al*., 2006). Singh *et al*. (2010) [31] also found increasing trend in non- reducing sugars in litchi cv. Rose Scented when stored upto 6th day of storage. Pulp pH, total soluble solids and total soluble solids to acid ratio increased with advancement of maturity [32].

Acids

At maturity, malic acid accounted for 80% of the acids, whereas citric, succinic, levulinic, glutaric, malonic and lactic acids were relatively minor constituents (Paull *et al*., 1984). Titratable acidity and total organic acids decrease during fruit development and ripening [33]. Ghosh *et al*. (1988) and Chakraborty *et al*. (1980) [34] observed that the pulp acidity varies from place to place, ranging from 0.26 to 0.57% in Punjab, 0.21 to 1.01% in U.P., 0.39 to 1.24% in West Bengal and 0.60 to 0.68% in Bihar. Titratable acidity (TA) decreases during development, while pH increases (Pauli *et al*., 1984 and Singh *et al*., 2013). The titratable acidity in cold-stored fruit rapidly decreased by 6 hour of shelf time and slowly decreased thereafter at 25°C. Thus, titratable acidity content tended to decline with extended storage duration [35].

Vitamins

Ascorbic acid being sensitive to light, oxygen and heat, might be oxidized easily in presence of oxygen by both enzymatic and non enzymatic reactions. The fruit may contain as much as 27.8 mg/100 g fruit weight of Vitamin C according to (Lee and Kader, 2000; Mozafar, 1994; Shewfelt, 1990) [36-38]. Revathy and Narasimham (1997) reported that the ascorbic acid content ranges from 17.2 to 32.4 mg/100 g of fresh pulp. In general, litchi fruit is also rich source of vitamins C, ranged from 21-36 mg/100g [39] and phenolic compounds that have antioxidant activities (Hu *et al*., 2010) but it may decrease after harvest [40]. The decreasing trend in ascorbic acid has also been reported by Ray *et al*. (2005) and Singh *et al*. (2013). Liu *et al*. (2011) observed that the ascorbic acid content decreased with self life increased during storage period at low temperature. Several antioxidant compounds such as phenolic acids, flavonoids and proanthocyanidins were recognized in different litchi cultivars (Hu *et al*., 2010). Due to the presence of significant amount of vitamins, minerals and phenolic compounds, the litchi fruit is highly cherished for its medicinal value (Hu *et al*., 2010). Its high attractive color, nutrients value, sweet flavor, abundance of vitamin C and phenolic compounds have won the litchi an important place in the export commodity list of the litchi producing countries [41].

Anthocyanins

Anthocyanin pigments were first detected on 40 days after full bloom and it continued to increase afterwards until harvest as reported earlier by Wang *et al*. (2002). The significant affect of red skin colour due to the embedded anthocyanin pigments [42,43]. The levels of anthocyanin and phenolics contents of the fruit decreased during storage [44]. In litchi fruit degradation of anthocyanins pigment is catalyzed by PPO [44,45]. Lin *et al*. (1988a) [46] also found an increase in anthocyanin content during the first few days of storage. With initial increase in anthocyanins, there is a gradual degradation associated with fruit senescence [47]. The anthocyanins are located in the vacuoles of the upper mesocarp tissue and to a lesser extent in the epidermis [48] and it is coincided with chlorophyll degradation, with the concentration of anthocyanins increasing progressively as the fruit [49].

The concentration of chlorophyll in the skin decreases at the beginning of litchi fruit maturation, coinciding with the synthesis of antho- cyanin, which accounts for the red pigmentation of the pericarp. Cyanidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-galactoside, malvidin 3-acetylglucoside, pelargonidin 3-glycosides and pelargonidin 3, 5-diglucoside have been isolated from the pericarp [50]. Zhang *et al*. (2000) and Sarni-Manchado *et al*. (2000) [51] identified the important coloured anthocyanins as cyanindin-3-rutinside, cyanidin-3-glucoside, quercetin-3-rutinosde and quercetin-3-glucoside by using high-performance liquid chromatography (HPLC). Recently, Zhang *et al*. (2004) [52] observed that the major anthocyanin is cyanindin 3-rutinside by using HPLC–mass spectrometry. Increases in anthocyanin concentration can occur during early storage (Lee and Wicker, 1991). Zhang *et al*. (2000) observed a decline in cyanidin-3-glucoside (major anthocyanin, representing 91.9% of the total anthocyanin) with increasing severity of browning during storage.

Browning mechanism and causes

Browning can be caused by a wide range of different stresses, such as climatic conditions prior to fruit maturation [53], desiccation [54,55], chilling [56], disease (Jiang *et al*., 2002), heat stress [57] and senescence [58]. All these factors disrupt cellular compartmentation, allowing polyphenol oxidase (PPO) located in the chloroplasts and other plastids to react with phenolic substrates located in the vacuole, forming brown polymers [59]. Peroxidase enzymes may also be involved in this process [60,61]. Desiccation-induced browning begins on the tips of the protuberances of litchi and subsequently spreads across the entire fruit surface, with browning localized in the epicarp and upper mesocarp (Underhill and Critchley, 1995). While the disappearance of the red pigments occurs at the same time as browning, the pericarp of litchi contains many phenols, and these are better substrates for PPO than are anthocyanins (Sarni-Manchado *et al*., 2000). The rapid post harvest pericarp desiccation and disease are by far the most common cause of browning. It is thought to be a rapid degradation of the red pigments by polyphenol oxidase, forming brown-colour byproducts (Jiang *et al*., 2004b; Underhill, 1992).

Li *et al*. (2005) [62-64] also reported that the incidence of anthracnose of the fruits was closely related to the browning of the pericarp, the infection of anthracnose fungus accelerated the pericarp browning and lesion larging. Pericarp browning has been considered the main postharvest problem which reduces litchi commercial value [65,66].

Beside desiccation, wounding or mechanical injury, storage of fruit at undesirable low temperature (chilling injury), pathogen or pest attack [67] and general senescence (Bagshaw *et al*., 1995) [68] may result in or promote pericarp browning. The litchi pericarp browning is due the oxidation of phenolic compounds, the degradation of anthocyanin by the enzymes polyphenol oxidase (PPO) or peroxidase (POD) resulting in polymeric browning pigments like O-quinones with PPO activity as the major factor (Huang *et al*., 1990; Underhill and Critchley, 1995). According to Sivakumar *et al*. (2008) [69], pericarp browning, postharvest decay and micro-cracking are the major constraints affecting the commercial quality of litchi during storage, transportation or at the consumer shelf. Litchi cultivars showed similar pericarp development, however, differences in the thickness of cuticle and spongy layers were observed between different cultivars [70]. The spongy tissue responsible for gas exchange in the pericarp was thought to be responsible for water loss [71].

Polyphenol oxidase (PPO) and peroxidase (PPD) activity

Jiang *et al*. (2004) reported that oxidation of pericarp phenolics to quinones and their polymerization to brown pigments, coupled with accelerated degradation of anthocyanins, have been reported to cause browning of litchi. Enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) have been reported to contribute to this browning process, by regulating the biosynthesis of phenolics and their oxidation [72]. Polyphenol oxidase activities measured during storage are often inconsistent. Lin *et al*. (1988a) demonstrated a rapid increase in PPO activity during the first 48 hours of storage, while Zauberman *et al*. (1991) observed no changes in PPO activity during the same period. Reactive oxygen species (ROS) including H2O2 accumulates during fruit ripening, which is recognized that its accumulation can accelerate the oxidation of phenolic compounds, resulting in pericarp browning of litchi fruit [73].

Water loss/ Dehydration

Jiang and Fu (1999a) [74] found that water loss from the litchi pericarp was more than 50% after 3 days of storage at 60% RH and 20°C. Hence, selective dehydration of the pericarp occurred with little movement of water between the aril and the pericarp. Eventually, the aril also loses water and the fruit becomes flaccid and bland (Underhill and Critchley, 1993; Underhill and Simons, 1993).

Micro-cracking

Micro-cracks were observed on the pericarp surface with increasing density after 12 hour (Underhill and Critchley, 1993; Underhill and Simons, 1993). These micro-cracks potentially increase the oxidation processes resulting in pericarp browning (Huang *et al*., 2004). The fluctuation of wet and dry periods at late fruit developmental stages can also aggravate fruit cracking. A relationship between fruit cracking and endogenous hormones or mineral nutrition (Ca, Mg and B) was reported by Qiu *et al*. (1999) [75] in cv. 'Nuomoci'. The contribution to cracking resistance by calcium is related to its structural role in the cell walls, and the availability of calcium during early fruit development is important for cracking resistance [76]. Peng *et al*. (2004) [77] also reported that fruit cracking could be reduced by foliar application of brassinolide, a plant growth activator, before blossom. Drought is another major cause of pericarp cracking during fruit development, which leads to a loss of pericarp extensibility [78]. Micro-cracking was also observed as a result of bad handling processes, and disruptions of the surface were observed in freshly harvested fruit. Fruit dropping during the separation process was observed to cause "splitting" damage in the pericarp. Commercial SO_2 fumigation was observed to intensify micro-cracking in the pericarp [79,80].

Rotting/decay

Litchi is very susceptible to postharvest decay caused by bacteria, yeasts and fungi (Liu, 1988; Sardsud *et al*., 1994; Wang, 1998). Control measures include the use of fungicides, irradiation, heat, controlled atmospheres and biological agents (Underhill *et al*., 1997; Jiang *et al*., 2002). Aril breakdown can be retarded by cold storage and controlled atmospheres (Zhou *et al*., 1997; Liang *et al*., 1998; Han *et al*., 2002; Lin *et al*., 2002d, e). It is probably associated with the degradation of the cell wall that is associated with natural fruit senescence (Shi, 1990; Han *et al*., 2002; Lin *et al*., 2002d) or pathogens (Liang *et al*., 1998). However, the exact mecha-nism is unclear (Lin *et al*., 2002d) and the relative roles of cell-wall metabolism, fruit senescence and infection require further investigation. Rapid pericarp browning and decay of litchi fruits during storage are the main problems that result in a great loss of its market value (Li *et al*., 2006). A wide range of fungi, such as Aspergillus, Penicillium and Rhizopus, can cause decay of litchi fruits, occurring during and after harvest through skin injury, whereas Colletotrichum and Botryodiplodia infects fruits either in the field or through the cut stem end during harvest or handling (Jiang *et al*., 2002; Scott *et al*., 1982). Li *et al*. (2005) who reported that the incidence of anthracnose of the fruits was closely related to the browning of the pericarp, the infection of anthracnose fungus accelerated the pericarp browning and lesion larging. Micro-cracks observed during fruit development and caused during postharvest handling can provide a port of entry for decay pathogens that colonize the fruit surface (Sivakumar *et al*., 2005).

Prevention of pericarp browning

Numerous postharvest techniques that have been investigated to maintain quality of harvested litchi fruit includes sulphur fumigation, sulphur padding and dipping treatments. Sulphur and fungicide treatments have been adopted commercially, whereas the other approaches require further development (Jiang *et al*., 2006).

Sulphur fumigation

Sulphur dioxide (SO_2) fumigation was once considered the most effective and practical treatment to reduce browning in litchis. Sulphur dioxide inhibits non-enzymatic formation of colourless quinone-sulfite complexes and enzymatic browning by inactivation of PPO [81,82]. One of the main concerns with SO_2 fumigation is that it leaves undesirable residues [83]. A maximum residue limit of 10 mg sulphur/g aril (fresh weight) has been set in Europe, Australia and Japan [84]. Fumigated fruit absorb about 20-30% of the SO_2 applied [85]. Sulphur residues are much higher in pericarp than in aril and decrease rapidly after fumigation (Paull *et al*., 1998). Several alternative treatments to SO_2 fumigation have been proposed, for example- pre-cooling [86], low temperature storage (Liu *et al*., 2011), bio-control agents (Sivakumar *et al*., 2007) but until now no method has been established commercially.

Storage at low temperature

Litchi cv. "Bombai" can be kept better at 4°C than 0°C [87]. The temperature increment from 3-5°C to 25°C induced marked increase in activities of lipase, phospholipase D (PLD) and lipoxygenase (LOX) (Liu *et al*., 2011). Kremer-Kohne and Lonsdale (1991) found that "Mauritius" (syn. "Tai So") browned more slowly after storage at 2°C than at 6°C and Archibald and Bower (2008) [88] observed that fruit stored at 5.5°C showed a statistically superior colour than those at 1°C and remained good for up to 40 day of storage. This higher storage temperature resulted better colour retention but greater incidence of disease. For long term storage, fruit can be stored at 4 to 5°C temperature [89]. Application of low temperature acclimation delayed the decrease in anthocyanin content, increases in PPO activity and membrane leakage, changes in colour, eating quality and partially inhibited decay of fruit [90]. Lee and Wicker (1991a) studied on changes in anthocyanin during refrigerated storage and found that anthocyanin level increased from 1.68 mg/g fruit weight (FW) at harvest to 2.06 mg/g FW after 15 days storage and decreased gradually to 0.73 mg/g FW.

Packaging

Selection of appropriate packaging material and packing method are equally important. Packaging should be such that provides protection, easy to handle, attractive and economical (Kore *et al*., 2013). There has been extensive experimentation into the use of various types of packaging to extend litchi shelf life. Modified atmosphere packaging (MAP) has the advantage of low cost and easy implementation at the commercial level [91]. Although MAP has been reported to prolong postharvest quality of litchi fruit, the detailed effects of MAP on physiological and biochemical alterations during storage have not been entirely defined or explained [92].

MAP provides three advantages: i) it helps to reduce browning; ii) it controls postharvest diseases and maintains a high humidity environment for fruit inside the sealed plastic film, and iii) preventing cross-contamination during transportation and storage (Pesis *et al*., 2002; Sivakumar *et al*., 2007). Although the use of plastic bags or liners combined with low storage temperature generally provided the best extension of shelf life (Singh, 2001; Wu *et al*., 2001) [93]. Modified atmosphere storage in plastic bags and sealed containers has been reported to reduce pericarp browning in litchis [94]. However, Pesis *et al*. (2002) found that litchis packed in micro-perforated polyethylene bags had less decay, but poorer taste, than fruit in non micro-perforated bags. Chaiprasart (2005) [95] reported that MAP using PVC/ PE film wrapping is more effective for extending the shelf life by reducing weight loss and maintaining the red colour of fruit. According to Archibald and Bower (2008) the packaging method significantly reduced fruit water loss, enhancing retention of fruit colour for up to 40 day. PPO activity was higher in the rind of fruit that had turned brown and lower in fruit with good colour retention. Fruits packed in corrugated fibre boards with perforated polyethylene and Cassia fistula leaves as cushioning materials were superior in maintaining the fruit quality [96]. Ramesh and Pal (2006) [97] found that the fruits packed in CFB boxes stored well up to five days after transport compared to those packed in wooden boxes. It is also helpful for high retention of anthocyanin, low rate of respiration and ethylene evolution.

Post harvest treatments to increase shelf life in litchi

Jiang *et al*. (2003) [98] reported that the sulphur dioxide fumigation has been the most effective post harvest treatment for control of pericarp browning in litchi fruit and used extensively in commercial situations. However, sulphur treatment can cause significant weight loss and reduce the commercial value of the fruit (Kremer-Kohne and Lonsdale, 1991). Fruits are to be covered with damp paper towel to ensure minimal moisture loss (Pathak and Chakraborty, 2005). The stored fruits were in good condition for 3 days irrespective of the treatments. Potassium metabisulfite (KMS) treated litchi fruits (cv. Bombai) became unmarketable within 5 days after treatment and untreated fruits were almost marketable even on the 9th day of storage inside the ice-lined foam box (Pathak and Chakraborty, 2005). It was also found that overall acceptability and the market value was the lowest in case of fruits treated with sulphur fumigation (20 to 30 minutes in a sealed container) after 5 days of storage at 32° C. Storage life was found to be the maximum (21 days) when sulphur fumigated fruits were packed in polythene bags and stored at 14°C [99-115].

Conclusion

Different cultivars of litchi fruit were subjected to different treatments like sulphur fumigation, micro-perforated polyethylene bags, non micro-perforated bags, corrugated fibre boards with perforated polyethylene, Cassia fistula leaves, litchi leaves as cushioning materials, modified atmosphere packaging and Control (without packaging and other treatments). Treated and untreated fruits packed were stored at different temperature like ambient condition and control condition.

Application of research: The treated fruits with sulphur with packed in perforated polyethylene with CFB box maintained all the quality parameters and long durability followed by fruit packed in perforated polyethylene with CFB box such as minimum PLW loss, browning and rotting, higher TSS, acidity, ascorbic acid, sugar and anthocyanin content than other treatments in low and ambient temperature during storage.

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