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An insight into postharvest pericarp browning in litchi (*Litchi chinensis* Sonn.)

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Abstract

Litchi (Litchi chinensis Sonn.), one of the most important fruits of tropical and subtropical world is highly prized for its perfectly blended sweet-acidic juicy pulp. The attractive bright red or pink colour of litchi pericarp which determines the consumer acceptance is attributed to the presence of a class of flavonoids, anthocyanin which is lost and turns brown within 2-3 days of harvesting if stored under ambient conditions. Post-harvest browning is an important factor limiting the quality and storage life of litchi fruits and has been attributed mainly to degradation of anthocyanins, along with the oxidation of phenolic compounds present in the pericarp by enzymes like polyphenol oxidase (PPO) and/or peroxidase (POD). Since, PPO, which is the main enzyme involved in browning has a low affinity to litchi pericarp anthocyanin, it is supposed that the anthocyanins are first hydrolysed by anthocyanase, resulting in formation of anthocyanidin which in turn is oxidized by PPO and/or POD resulting in oxidative browning. The current research endeavors focuses on prolonging the shelf life of litchi fruits using different post-harvest treatments which can maintain the pericarp membrane integrity thus, avoiding loss of compartmentalization between litchi pericarp oxidase enzymes and their substrates and in turn preventing browning.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.), belonging to the family Sapindaceae, is one of the most important subtropical fruits of the world native to South-east Asia (Hwang *et al.,* 2013). Litchi fruits, characterized by attractive bright red pericarp enclosing a perfectly blended sweet-acidic juicy pulp are of high commercial value in the international trade. By far China is the largest producer of litchi in the world accounting for about 80% of the plantings worldwide. It is mainly distributed in Southeast Asian regions like China, Vietnam, Indonesia, Thailand, and Philippines, but recently its cultivation has stretched throughout the American subtropics, Burma (Mayanmar), India, Southern Hemisphere (Madagascar, Mauritius, and South Africa), Australia, Brazil, Honduras, Israel, Mexico etc. owing to its increasing demand in domestic as well as export market. One of the most important constraints limiting the exploitation of full potential of this crop is its short shelf life. Once harvested, litchi fruits are highly perishable and can lose their bright red pericarp colour rapidly, turning brown within 72 hours if stored at ambient temperatures. The attractive bright red or pink colour of litchi pericarp is attributed to the presence of a class of flavonoids, anthocyanin. In many cultivars of China and Israel, Cyanidin-3-O-rutinoside has been reported to be the

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principal anthocyanin pigment contributing about 67% to >95% of total anthocyanin. Cyn-3-O-rutinoside, Cyn-3,5-diglucoside, Cyn-3-O-glucoside and Cyn-3-O-galactoside are the major anthocyanin pigments identified from the pericarp of litchi cultivar Culcuttia with Cyn-3-O-rutinoside contributing about 71-96% of the total anthocyanin pool (Wei *et al.*, 2011). Post-harvest browning is an important factor limiting the quality and storage life of litchi fruits and has been attributed mainly to degradation of anthocyanins, along with the oxidation of phenolic compounds present in the pericarp by enzymes like polyphenol oxidase (PPO) and/or peroxidase (POD) (Zhang and Quantick, 1997). Understanding of the mechanism involved in browning and identification of the enzymes and substrates responsible for it is a paramount for undertaking efficient measures to alleviate this problem.

Enzymes involved in pericarp browning

Polyphenol oxidase

Polyphenol oxidase (PPO) also referred to as catechol oxidase, tyrosinase, catecholase or o-diphenol oxygen oxidoreductase are copper containing enzymes involved in the oxidation of phenolic compounds and degradation of anthocyanin pigments of litchi pericarp. The enzyme has an optimum pH of 6.5 with temperature optima of 70°C (Jiang *et al.,* 1997; Jiang *et al.,* 1999). PPO is the most crucial enzyme which is associated with the pericarp browning in litchi. Higher activities of this enzyme are present in the tissues like exocarp and upper-mesocarp where browning is noticed first. Presence of higher concentration of PPO in fruits more susceptible to browning as compared to those which are comparatively less susceptible also affirms its involvement in pericarp browning. PPO activity can be inhibited by antioxidants, like glutathione and L- Cysteine, and accelerated by divalent cations, such as Mn^{2+} and Ca^{2+} (Jiang et al., 1998).

Peroxidase

Peroxidase (POD) is a heme-containing glycoprotein which exhibits increased activity during browning of litchi and is highest in exocarp along with the vascular tissues of mesocarp located in the cell wall. POD from litchi pericarp is capable of oxidizing phenolic compound, 4-methylcatechol, in the presence of H_2O_2, producing brown pigment polymers (Gong and Tian, 2002). A consistent increase in the activity of POD in litchi pericarp has been reported along with skin browning index during storage suggesting its potential role in the browning process (Zhang et al., 2005).

Anthocyanase

It is important to know that PPO cannot oxidize anthocyanins directly but can oxidize anthocyanin degradation products producing brown-coloured substances. PPO yields an oxidative product, 4-methylcatechol, which can accelerate anthocyanin degradation (Jiang, 2000). Activities of enzyme anthocyanase (anthocyanin-b-glucosidase) present in litchi pericarp are high during the entire storage period when the fruits are stored at ambient temperature. This enzyme promotes loss of anthocyanins by catalyzing the hydrolysis of sugar moieties from anthocyanin and yields anthocyanidin, a degradation product that can further be oxidized by PPO or PDO (Zhang, 2001). Hence, anthocyanase seems likely to accelerate the process of litchi pericarp browning by facilitating the availability of major phenolic constituents like anthocyanins to POD or PPO for oxidation. Heat and chilling injuries enhance anthocyanase activities whilst

cool temperature and treatment with ABA, 1-MCP or HCl inhibits anthocyanase activities promoting and inhibiting the loss of anthocyanin respectively (Hu, et al., 2005).

Phenylalanine ammonialyase

Phenylalanine ammonia lyase (PAL) is the key regulatory enzyme involved in the synthesis of phenolics. It catalyzes the deamination of phenylalanine the phenylpropanoid pathway to form cinnamic acid from which different phenolic compounds such as (-) - epicatechin which is the main substrate for PPO are synthesized (Camm and Towers 1973). PAL activity largely regulates the (-)-Epicatechin content thus regulating the overall process of browning. The phenolic compounds synthesized during phenylpropanoid pathway can be further converted to other phenolic compounds via coumarate, such as flavonols, anthocyanins, chlorogenic acid and caffeic acid derivatives which serve as browning substrates in some other plant tissues

Substrates involved in litchi pericarp browning

Pigment

Anthocyanins are the pigments responsible for the red colour of litchi pericarp. Several types of anthocyanin pigments like cyanidin-3- rutinoside, cyanidin-3-glucoside, cyanidin-3-galactoside, malvidin-3-acetylglucoside, pelargonidin-3-glycoside, and quercetin-3-rutinosde have been reported from litchi pericarp out of which cyanidin-3-rutinoside is the major anthocyanin pigment present. Loss of anthocyanins results in the browning of pericarp but they cannot be oxidized directly by enzymes like PPO and POD. However being unstable in nature anthocyanins can be degraded both non-enzymatically and enzymatically. There are two possible mechanisms for the nonenzymatic degradation of anthocyanin firstly the hydrolysis of the 3-glycosidic linkages producing more labile aglucone, and hydrolytic opening of the pyrylium ring to form a substituted chalcone. Enzymatically, anthocyanins are degraded by anthocyanase to form anthocynindin which is structurally similar to catechol, a good substrate for polyphenol oxidase, thus making anthocyanidin a direct substrate for PPO oxidation or indirect substrate for POD as it can be degraded by POD if reacted together with guaiacol and H_2O_2.

Phenolic compounds

Since PPO and POD do not have affinity for anthocyanin as a substrate, there must be some non-anthocyanin substrates on which PPO and POD can act. Several phenols are present in litchi pericarp that acts as direct substrate for PPO. Monomers and dimers of flavan-3-ol are the main phenolic compounds present in litchi pericarp which comprises nearly 87.0% of its total phenolic extracts. Among these (-)-epicatechin functions as the direct substrate of PPO. The oxidation of (-) - epicatechin by PPO involves a direct contact of the substrate with the enzyme. Consistent decline in the content of most species of phenol during storage suggests that they are an important substrate for enzymes involved in litchi pericarp browning.

Membrane integrity and browning

The substrates and enzymes involved in oxidation reaction are present in different compartments of pericarp cell and their interaction with each other under normal condition is restricted by this sub-cellular compartmentalization, thus preventing browning. However, any loss in this cellular compartmentation may result in interaction between the oxidative enzymes

like PPO and their substrates leading to the browning of litchi fruit (Liu *et al*., 1991). When litchi fruits are harvested at completely matured, bright red stage, active metabolic activities continues in pericarp tissues and the integrity of membrane is maintained. Prolonged storage results in accelerated activities of enzymes like lipoxidase (LOX) and xanthine oxidase and increased accumulation of superoxide anions in pericarp while the content and activities of anti-oxidant enzymes like super-oxide dismutase and ascorbic acid start declining. This further result in increased production of free radicals and their accumulation causes severe damage to the cell membranes. Gradually the integrity of the membrane system is lost and membrane permeability that can be used as an indicator of membrane integrity, increases. Loss of membrane integrity can be attributed to membrane lipid peroxidation, as indicated by increase in malondiadehyde, peroxides contents and membrane fluidity in pericarps, which is a result of decreased ability of the cells to scavenge reactive oxygen species (ROS). Loss of membrane integrity is accompanied with the loss of compartmentation in pericarp which in turn allows various enzymes to act on their substrates eventually leading to reactions that result in pericarp browning (Jiang et al., 2004).

Postharvest management of pericarp browning in litchi

Maintenance of membrane integrity

Keeping the membrane integrity intact is crucial in order to maintain the fruit quality and shelf life of litchi fruits since disintegration of membrane integrity accelerates the formation of deleterious reactive oxygen species, thus, enhancing the rate of enzymatic browning. One of the key enzymes responsible for the disintegration of membrane integrity is Phospholipase D (PLD) which acts as a catalyst in the hydrolysis of phosphatidylcholine to form phosphatidic acid. An inhibitor or PLD like n-butanol is likely to be a potential agent in enhancing the shelf life by inhibiting membrane lipid disintegration (Motes et al., 2005). The membrane integrity achieved after treatment with n-butanol slows down the de-compartmentalization of polyphenol oxidase and its substrates in vacuolar membranes thus, preventing the contact between them. This ultimately results in reduced rate of enzymatic browning reactions. Litchi anthocyanins also exhibit excellent antioxidant activity and prevent peroxidation of membrane lipid. Exogenous ATP supply has also been reported to be helpful in inhibiting pericarp browning (Yi *et al*., 2010).

Sulphur treatment

SO_2 fumigation is considered to be one of the most effective postharvest treatments for retention of red colour of litchi fruit (Kremer-Kohne and Lonsdale, 1991). However, one of the main issues with this treatment is the bleaching action of sulphur on the pericarp surface due to the formation of a colourless anthocyanin-SO_3H complex. Sulphur residues in fumigated fruit are higher in the pericarp than in the aril, and declines gradually within few days of fumigation (Swarts, 1985.). Additionally dipping of fruits in sodium metabisulfite solution is also effective against pericarp browning but its effectiveness is comparatively less as compared to SO_2 treatment (Duvenhage, 1994). However in past few years, due to increased concern about sulphur residues in the treated fruits and its implications on human health, treatment of fruits with sulphur has been discouraged.

Acid treatment

During storage, litchi pericarp is gradually loses its red colour, probably owing to an increase in the pH of cell sap. Dipping litchi fruit in diluted HCl solution is an effective way of restoring the lost colour of litchi pericarp after fumigation using SO_2 (Duvenhage, 1994). This treatment works by stabilizing anthocyanin pigments in the litchi pericarp as a result of co-pigmentation of anthocyanins which is caused by the treatment of acids after SO_2. However acid can have an adverse effect on fruit quality and the efficacy of this treatment in combating the issue of pericarp browning highly relies on the time of treatment and the concentration of acid used. Treating fruits with 1% HCl for 6 min was reported to be able to preserve the red colour of litchi pericarp by inhibiting PPO activity and thus extend the shelf life of fruits (Jiang et al., 2004). Use of 1% HCl has been recommended for commercial application to prevent browning of frozen litchi fruit and maintaining its quality during storage and marketing (Jiang et al., 2004).

Wax coatings

Use of wax coatings is not much prevalent in litchi as its efficiency is reduced by continuous dehydration and discolouration of pericarp of litchi fruits once harvested. Chitosan, an edible wax generally used in coating of various fruits is a high molecular weight cationic polysaccharide which is soluble in dilute organic acids like 2% acetic acid; dipping litchi fruits in acidic chitosan solution inhibit PPO activity and can effectively delay pericarp browning of litchi fruits. Coating with chitosan has been reported to delay the changes in contents of substrates of enzymatic browning like anthocyanin, flavonoid, and total phenolics. It also reduces PPO activity and causes partial inhibition of POD activity (Zhang and Quantick, 1997). Use of Ascorbic acid, a potent anti-oxidant in combination of chitosan results in increased shelf life of litchi fruits owing to the reduced activities of browning enzymes (PPO and POD) along with maintenance of membrane integrity.

Packing and storage

Moisture loss or desiccation from the pericarp together with mechanical injury owing to improper handling of fruits results in development of micro-cracks on pericarp of litchi fruit which may act as entry point for microbes during storage or transportation thus leading to pericarp browning and eventually deterioration of fruit quality (Shivakumar et al., 2007). Low moisture causes skin dehydration and eventually browning of pericarp. Dehydration of pericarp can result in about 40% decrease in water content of litchi fruits within 48 hours of storage if stored at a temperature of 25°C and 60% relative humidity. This causes rapid cell damage which increases the respiration rate triggering faster deterioration of tissues. This can further result in increased contact between enzymes (PPO and POD) from damaged chloroplast, leucoplast and substrates (anthocyanins and phenols) from vacuole, accelerating the reactions of phenolic oxidation.

Prevention of dehydration is hence of paramount to preserve litchi fruit quality. Use of plastic bags or sealed containers for packing effectively reduces the rate of discolouration of pericarp. Packing litchi fruits in moisture proof, sealed polyethylene bags with subsequent storage at low temperature of 1- 3°C has been reported to keep the fruits marketable for 40 days as compared to un-bagged fruits which became unmarketable within 20 days at the same temperature (Chen et al., 1986). This can be attributed to the reduction in moisture loss and rate of

respiration of fruits stored in such conditions thus enhancing their shelf life. Controlled atmosphere (CA) storage at 4 to 6% O_2 and 6 to 8% CO_2 is found to prolong the shelf life and preserve quality of the fruit along with reducing browning in litchi cultivar 'Huai zhi' (Jiang and Fu 1999).

CONCLUSION

Pericarp browning in litchi is mainly attributed to the activities of the oxidative enzymes like PPO, POD and anthocyanase and is considered to be the main limitation to long term marketing and export of litchi fruit. Several methods have been used to overcome this problem which include exogenous treatment of harvested litchi fruits with ascorbic acid, 1-methylcyclopropene, hydrochloric acid, oxalic acid, irradiation, salicylic acid, pyrogallol, Potassium metabisulfite, apple polyphenols, tea seed oil, biocontrol bacteria, novel chitosan formulation and methionine. Since, attractive red pericarp of litchi is one of the major quality traits of litchi, pericarp browning deserves an extensive research for understanding and alleviating this problem. PPO substrates present in litchi pericarp (_)-epicatechin and procyanidin A2 possess a good antioxidant activity and can be used as potential antioxidants in litchi waste opening up a new avenue for litchi waste utilization.

REFERENCES

Camm, E. L. and Towers, G. H. N. 1973. Review article, phenylalanine ammonialyase. Phytochemistry, 12: 961–973.

Chen, F., Li, Y. B., and Chen, M. D. 1986. Production of ethylene by litchi fruits during storage and its control. Acta Hort Sin. 13: 151–156.

Duvenhage, J. A. 1994. Control of postharvest decay and browning of litchi fruit by sodium metabisulphite and low pH dips − an update. Yearbook South African Litchi Grow. Asso., 6: 36–38.

Hu, W. R., Pang, X. Q., Liu, S. Z., Zhang, Z. Q. and Ji, Z. L. 2005. Effect of postharvest handling on the anthocyanin content and anthocyanase activity in litchi pericarp. Journal of Fruit Science, 22(3): 224-228.

Hwang, J. Y., Lin, J. T., Liu, S. C., Hu, C. C., Shyu, Y. S. and Yang, D. J. 2013. Protective role of litchi (Litchi chinensis Sonn.) flower extract against cadmium- and lead-induced cytotoxicity and transforming growth factor 1-stimulated expression of smooth muscle–actin estimated with rat liver cell lines. Journal of Functional Foods, 5: 698–705.

Jiang, Y. M. 2000. Role of anthocyanins, polyphenol oxidase and phenols in lychee pericarp browning. Journal of the Science of Food and Agriculture, 80: 305-310.

Jiang, Y. M. and Fu, J. R. 1999. Postharvest browning of litchi fruit by water loss and its control by controlled atmosphere storage at high relative humidity. Lebensm.-Wiss. U.-Technol. (Food Sci. Technol.), 32: 278−283.

Jiang, Y. M., Zauberman, G. and Fuchs, Y. 1997. Partial purification and some properties of polyphenol oxidase extracted from litchi pericarp. Postharvest Biology and Technology. 10: 221-226.

Jiang, Y. M., Zauberman, G., Fuchs, Y. and Fu, J. R. 1999. Purification of polyphenol oxidase and the

browning control of litchi fruit. Journal of the Science of Food and Agriculture, 79: 950-954.

Jiang, Y. M., Zauberman, G., Fuchs, Y. and Fu, J. R. 1998. Analysis of litchi polyphenol oxidase activity under different conditions. Wuhan Journal of Botany, 16: 207-212.

Jiang, Y., Li, Y. and Li, J. 2004. Browning control, shelf life extension and quality maintenance of frozen litchi fruit by hydrochloric acid. Journal of Food Engineering, 63(2): 147-151.

Kanaho, Y., Nakai, Y., Katoh, M. and Nozawa, Y. 1993. The phosphatase inhibitor 2,3-diphosphoglycerate interferes with phospholipase D activation in rabbit peritoneal neutrophils. Journal of Biological Chemistry, 268: 12492–12497.2

Kremer-Kohne, S. and Lonsdale, J. H. 1991. Maintaining market quality of fresh litchis during storage. Part 1: Control of browning. Yearbook South African Litchi Grow. Asso., 3: 16−17.

Liu, X. S., Jiang, Y. M., Chen, F., Zhang, D. L. and Li, Y. B. 1991. The relationship between the browning in pericarp of litchi (Litchi chinensis Sonn.) fruit and polyphenol oxidase, peroxidase, phenolics and their compartmentation. Acta Botanica Austro Sinica, 7: 95-98.

Motes, C. M., Pechter, P., Yoo, C. M., Wang, Y. S., Chapman, K. D. and Blancaflor, E. B. 2005. Differential effects of two phospholipase D inhibitors 1-butanol and N acylethanolamine, on in vivo cytoskeletal organization and Arabidopsis seedling growth. Biomed. Life Science, 226: 109–123.

Peters, N. T., Logan, K. O., Miller, A. C. and Kropf, D. L., 2007. Phospholipase D signaling regulates Microtubule organization in the fucoid alga Silvetia compressa. Plant Cell Physiology, 48: 1764–1774.

Sivakumar, D., Korsten, L. and Zeeman, K. 2007. Postharvest management on quality retention of litchi during storage. Fresh Produce, 1(1): 66-75.

Swarts, D. H. 1985. Sulphur content of fumigated South African litchi fruit. Subtropica, 6: 18−20.

Wei, Y. Z., Hu, F. C., Hu, G. B., Li, X. J., Huang, X. M. and Wang, H. C. 2001. Differential expression of anthocyanin biosynthetic genes in relation to anthocyanin accumulation in the pericarp of Litchi Chinensis Sonn. PLoS One, 6 (4): e19455-10.1371/journal.pone.0019455.

Yi, C., Jiang, Y., Shi, J., Qu, H., Xue, S., Duan, X. and Prasad, N. K. 2010. ATP-regulation of antioxidant properties and phenolics in litchi fruit during browning and pathogen infection process. Food Chemistry, 118(1): 42-47.

Zhang, D. L. and Quantick, P. C. 1997. Effect of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (Litchi chinensis Sonn.) fruit. Postharvest Biology and Technology, 12: 195-202.

Zhang, Z. Q., Pang, X. Q., Ji, Z. L. and Jiang, Y. M. 2001. Role of anthocyanin degradation in litchi pericarp browning. Food Chemistry, 75: 217-221.

Zhang, Z., Pang, X., Xuewu, D., Ji, Z. and Jiang, Y. 2005. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. Food Chemistry, 90(1-2): 47-52..