Review

Postharvest physiology and technology of loquat (*Eriobotrya japonica* Lindl.) fruit

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Abstract

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical evergreen tree whose fruit is consumed both fresh and processed. Loquat fruit is a good source of minerals and carotenoids, while the kernel is rich in protein and carbohydrates. It has been considered a non-climacteric fruit, but there is evidence that some cultivars have a ripening pattern similar to that of climacteric fruits. The fruit has a short postharvest life at ambient temperatures and is susceptible to physical and mechanical damage, loss of moisture and nutrients, and decay. Low-temperature storage extends the shelf life of loquat fruit, but some cultivars are severely affected by chilling injury and flesh browning during cold storage. Purple spot, browning and leatheriness are major postharvest disorders. The shelf life of loquat can be extended by modified or controlled atmosphere storage as well as by postharvest treatment with 1-methyl cyclopropene or methyl jasmonate.
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Keywords: *Eriobotrya japonica*; postharvest; maturity; quality; diseases; shelf life; storage

INTRODUCTION

Loquat is a subtropical evergreen fruit tree native to the southeast of China and belonging to the subfamily Maloideae of the Rosaceae. Loquat is cultivated in China, Japan, India, Pakistan, Cyprus, Egypt, Greece, Israel, Italy, Spain, Tunisia, Turkey and many other countries.[1–4] Its golden fruit is round or oval in shape and has a sweet taste. The fruit typically has many seeds, like pome fruits, although seedless triploids are now being produced.[5] Although different from stone fruits, the seeds are relatively large and usually account for about 20–30% of the total fruit weight, with individual fruits containing three to five seeds on average.[6,7] Medicinal value of loquat fruit is found in traditional Chinese medicine.[8] In traditional Japanese lore, loquat seed is called 'good for health', and villagers soak the seeds in alcoholic drinks.[9] Loquat fruit is largely consumed fresh, but significant amounts are now being processed in China and Japan.[9] The fruit contain nearly all the essential nutrients, being particularly rich in minerals and carotenoids,[10] while the kernel is very rich in protein (22.5%) and carbohydrates (71.2%). Waste loquat kernels may be used as a general fermentation substrate in submerged culture for various molds.[11] In traditional medicine, loquat fruit has been considered to possess anti-inflammatory, hypoglycemic, antioxidant, antitumor, antiviral, cytotoxic, antimutagenic and hypolipidemic activities, and beneficial effects have been suggested against chronic bronchitis and nephropathy.[12]

After harvest, loquat fruits are very perishable and prone to mechanical injury and microbial decay. Chilling injury, browning and purple spot are major problems, and the fruits are susceptible to various postharvest diseases, especially following mechanical injury. The goal of this paper is to review the physiological and biochemical changes during fruit maturation and ripening, postharvest handling and technologies, and postharvest disorders and pathologies of loquat fruit.

NUTRITIVE VALUE OF LOQUAT

Loquat is popular all over the world owing to the mild, subacid and sweet taste of its fruit. In addition to vitamins and minerals, loquat fruit is rich in phenolics and carotenoids[13] and has been shown to inhibit low-density lipoproteins.[9] The bioactive components of loquat fruit include flavonoids,[14] triterpenic acids[15] and carotenoids[16] and show remarkably high scavenging activity against chemically generated radicals, thus making it effective in inhibiting oxidation of human low-density lipoproteins (Table 1).[9,17–19] The phenolic profile of loquat fruit varies with cultivar. Flavonoids are found only in the peel. 'Mizauto' has a very high content of phenolics,[20] with concentrations of phenolics and flavonoids averaging 33.6 and 24.3 mg per 100 g fresh weight (FW) respectively.[21] Ferreres *et al.*[20] studied fruits (peel and flesh) of six improved cultivars ('Mizuho', 'Néctar de Cristal', 'Mizauto', 'Mizumo', 'Centenária' and 'NE-3') of loquat for their

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Table 1.	Nutrient value of loquat fruit
Constituent	Content (per 100 g fruit)
Water (g)	86.5 – 88.2
Calories (kcal)	47 – 168
Carbohydrate (g)	9.6 – 43.3
Total dietary fiber (g)	0.8 – 1.7
Protein (g)	0.43 – 1.4
Fat (g)	0.2 – 0.7
Ash (g)	0.4 – 0.5
Calcium (mg)	16 – 70
Iron (mg)	0.28 – 1.4
Magnesium (mg)	13
Phosphorus (mg)	20 – 126
Potassium (mg)	266 – 1216
Sodium (mg)	1
Vitamin C (mg)	1.0 – 3.0
Vitamin A (IU)	1528 – 2340
Total carotenoids (µg)	196 – 3020
Carotene (µg)	559
Total phenolics (mg)	33.6
Total flavonoids (mg)	24.3

Sources: Morton,[17] Barreto *et al.*,[21] Faria *et al.*[26] and USDA.[19]

phenolic composition and found that 3- and 5-caffeoylquinic and 5-feruloylquinic acids were the major compounds, with the 'Mizauto' cultivar presenting the highest phenolic content.

Hasegawa *et al.*[22] determined the composition of five cultivars of loquat fruit and found that total soluble solids (TSS), total organic acids and total dietary fibers ranged from 4.32 to 11.48 g per 100 g, from 0.76 to 1.11 g per 100 g and from 1.19 to 1.41 g per 100 g respectively, while total lipids were less than 0.2 g per 100 g. The predominant sugars are fructose and sucrose, while the main organic acids are malic, succinic and citric acids. Recently, the physicochemical composition of loquat fruit was reported as 13.25% carbohydrates, 0.51% protein, 0.21% lipids and 0.34% ash.[23]

When fully ripe, loquat fruits are yellow to orange/red in color owing to the presence of carotenoids. Carotenoid composition has been measured by thin layer chromatography (TLC), open column chromatography (OCC), mass spectrometry (MS) and high-performance liquid chromatography (HPLC).[15,24,25] The major carotenoids are β-cryptoxanthin and β-carotene. Faria *et al.*[26] determined the carotenoid composition of five loquat cultivars ('Mizauto', 'Mizumo', 'Centenaria', 'Mizuho' and 'Néctar de Cristal') by HPLC-PAD/MS/MS (HPLC-pulsed amperometric detection coupled with tandem MS) and reported the main carotenoids to be *trans*-β-carotene (19 – 55%), *trans*-β-cryptoxanthin (18 – 28%), 5,6:5′,6′-diepoxy-β-cryptoxanthin (9 – 18%) and 5,6-epoxy-β-cryptoxanthin (7 – 10%). Total carotenoid contents ranged from 196 µg per 100 g FW ('Néctar de Cristal') to 3020 µg per 100 g FW ('Mizumo'). Cultivars 'Mizauto', 'Mizuho', 'Mizumo' and 'Centenaria' showed provitamin A values ranging between 89 and 162 µg retinol activity equivalent (RAE) per 100 g and can thus be considered a good source of this provitamin. β-carotene and lutein were found to be the major carotenoids in the flesh. Other carotenoids such as neoxanthin, violaxanthin, luteoxanthin, 9-*cis*-violaxanthin, phytoene, phytofluene and ζ-carotene were also identified. In another study, vitamin A values of loquat flesh were found to be 0.49 and 8.77 µg retinol

equivalent (RE) g^{-1} dry weight (DW) (8.46 and 136.41 µg RE g^{-1} FW) on average for white- and red-fleshed cultivars respectively.[27]

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING FRUIT RIPENING

Development of loquat fruit occurs in two phases: a growth phase characterized by the growth of the seed, and a maturation phase, the later part of which is characterized by ripening-related changes such as decreasing organic acid content, color development and softening of the pulp tissue. Sugar accumulation and a rapid increase in the fresh weight of the pulp tissue are also observed during maturation.[28] Loquat quality, including color, flavor, aroma and chemical compounds, is highly dependent on the ripening degree at harvest.[29]

Respiration and ethylene production

There is a controversial assessment concerning ethylene production *versus* fruit maturity and ripening. Some authors found ethylene production and an increase in respiration rate prior to color break,[28,30 – 33] leading Amoros *et al.*[33] to define loquat as a climacteric fruit. Hirai[28] also observed a marked increase in respiration during fruit maturation, and this observation may have contributed to the reclassification of loquat as a climacteric fruit by some authors, which is still controversial.[22] However, others reported that, although there is a well-defined peak of ethylene production, it is insufficient to define loquat as a climacteric fruit, since there is no concomitant increase in respiration rate, which often appears irregular.[31,34 – 37] Therefore it is rash to conclude that loquat is a climacteric fruit, because (1) there is no general response to ethephon to promote fruit ripening,[38,39] (2) there are discrepancies in the CO_2–C_2H_4 production relationship, despite this relationship being reported in many fruits,[28,30 – 33,40] (3) there is no starch translocation at stage II of fruit development, although the extra sugars accumulated are thought to be derived from other parts of the plant by starch hydrolysis,[28,41] and (4) increases in hemicellulase, cellulase and pectin-disrupting enzyme activities linked to ethylene production have not been confirmed.

Ethylene biosynthesis and the expression of related genes in loquat at different developmental and ripening stages were studied by Jiang *et al.*[42] Three ethylene biosynthesis-related genes, *EjACS1*, *EjACO1* and *EjACO2*, were cloned from 'Luoyangqing' loquat fruit. Real-time quantitative polymerase chain reaction (Q-PCR) analysis showed the specific expression of *EjACS1* and *EjACO1* in fruits, whereas *EjACO2* was also expressed in leaves and petals. The expression pattern of *EjACO2* was consistent with ethylene production during fruit development, which reached a peak when the fruit color was turning. *EjACS1*, *EjACO1* and *EjACO2* all showed low transcript levels throughout storage at 20 °C during postharvest ripening of loquat. Furthermore, climacteric increases in ethylene production and respiration rate were observed during the development of loquat fruit, and it is suggested that *EjACO2* might play an important role in this process.[42]

Different respiration profiles during fruit ripening were produced in five loquat cultivars ('Centenaria', 'Mizuho', 'Mizumo', 'Néctar de Crystal' and 'Mizoto'), which also differed in relation to the peak of CO_2 release. The lowest levels of CO_2 were released by 'Centenaria' ($\sim$30 mg kg^{-1} h^{-1}) throughout ripening, with virtually no change. The highest levels of respiration were recorded for 'Mizauto' and 'Mizumo', in which respiratory peaks were detected on the second day after harvest (238 and 240 mg CO_2 kg^{-1} h^{-1}

respectively), decreasing thereafter. In contrast, the respiration rate of 'Néctar de Crystal' was low (11 mg CO_2 kg^{-1} h^{-1}) on the first day after harvest.[33]

Sugars

Sugar accumulation is most rapid at the beginning of the maturation phase. Sucrose accumulates faster than any other sugar during this phase and is a major sugar in the ripe fruit. Sorbitol is a predominant component in young fruit, and its content increases during fruit development, but its percentage relative to total sugars decreases and it is only a minor component in ripe loquat fruit. Glucose and fructose contents increase with the advancement of ripening.[43] It was noted that 90% of the sugar present in the ripe fruit accumulates within 2 weeks of maturation.[28]

Glucose, fructose and sucrose are the dominant sugars in ripe loquat flesh, with sorbitol present in small quantity.[33] Galactose has also been found in small quantity.[35] However, the distribution of these sugars depends on the cultivar. Ding et al.[35] reported that the major soluble sugars and sugar alcohols in 'Mogi' loquat fruit were fructose (3.9 mg per 100 g FW), sucrose (2.4 mg per 100 g FW) and glucose (2.5 mg per 100 g FW). Hamauzu et al.[29] reported that the most dominant sugar in 'Mogi' fruit at the ripe stage was fructose, while the most dominant sugar in 'Tanaka' was sucrose. Xu and Chen[13] identified sucrose, fructose, glucose and sorbitol as major sugars in 12 cultivars of loquat fruit. The percentage of sucrose among total soluble sugars was found to be higher in white-fleshed cultivars than in orange-fleshed ones.

Ding et al.[35] observed that sucrose declined rapidly in 'Mogi' loquat fruit and that the rate of decline was greater with an increase in storage temperature, while fructose and glucose changed only slightly during storage and showed similar concentrations at different temperatures. Cao et al.[44] reported that, during cold storage at 1 °C, levels of sorbitol and sucrose in 'Dahongpao' and 'Ninghaibai' decreased with increasing storage time. During storage of 'Dahongpao' and 'Jiefangzhong', total sugar decreased gradually when the fruits were stored at 20 °C. At the beginning of storage, sucrose declined rapidly, which led to an increase in reducing sugar.[45] The activities of sucrose-metabolizing enzymes were found to be closely related to the difference in soluble sugar accumulation between these two groups.[46]

Organic acids

High fruit acidity has been a major factor lowering fruit quality and commodity value in commercial loquat production, as well as in other fruits. The predominant organic acid in unripe loquat fruit is malic acid, which accounts for 90% of all acids, and there are small amounts of citric, succinic and fumaric acids.[35,47] During ripening, the concentration of these organic acids decreases, because they are used as a source of energy for respiratory metabolism and may also be used as a source of carbon for sugar production, which contributes to the sweetness of the fruit. Chen et al.,[46] on the other hand, reported that malate and quinate are the major organic acids in the pulp of developing loquat. In addition, they also reported small quantities of isocitrate, α-ketoglutarate, fumarate, oxaloacetate, tartrate, ferulate, cis-aconitate and β-coumarate. He et al.[48] analyzed two loquat fruit cultivars ('Jiefangzhong' and 'Zaozhong 6') and detected six different organic acids. The concentrations of these organic acids in 'Jiefangzhong' were 7.435 mg malic acid, 0.583 mg lactic acid, 0.259 mg oxalic acid, 0.245 mg tartaric acid, 0.031 mg pyruvic acid and 0.686 µg fumaric acid g^{-1} FW. Among them, malic acid (~85%) was the main organic acid.

Phenolic acids

Total phenolic compounds also varied significantly during growth and maturation of loquat fruit. Neochlorogenic acid was dominant in the early stages of loquat fruit development,[47] and total phenolic compounds decreased during growth, but the concentration of chlorogenic acid increased during ripening and became predominant in ripe fruit.[35,47] It was thus suggested that a rise in chlorogenic acid concentration could be a characteristic of loquat fruit ripening.[35,47] On the other hand, during the ripening period, total phenolics and chlorogenic acid in fresh pulp of loquat fruit increased 2.2 and 8.2-fold respectively, while the concentrations of three other phenolic compounds varied little.[47] During ripening, chlorogenic acid increased from 13.7 to 52.0% of total phenolics, indicating that the chlorogenic acid increase contributed to the increase in total phenolics during fruit ripening.

Pigments

Loquat is usually divided into white flesh and orange/red flesh groups. The flesh color of the former is pale yellow or ivory white, while that of the latter is saffron yellow or orange. Unripe loquat fruits are green, and most change to orange or yellow once they ripen. The pulp of loquat fruit contains carotenoids and also vitamins B1 and B2 and nicotinamide.[35,49] Carotenoids are mainly responsible for the color of loquat fruit, the most abundant ones being β-carotene, β-cryptoxanthin, lutein, violaxanthin, α-carotene and γ-carotene.[24,50] However, the concentrations of cryptoxanthin and β-carotene were reported to increase during storage for 60 days at 20 °C and during the first 30 days of storage at 1 or 5 °C.[35] The concentration of cryptoxanthin increased from 0.7 to 1.7 mg per 100 g FW at 20 °C and from 0.7 to 0.8 and 0.9 mg per 100 g FW at 1 and 5 °C respectively, while that of β-carotene increased from 0.7 to 1.17 mg per 100 g FW at 20 °C and from 0.7 to 1.1 and 1.2 mg per 100 g FW at 1 and 5 °C respectively.

The expression of 12 carotenoid biosynthetic genes in the flesh of red-fleshed 'Luoyangging' and white-fleshed 'Baisha' loquat cultivars was compared by Fu et al.[51] Transcripts of lycopene ϵ-cyclase and violaxanthin de-epoxidase were not detected in the flesh of either cultivar. The high expression of carotenoid cleavage dioxygenase gene and the low expression of some biosynthetic genes might account for the low accumulation of carotenoids in 'Baisha' fruit.

Firmness

The firmness of loquat fruit gradually increases during storage, which is different from most other fruits. The phenomenon is accentuated when loquat fruits are stored at low temperature (8 °C).[45] Cai et al.[52] analysed the firmness of loquat flesh using a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, UK) with a probe 5 mm in diameter, a penetration depth of 4 mm and rate of penetration of 1 mm s^{-1}. They reported that the firmness of loquat ('Luoyanqing') fruit flesh increased during stored at 20 °C and that this increase was positively correlated with increase in lignin content and caused by the enhanced activities of related enzymes such as phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and peroxidase (POD).[52]

Proteins, enzymes and fruit ripening

Proteins and enzyme activities during loquat ripening were also investigated by some authors. For example, Yang et al.[53] partially isolated cDNAs encoding phosphoenolpyruvate carboxylase (PEPC), nicotinamide adenine dinucleotide phosphate malic

enzyme (NADP-ME), nicotinamide adenine dinucleotide malate dehydrogenase (cyNAD-MDH), mitochondrial nicotinamide adenine dinucleotide malate dehydrogenase (mNAD-MDH), tonoplast adenosine triphosphatase A (V-ATPase A) and tonoplast pyrophosphatase (V-PPiase) from loquat pulp. The expression of all six genes was developmentally regulated in low-acid ('Changhong 3') and high-acid ('Jiefangzhong') cultivars. NADP-ME, mNAD-MDH, V-ATPase A and V-PPiase displayed basically similar expression patterns in both cultivars, but the expression level of NADP-ME in the high-acid cultivar was higher than in the low-acid one, while the expression levels of mNAD-MDH, V-ATPase A and V-PPiase were higher in the low-acid cultivar. In contrast, the expression patterns of PEPC and cyNAD-MDH differed between the low- and high-acid cultivars.[53] Moreover, it was noted that the activities of sorbitol-6-phosphate dehydrogenase and NADP-malic enzyme were also enhanced during natural ripening of loquat.[54,55]

Jin *et al.*[56] investigated the possible association of loquat ROPs (rho-related GTPase) with flesh lignification under different temperatures. Four ROP cDNA fragments, *EjROP1.1*, *EjROP1.2*, *EjROP2* and *EjRROP3*, were isolated from 'Luoyangqing' loquat fruit, and all of them shared over 80% nucleotide identity with known ROPs from other plants. An increase in expression of *EjROP1.1*, *EjROP2* and *EjROP3* was observed during the first 4–6 days of storage at 20 °C and was positively correlated with the increase in flesh firmness.[56] The increase in firmness as a result of lignification in ripening red-fleshed 'Luoyangqing' fruit was confirmed, whereas white-fleshed 'Baisha' fruit softened without lignification. Six cDNAs associated with the lignification pathway, i.e. *EjPAL1*, *EjPAL2*, *Ej4CL* (4-coumarate:coenzyme A ligase), *EjCAD1*, *EjCAD2* (CAD) and *EjPOD* (POD), were cloned from flesh tissue of 'Luoyangqing' fruit. Expression profiles of the six corresponding genes differed greatly in different tissues and during fruit development and ripening of both 'Luoyangqing' and 'Baisha' cultivars. CAD and POD enzyme activities and the expression of *EjCAD1* and *EjPOD* genes were most closely associated temporally with lignification of loquat flesh tissue. Levels of *EjCAD1* transcripts were particularly aligned with changes in lignification during ripening as modified by either ethylene treatment or low-temperature conditioning.[57]

POSTHARVEST HANDLING AND TECHNOLOGY

Low-temperature storage

As for any other commodity, low-temperature storage is widely used to extend loquat shelf life, reduce decay and maintain quality,[58–60] and ripe fruit showed superior storage capacity compared with fruit harvested mid-ripe or over-ripe.[60] The optimal storage temperature for loquat fruit is dependent on cultivar susceptibility to chilling injury. The minimum safe temperature to avoid chilling injury ranges from 0 to 10 °C. For example, 'Jiefangzhong' is usually kept at 6–8 °C and 'Zhaozhong' at 8–10 °C,[61] while 'Wuxing' may be stored at 1 °C for 30 days.[60] Ding *et al.*[35] reported that fresh fruit quality of 'Mogi' loquat could be maintained for up to 30 days at 1 or 5 °C, with the shelf life limited by high weight loss, internal browning and breakdown of the whole fruit. The storage life was only 15 days at 10 °C and 10 days at 20 °C owing to high respiration rates of 40.0 and 15.3 mL CO_2 kg^{-1} h^{-1} respectively. The high respiration rates result in high CO_2 concentrations in the core or the pulp tissue, which may lead to breakdown in fruits stored at higher temperatures. As reported above, organic acids are major compounds of loquat, and when the storage temperature decreased, malic acid concentration was

reduced further (decreasing to 200 mg per 100 g FW at 20 °C after storage for 10 days), while fumaric acid decreased less at higher storage temperatures (decreasing to 2.9 and 2.7 mg per 100 g FW after storage at 20 and 1 °C for 10 days respectively).[35]

Modified atmospheres (MA) and controlled atmospheres (CA)

Loquat fruit responds well to MA conditions. The combination of MA and low-temperature storage could be ideal to prolong loquat shelf life and reduce fruit disorders. Modified atmosphere packaging (MAP) using films with low permeability to CO_2 under ambient conditions may lead to high-CO_2 atmospheres surrounding the fruit, causing internal browning and increased incidence of decay.[62–64] Ding *et al.*[35] showed that loquat fruits retained their initial quality and chemical components for 30 days during storage in microperforated polyethylene (PE) film packaging at 1 and 5 °C. Ding *et al.*[64] found that bagging loquats in 20 μm thick PE film at 5 °C with an in-bag atmosphere of approximately 4 kPa O_2 and 5 kPa CO_2 resulted in the highest scores for appearance and chemical compounds. Under these MA conditions, fruits showed less weight loss and retained organic acids, without any effect on sugars, and could be stored for 2 months with higher quality and minimal risk of internal browning.[64] Loquat cultivars 'Golden Nugget' and 'Sayda' were overwrapped with 12.5, 14 or 16 μm thick poly(vinyl chloride) (PVC) films and kept at 0 °C for 60 days. Overwrapping with 12.5 μm thick PVC film resulted in higher weight loss in both cultivars. Incidence of skin browning was higher in fruits overwrapped with 16 μm thick PVC film, and this browning with decay and off-flavor limited the storage life of the fruits. 'Golden Nugget' and 'Sayda' loquat fruits overwrapped with PVC films could be stored for 30 days at 0 °C.[65] However, the composition of the atmospheres inside the packages was not reported in either of the above studies. Zheng and Xi[66] evaluated the effect of MAP with different thicknesses (0.01, 0.02 and 0.03 mm) of PE films for loquat fruit storage and observed that fruit weight loss and quality deterioration were significantly inhibited by the MAP, although the resulting atmospheres were not reported. MAP using a specific film that developed an atmosphere of 4.8% CO_2 and 11.5% O_2 reduced the respiratorion rate and delayed quality deterioration of loquat.[67] However, in a study of MAP using microperforated polypropylene (PP) film, the most suitable atmosphere for loquat storage was found to be around 2–4 and 16–18 kPa for CO_2 and O_2 respectively.[68] Loquat fruits also retained higher quality at 3 °C for 40 days when Na_2CO_3 was added into 0.06 mm thick PE storage bags to decrease CO_2 accumulation.[69]

Loquat fruit in CA with 10 kPa O_2 + 1 kPa CO_2 could be stored for 50 days at 1 °C with normal flavor and low decay incidence.[60] CA containing 12 kPa CO_2 with either air or 2 kPa O_2 was reported to induce severe internal browning in loquat fruit.[64] Short-term high-O_2 (70 kPa) treatment for 24 h followed by storage in CA with 10% O_2 + 1% CO_2 at 1 °C had little effect on fruit flavor but stimulated ethanol accumulation in loquat fruit and reduced activities of endo-PG (polygalacturonase) and exo-PG. CA conditions (10% O_2 + 1% CO_2 at 1 °C) also reduced polyphenoloxidase (PPO) activity and increased POD activity but had little effect on PAL activity, leading to reduced flesh browning. Thus control of tissue browning in loquat fruit through inhibition of oxidases and mitigation of oxidative stress during low-temperature storage is important and merits being associated with the CA storage of loquat fruit.[60,70] High-O_2 atmosphere (30–80 kPa) was also reported to overcome the disadvantages of low-O_2

atmosphere (hypoxia), e.g. fermentation, off-flavors and anaerobic microorganism growth, and was confirmed to be particularly effective in inhibiting enzymatic discoloration and preventing anaerobic fermentation reactions, undesirable moisture and odor losses.[71] Exposure of loquat fruit to high O_2 ($>90\%$) significantly reduced the incidence of internal browning and inhibited PPO activity during storage at $1\,^{\circ}C$.[72]

Hypobaric storage has also been shown to influence loquat fruit quality positively. Loquat fruit stored for 49 days at 40–50 kPa pressure (equivalent to 1.1–1.4 kPa O_2) and 2–4 $^{\circ}C$ had lower decay, reduced respiration and ethylene production rates and inhibited activities of PPO and POD, resulting in higher titratable acidity and ascorbic acid content.[73] Therefore hypobaric storage also has potential to control fruit decay and flesh leatheriness development in loquat fruit stored at low temperature. It is evident that MA supplemented with low temperature helps to maintain loquat fruit quality during long-term storage and also helps to control tissue lignification, internal browning and decay.[60]

Effect of 1-methylcyclopropene (1-MCP)

Since 1-MCP is non-toxic and odorless, it is potentially of commercial value to control ethylene-dependent postharvest disorders, and postharvest 1-MCP treatment could reduce the development of chilling injury (CI) in loquat fruit.[74–76]

Loquat fruit treated with 1-MCP (50 nL L^{-1} for 24 h at 20 $^{\circ}C$ and stored at 1 $^{\circ}C$ for 35 days) exhibited a lower incidence of decay and higher levels of TSS, titratable acidity (TA), fructose, glucose, sucrose, malic and lactic acids, total phenolics and total flavonoids than control fruit during storage.[77] 1-MCP inhibited the activities of the ethylene-induced enzymes PAL, CAD, 4-coumarate:coenzyme A ligase, POD and PPO. Reduction of CI in loquat associated with 1-MCP was primarily due to inhibition of lignin accumulation, higher PG/PME (pectin methyl esterase) ratio and enhanced solubilization of pectin in the cell wall.[78]

Internal browning and flesh leatheriness are major problems of loquat fruit during cold storage. When freshly harvested loquat fruits were exposed to 50 nL L^{-1} 1-MCP for 24 h at 20 $^{\circ}C$ and then stored for 6 weeks at 5 $^{\circ}C$, internal browning was effectively controlled for up to 5 weeks.[79] The 1-MCP treatment inhibited PPO activity, which may account for the inhibited internal browning. Treatment with 1-MCP also reduced the decline in both TSS and TA contents.[79]

1-MCP treatment significantly alleviated CI in 'Fuyang' loquat fruit.[76] The treatment markedly inhibited the accumulation of malondialdehyde, superoxide radicals and hydrogen peroxide (H_2O_2) and the increase in electrolyte leakage. 1-MCP-treated fruit exhibited significantly higher catalase (CAT) activity and lower lipoxygenase (LOX) and phospholipase C (PLC) activities than control fruit during storage.[76] Modifications of fatty acid and cell wall polysaccharide composition are associated with CI development in loquat, and 1-MCP treatment modulated the changes that seem to regulate the strength of the cell wall and so alleviate CI.[75]

Chitosan coating

Chitosan coating extends the postharvest life of loquat fruit.[80] When loquats were treated with 0, 0.25, 0.5, 0.75 and 1% (w/v) chitosan solutions and stored at 7 $^{\circ}C$ and 88 ± 2% relative humidity (RH) for 28 days, the chitosan coating significantly reduced weight loss and suppressed browning compared with the control, with the most effective chitosan concentration being 0.75%. Chitosan coating also induced total polyphenols and phenolic compounds such as catechin and quercetin and maintained antioxidant capacity in the fruit during cold storage. Among chitosan (0.6%) and sucroester fatty acid (1%) coatings, chitosan was found to be more efficient in reducing weight loss, respiration rate and ethylene production, although both substances contributed to maintain flesh firmness, organoleptic characteristics and fruit presentation.[81]

Sulfur treatment

Zheng et al.[82] found that treatment of loquat fruit with 2–4 g kg^{-1} SO_2 releaser (SO_2-releasing pad) significantly inhibited the incidence of decay and browning of internal tissue. TSS and TA contents were found to be higher than in untreated fruit. Zheng et al.[83] observed that treatment with SO_2 retarded the decrease in TA and percentage juice and retained acceptable fruit quality after 35 days of storage at 1 $^{\circ}C$.

Heat treatment

Pre-storage heat treatment with hot air at 38 $^{\circ}C$ for 5 h was effective in reducing CI symptoms, including internal browning, in loquat fruit.[84] Heat treatment delayed the occurrence of internal browning and inhibited the increase in internal browning index in 'Jiefangzhong' loquat fruit. Heat treatment also maintained lower levels of electrolyte leakage and malondialdehyde content, inhibited the increases in palmitic, stearic and oleic acid levels and delayed the decreases in linoleic and linolenic acid contents, thus maintaining higher unsaturated/saturated fatty acid ratio than the control, resulting in the reuction of internal browning and CI.[85]

Calcium treatment

Dipping loquat fruits in an aqueous solution of 1% $CaCl_2$ did not significantly affect any quality parameters, while loquats treated with 2% $CaCl_2$ had higher firmness and relative electrical conductivity after storage for 10 weeks at 4 $^{\circ}C$. Dipping fruits in 3% $CaCl_2$ retained maximum TSS and firmness and also delayed internal browning and weight loss for up to 4–5 weeks at 4 $^{\circ}C$.[86]

Effect of nitrogen treatment

Loquat fruits exposed to temporary anoxia (100% N_2) for 6 h at 20 $^{\circ}C$ and then stored in air at 5 $^{\circ}C$ for 35 days showed a significant delay in the increase in fruit decay rate and decreased TSS and TA, thereby maintaining better eating quality and extended storage life. Short-term N_2 treatment also markedly delayed the increases in membrane permeability, malondialdehyde content and superoxide anion production rate. N_2-treated fruits exhibited significantly higher superoxide dismutase (SOD) and CAT activities and lower LOX activity than control fruits, resulting in the reduction of CI.[87]

PHYSIOLOGICAL DISORDERS

Chilling injury (CI)

Loquat fruits are perishable and their postharvest life is approximately 10 days at 20–25 $^{\circ}C$ because of microbial decay following mechanical damage, as well as moisture and nutritional losses. Low-temperature storage is commonly used for loquat to inhibit decay and extend postharvest life. However, red-fleshed 'Luoyangqing' loquat fruit is prone to CI, including stuck peel, firm and juiceless texture (flesh leatheriness) and internal browning, while white-fleshed 'Baisha' loquat fruit is resistant to CI.[88] Fruits

of the red-fleshed cultivar 'Luoyangqing' stored at 5 °C retained acceptable quality for up to 39 days, while fruits stored at 0 °C developed CI after 4 days. The symptoms were tissue browning and lignification, a decrease in juice content and increases in superoxide free radical production, electrical conductivity and activities of lignification enzymes, including PAL, CAD and G-POD (guaiacol peroxidase). These CI symptoms became more severe after the fruits were moved to 20 °C.[89]

The role of ethylene signaling in the development of CI was investigated in fruits of both chilling-sensitive red-fleshed ('Luoyangqing') and chilling-tolerant white-fleshed ('Baisha') cultivars. Three ethylene receptor genes, one *CTR1*-like gene and one *EIN3*-like gene were isolated and characterized in ripening fruit. All of these genes were expressed differentially within and between fruits of the two cultivars. Transcripts either declined over fruit development (*EjERS1a* in both cultivars and *EjEIL1* in 'Luoyangqing') or showed an increase in the middle stages of fruit development before declining (*EjETR1*, *EjERS1b* and *EjCTR1* in both cultivars and *EjEIL1* in 'Baisha'). The main cultivar differences were in levels rather than in patterns of expression during postharvest storage. The *EjETR1*, *EjCTR1* and *EjEIL1* genes showed increased expression in response to low temperature, and this was particularly notable for *EjETR1* and *EjEIL1* during CI development in 'Luoyangqing' fruit. The genes were also differentially responsive to ethylene treatment, 1-MCP and low-temperature conditioning, confirming a role for ethylene in the regulation of CI in loquat fruit.[90]

Oxidative stress and a decrease in lipid unsaturation might be involved in the development of CI in red-fleshed loquat fruit.[75,76] There were no CI symptoms in the fruit of 'Qingzhong' cultivar during storage for 35 days, whereas CI in 'Fuyang' fruit increased sharply after 21 days at 1 °C. Chilling-resistant 'Qingzhong' fruit had lower levels of superoxide radical and H_2O_2, in addition to lower LOX activity, but higher membrane lipid unsaturation and higher activities of SOD and CAT than chilling-susceptible 'Fuyang' fruit. 'Qingzhong' fruit also showed higher activities of antioxidant enzymes involved in the ascorbate/glutathione cycle and higher levels of ascorbic acid and reduced glutathione. Development of CI in loquat fruit was associated with a reduction in unsaturated/saturated fatty acid ratio, and postharvest methyl jasmonate (MeJA) or 1-MCP treatment delayed the decrease in this ratio, thereby increasing chilling resistance.[75,76] Therefore the higher membrane lipid unsaturation and the more efficient antioxidant system were both suggested to be beneficial in enhancing the resistance of loquat fruit to CI.[91]

Various treatments such as low-temperature conditioning and the application of polyamines, salicylic acid or 1-MCP can reduce chilling-related disorders in loquat.[45,74,89,92,93] Benzothiadiazole (BI) was reported to activate systemic acquired resistance (SAR), reduce CI and maintain fruit quality of loquat stored at low temperature.[42] Postharvest application of 1 mmol L^{-1} aqueous acetylsalicylic acid to loquat fruit significantly alleviated CI symptoms, inhibited the accumulation of superoxide free radical and reduced PAL, CAD and G-POD activities. Acetylsalicylic acid treatment impairing the accumulation of superoxide free radical may prevent CI and lignification in loquat.[89]

Low temperature (1 °C) triggered a marked increase in endogenous nitric oxide (NO) levels in loquat fruit during postharvest storage. Pretreatment of fruit with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) not only abolished endogenous NO accumulation but also aggravated CI symptoms in fruit stored at 1 °C and 95% RH. Moreover, the cold-stored fruit in which NO accumulation was abolished by cPTIO exhibited significantly higher membrane permeability, lipid peroxidation, superoxide anion (O_2^-) production rate and H_2O_2 content than the control fruit. Abolition of endogenous NO accumulation significantly reduced activities of SOD, CAT, ascorbate peroxidase (APX) and POD in the fruit during cold storage. Cold-induced endogenous NO generation in loquat fruit during postharvest storage plays a critical role in alleviating CI symptoms by affecting the antioxidative defense systems in the fruit.[94]

Application of MeJA to loquat fruit inhibited the incidence of CI manifested as internal browning and increased ascorbic acid and reduced glutathione contents owing to the inhibition of ascorbate oxidase activity and the enhancement of monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase activities. MeJA also enhanced activities of APX, glutathione peroxidase and glutathione-*S*-transferase. MeJA can regulate the ascorbate and glutathione metabolism and has important roles in alleviating oxidative damage and enhancing chilling tolerance in loquat fruit.[95,96] The MeJA-treated fruit (10 μmol L^{-1} and stored at 1 °C for 35 days) exhibited lower levels of respiration rate, ethylene production and PAL and PPO activities and higher levels of sugars, organic acids, total phenolics and total flavonoids than the control fruit. The treatment also maintained higher antioxidant activity as measured by the scavenging capacity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide and hydroxyl radicals.[97] MeJA treatment at 10 μmol L^{-1} was effective in alleviating CI of cold-stored loquat fruit,[95] and the reduction of CI by MeJA might be due to enhanced antioxidant enzyme activity and higher unsaturated/saturated fatty acid ratio.[97] Cao *et al.*[98] found that MeJA could also increase proline and γ-aminobutyric acid contents, which was associated with the induced chilling tolerance.

Lignin contributes to the development of CI in cold-stored loquat fruit, and its accumulation confers high rigidity and compression resistance to the cell wall.[45] Shan *et al.*[57] suggested that the different patterns of lignin accumulation in red- and white-fleshed loquat cultivars is related to the development of CI. The involvement of lignin synthesis in CI and its related enzymes were analyzed.[99] It was observed that MeJA treatment modified the time course of this response and reduced its magnitude. In all cases of lignin accumulation, increased activities of PAL, POD and PPO were detected.[99] The activation of these three enzymes in loquat fruit has been reported in response to chilling stress.[57] These studies suggested that the effect of MeJA in reducing the development of CI was correlated with the inhibited activities of the enzymes involved in lignin synthesis.

Treatments such as acetylsalicylic acid and 1-MCP that reduce CI have been shown to decrease PAL, POD and PPO activities,[89,93,99] and 1-MCP treatment also significantly alleviated CI in 'Fuyang' loquat fruit. The treatment markedly inhibited the accumulation of malondialdehyde, superoxide radicals and H_2O_2 and the increase in electrolyte leakage. 1-MCP-treated fruit exhibited significantly higher CAT activity and lower LOX and PLC activities than control fruit during storage. This inhibition of LOX and PLC activities and alleviation of oxidative damage by 1-MCP reduces the CI buy slowing down the internal browning process.[76] The CI symptoms were reduced by 1-MCP treatment (2.32 nmol L^{-1}). 1-MCP treated fruit exhibited higher levels of linoleic and linolenic acids and higher unsaturated/saturated fatty acid ratio than control fruit during storage. Modification of fatty acid and cell wall polysaccharide composition was associated with CI development in loquat, and

1-MCP treatment modulates the changes that seem to regulate the lignification of the cell wall and therefore alleviate CI.[75]

Purple spot

Loquat fruit is highly sensitive to purple spot, a physiological disorder that affects crops in Taiwan,[100] Brazil[101] and Spain.[102] In Spain, 15% of loquat fruits are damaged, decreasing the commercial value by 40–50%. This disorder appears at fruit color break and mainly affects early cultivars and the earliest orchards, when fruits have the highest commercial value.[38,103] The origin of purple spot of loquat fruit is related to an alteration in the water relationship between the flesh and the rind caused by the simultaneous occurrence at fruit color break of a period of high sugar accumulation in the flesh in addition to a high fruit growth rate. The dehydration process is enhanced by cultivation practices (thinning intensity) and environmental factors (low temperature and sunlight exposure) that affect sugar and mineral assimilation and partitioning in favor of the flesh, increasing the gradient of solute concentration between flesh and rind tissues.[104]

Purple spot is characterized by an extensive area of slightly depressed and irregularly shaped surface with purple color that affects up to 30% of the exposed surface of the fruit. The disorder only affects the epidermal tissue,[102] and localized fruit Ca deficiency is the most accepted cause of the damage.[102,104] The Ca concentration decreases from 2.01 to 0.57% during loquat fruit development.[102] On the other hand, there is evidence that sudden changes in fruit water potential components at color break might cause epidermal cell dehydration, which in turn might be responsible for the purple spot.[38] Purple spot initially appears at the deepest rind cell layers as a fringe of compact and empty cells. As the purple spot intensity increases, the number of affected cells also increases to include all rind tissue. The cuticle of affected fruit shows no sign of damage, and the water permeability of isolated cuticles does not show any consistent differences between injured and healthy fruits. In epidermal tissue, concentrations of K, Fe and Cu were higher in affected fruit than in healthy fruit.[38] Fruit thinning increased the sugar content, and the total sugar concentration was significantly positively correlated with the proportion of purple spot in the affected fruit.[105]

The highest incidence of purple spot occurs at the beginning of harvest time, affecting 25–30% of fruits, followed by a sudden decrease to about 5–10%. Early-maturing orchards had higher purple spot incidence at the beginning of harvest time (60%) than late-maturing orchards (<30%).[106,107] Low day temperature at fruit color break was the main environmental factor. Heating plants in a greenhouse during the night greatly reduced the incidence of the disorder. Sunshine also affects purple spot occurrence. The amount of affected fruit was significantly reduced or even nullified by keeping direct sunlight off the fruit. Fruit thinning, cool nights and exposure to the sun modify the flesh/rind sugar partitioning in favor of the flesh, increase the gradient of total sugar concentrations between the two tissues and increase the incidence of purple spot.[38]

Calcium nitrate, calcium chloride, Ca-EDTA, ammonium nitrate and potassium nitrate at a concentration of 150 mmol L^{-1} applied 2 weeks before fruit color break cause a reduction of water potential in the epidermal tissue that allows it to retain water, resulting in a significantly reduced incidence of purple-spotted fruit. The most favorable date of treatment was during the 2 weeks prior to fruit color break.[108]

In flesh tissue, the K concentration significantly increased and the Fe concentration significantly decreased at color break in response to thinning. In rind tissue, N, K, Mg and Fe concentrations diminished at color break, depending on the thinning intensity, down to 23, 21, 27 and 41% respectively, for one fruit per panicle treatment. Changes in the mineral composition of individual fruits caused by thinning significantly increased the gradients of concentrations of N, K, Ca and Mg between rind and flesh tissues. This increase in mineral gradients was positively and significantly correlated with the percentage of purple spot.[109]

Flesh browning

Flesh browning is a serious problem for postharvest storage and processing of loquat fruit. The fruit turns brown rapidly when peeled or crushed. During storage, fruit browning occurs from the core area and is accompanied by lignification of the flesh tissue.[35] Browning is mainly caused by enzymatic oxidation of endogenous polyphenols into quinones, which are then polymerized with other quinones and amines to form brown pigments.[110] Polyphenolic compounds and PPO are considered to be directly responsible for the enzymatic browning.[110] The main phenolic compounds in ripe loquat fruit are chlorogenic acid, neochlorogenic acid, hydroxybenzoic acid and 5-feruoylquinic acid.[64] Sulfites, ascorbic acid and its derivatives, and cysteine have been shown to prevent flesh browning in loquat fruit.[110]

CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

Loquat fruit ripening has been studied mainly on the basis of biochemical changes and to a lesser extent on the basis of enzymatic changes. The roles of some enzymes such as cell wall hydrolases (polygalacturonase, pectin esterase and β-galactosidase), pathogenesis related (PR)-related enzymes (CAT, SOD and PAL) and antioxidant enzymes (CAT and SOD) have been described during the softening of harvested fruits; however, these enzymes may not be the sole determinants of fruit ripening. Therefore genetic control factors, studies on chilling-associated expansin genes, proteomics and other molecular approaches will be helpful in investigating the mechanisms of ripening, senescence and resistance to CI in loquat fruit. Moreover, proteomics approaches have been used to study the mechanisms of ripening regulation and resistance to CI in some temperate and tropical fruits.

Global marketing of loquats is limited by their relatively short postharvest life even under optimal temperature (0–5 °C, depending on cultivar) and RH (90–95%) and by the variability in flavor quality and consumer acceptance among cultivars. MAP has shown interesting results when combined with low temperature and adequate humidity for good postharvest handling and storage of loquat fruit. However, little is known about the real sensitivity of loquat fruit to CI. Cold storage is effective in prolonging loquat shelf life, but it can increase flesh leatheriness incidence, which is the major problem of postharvest loquat fruit. In addition, chilling susceptibility differs among the different cultivars at low temperatures. Therefore the degree of chilling susceptibility and minimum safe temperature for each cultivar should be determined and strategies to bring to market the highest-quality fruits should be pursued. Meanwhile, more research is needed to elucidate physiological, biochemical and molecular responses of loquat to flesh leatheriness and on effective techniques for storage and transportation. However, for a long-term solution to the problem, basic research to understand the genetic and biochemical basis of

genetic control by using available molecular genetic technologies is also needed.

On the other hand, the use of protective packaging has also shown interesting results, and appropriate packaging significantly reduces bruising and associated browning of loquat fruit. Chemicals can also be used to extend the shelf life of loquat fruit; however, they need to be approved either by regulatory agencies or national authorities before their commercial application, although the use of chemicals is not well accepted by consumers.

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