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Evolution of phenolics and polyphenoloxidase isoenzymes in relation to physical-chemical parameters during loquat (*Eriobotrya japonica* cv. *Algerie*) fruit development and ripening

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SUMMARY – Polyphenoloxidase (PPO) and phenolics are directly responsible for the enzymatic browning. In intact cells PPO and its phenolic substrates are physically separated in chloroplasts and vacuoles respectively thus precluding oxidation of phenolics and thus enzymatic browning. Cell disruption by injury or cell disassembly during ripening brings PPO into contact with phenolics and enzymatic browning occurs. In this work, we have monitored the co-evolution of PPO isoenzyme levels and phenolics during loquat cv. *Algerie* development and ripening, as well as fruit juice physical-chemical parameters (pH, sugars as total soluble solids, acidity and conductivity) which evolve according to the ripening stage. The onset of ripening is accompanied by a drastic increase of juice pH (0.4 units), sugars (from 3.5% to 4.5%) and a drastic decrease of acidity (from 1.4% to 0.7% of malic acid) while conductivity decreased uniformly during fruit development and ripening. Changes in PPO resemble those of pH, sugars and acidity as judged by drastic changes at onset of ripening, while phenolics increased uniformly during fruit development and ripening.

Key words: Polyphenol oxidase, enzymatic browning, phenolics, loquat.

RESUME – "Evolution des phénols et isoenzymes polyphénoloxidasiques en liaison avec les paramètres physico-chimiques pendant le développement et la maturation des nêfles (*Eriobotrya japonica* cv. *Algérie*)". La polyphénoloxydase (PPO) et les phénols sont directement responsables de la noircissure enzymatique. Dans les cellules intactes les PPO et leurs substrats phénoliques sont physiquement séparés dans les chloroplastes et vacuoles, excluant ainsi l'oxydation des phénols et la noircissure enzymatique. La rupture de la cellule par défaut ou cellule dissociée pendant la maturation fait entrer en contact avec les phénols et la noircissure enzymatique a lieu. Dans ce travail, nous avons dirigé l'évolution conjointe au niveau des isoenzymes PPO et des phénols pendant le développement et la maturation des nêfles cv. *Algérie*, ainsi que les paramètres physiques et chimiques du jus de fruit (pH, sucres ainsi que solides solubles totaux, acidité et conductivité) qui évoluent pendant l'étape de maturation. Le début de la maturité est accompagné d'une augmentation brutale du pH du jus de fruit (0,4 unités), des sucres (de 3,5 à 4,5%) et d'une brutale baisse de l'acidité (de 1,4 à 0,7% d'acide malique) alors que la conductivité diminue uniformément pendant la croissance et la maturation du fruit. Les changements des PPO ressemblent à ceux du pH, les sucres et l'acidité sont calculés avec des changements brutaux au début de la maturité alors que les phénols augmentent uniformément pendant la maturation et la croissance du fruit.

Mots-clés : Polyphénoloxydase, noircissure enzymatique, phénols, nêfle.

Introduction

Polyphenoloxidase (PPO) and phenolics are directly responsible for the enzymatic browning in fruits and vegetables (Mayer and Harel, 1979). In intact plant cells PPO and its phenolic substrates are physically separated in chloroplasts and the vacuole respectively thus precluding the oxidation of phenolics and eventually the enzymatic browning. Cell disruption by injury or cell disassembly during ripening brings into contact PPO with phenolics and then the enzymatic browning occurs. In loquat fruit it is manifested as brown discoloured areas in the flesh and the epidermis.

The levels of PPO and phenolics may change during the fruit development and ripening that may influence the potential damage that loquat fruit may undergo by enzymatic browning (Vamos and

Nadudvari, 1982; Sánchez-Ferrer, 1989a,b). Ding *et al.* (1998) studied the coevolution of loquat phenolics and PPO of the Mogi cultivar and showed a sharp decrease of both parameters during fruit development and ripening.

In this work we have monitored the co-evolution of PPO isoenzyme levels and phenolics during loquat cv. Algeria development and ripening, as well as fruit juice physical-chemical parameters (pH, sugars, titrable acidity and electrical conductivity) which evolve according to ripening stage. Results are compared and discussed with reference to Mogi variety (Ding *et al.*, 1998).

Materials and methods

Biological materials

Loquat fruits (*Eriobotrya japonica* cv. Algeria) from trees cropped in the experimental orchards of Cooperativa Agrícola de Callosa d'En Sarriá were picked weekly from March 25th to April 26th of 2000.

Analytical procedures

Loquat flesh was homogenized in 50 mM phosphate buffer containing 10 mM ascorbic acid and filtered through eight layers of gauze. The filtrate was centrifuged at 5000 g for 15 minutes. The clear supernatant was used for soluble isoenzyme polyphenol oxidase activity and total phenols determination. The pellet was treated with the detergent Triton X-114 to extract the particulate isoenzyme polyphenol oxidase (Bru *et al.*, 1995). Polyphenol oxidase activity was determined using tert-butyl catechol as a substrate by following the appearance of yellow-coloured tert-butyl quinone which has an absorption band at 390 nm (Bru *et al.*, 1990). Total phenols were determined according to the colorimetric method of Singleton and Rossi (1965).

For the determination of physical-chemical parameters, the loquat flesh was homogenized with water and filtered through eight layers of gauze. The filtrate was used for determination of pH, electrical conductivity, titrable acidity as % malic acid and total sugars by refractometry.

Results and discussion

As shown in Fig. 1, the loquat development and ripening is accompanied by changes in the fruit juice physical-chemical characteristics as represented by pH, titrable acidity, electrical conductivity and total sugars. All of these parameters with the exception of conductivity inverted their trend at the moment of colour break, when the fruit color changes from green to the typical orange-yellow of the ripe fruit. The onset of ripening is accompanied by a drastic increase of juice pH (0.4 units) and sugars (from 3.5% to 4.5%) and a drastic decrease of acidity (from 1.4% to 0.7% of malic acid) while conductivity decreased uniformly during fruit development and ripening. The evolution of these parameters is similar to that found in variety Mogi (Ding *et al.*, 1998).

PPO activity is present in two fractions of the loquat flesh filtrate: a soluble fraction and a particulate fraction that can be recovered as a supernatant and pellet after centrifugation respectively. The soluble isoenzyme can be determined directly from the supernatant but to further extract the particulate PPO it is necessary to use detergents (Sánchez-Ferrer *et al.*, 1989a; Bru *et al.*, 1995) thus meaning this isoenzyme is associated with membranes.

The soluble PPO isoenzyme displays almost the same level of activity when determined at pH 7.0 or 4.5 while the particulate isoenzyme displays very low activity levels at pH 7.0. Many PPOs have been described to be latent at neutral pH and that the addition of ionic detergents such as SDS to the reaction medium fully activates the enzyme (Sánchez-Ferrer *et al.*, 1989b). As shown in Fig. 2, this was the case with the particulate isoenzyme but SDS did not cause a further increase in activity of the soluble isoenzyme at pH 7.0.

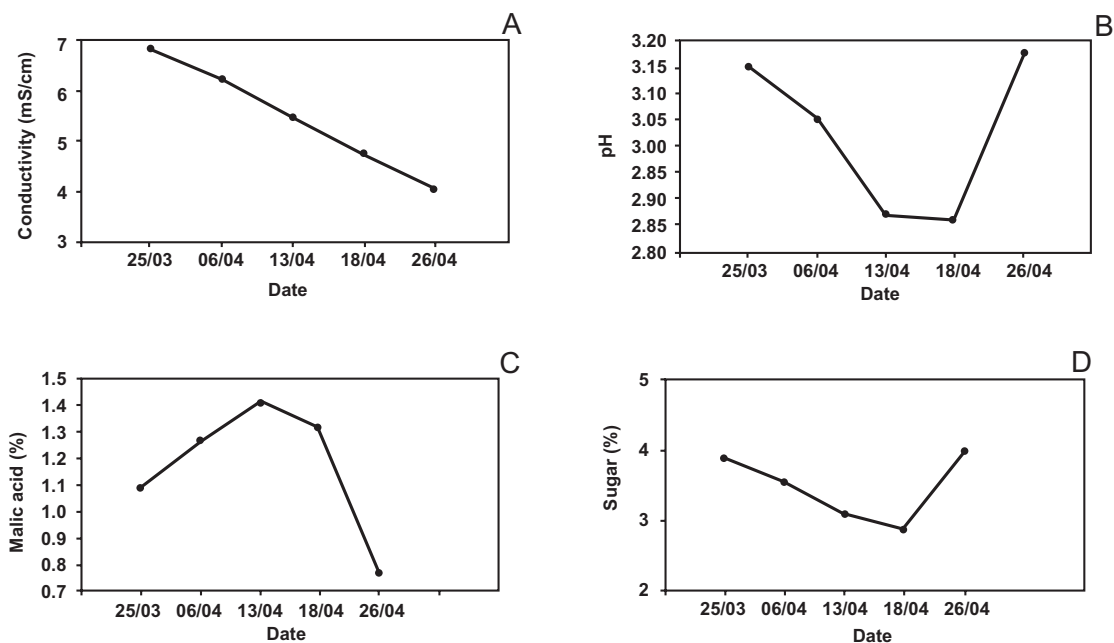


Fig. 1. Evolution of loquat fruit juice physical-chemical parameters during fruit development and ripening up to harvest. (A) electrical conductivity, (B) pH, (C) titrable acidity expressed as % malic acid, (D) total sugars as determined by refractometry.

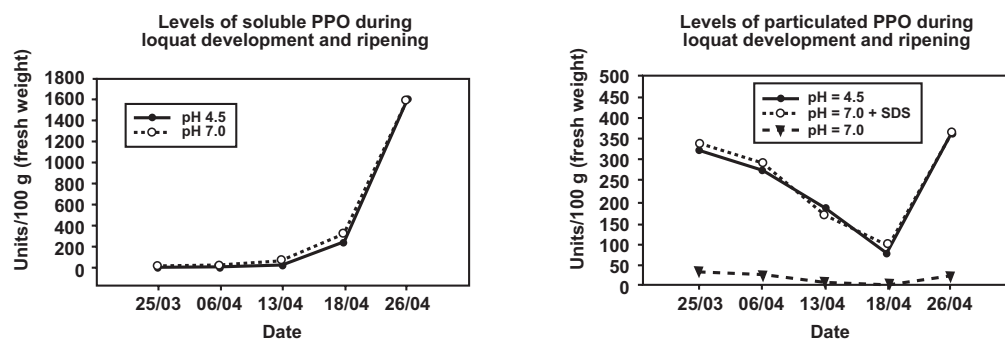


Fig. 2. Evolution of loquat flesh PPO isoenzymes during fruit development and ripening. The PPO activity for each fraction has been determined at pH 4.5 and 7.0, in the absence or presence of the detergent SDS. Such detergent had activating effect only on the particulate isoenzyme at pH 7.0.

Figure 2 also show the evolution of soluble and particulate PPO isoenzymes with loquat development and ripening up to the harvest date. Before colour break levels of soluble PPO are very low and increase dramatically after fruit set, reaching very high levels. The level of the particulate PPO is moderate in the green unripe fruit and decreases as the fruit develops. Minimal levels are found at colour break and then they undergo a sharp increase to restore the initial levels around the harvest date.

Figure 3 shows that total phenolics accumulate steeply along the development and ripening, increasing their concentration 5-fold during the last month of fruit development. According to these results, the highest levels of PPO and phenolics together occur in the moment of harvest. This coincidence makes the fruits more susceptible to enzymatic browning.

These results contrast with those obtained for the variety Mogi (Ding *et al.*, 1998) in several aspects. First, PPO activity was measured as total, not in different fractions; second, PPO activity decreased as the Mogi fruit developed, being lowest at harvest; and third, levels of total phenolics decreased having a minimum at colour break. Thus, since the evolution of phenols and PPO depends on the loquat variety, it is expected that their levels at harvest are directly related to the susceptibility of the variety to bruising.

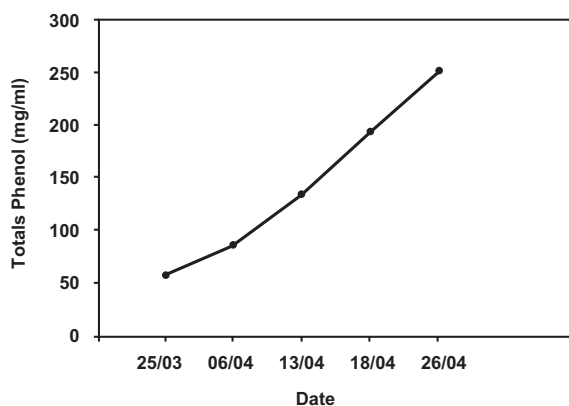


Fig. 3. Evolution of total phenolics in loquat flesh during fruit development and ripening.

Conclusions

The levels of PPO in loquat cv. Algeria undergo drastic changes at the onset ripening as judged by ripening-dependent parameters such as juice pH, acidity and sugar content. The levels of PPO and phenolics are the highest at harvest time in the Algeria variety, in contrast to Mogi (Ding *et al.*, 1998), that may have a direct effect on susceptibility to bruising.

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