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Fruit sampling methods to quantify calcium and magnesium contents to predict bitter pit development in 'Fuji' apple: A multivariate approach

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ABSTRACT

Bitter pit (BP) is a physiological disorder in apple commonly associated with high Mg/Ca ratio in fruit tissue. This work was carried out to identify the best fruit tissue sampling method for Ca and Mg assessment to discriminate 'Fuji' apples regarding the occurrence of BP. After six months under controlled atmosphere storage (2.0 kPa O_2 + 0.5 kPa CO_2, at 0.5 °C/90–95% RH), fruit without or with BP were submitted to Ca and Mg analysis (mg kg^{-1} of fresh weight) in the total fraction (TF) of peel + flesh, and in the TF and soluble fraction (SF) of flesh or peel tissues. For the peel + flesh, a wedge-shaped segment was cut longitudinally from the fruit (with 1 cm wide at the equatorial region), discarding the core tissue. For individual sampling of the peel (thickness of 0–2 mm) or flesh (thickness of 2–8 mm), the fruit were cut along the equatorial region, so that only the distal end was used. In this portion of the fruit, Ca and Mg contents were higher in the peel than in the flesh in the TF, and lower in the peel than in the flesh in the SF, while the Mg/Ca ratios in TF and SF fractions were higher in the flesh than in the peel, regardless the occurrence of BP. Calcium and Mg contents in the SF relative to the TF were very low in both peel and flesh tissues. Calcium in the SF represented 0.36% and 2.79% of its content in the TF for peel and flesh, respectively. Magnesium content in the SF represented 0.70% and 3.74% of that in the TF for peel and flesh, respectively. Fruit without BP showed higher Ca content and lower Mg/Ca ratio in the TF of peel + flesh, peel or flesh, and in the SF of peel or flesh, compared to fruit with BP. Fruit without BP also showed a higher percentage of Ca in the SF in relation to TF in the peel (0.42%) compared to fruit with BP (0.31%). Fruit without BP also showed lower Mg content in TF of peel + flesh and peel, and in the SF of the flesh. There was no difference between fruit with and without BP only for Mg contents in TF of the flesh and SF of the peel. The canonical discriminant analysis showed that the Mg/Ca ratio in the SF of the peel tissue at the distal end of the fruit provides the best method to discriminate fruit without and with BP, which can be potentially used as a tool to predict BP development in 'Fuji' apples.

1. Introduction

Bitter pit (BP) is a physiological disorder in apple fruit that occurs mainly during storage, but it can also develop before harvesting in severe cases. This disorder is characterized by the collapse of flesh cells just below the peel, causing slight darkened depressions on fruit surface, mainly at the distal end. Apples with low calcium (Ca) and high magnesium (Mg) contents in their flesh have a high risk of manifesting BP (Ferguson and Watkins, 1989; Saure, 2005; Amarante et al., 2006b, 2009). In Brazil, the occurrence of BP has been reported to cause losses up to 30%, in cultivars susceptible to this disorder and in fruit harvested from orchards with high risk and/or in growing seasons that predispose the manifestation of BP (Miqueloto et al., 2011).

Calcium plays an important role in cell wall structure and stability. It binds to carboxyl groups of galacturonic acid chains on pectins, aiding to preserve flesh firmness during storage (Ferguson and Watkins, 1989). In addition, Ca contributes to cellular

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membrane structure and function by binding to phospholipids and proteins at the membrane surface (Hanson, 1960; Clarkson and Hanson, 1980; Hirschi, 2004). Calcium binding to the membrane also delays phospholipid and monogalactosyldiacylglycerol catabolism, which preserves membrane integrity by reducing senescence-related membrane lipid changes, and by increasing membrane restructuring processes (Picchioni et al., 1996, 1998). The maintenance of proper membrane structure and function has been reported to be dependent on apoplastic pool of free Ca^{2+} (Hanson, 1960; Freitas et al., 2011). Therefore, Ca depletion in the apoplast, which can weaken plasma membrane structures, might lead to cell death and the manifestation of BP symptoms (Freitas et al., 2010, 2011).

Calcium concentration in the fruit alone may not be informative in relation to BP development. It is believed that a high Mg content exacerbates the potential for apple fruit to initiate the chain of reactions involved in expressing BP (Burmeister and Dilley, 1994; Amarante et al., 2009). Due to the ionic similarity between Ca and Mg, these two minerals have strong competition in various cellular processes, such as at activation sites of enzymes and binding on phosphorylated cell membranes (White and Broadley, 2003). Although there are ionic similarities between these ions, Mg cannot replace the role of Ca in cellular processes, and competition between these ions for binding sites may explain the occurrence of physiological disorders related to Ca deficiency in response to high Mg/Ca ratios in fruit tissues (Freitas et al., 2010). Thus, the quantification of Ca and Mg contents in fruits represents an important approach to predict BP development in apple fruit (Amarante et al., 2009).

The Ca and Mg concentrations vary according to the fruit tissue (Amarante et al., 2006a,b, 2011; Miqueloto et al., 2011) and mineral fraction (total or soluble) analyzed (Pavicic et al., 2004). Although a few approaches have been developed to predict BP development in apple fruit based on Ca and Mg contents, there are limited studies focused on determining the most appropriate tissue and mineral fraction (total or soluble) to be sampled for that purpose. The sampling method most used today is the removal of a longitudinal slice, containing peel + flesh tissues (Perring and Wilkinson, 1965; Argenta and Suzuki, 1994), involving different parts of the fruit (proximal, medial and distal), which reduces the ability to predict the occurrence of BP in apples (Amarante et al., 2006a,b, 2011). As BP symptoms occur mainly at the distal end of the fruit, the most suitable approach is to sample this part and quantify Ca and Mg contents in tissues of the peel and flesh separately (Amarante et al., 2009; Miqueloto et al., 2011). In addition, Ca and Mg contents can be quantified in water soluble and insoluble fractions, which have different physiological meanings (Pavicic et al., 2004; Saks et al., 1990). In the insoluble fraction, Ca is bound to polysaccharides, lipids and proteins in the cell wall, and accumulates inside the vacuole, being considered unavailable for many cellular functions (White and Broadley, 2003). In the soluble fraction, Ca is associated with organic acids, chlorides and nitrates, or in its ex-changeable form, Ca is adsorbed to soluble pectin and proteins, being considered physiologically active (Manganaris et al., 2006, 2007) and therefore it is able to act in different cellular needs and potentially prevent the occurrence of BP (Pavicic et al., 2004; Saure, 2005) and senescent breakdown (Saks et al., 1990) in apples. During cold storage, soluble and pectic Ca contents declines, while insoluble Ca in the vacuole (mainly represented by calcium phosphate, calcium oxalate and calcium silicate) increases, and this has been associated with increased loss of membrane functionality and BP occurrence (Jian-Hui and Wei, 2004). For this reason, threshold total Ca levels in the fruit above which BP will not develop are not applicable in all cases (Wills et al., 1976; Ferguson and Watkins, 1983), while soluble Ca is strongly related to postharvest physiological disorders and this relationship is not disturbed by season or harvesting date (Saks et al., 1990).

Analysis of variance (ANOVA) is commonly used to analyze differences in terms of mineral attributes between apple fruit without and with BP. However, ANOVA does not show how these groups compare when all the mineral attributes are considered together, or how those attributes may be inter-related. This is relevant, for example, when the main objective is to identify the best mineral attribute and/or tissue sampling method to discriminate between fruit without and with BP. Multivariate analysis techniques, such as canonical discriminant analysis (CDA), can be used to identify the best mineral attribute and tissue sampling method to discriminate between fruit without and with BP. CDA finds linear functions of quantitative attributes that maximally separate these two groups of fruit while keeping variation within groups as small as possible. This approach distinguishes several uncorrelated canonical discriminant functions (CDFs). These are linear combinations of the original attributes that best separate the means of groups of fruit relative to withingroup variation (Rencher, 1992). CDA provides standardized canonical coefficients (SCC), which are used to rank attributes in order of their contribution to the separation of groups and to characterize the CDFs, and canonical correlation (r) between CDFs and the original attributes. While SCC provides information about the attributes contributing jointly (multivariate contribution), r shows the importance of each attribute independent of the others (univariate contribution) to the separation of groups (such as fruit without and with de disorder) (Cruz-Castillo et al., 1994). The use of parallel discriminant ratio coefficient (DRC), a product of SCC and r, has also been suggested to assess the relative importance of attributes in a CDF, with attributes having large and positive DRC's having more power in discriminating groups/treatments (Thomas, 1992; Thomas and Zumbo, 1996).

This work was carried out to identify the best fruit tissue sampling method for Ca and Mg assessment to discriminate 'Fuji' apples regarding the occurrence of BP, by means of CDA.

2. Materials and methods

'Fuji' apples were harvested in 2010, in commercial orchards located in three regions of Southern Brazil: São Joaquim (three orchards), Fraiburgo (six orchards) and Vacaria (three orchards). Fruit were harvested at commercial maturity, and then stored in controlled atmosphere (CA) storage (2.0 kPa of O_2 + 0.5 kPa of CO_2, at 0.5 °C and 90–95% RH) for six months. After removal from CA storage, following five days at ambient condition (20 °C and 60–70% RH), fruit were separated in lots without and with BP. Both lots of fruit were washed with distilled water and kept under environmental conditions for 15 min for drying before being subjected to tissue sampling for mineral analysis.

A longitudinal wedge-shaped segment (1 cm wide at the equatorial region, with peel and fresh tissues), without core tissue, was used for peel + flesh sampling method, as suggested by Perring and Wilkinson (1965). For individual sampling of the peel (thickness of 0–2 mm) and flesh (thickness of 2–8 mm), fruit were cut along the equatorial region, so that only the distal end was used. In the same fruit, samples were removed by the three different methods. Calcium and Mg contents (mg kg^{-1} fresh weight) in the total fraction (TF) of the tissues of peel + flesh, peel and flesh were quantified with an inductive coupled plasma (ICP) spectrophotometer, according to the method described by Miqueloto et al. (2011).

Calcium and Mg contents in soluble fraction (SF; mg kg^{-1} fresh weight) were quantified only in tissues of the peel and flesh obtained at the distal end of the fruit. Extracts (juice) were obtained from peel samples (~200 g) and flesh samples (~150 g) previously weighed, using a NKS multiprocessor model TSK 949 (with a power