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Multivariate analysis of mineral content associated with flesh browning disorder in 'Fuji' apples produced in Southern Brazil

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ABSTRACT: Flesh browning is a physiological disorder that occurs in 'Fuji' apples during storage, which causes considerable postharvest losses of fruit produced in Southern Brazil. This work aimed to assess the mineral attributes [Ca; Mg and K contents and the Mg/Ca; K/Ca and (K + Mg)/Ca ratios] associated with the flesh browning disorder incidence, as well as to identify which of these mineral attributes better discriminate the differences in the degree of susceptibility to flesh browning disorder in 'Fuji' apples stored under controlled atmosphere (CA; 1.2 kPa O_2 + 2.0 kPa CO_2 and 1.2 kPa O_2 + < 0.5 kPa CO_2; 0.5 ± 0.1 °C and 96 ± 2% RH, during an 8-month period). Apples from 2 orchards in Fraiburgo, Santa Catarina, 3 orchards in São Joaquim, Santa Catarina, and 3 orchards in Vacaria, Rio Grande do Sul were used. The fruit with flesh browning disorder has lower levels of Ca and a higher Mg/Ca ratio when compared to the fruit without flesh browning. The Mg and K contents were not related to the physiological disorder. The canonical discriminant analysis (CDA) showed that the isolated Ca content better discriminated the fruit with and without flesh browning disorder. 'Fuji' apples with Ca contents < 80 mg·kg^{-1} in the flesh present a greater risk of developing this disorder in the Southern Brazil production region.

Key words: Malus domestica, physiological disorder, canonical discriminant analysis, calcium.

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INTRODUCTION

Flesh browning is a physiological disorder that occurs in 'Fuji' apples during storage, which causes considerable postharvest losses in Southern Brazil (Brackmann et al. 2005). Usually, it is associated with high partial pressures of CO_2 during storage under controlled atmosphere (CA) (Corrêa et al. 2010). However, this CO_2 sensitivity may be related to preharvest factors, such as mineral nutrition (Hunsche et al. 2003; James and Jobling 2009) and the fruit maturation stage (Brackmann et al. 2001).

Several authors relate the fruit's mineral contents, such as low Ca content, to the occurrence of physiological disorders (Amarante et al. 2012; Andziak and Tomala 2004; Miqueloto et al. 2011), since the nutrient guarantees cell structure, functionality, and stability (James and Jobling 2009). High K contents have also been associated with flesh browning disorder in 'Braeburn' apples (Neuwald et al. 2008). The nutrient Mg may also have some connection with physiological disorders in fruit, because it competes directly with Ca in the plasma membrane binding sites, although, like K, it does not perform the same function as Ca (Freitas et al. 2010).

The relationship between nutrient levels in the flesh may also influence the flesh browning disorder (Hunsche et al. 2003). Andziak and Tomala (2004) pointed out that apples with low Ca/K presented a high incidence of several physiological disorders.

In order to identify the nutrient that may be associated with physiological disorders at postharvest, some statistical tools are highly valuable, such as the use of the canonical discriminant analysis (CDA), a multivariate analysis technique. According to Amarante et al. (2006a), CDA allows for the identification of the most relevant nutritional attributes for the discrimination of apples with different degrees of bitter pit severity. In terms of flesh browning disorder in 'Fuji' apples, there is not any information about the nutrient that is most often associated with this physiological disorder, or that best discriminates fruit with and without flesh browning. CDA can be a valuable tool for mineral attribute studies associated with flesh browning disorder in fruit.

This work aimed at assessing the relationship of mineral attributes with the occurrence of flesh browning disorder, as well as identifying which of these attributes best discriminates the differences in the degree of susceptibility to flesh browning disorder in 'Fuji' apples produced in Southern Brazil and stored under CA.

MATERIAL AND METHODS

The experiment was carried out with 'Fuji' apples from 2 commercial orchards in Fraiburgo, Santa Catarina (lat 27°01′04.96″S, long 50°55′56.99″W, and altitude of 1,022 m), 3 commercial orchards in São Joaquim, Santa Catarina (lat 28°21′42″S, long 49°59′42.60″W, and altitude of 1,364 m), and 3 commercial orchards in Vacaria, Rio Grande do Sul (lat 28°30′36.50″S, long 50°55′49.32″W, and altitude of 900 m). In all orchards, 10 sprays with 0.6% of calcium chloride were performed onto each crop 14 days apart, from 30 days after full bloom.

After the harvest, the fruit was selected by eliminating the units with defects or damages followed by the homogenization of the experimental units. The fruit selected from the 3 locations were stored under 2 CA conditions (1.2 kPa O_2 + < 0.5 kPa CO_2 and 1.2 kPa O_2 + 2.0 kPa CO_2) for 8 months at −0.5 ± 0.1 °C and 96 ± 2% relative humidity; 450-L capacity experimental mini chambers were used for storage. The O_2 partial pressures were obtained from the dissolution of O_2 in the storage environment with the injection of N_2 from an N_2 generator with pressure swing adsorption (PSA) technology. The CO_2 partial pressure in the treatment with high CO_2 (2.0 kPa) level was obtained from the injection of this gas in high pressure cylinders. The desired partial pressures of the gases, which varied according to the fruit respiration, was maintained daily in both storage conditions by gas control automatic equipment (Kronenberger/Climasul). When the CO_2 and O_2 levels were unsatisfactory, the equipment corrected the partial pressures to the appropriate levels preset in the treatments. The O_2 consumed in respiration was replaced by atmospheric air injection in the chambers, while the excess of CO_2 (under the 2.0 kPa condition) was absorbed by a potassium hydroxide solution (40%) through which the air circulated the storage environment. The low CO_2 (0.5 kPa) partial pressure was maintained by adding 0.1 kg of hydrated lime ($Ca(OH)_2$) per kilogram of fruit.

Prior to storage, 4 samples of 40 fruit units from each location were assessed in terms of their respiratory and ethylene production rates, iodine test for starch, density, diameter, red color index, skin color, flesh firmness, texture attributes (skin rupture and flesh penetration forces), titratable acidity (TA), and soluble solids (SS) concentration.

The respiratory (nmol $CO_2 \cdot kg^{-1} \cdot s^{-1}$) and ethylene (pmol $C_2H_4 \cdot kg^{-1} \cdot s^{-1}$) production rates were quantified with →

gas chromatography. Six to 8 fruit units from each sample were set aside in a hermetically sealed plastic container with a 4,100 mL volume. The respiration rate was obtained from the difference between the CO_2 concentration inside the container immediately after its closure and after 1 h. Aliquots of gas (1 mL) were taken from the container with a dissepiment and injected into a gas chromatograph (Varian® CP-3800, Palo Alto, CA, USA) equipped with a 3 m long Porapak N® column (80 – 100 mesh), methanator, and flame ionization detector. The temperatures of the column, detector, methanator, and injector were 45; 120; 300 and 110 °C, respectively. The nitrogen, hydrogen, and synthetic air flows were 70; 30 and 300 mL·min^{-1}, respectively.

The iodine test for starch was determined by means of comparison between the half stems of the fruits treated with an iodine solution with the aid of a table of photographs, where index 1 is the maximum starch and index 5 is the completely hydrolyzed starch.

The density, expressed in g·mL^{-1}, was calculated by dividing the fruit mass (obtained from a precision balance) by its volume (obtained by the mass increase after complete immersion of the fruit in water in an 800-mL container). The diameter (mm) of the fruit was measured twice in the cross-section with a caliper rule, followed by a calculation of the average diameter.

The red color index was determined by the assessment of the coated surface of the fruit, assessed with grades from 1 to 4 (0 – 25; 26 – 50; 51 – 75 and 76 – 100% of the fruit surface were pigmented in red for grades 1; 2; 3 and 4, respectively).

The skin color (hue angle — h°) was determined by a colorimeter (Minolta CR 400, Japan) in the equatorial region of the fruit. The h° defines the basic coloration, where 0° = red, 90° = yellow, and 180° = green.

The flesh firmness (N) was determined in the equatorial region of the fruit on 2 opposite sides after the removal of a small portion of the skin with an 11 mm pointed tip penetrometer.

The texture attributes (N) were assessed by an electronic texturometer TAXT- plus® (Stable Micro Systems Ltd., United Kingdom) in terms of the necessary forces for skin rupture and penetration. In order to quantify the necessary forces for skin rupture and penetration, the 2 mm diameter PS2 tip was introduced into the flesh to a 5 mm depth with pre-test, test, and post-test speed of 30, 5, and 30 mm·s^{-1}, respectively.

The TA values (mEq 100·mL^{-1}) were obtained from a 10 mL sample of juice extracted by a centrifuge from cross-sections removed from the equatorial region of the fruit. This sample was diluted into 90 mL of distilled water and titrated with a NaOH 0.1 N solution until it reached the pH 8.1.

The SS contents (°Brix) were extracted by refractometry with temperature effect correction. After 8 months stored and 7 days exposed to ambient temperature (20 ± 2 °C), to simulate an actual commercialization period, the fruit from all treatments was assessed in terms of incidence and severity of flesh browning disorder. The flesh browning disorder incidence (%) was assessed by a cross-sectional cut of the fruit. Afterwards, the fruit which presented any kind of darkening in the internal regions, humid or dry and spongy aspect, as well as small cavities in the flesh, were counted. Then, the fruit were grouped according to the severity, where 1 = no flesh browning disorder incidence, 2 = slight flesh browning disorder incidence, with an affected area of the flesh up to 10%; 3 = moderate flesh browning disorder incidence, from 11 to 30% of the area affected; and 4 = severe flesh browning disorder incidence, with more than 30% of the flesh affected.

The fruit was separated according to the severity scale described to determine the Ca, Mg, and K contents (mg·kg^{-1} fresh mass) in the flesh. For such, a longitudinal wedge-shaped segment (1 cm wide at the equator region) of the flesh, without core and peel tissues, from the fruit from each severity stage was used (Amarante et al. 2013b). These samples were crushed and homogenized with a RI 6720 multiprocessor and a Braun Multiquick MR40 mixer, respectively. Then, ~ 5 g of flesh samples were weighted with a Tecnal Mark-5000 analytical scale, stored in an M-2 porcelain crucible, and incinerated in a muffle furnace to a 600 °C temperature for 4 h. After cooling, the ashes of each sample were solubilized with the addition of 16 mL of HCl 1.8 M, as described in Corrêa et al. (2012).

In order to determine the Ca content, an aliquot of 5 mL of the original extract was removed and added to 5 mL of lanthanum inside a 15 mL Falcon tube, so that the reading could be carried out with an atomic absorption spectrophotometer (Aanalyst 100 PerkinElmer).

To determine the Mg content, 2 mL of the original extract were removed, to which 10 mL of distilled water were added; 3 mL of this diluted solution (2 mL of extract + 10 mL of distilled water) were pipetted, to which 3 mL of lanthanum were added. Then, the reading was carried out with the same spectrophotometer used to read the Ca content.

In order to quantify the K content, an aliquot of 3 mL of the original extract was removed, followed by the addition →

of 20 mL of distilled water and homogenization. The quantification was carried out with a Digimed DM-61 flame photometer.

The experimental design adopted was completely randomized and replicated 4 times, each experimental unit having 40 fruit units. The data obtained from the mineral and flesh browning analysis were submitted to a CDA with SAS software version 9.0, aiming at assessing the mineral attributes that best discriminate the differences in the degree of susceptibility to flesh browning disorder in 'Fuji' apples stored under CA. The data concerning the maturation attributes was submitted to the Pearson's correlation test with the flesh browning incidence and severity after CA storage.

RESULTS AND DISCUSSION

The correlation between maturation attributes at harvest (flesh firmness 72.5; 78.5; 73.9 N; TA 4.11; 4.81; 4.79; SS 13.1; 12.77; 13.45 and iodine test for starch 4.15; 4.00; 4.10 for Vacaria, Fraiburgo and São Joaquim, respectively), the density and diameter of the fruit, and the incidence and severity of flesh browning disorder were not observed (data not presented). This is due to the lack of substantial differences among the orchards in the 3 municipalities in terms of maturation attributes, which proves that the maturation stage of the fruit among the orchards was similar at the time of harvest.

In terms of mineral contents, the fruit with flesh browning disorder presented lower contents of Ca (Table 1). Andziak and Tomala (2004) have also verified lower Ca contents in the flesh of fruit with flesh browning disorder compared to the fruit without the disorder. According to Saquet et al. (2003), the occurrence of flesh browning disorder is characterized by a reduction in the integrity of membranes, causing cell compartmentalization; Ca aids maintaining the cell structure, functionality, and stability (James and Jobling 2009), playing an important role in the compartmentalization of cells. Bitter pit is also a physiological disorder associated with Ca deficiency in apples (Amarante et al. 2006a,b; 2009; 2011; 2013a,b). Studies point out that the nutrient is accumulated in the vacuole and its connection to the cell wall, which causes a decrease in Ca content in the apoplast, resulting in an increase in membrane permeability, plasmolysis, and, ultimately, cell death (Freitas et al. 2010). The mechanism that explains how membrane integrity occurs in apples with bitter pit may be similar to the one affecting fruit with flesh browning disorder, considering that both physiological disorders are associated with lower Ca contents in the tissues (Andziak and Tomala 2004; Miqueloto et al. 2011).

The Mg content remained the same in fruit with and without flesh browning disorder (Table 1). According to Freitas et al. (2010), the Mg content may be related to the incidence of physiological disorders in fruit because, due to the ionic similarity between Ca and Mg, they compete in several cell processes, such as in sites of activation of certain enzymes and of cell membrane binding (White and Broadley 2003). Nevertheless, in spite of the ionic similarity, Mg does not replace Ca in some cell processes, which may explain the occurrence of physiological disorders related to Ca deficiency in response to the high Mg/Ca ratio in fruit tissues (Freitas et al. 2010). In this work, fruit with flesh browning presented a higher Mg/Ca ratio (Table 1) in comparison with fruit without the disorder. This result is in accordance with those found by Miqueloto et al. (2011), since the authors observed that high Mg/Ca ratios may be associated with the incidence of physiological disorders. However, in this paper, the higher Mg/Ca ratio owes to the lower Ca content in these fruits, as opposed to being caused by a higher Mg content. Although the Mg content is not higher in fruit with flesh browning, the higher Mg/Ca ratio may have caused more competition between these nutrients in the sites of binding →

Table 1. Mineral attributes in the flesh of 'Fuji' apples harvested in São Joaquim, Santa Catarina, Fraiburgo, Santa Catarina, and Vacaria, Rio Grande do Sul, with and without flesh browning disorder after storage under 2 CA conditions (1.2 kPa O_2 + 2.0 kPa CO_2 and 1.2 kPa O_2 + 0.5 kPa CO_2; −0.5 ± 0.1 °C and 96 ± 2% RH) during 8 months.

Flesh browning disorder	Ca	Mg	K	Mg/Ca	K/Ca	(K + Mg)/Ca
	(mg·kg^{-1} of fresh mass)					
Without	80.3	51.2	776.0	0.64	10.9	10.3
With	66.6	50.3	704.6	0.77	9.9	11.3
Probability	< 0.0001	ns	ns	< 0.0001	ns	ns
CV (%)	15.6	14.8	24.4	18.9	31.3	24.4

ns = Non-significant ($p > 0.05$); CV = Coefficient of variation.

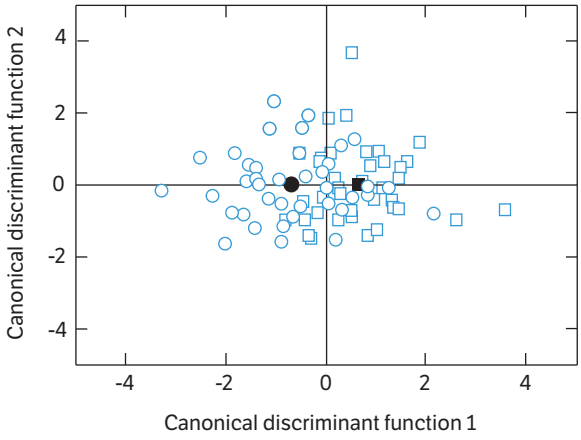
in fruits with the physiological disorder compromising the cell membranes structure, functionality, and stability (James and Jobling 2009).

The fruit with and without flesh browning did not present different K contents as well as K/Ca and (K + Mg)/Ca nutritional ratios (Table 1). However, Neuwald et al. (2008) observed in 'Braeburn' apples a strict connection between the K concentration in the flesh and the incidence of flesh browning disorder. Similarly, Andziak and Tomala (2004) observed a higher occurrence of flesh browning disorder in 'Janagold' apples with a high K/Ca ratio. The incompatibility of these results with those obtained in this work can reflect differences among apple cultivars. According to Volz et al. (2006), there are variations in the concentration of K and in the K/Ca ratio with the flesh browning disorder incidence in 'Braeburn' and 'Janagold', respectively, but not in 'Fuji' apples.

The CDA was used to assess the discriminative power of mineral attributes (Ca content as well as K/Ca and Mg/Ca ratios) to segregate fruits with and without flesh browning disorder obtained from both CA conditions independently of the production region. Since only 2 groups were separated (fruits with and without flesh browning), only 1 canonical discriminant function can be used (CDF1) (Cruz-Castillo et al. 1994). In the CDA, the Wilks' lambda statistical multivariate test showed significant differences ($p < 0.0001$) between fruit with and without flesh browning disorder in terms of the mineral attributes assessed for the CDF1. The CDF1 presented a canonical correlation of 0.5621, indicating a considerable association between mineral attributes and the flesh browning disorder.

The graphic representation of the standardized canonical coefficients (SCC) for CDF1 clearly indicates the separation between the fruit with and without flesh browning disorder (Figure 1), showing that the CDA was efficient in discriminating the fruit in terms of occurrence of the disorder.

The parallel discrimination rate (PDR) coefficient was used to identify which nutritional attribute best discriminates the fruit with and without flesh browning disorder in each municipality. This coefficient is obtained from the product between the values of SCC and the canonical correlation coefficients (r) (Amarante et al. 2006a). The Ca contents presented higher PDR coefficient values for fruits from Fraiburgo and São Joaquim (Table 2), indicating that this nutrient best discriminates the fruit with and without flesh browning disorder for the orchards from these 2 municipalities. Several tests showed that the CDA represents an important tool to indicate which →



Black-filled symbols represent the average value of SCC for fruits without and with flesh browning disorder. As the maximum number of canonical discriminant functions that could be obtained in the first case is 1, a dummy canonical discriminant function 2 was created with random observations from N(0,1) for plotting purposes.

Figure 1. Standardized canonical coefficients (SCC) of the discriminant canonical functions 1 and 2 in 'Fuji' apples with and without flesh browning disorder after storage under 2 CA conditions (1.2 kPa O_2 + 2.0 kPa CO_2 and 1.2 kPa O_2 + 0.5 kPa CO_2; −0.5 ± 0.1 °C and 96 ± 2% RH) during 8 months, considering the Ca content in the flesh of fruit from São Joaquim, Santa Catarina, Fraiburgo, Santa Catarina, and Vacaria, Rio Grande do Sul.

Table 2. Standardized canonical, canonical correlation, and parallel discrimination rate coefficients for the discriminant canonical function 1 referring to the Ca (mg·kg^{-1}) and the K/Ca and Mg/Ca contents in 'Fuji' apples from 3 sites of apple production in Southern Brazil in the discrimination of lots with and without flesh browning disorder after storage under 2 CA conditions (1.2 kPa O_2 + < 0.5 kPa CO_2 and 1.2 kPa O_2 + 2.0 kPa CO_2; −0.5 ± 0.1 °C and 96 ± 2% RH) during 8 months.

Mineral atribute	SCC	r	PDR
Fraiburgo			
Ca	1.42	0.73	1.0444
K/Ca	0.93	−0.001	−0.0017
Mg/Ca	0.09	−0.47	−0.0427
Vacaria			
Ca	0.31	0.84	0.2681
K/Ca	−0.50	−0.80	0.4030
Mg/Ca	−0.42	−0.76	0.3287
São Joaquim			
Ca	0.60	0.92	0.5624
K/Ca	−0.02	−0.53	0.0149
Mg/Ca	−0.48	−0.87	0.4226

SCC = Standardized canonical coefficients; *r* = Canonical correlation; PDR = Parallel discrimination rate.

nutrient or nutrient ratio is best capable of discriminating apples more or less susceptible to bitter pit occurrence (Amarante et al. 2006a,b; 2009; 2011). Although the occurrence of bitter pit in apples has been attributed to the low Ca content in the fruits (Amarante et al. 2006a, b; 2009; 2011; Miqueloto et al. 2011; 2014; Sestari et al. 2009), some authors indicate through CDA the K/Ca ratio (Amarante et al. 2006a; Miqueloto et al. 2011) and Mg/Ca ratio (Amarante et al. 2006b; 2013a,b) as the best attributes to discriminate the fruit with and without bitter pit. Nonetheless, in relation to the flesh browning disorder in 'Fuji' apples, there are no previous tests which indicate the best mineral attribute to discriminate the fruit with and without the disorder.

Hunsche et al. (2003) verified that the flesh browning disorder in 'Fuji' apples stored under CA is associated with K contents, where the lower the content in the fruit flesh, the higher the incidence of flesh browning disorder. However, in 'Braeburn' apples, Neuwald et al. (2008) observed a positive correlation between the K contents and flesh browning disorder. However, in this test, the nutrient did not present a correlation with the flesh browning disorder in the orchards under study.

In the orchards of Vacaria, the K/Ca ratio presented a higher PDR than the Ca content and the Mg/Ca ratio (Table 2), which partly coincides with the results found by Neuwald et al. (2008). The higher PDR coefficient for the K/Ca ratio may be due especially to the Ca content, which presented a higher canonical correlation coefficient (r) (Table 2). According to Amarante et al. (2006a,b; 2009; 2011), the K/Ca ratio presented the best results to discriminate the fruit with and without bitter pit, coinciding with the results obtained for flesh browning disorder in this work. The nutrient K can compete with Ca in the plasma membrane binding sites, however, without replacing Ca in terms of its membrane structuring and stabilization role (Freitas et al. 2010). This can explain the higher occurrence and severity of flesh browning disorder in response to a higher K/Ca ratio in Vacaria.

Since Ca was the only nutrient which presented a closer relation with the flesh browning disorder incidence in 'Fuji' apples after storage, it was possible to explain the severity of the physiological disorder with the concentration of this nutrient in the flesh tissues of the fruits of all apples production sites evaluated in Southern Brazil. It was observed that, the more severe the flesh browning disorder, the lower the Ca contents in the fruit, having occurred a linear relation for Vacaria and São Joaquim and an exponential relation for Fraiburgo (Figure 2).

James and Jobling (2009), in their work with 'Cripps Pink' apples from different regions and years of production, found a significant correlation between the flesh browning disorder and Ca, Mg, and K contents in the fruit, with a →

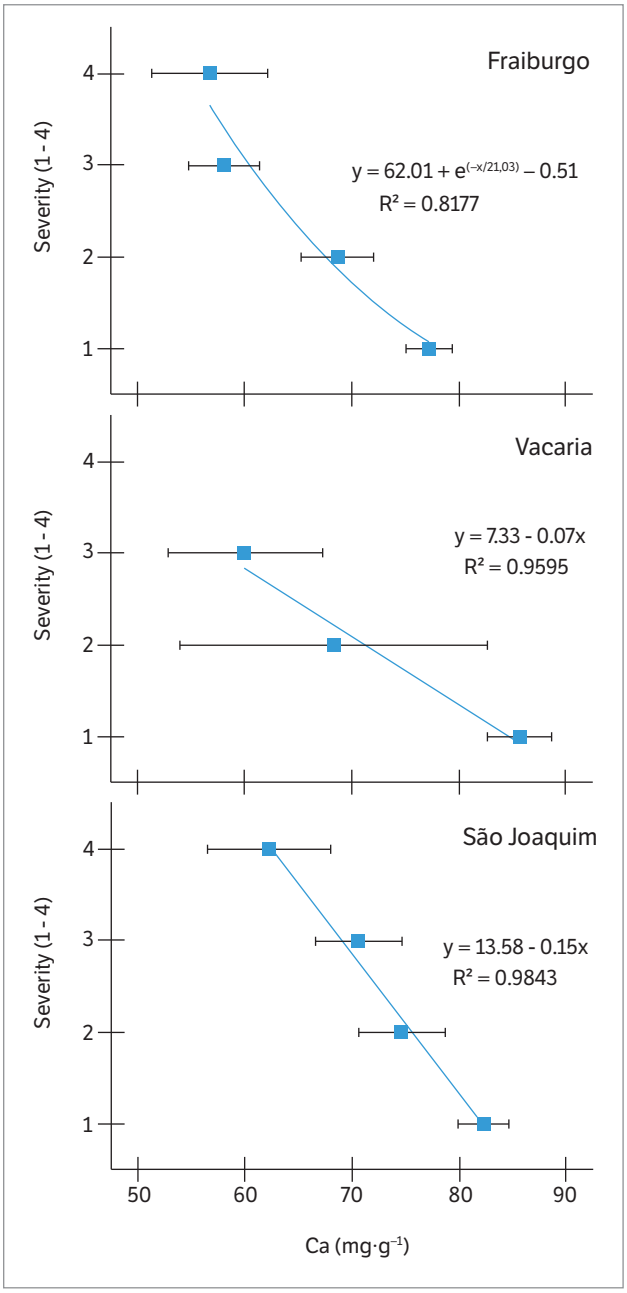


Figure 2. Correlation between Ca contents and the severity of flesh browning disorder in 'Fuji' apples from orchards in Fraiburgo, Vacaria, and São Joaquim stored under 2 CA conditions (1.2 kPa O_2 + < 0.5 kPa CO_2 and 1.2 kPa O_2 + 2.0 kPa CO_2; −0.5 ± 0.1 °C and 96 ± 2% RH) during 8 months.

different behavior according to the region. Nevertheless, for these authors, Ca is the nutrient most related with flesh browning disorder in apples stored for long periods, since its deficiency is associated with the degradation of the middle lamella, which affects the cell wall stability during storage, in accordance with the results found in this work. Additionally, low Ca levels in apples can cause the plasma membrane rupture and promote the occurrence of physiological disorders as bitter pit (Freitas et al. 2010) and flesh browning disorder (Andziak and Tomala 2004).

The results obtained show that 'Fuji' apples with low Ca content in the flesh present a higher susceptibility to the flesh browning disorder incidence during storage under CA. Fruits with Ca contents higher than 80 $mg \cdot kg^{-1}$ presented low susceptibility to flesh browning disorder, stored in both low (< 0.5 kPa) and high (2.0 kPa) CO_2.

CONCLUSION

The occurrence of flesh browning disorder in 'Fuji' apples stored under controlled atmosphere is associated with low Ca contents in the fruit. Ca concentration is the best attribute to discriminate 'Fuji' apples with and without flesh browning disorder.

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