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Can Mineral Analysis be Used as a Tool to Predict 'Braeburn' Browning Disorders (BBD) in Apple in Commercial Controlled Atmosphere (CA) Storage in Central Europe?

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Abstract 'Braeburn' apples stored in controlled atmosphere (CA) frequently present internal flesh browning physiological disorder which is commonly referred as 'Braeburn' Browning Disorder (BBD). Apples from different orchards, years or site conditions can vary considerably in their sensitivity. The aim of this research was to evaluate the relationship between the mineral status of 'Braeburn' apples before-harvest (18 days) and at early and normal harvest, to correlate the data with the BBD incidence found in apples post storage and to investigate possible reasons for differences in disorder sensitivity. Fruits from seven orchards in the Lake Constance area (South-Western Germany) were harvested at two picking dates and the mineral content was measured before-harvest, at-harvest and during storage. Fruit were stored at 1.5 °C under CA conditions (1 kPa O_2 and 0.5 kPa CO_2) using either a 10 days or a 24 days delayed establishment of CA conditions. Fruit were evaluated after 6 months of storage plus 10 days of shelf life at 18 °C for mineral status and the browning disorder incidence. Results indicate no significant changes of the mineral concentrations in the fruit during CA-storage. Significant correlations between the post storage BBD incidence with K, and in some cases also for the K/Ca ratio and for P at-harvest were found.

Keywords Braeburn Browning Disorder (BBD) · Controlled atmosphere (CA) · Fruit quality · *Malus domestica* · Physiological storage disorders · Internal flesh browning

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Ermöglichen Mineralstoffanalysen eine Prognose der Fleischbräune-Anfälligkeit im CA-Lager bei der Apfelsorte 'Braeburn' unter mitteleuropäischen Anbaubedingungen?

Zusammenfassung 'Braeburn' Äpfel besitzen bei Lagerung unter kontrollierter Atmosphäre (CA) eine erhöhte Anfälligkeit für Fleischbräune, eine physiologische Erkrankung, die auch als 'Braeburn' Browning Disorder (BBD) bezeichnet wird. Äpfel aus verschiedenen Obstanlagen, Jahren oder Standortbedingungen können stark in ihrer Empfindlichkeit gegenüber Fleischbräune variieren. Ziel der vorliegenden Arbeit war es, den möglichen Zusammenhang zwischen der Mineralstoffversorgung der Frucht vor der Ernte (18 Tage), bei frühem sowie bei praxisüblichem Erntetermin und dem Auftreten von Fleischbräune an `Braeburn` genauer zu untersuchen. Zudem sollten mögliche Ursachen für die unterschiedliche Verbräunungs-Anfälligkeit genauer betrachtet werden. Früchte aus sieben verschiedenen Obstanlagen in der Bodenseeregion (Süd-West- Deutschland) wurden an zwei Terminen geerntet und die Mineralstoffkonzentration vor der Ernte, zu beiden Ernteterminen sowie während der Lagerung untersucht. Die Äpfel wurden bei 1,5 °C im CA-Lager (1 kPa O_2 und 0,5 kPa CO_2) gelagert, wobei das Einstellen der CA-Bedingungen mit einer Verzögerung von 10 Tagen oder 24 Tagen erfolgte. Im Anschluss an sechs Monate Lagerung wurden die Früchte einer 10-tägigen Nachlagerung bei 18 °C unterzogen. Anschließend erfolgten erneut Mineralstoffanalysen sowie die Bonitur der Äpfel auf Fleischbräune. Die Ergebnisse zeigten keine signifikanten Veränderungen der Mineralstoffkonzentration während der CA-Lagerung. Signifikante Korrelationen konnten zwischen dem Fleischbräune-Befall und dem Kalium-Gehalt der Äpfel sowie in einigen Fällen mit dem K/Ca-Verhältnis und dem Phosphor-Gehalt zur Ernte gefunden werden.

Schlüsselwörter Braeburn Browning Disorder (BBD) · Kontrollierte Atmosphäre (CA) · Fruchtqualität · *Malus domestica* Borkh · Physiologische Lagererkrankung · Fleischbräune

Introduction

'Braeburn' apple is an important cultivar for the fresh market in many apple producing countries and it has a high storage potential. However, in controlled atmosphere (CA) storage 'Braeburn' apples frequently show internal flesh browning, a physiological disorder which is commonly referred to as 'Braeburn' Browning Disorder (BBD). The rapid establishment of CA conditions improves post-storage quality of many apple cultivars, however affects negatively the storability of 'Braeburn' apples increasing BBD during CA storage period (Saquet et al. 2003). BBD symptoms include the formation of cavities and internal tissue browning, but only in very severe cases the symptoms appear visible on the outside of the fruit (Elgar et al. 1999). BBD occurrence is influenced by pre-harvest orchard factors (e.g. climate, harvest maturity, nutritional conditions, etc*)* and post-harvest factors such as storage temperature, and storage O_2 and/or CO_2 concentrations, where low internal O_2 and/or higher CO_2 concentrations in the fruit lead to a higher incidence of the disorder (Curry 1998; Bangerth 2008; Neuwald et al. 2008; Ho et al. 2011; Herremans et al. 2013). BBD incidence can vary markedly between different orchard rows (Elgar et al. 1999). The susceptibility to BBD is known to increase with advanced fruit maturity (Curry 1998; Herremans et al. 2013). The disorder is low O_2 and/or high CO_2 related, where low internal O_2 and/or higher CO_2 concentrations in the fruit lead to a higher incidence of the disorder (Curry 1998; Ho et al. 2011; Herremans et al. 2013). Further studies regarding flesh browning have been conducted with 'Conference' pears including analysis at a proteomics and metabolomics level (Pedreschi et al. 2009). Ho et al. (2011) observed that 'Braeburn' apples show a high O_2 and CO_2 gradient within the tissue which could promote the occurrence of internal browning in 'Braeburn' apples, and also in pears. In summary the low O_2 and high CO_2 partial pressures can cause an oxidative stress, as well as changes in normal cellular, physiological and biochemical metabolism. For example, the respiratory rate can be decreased to a level insufficient for the maintenance of cellular metabolic processes (Saquet et al. 2003; Franck et al. 2007; Bangerth 2008; Herremanns et al. 2013). Under anaerobic conditions energy supply continues by anaerobic respiration, although the energy provided by fermentation processes is limited and generally not sufficient to maintain cellular metabolism and to restore membrane damages caused by reactive oxygen species (Franck et al. 2007; Herremanns et al. 2013). The same authors observed that damages in the membranes provide a break in the compartmentalization of the cell (e.g. vacuole), placing phenolic substances in the cytoplasm which can be then enzymatically oxidized. At more severe levels water may be used for transpiration which can lead to the formation of cavities in the tissue (Franck et al. 2007). Saquet and Streif (2002) found high energetic status when 'Braeburn' apples and 'Conference' pears were stored under delayed CA conditions, where fruits show a lower susceptibility to internal browning compared to those from immediate CA establishment. Jung and Watkins (2011) observed higher PPO (polyphenol oxidase) activity in flesh browned apples when evaluated the effect of ethylene in flesh browning development of CA storage by 'Empire' apple. Milani and Hamedi (2005) as well as Castro et al. (2008) found a correlation between CO_2-induced flesh browning and (anti-)oxidant activity of specific browning linked enzymes like PPO. Common practice in 'Braeburn' storage, in temperate zones like central Europe, is to apply a delayed establishment of CA conditions of about 20 days at the beginning of harvest, during which period an increase of CO_2-level as well as a decrease of O_2-level are avoided (Saquet et al. 2003).

Altherr et al. (1995) observed in 'Cox's Orange Pippin' and 'Jonagold' apples that internal browning disorders were correlated with the concentration of some mineral nutrients in the fruit flesh, mainly with Ca, K, P and Mg and their ratios, for example, K/Ca or (K+Mg)/Ca. Bangerth (1979) found that the development of physiological browning disorders can be minimized through the application of calcium. A low content of calcium in the fruit tissue predisposes the apple to BBD (Brackmann and Ribeiro 1992) and variation in mineral content of different orchard locations can have a high impact on the incidence of physiological disorders in apple and pears (Wilkinson and Perring 1964). Marschner (2012) stated that the occurrence of physiological disorders is related to a nutritional imbalance, especially to a Ca deficiency (Wilkinson and Perring 1964). Calcium can be exchanged for other cations due to their chemical similarities, but Mg and K will not have the same functions as the Ca in the cell or fruit tissue (Marschner 2012).

There is already an understanding of the biochemical and physiological basis of BBD (Bangerth 2008) but the disorder development still appears too complex to allow a reliable BBD prediction method. Trials with Mg infiltration were performed to predict the occurrence of physiological disorder, mainly related to a Ca deficiency in the tissue (bitter pit, lenticel spot, internal breakdown). The Ca uptake and action can be influenced by minerals which have a very similar electrochemical structure, such as Mg and K (Sestari et al. 2009). As BBD incidence is also believed to be partly related to the level of calcium within the fruit, Mg infiltration could be used as a test at-harvest to predict the susceptibility of the fruit to the occurrence of BBD during storage, however for internal physiological disorders, like for BBD, no success has been reported with this method so far.

The aim of this research was to evaluate the relationship between the mineral status of 'Braeburn' apples before-harvest (18 days) and at early and normal harvest, to correlate the data with the BBD incidence found in apples post storage and to investigate possible reasons for differences in disorder sensitivity.

Material and Methods

Fruits from seven orchards were sourced from the Lake Constance apple growing area, South-Western Germany. Early harvested 'Braeburn' apples were picked on 10th October, with starch pattern index 2.4–3.4 on a scale of 1–10 and firmness values from 88 to 105 N (Newton) and normal harvested fruits on 23rd October, with starch pattern index 3.9–5.2 and firmness values from 87 to 98 N. These parameters are according to the values recommended by the post-harvest research group in the Lake Constance region (Streif et al. 2012), which recommend for 'Braeburn' apples for long term storage firmness values between 75–90 N and starch pattern index between 4 and 5. Fruit were stored for 6 months at 1.5 °C (±0.5 °C) under CA-conditions (1.0 kPa O_2 and 0.5 kPa CO_2) using either a short (10 days) or normal delayed (24 days) establishment of CA-conditions. For CA-storage, approximately 100 fruits per orchard and per treatment were placed in 560-liter CA containers and the CO_2 and O_2 concentration were continually monitored by computer controlled gas analysers. Fruit were evaluated for mineral status before harvest (18 days), at early and normal harvest, and after 6 months of storage. The browning disorder incidence after 6 months storage was correlated to the initial mineral analysis (K, P, Mg and Ca).

The mineral content of K, Ca, Mg and P was assessed as follows: vertical slices were cut from 24 fruits/sample, crushed, homogenized, weighted (~100 g), freeze-dried and the dry substance was calculated. Approximately 1,5 g of the dried sample was weighed and ashed by 480 °C during 6 h, added 2 ml hydrochloric acid (20 %) containing 0.1 % lanthanum to a 100 ml volume. Potassium, calcium and magnesium was determined by atomic absorption spectrometry and determined by flame Absorption Spectroscopy (GBC908AA). Standards were prepared corresponding to average concentrations in the tissue (e.g. 0, 2, 4, 6 and 8 ppm of Ca; 0, 20, 40, 50, 70 and 100 ppm of K and 0, 3, 6, 8, 10 and 12 ppm Mg) which was measured together with fruit samples. The determination of phosphorus was realized by a molybdenum blue colorimetric procedure. The standard was prepared with the average of this element in the tissue (0, 1, 2, 3, 4, 5, 7 and 10 ppm). The result of Ca, K, Mg and P was calculated in mg of each element/100 g FW (fresh weight) by the following formula:

$$\text{Ca, K, Mg or P}\left(\frac{\text{mg}}{100\text{g}}\text{FW}\right) = \frac{\text{Measured element (ppm)} \times 100\ \text{(g)} \times \text{drying substance (\%)}}{\text{dried sample weighed (e.g. 1,5g)}}.$$

To monitor the physiological disorders fruits were cut transversally through the equatorial region and scored for flesh browning and cavities. The disorders were scored by a scale from 0 to 10 (Neuwald et al. 2008), where 0 describes no visible symptoms and 10 a maximal occurrence of the symptoms.

Statistical analysis: The before-harvest and at-harvest mineral status from all 7 orchards and harvest date combinations were subjected to a correlation analysis together with the physiological disorder indices and tested for significance (T test). Regression analysis was used to test the relationship between the potassium content and BBD. The mean was compared with Tukey's test using to assess the fruit nutritional status during the study period (18 days before, at early and normal harvest, and 6 months of storage). The statistical program SISVAR was used (Ferreira 2008).

Results and Discussion

Delayed CA (24 days) storage reduced the occurrence of BBD in the apples after 6 months storage (Fig. 1**a**–**d**). In the early harvested fruits, the 24 days delayed CA treatment reduced the internal browning disorders incidence com-

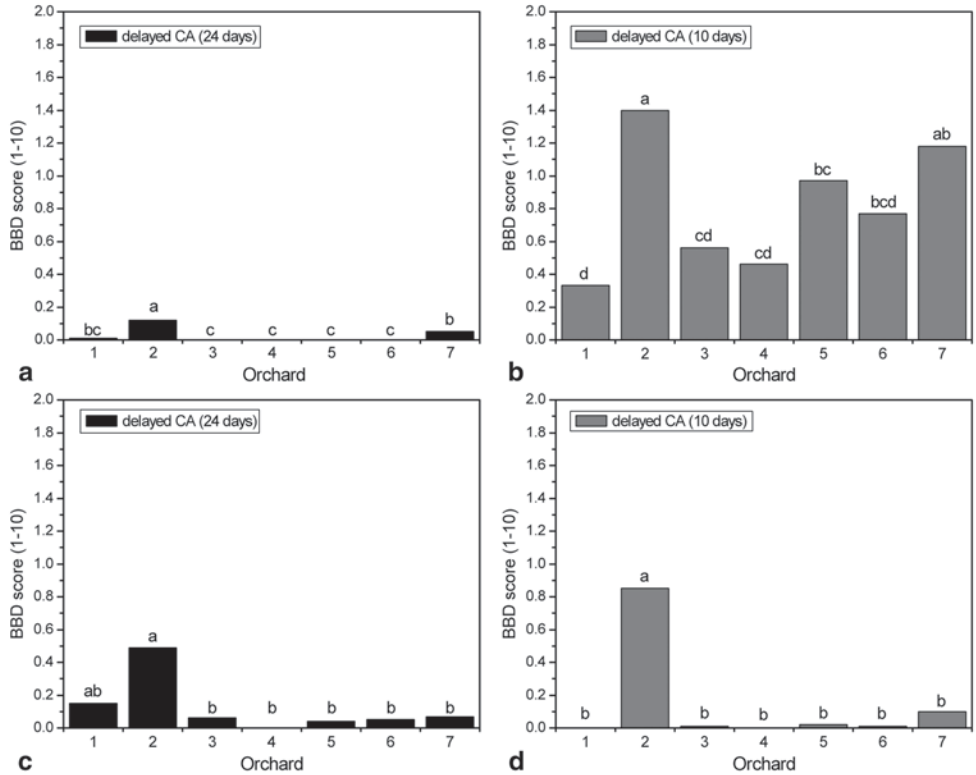

Fig. 1 Occurrence of BBD in 'Braeburn' apples from 7 different orchards after 6 months CA-storage. **a** Early harvested and 24 days delayed CA. **b** Early harvested and 10 days delayed CA. **c** Normal harvested and 24 days delayed CA. **d** Normal harvested and 10 days delayed CA. Means followed by equal letters do not differ by Tukey's test, at $p \leq 0.01$

pared with 10 days delayed CA. Saquet et al. (2003) also reported that a 20 days delayed CA establishment reduces the risk for BBD. A possible reason could be an adaptation of the fruit to a gradual slowing of its metabolism, first by reduced temperature and then by the CA establishment. The delayed establishment of CA conditions possibly leads to a reduced physiological stress to the fruit when compared with a simultaneous reduction of temperature and O_2 concentration together with an increase of CO_2 levels at the beginning of storage. This condition could cause an oxidative stress, as well as, a deviation in cellular physiological and biochemical metabolisms (Franck et al. 2007; Bangerth 2008; Herremanns et al. 2013). Fruits under 10 days delayed CA showed a higher incidence of BBD as 24 days delayed CA, a consequence of a higher stress impact possibly due to a shortage of O_2 and extreme restriction in the energy metabolism causing an accumulation of ethanol, acetaldehyde and ethyl acetate, products of fermentation (Saquet et al. 2003). In this case the fermentation process maybe is not sufficient to supply the energy needed for the processes of cell maintenance and for the reparation of membrane damages caused by reactive oxygen species (Franck et al. 2007; Herremanns et al. 2013).

Fruit from second (normal) harvest date with a CA delay of 10 days showed a lower browning disorder incidence after storage than the earlier harvested and also 10 days CA-delayed apple (Fig. 1**b**, **d**). This unexpected result is in contrast with previous research (Saquet et al. 2003). Other researchers have shown that normal harvested 'Braeburn' are susceptible to a higher incidence of BBD compared to early harvested fruit (Curry 1998). The higher browning incidence of early picked apples in our experiment may be only explained by specific climate influences of the growing season, e.g. temperature below average during fruit development. However, normal harvest in this work describes fruit harvested actually at the optimum ripening stage for long term storage as recommended for the region (Streif et al. 2012).

From all the minerals tested and ratios of different minerals the K concentration showed the best correlation with BBD for all harvest dates and storage regimes (Table 1). K is an antagonist of Ca, so fruit K concentration or K/Ca ratio could be used as a possible BBD predictor (Altherr et al. 1995; Marschner 2012). P could possibly also be a good alternative for BBD prediction, as it is involved in the energy metabolism that is possibly linked to the appearance of the BBD symptoms (Saquet and Streif 2002). Physiological processes associated with this mineral change might include a modification of the enzymes phosphorylation status and/or in the action of the Ca as a second messenger (Larrigaudiere et al. 1996). Milani and Hamedi (2005) also reported that some apple cultivars are sensitive to enzymatic browning (Castro et al. 2008; Jung and Watkins 2011), and P and K are used possibly by the fruit as co-factors to activate certain enzymes, that could cause BBD. Currently there are scientific intentions to find genetic indicators which can be used to predict the susceptibility of the fruit to physiological dis-

Table 1 Correlation (r^2) between mineral content and browning disorder in 'Braeburn' apples of different harvest times and storage regimes

	Early harvest		Normal harvest	
	24 days delay	10 days delay	24 days delay	10 days delay
K/Ca	0,84*	ns	ns	0,88*
K	0,83*	ns	0,82*	0,76*
P	ns	ns	ns	0,75*
Ca	ns	ns	ns	ns
Mg	ns	ns	ns	ns

ns not significant

*$p \leq 0.05$

orders prior to the start of CA storage. The regression curves in Fig. 2 show the relationship between K concentration and BBD incidence. A K concentration of around 100 mg/100 g FW resulted in the lowest BBD incidence for both the 10 days and 24 days delayed CA storage regime.

During a 6-month storage period, there was no change in the mineral content (data not shown) compared with analysis 18 days before harvest or at early and normal harvest. These results are in accordance with findings of Wilkinson and Perring (1964), Chardonnet et al. (2003) who did not find any changes in the mineral status of whole apples, but they measured some translocation within different parts of a fruit during storage. Figure 3 shows that there is a large variation in the mineral concentrations of K, Ca and P levels and K/Ca ratio mainly between orchard 2 and the other orchards. The probability of BBD occurrence is increased with K concentrations over 110 mg/100 g FW (Fig. 2). Data not shown, it was observed, that there was no alteration in the mineral levels during CA storage in 'Braeburn' apples (Wilkinson and Perring 1964; Chardonnet et al. 2003).

Analyzing Fig. 1 (**a**–**d**) and Fig. 3 (**a**, **c**, **e**) it can be observed, that the fruits of orchard 2 showed higher BBD occurrence (Fig. **a**–**d**) compared to the other six orchards. At the same time fruits from orchard 2 had the lowest Ca level, as well as, the highest K level, and consequently the highest ratio K/Ca (Fig. 3**a**, **c**, **e**). The results are in accordance with findings from Brackmann and Ribeiro (1992) and Bangerth (2008) who mention an interaction between the Ca level and the susceptibility to some physiological disorders. Bangerth (2008) reported that besides the mentioned structural effect of Ca, it can influence considerably parts of ripening metabolic, hormonal and also gene expression reactions. The main effect of Ca in fruit metabolism can be found on fruit firmness and ethylene production. The BBD occurrence is an interaction between several factors. The most important factors are the climate conditions, orchard management (nutritional conditions of fruit and/or plant), harvest dates and storage conditions. Because of the complexity of these factors, it is quite difficult to predict the BBD susceptibility.

The results obtained with 'Braeburn' in this work are confirmed by our studies with the internal browning sensitive apple variety 'Santana', where fruits with a high percentage of internal browning after pack out from a commercial storage room have shown a higher potassium content and a higher K/Ca ratio compared to those fruits in the same room from orchards without internal browning disorder (Fig. 4). This confirms the hypothesis that besides the information about the ripening stage at harvest the mineral status of the fruit can be a helpful tool to predict at least partly the susceptibility of a batch to physiological disorders during storage (Ferguson and Triggs 1990).

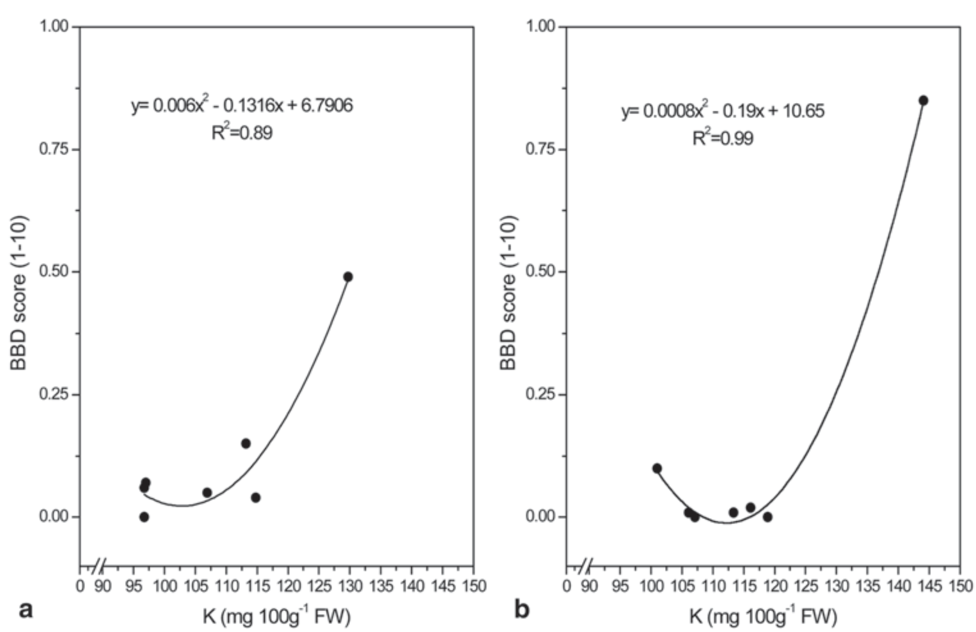

Fig. 2 Regression between K concentrations at harvest and BBD incidence in optimal harvest date harvested 'Breaburn' apples from 7 orchards after 6 months CA-storage. **a** 24 days delayed CA. **b** 10 days delayed CA

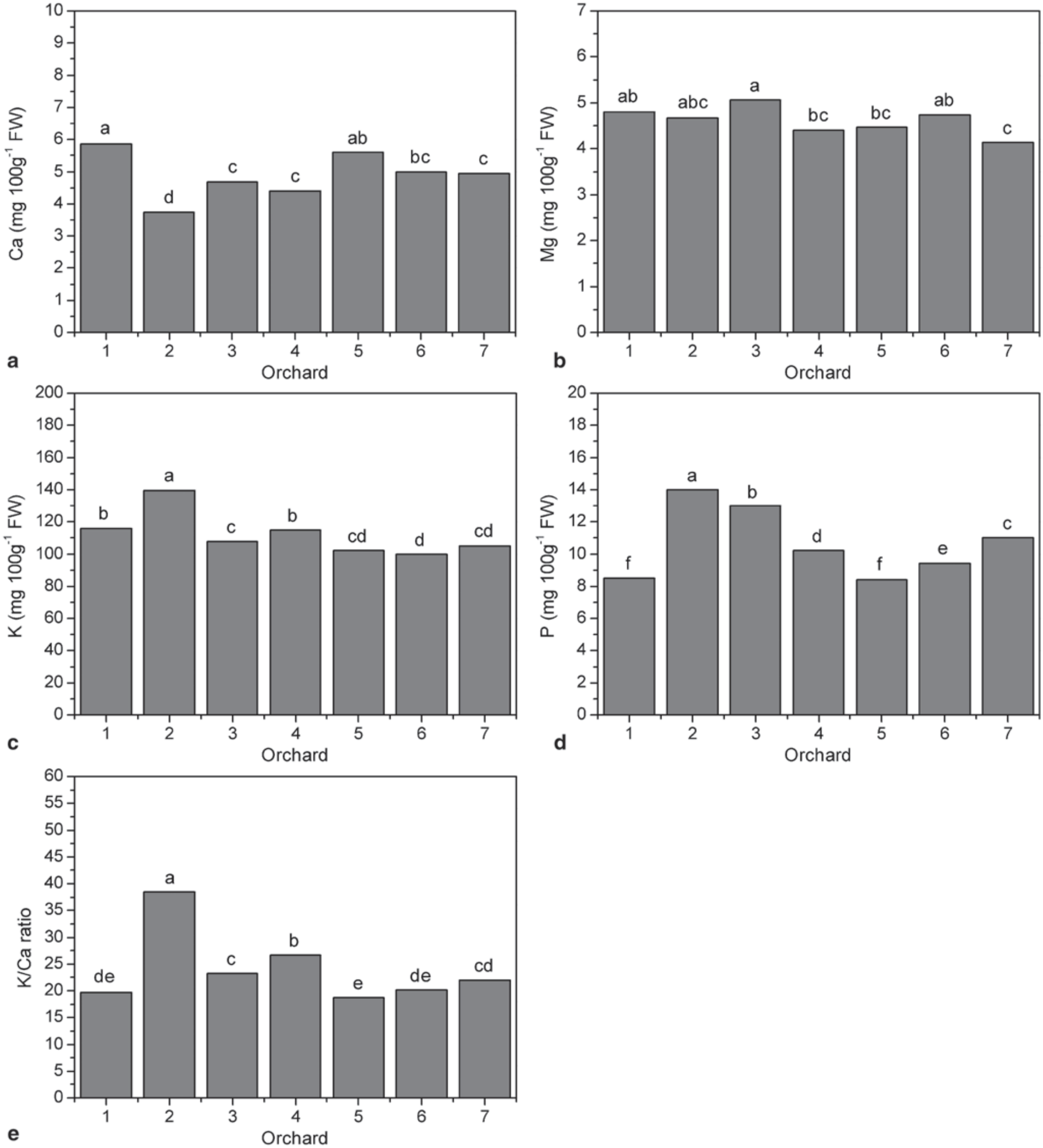

Fig. 3 Mineral concentrations of Ca, Mg, K, P (mg/100 g FW) and K/Ca ratio in 'Braeburn' apple fruits from 7 orchards, different harvest times and storage regimes stored during 6 months under CA-conditions. Means followed by equal letters do not differ by Tukey's test, at $p \leq 0.01$

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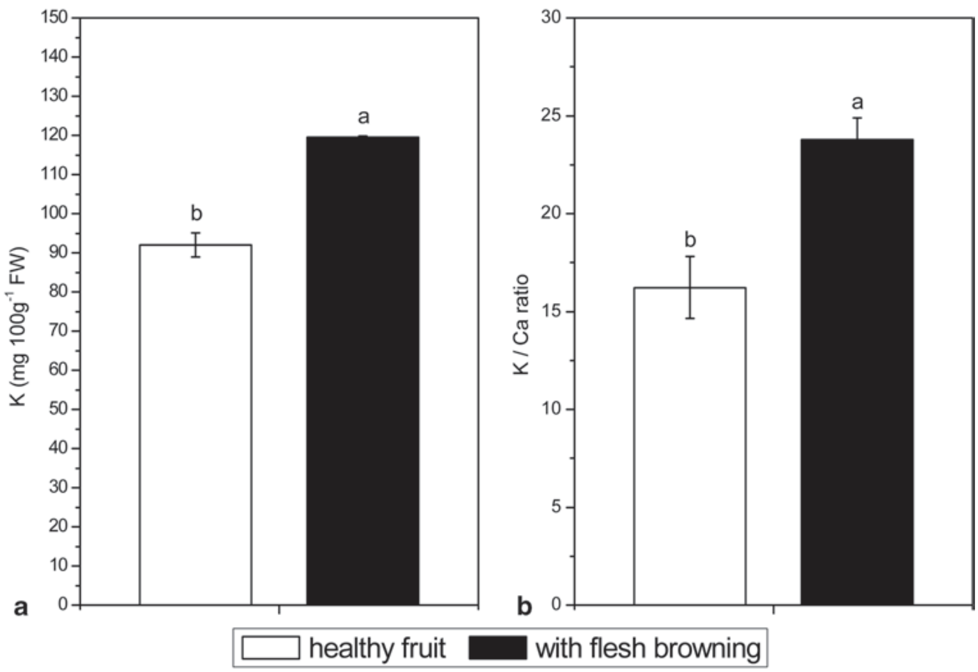

Fig. 4 K concentration (**a**) and K/Ca ration (**b**) of 'Santana' apples storage in commercial CA room, in Lake Constance region, during 6 months with flesh browning occurrence fruits and healthful fruits. Means followed by equal letters do not differ by Tukey's test, in the same graphic, at 1 % error probability

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