Erwerbs-Obstbau (2017) 59:171–183
DOI 10.1007/s10341-017-0336-8

ORIGINAL ARTICLE

Dietary Elements and Quality Parameters of 34 Old and Eight Commercial Apple Cultivars Grown at the same Site in South Tyrol, Italy

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Received: 9 September 2016 / Accepted: 4 May 2017 / Published online: 19 May 2017

Abstract Apple is one of the most widely produced and consumed fruits worldwide and hence, complete data of apple composition are important for human diet. Currently, a limited number of cultivars dominate the market, while many others, with a potentially higher nutritional value, are neglected by consumers. The present work reports the content of the dietary elements potassium (K), phosphorus (P), calcium (Ca) and magnesium (Mg) as well as the content of the macroelement nitrogen (N) of 34 old cultivars grown at the same site under identical conditions in South Tyrol, Italy. Their elemental composition was assessed along with quality parameters such as fruit weight, firmness, and soluble solid content and total acidity at harvest and post storage. For selected cultivars the measurements were performed over two or even three different harvest years. Comparison with eight commercial cultivars chosen to represent the fruit currently dominating the market was performed.

Besides offering a valuable insight in the variation of dietary elements among old and commercial apple cultivars in up to three harvest years, this study, that complements current nutritional databases, recommends several old cultivars with high content of dietary elements for further study and eventual re-introduction in niche markets.

Keywords Apple cultivars · Elemental composition · Dietary elements · Macroelements · Biodiversity and nutrition · Fruit quality

Makroelemente und Qualitätsparameter von 34 alten und acht kommerziellen Apfelsorten angebaut am selben Standort in Südtirol, Italien

Zusammenfassung Der Apfel gehört zu den weltweit am meisten produzierten und konsumierten Früchten, weswegen umfangreiche Daten zu dessen Zusammensetzung für die menschliche Ernährung von Bedeutung sind. Aktuell dominieren einige wenige Sorten den Markt, während viele andere Sorten mit einem potentiell höheren Nährwert dem Konsumenten kaum zugänglich sind. Die vorliegende Arbeit beschreibt den Gehalt der Mengenelemente Kalium (K), Phosphor (P), Kalzium (Ca) und Magnesium (Mg) als auch den Gehalt des Grundelements Stickstoff (N) in 34 alten Apfelsorten, welche unter identischen Bedingungen am selben Standort in Südtirol (Italien) angebaut wurden. Der Mineralstoff- und Stickstoffgehalt sowie Qualitätsparameter wie Fruchtgewicht, Fruchtfestigkeit, Zucker- und Säuregehalt wurden sowohl bei der Ernte als auch nach

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Electronic supplementary material The online version of this article (doi: 10.1007/s10341-017-0336-8) contains supplementary material, which is available to authorized users.

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der Lagerung erhoben. Für ausgewählte Sorten wurden die Analysen in einem zweiten bzw. dritten Erntejahr wiederholt. Ein Vergleich mit acht kommerziellen Sorten, welche den aktuellen Markt dominieren, wurde durchgeführt.

Diese Arbeit ergänzt bestehende Nährstoffdatenbanken und eröffnet wertvolle Einblicke in die Mineralstoffzusammensetzung von alten und kommerziellen Apfelsorten in bis zu drei Erntejahren. Nicht zuletzt zeigt sie alte Apfelsorten mit hohem Mineralstoffgehalt auf, welche für Nischenmärkte empfohlen werden können.

Schlüsselwörter Apfelsorten · Fruchtqualität · Mineralstoffgehalt · Makroelemente · Biodiversität und Ernährung

Introduction

Apple (*Malus domestica* Borkh.) is one of the most frequently consumed fruits worldwide and is recognized as an excellent source of carbohydrates, vitamins, minerals, dietary fibre, pectin and different classes of phenolics, all contributing to its overall beneficial effects and consumer acceptance (Boyer and Liu 2004; Ferretti et al. 2014; Hyson 2011; Jensen et al. 2009). The relative contents of these compounds, which define the nutritional value of apple fruits, depend on the genotype, fruit tissue, ripeness, growing conditions and growing area, cultural practices and postharvest storage conditions (Amiri et al. 2014; Ciesa et al. 2013; Dal Cin et al. 2008; Drogoudi and Pantelidis 2011; Durrani et al. 2010; Henríquez et al. 2010; Moor et al. 2006; Stopar et al. 2002; Szalay et al. 2013; Veberic et al. 2010).

South Tyrol, with an apple growing area of approximately 18,500 hectares and an annual production of up to 1.2 million tons accounts for more than half of the total apple harvest of Italy and holds the largest contiguous apple growing area of Europe (Dalla Via and Mantinger 2012). With several thousand known cultivars, the biodiversity in apple is remarkable; however, the demand for intensive cultivation, long storage time and consumer preferences have led to a progressive varietal impoverishment towards few commercially available apple cultivars (Donno et al. 2012; Storti et al. 2012). In South Tyrol, for example, the three cultivars, Golden Delicious, Gala, and Red Delicious, account for more than 65% of the total commercial production of the region (Dalla Via and Mantinger 2012). This decrease in the number of commercially available apple cultivars is also accompanied by a decrease of knowledge in the identification of the old cultivars over the last decades. The traditional characterisation of apple cultivars is based on morphological and agronomic traits, which depend on environmental factors and are susceptible to misidentification. Thus, an accurate identification of apple cultivars by molecular genetic tools is an important prerequisite (Baric et al. 2011), in order to avoid an incorrect assignment of the results.

In the present study, the major quality parameters and a set of four dietary elements along with nitrogen of 42 accurately identified apple cultivars from the Limburg varietal collection were investigated both at harvest and after storage. Eight of them, accounting for the 94% of the apple production of South Tyrol (Dalla Via and Mantinger 2012; Relation agrarian e forestalled 2014), were chosen to represent the fruit commercially available on the market, and 34 were old or local cultivars formerly grown in the area. The quality attributes and elemental compositional data were obtained from an experimental design that mimicked commercial practises.

Materials and Methods

Plant Material

All apple (*Malus x domestic* Bork.) cultivars included in this study were part of the Laimburg Research Centre varietal collection. The trees were grown in an experimental orchard in Patten/Cadena (South Tyrol, Italy) at 220 m a.m. on M9 rootstocks following the guidelines for integrated fruit production in South Tyrol (AGRIOS 2014).

Approximately 50 fruits were picked at commercial harvest time from the outer layer of 5 trees for each cultivar, avoiding the tops and bottoms of the tree. The investigation was conducted in the 2008 season and comprised 42 apple cultivars, eight of which were commercial and 34 old or local ones. Apple fruits were analyzed at harvest and after 60 days of cold storage (2 °C and 90% RH, relative humidity, ambient air). For selected cultivars (33 in 2009 and 11 in 2010), the experiment was repeated in the following seasons harvest (Table 1).

The genetic identity of all accessions included in the study was certified to be 'true to type' with microsatellite markers (Baric et al. 2008, 2009; Storti et al. 2012) involving references from European germplasm collections except for Alter Wildling, Boznerapfel, Rosa di Seio, Roter Gestreifter Astrachan, and Süßapfel, which were single accessions with no correspondence in international germplasm collections.

Fruit Quality Parameters

Standard quality parameters, including fruit weight, firmness, total soluble solid content (TSS) and titratable acidity (TA) were measured for each cultivar using the 'Pimprenelle' semiautomatic instrument (Setop Giraud Technology, France). Weight (g) and fruit firmness (kg/cm^2) were determined for ten individual apples. Total soluble solid content (TSS, °Brix) was determined refractometrically on the juice of nine apples. Titratable acidity (TA, g/L malic acid) was obtained by neutralization of the juice of nine apples with 0.1 M NaOH. The starch content was determined by iodine-starch test performed on ten apples per cultivar. A 1 cm thick equatorial disk was dipped for one minute in an aqueous Lugol's solution (10 g/L KI + 3 g/L I_2) and the starch index was visually assessed by comparison with the 1 (100% starch) to 10 (0% starch) CTIFL starch conversion color chart for apples (DeLong et al. 1999; Peirs et al. 2002; Streif, 1996; Szalay et al. 2013).

Determination of Macroelements

For the determination of the contents of the macroelements N, K, P, Ca and Mg apples were peeled, cored and homogenized. Approximately 8 g of the homogenized apple pulp were digested at 390 °C for 3–4 h using a modified Kjeldahl solution obtained by mixing 20 mL of concentrated H_2SO_4, 8 mL of H_2O_2 (30% w/w), 6.88 g Na_2SO_4 and 0.112 g $CuSO_4$ $5H_2O$ with water to a final volume of 250 mL. *N* was measured with an AutoAnalyzer 3 (SEAL Analytical Ltd., Hampshire, UK). For the determination of K, P, Ca. and Mg an ICP-OES (Vista-MPX Varian Inc.), equipped with an autosampler (ASX-510 Series Varian Inc.) was used.

For the harvest year 2008, a portion of the superior equatorial slice from the pulp of ten individual apples was analyzed for each cultivar both at harvest and after storage, while for the cultivars assessed in 2009 and 2010 three technical replicates of a bulk of the pulp from five apples were measured at harvest.

Data Analysis

Statistical analysis was performed using R (R Development Core Team, Vienna, Austria).

For macroelements, firmness, TSS and apple weight measurements related to the harvest year of 2008 ANOVA was conducted to test the effect of storage. The model included the effect of storage, the effect of cultivar and the interaction between the two of them.

For samples measured at harvest, the difference between harvest year (2008, 2009 and 2010) was also tested using ANOVA with regard to firmness, weight and TSS. The model included the effect of harvest year, the effect of cultivar and the interaction between the two of them.

Since the analysis of the macroelements was only carried out with biological replicates in 2008 the comparison of the difference between the three years was performed using ANOVA from sufficient statistics using the R package HH (Heiberger 2015). The variance for the 2009 and 2010 samples was in fact set to the maximum variance among all cultivars observed in 2008 to achieve the most conservative test. The ANOVA model was the same as described above.

Multiple comparison testing was conducted according to the Tukey–Kramer method using the R package multcomp (Hothorn et al. 2008).

For the heatmap in Fig. 5, the data were scaled to unit variance and values outside the range of 3 standard deviations were reassigned to 3. The distances in the dendrogram were calculated as '1 – the Pearson correlation coefficient' and the complete linkage method was used for hierarchical clustering.

Results and Discussion

Fruit Quality Parameters: Characterization of Cultivars and Storage Effect

Established quality parameters like size, color, firmness, starch content, total soluble solids (TSS) and titratable acidity (TA) are measured routinely on apples to determine the overall physiological state of the fruit. Furthermore, the kinetics of these parameters during storage define the post-storage fruit quality and ultimately marketability potential of a certain cultivar.

In the present study physicochemical properties of eight well-known commercial apple cultivars have been evaluated along with 34 old cultivars both at commercial harvest and after two months of cold storage (Table 1). Results are reported in full in the supplementary information (Table S1 and Table S7) and boxplots for each parameter at harvest and after storage are shown in Fig. 1.

The studied cultivars were found to cover a wide range of fruit sizes, from small (Rosa di Seio, 118 g; Kalterer Böhmer, 121 g; Steinpepping and Tiroler Spitzlederer, both 137 g) to large (Bismarckapfel, 329 g; Jonagold, 285 g; Weisser Winterkalvill, 281 g) apples. The commercial cultivars were distributed in the interval from medium to large size while the first lower quartile of the dataset contained only old cultivars, characterized by small-sized fruits. As expected, the average weight slightly decreased after storage (Fig. 1). Jan and Rab (2012) reported a decrease in weight of about 2–3% due to water loss and respiration during storage depending on the skin structure and the nature of the surface waxes. However, the biological variation among apples from the same cultivar (relative standard deviation, RSD, from 8 to 28%) averaged the known weight loss during storage.

Firmness is a key factor to determine consumer preferences, as firmer fruits are generally considered to have better eating quality than softer ones. A minimal firmness

Table 1 Overview of the cultivars included in the study reported in alphabetic order

Alter Wildling[a]	*Gala*[c]	*Red Delicious*[c]
Ananas Renette[b]	Gelber Bellefleur[c]	Rosa di Seio[a]
Baumanns Renette[a]	Gelber Edelapfel[b]	Roter Gestreifter Astrachan[b]
Bismarckapfel[a]	*Golden Delicious*[c]	Schmidtberger Renette[c]
Boiken Apfel[a]	Goldrenette von Blenheim[a]	Schöner von Boskoop[b]
Boznerapfel[a]	Grahams Jubiläumsapfel[a]	Schöner von Nordhausen[b]
Braeburn[c]	*Granny Smith*[c]	Steinpepping[b]
Brixner Plattling[b]	*Jonagold*[a]	Süßapfel[b]
Böhmischer Brünnerling[b]	Kalterer Böhmer[b]	Tiroler Spitzlederer[b]
Champagner Renette[b]	Kronprinz Rudolph[b]	Wagnerapfel[b]
Cripps Pink (Pink Lady®)[c]	Lavanttaler Bananenapfel[b]	Weißer Rosmarin[c]
Edelböhmer[b]	Minister von Hammerstein[b]	Weißer Winterkalvill[c]
Falchs Gulderling[b]	Ontario[b]	Weißer Wintertaffet[b]
Fuji[b]	Osnabrücker Renette[c]	Winter Bananenapfel[b]

Commercial cultivars are reported in italics
[a]Cultivars analyzed in 2008 both at harvest and post storage
[b]Cultivars analyzed also in 2009 at harvest
[c]Cultivars analyzed also in 2010 at harvest

of 6–7 kg/cm^2, maintained during storage, is generally required for commercial cultivars (Šavikin et al. 2014).

In our study, firmness at harvest varied from 5.8 (Weisser Winterkalvill) to 10.9 (Edelböhmer) kg/cm^2. The commercial cultivars were generally characterized by a lower firmness at harvest (from Jonagold, 6.5 to Granny Smith 7.7 kg/cm^2), with the exception of Braeburn and Cripps Pink (8.9 kg/cm^2). Only old cultivars were found in the last upper quartile of the dataset characterized by higher firmness. After two months of cold storage, a clear decrease in this attribute was observed. The range of variation after storage spanned from 4.7 (Jonagold) to 8.7 (Tiroler Spitzlederer) kg/cm^2, with an average loss in firmness varying from negligible if compared to biological variation (Fuji, +4%; Granny Smith and Osnabrücker Renette, –4%) to more than 35% (Champagner Renette and Süßapfel, –36%; Lavanttaler Bananenapfel, –38%; Grahams Jubiläumsapfel, –40% and Gelber Edelapfel, –42%). Fig. 1 shows the impact of storage on the relative distribution of firmness in commercial and old cultivars compared to harvest. Old cultivars like Tiroler Spitzlederer, Schmidtberger Renette, Edelböhmer and Baumanns Renette, characterized by high firmness at harvest, remained firm after storage but, while the first two lost 7% of their firmness on average, the latter two lost notable 25%. Gelber Edelapfel and Champagner Renette, which were also characterized by high firmness at harvest, were found in the second quartile of the dataset after storage, with mean firmness losses of 41.5% and 36.2%, respectively. As expected, the firmness of commercial cultivars was less affected by storage. Jonagold displayed the largest change in firmness after storage with an average loss of 27%, followed by Braeburn with 21%. Cripps Pink and Granny Smith maintained firm fruits, the first cultivar due to a high firmness at harvest (8.9 kg/cm^2 compared to 7.4 kg/cm^2 post storage, PS) and the second because of the negligible softening (7.7 kg/cm^2 vs 7.4 kg/cm^2). Thirteen old cultivars and two commercial ones (Jonagold and Golden Delicious) fell below 6 kg/cm^2 after storage. As expected, storage had a major effect on fruit firmness, with old cultivars being more susceptible to softening.

Total soluble solids (TSS) is an established indicator for sugar content. Once a minimum firmness is reached, TSS together with titratable acidity are most relevant for consumer acceptance (Šavikin et al. 2014). In this dataset TSS varied from 10.1 (Granny Smith) to 16.1 °Brix (Ananas Renette), both values being considered extremes in the opposite direction (Fig. 1). All commercial cultivars apart from Granny Smith had TSS values higher than the median and were thus found in the upper half of the boxplot, between Golden Delicious (12.6 °Brix) and Cripps Pink (14.6 °Brix). This picture changed after storage as a result of cultivar-dependent ripening and sugar biosynthesis (Berüter 2004). Granny Smith and Winter Bananenapfel, which were at the lower end of the boxplot at harvest, but also Osnabrücker Renette, Süßapfel and Schöner von Boskoop, which were in the upper quartile, all increased TSS by more than 15%, ranging from 11.5 to 16.5 °Brix after storage. For all other cultivars, including the commercial ones, apart from Granny Smith, TSS increased up to 15%. The biological variation for this parameter was found in a range from 1 to 10% expressed as relative standard deviation, RSD. As a general trend, cultivars with a low starch index (high level of starch content) at harvest showed a higher TSS increase during storage. This can be explained with the conversion of starch to sugar during storage. However, it has to be considered that the reported starch deter-

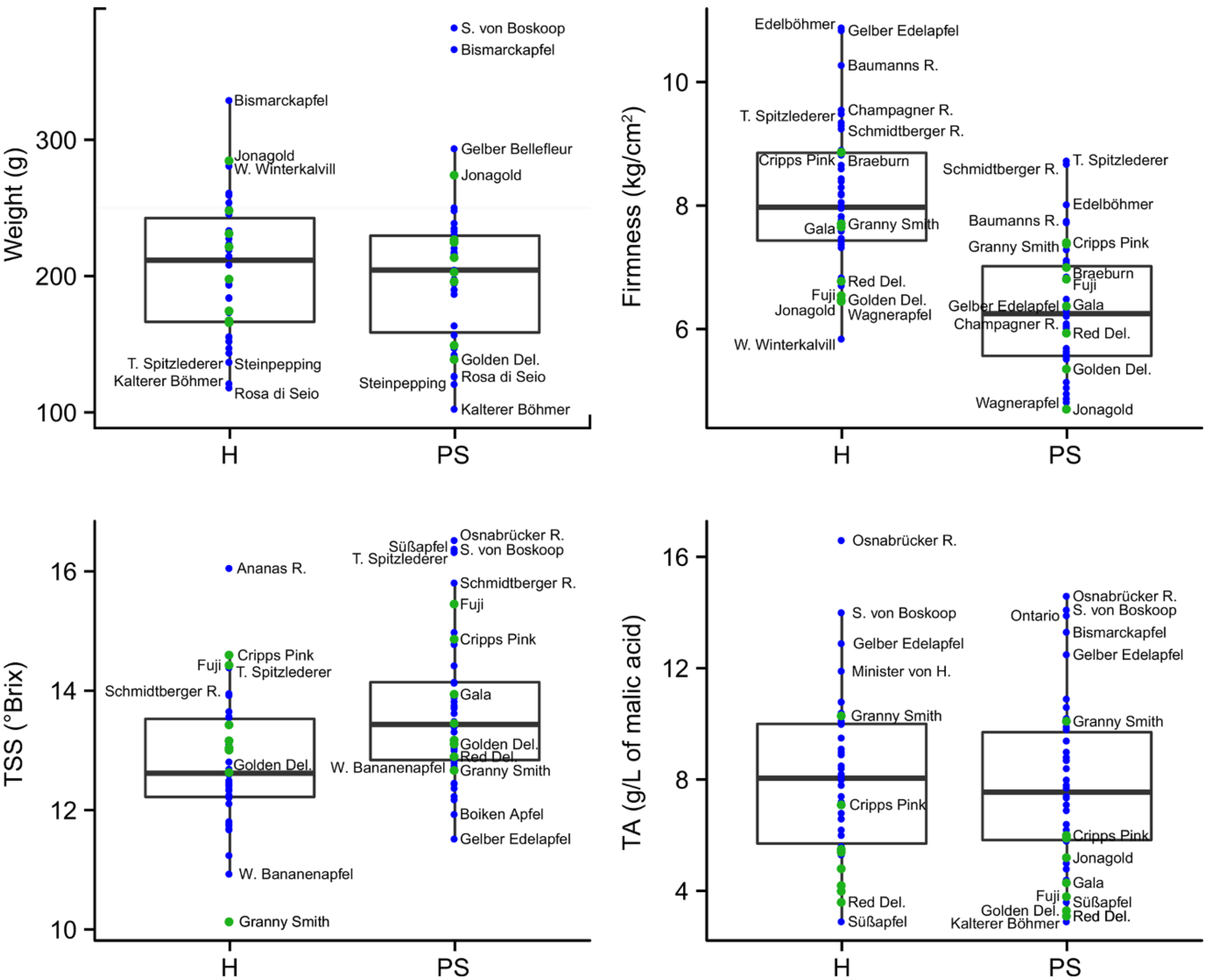

Fig. 1 Apple quality parameters for 34 old (*blue dots*) and eight commercial (*green dots*) cultivars: boxplots for weight, firmness, total soluble solid (TSS) content and titratable acidity (TA) are reported at harvest (*H*) and post-storage, (*PS*, two months of cold storage) in 2008. Each dot represents the mean of ten measured apples

mination is based on a visual assessment with intrinsic bias by the subjective perception (Peirs et al. 2002). After storage, the commercial cultivars were more evenly distributed over the TSS range from Granny Smith (12.7 °Brix) to Fuji (15.5 °Brix). Notably, seven and five old cultivars were found at the lower and higher end of the TSS range respectively, resulting in a final range of variation for TSS from 11.5 to 16.5 °Brix. ANOVA analysis revealed a significant difference for cultivars, but also for storage.

Titratable acidity (TA) was performed on the juice of nine apples for each cultivar. The values ranged from 2.9 (Süßapfel) to 16.6 g/L malic acid (Osnabrücker Renette) at harvest. As expected, Granny Smith was the sourest commercial cultivar in our study with a TA of 10.3 g/L malic acid. All other commercial cultivars were found in the lower part of the range of variation from 3.6 (Red Delicious) to 7.1 g/L (Cripps Pink). The old cultivars, in contrast, were mostly found in the upper part of the range of variation. TA has been shown to decrease during storage (Jan and Rab 2012) and our data confirm this, however, exceptions were detected.

Fruit Quality Attributes: Effect of Harvest Year

33 cultivars (Table 1) were reanalysed in a second harvest year (2009). Detailed data and relative changes compared to 2008 are reported in the supplementary information (see Table S2 and Table S8, respectively). ANOVA analysis was performed, and significant differences were found between cultivars but also between harvest years. However, the changes between the two years were not uniform for all cultivars. The weight was on average 20% higher in 2009

for 24 out of 33 cultivars. Firmness showed an increase for 20 out of 33 cultivars, while TSS was, instead, lower in 2009 for the majority of the cultivars (23 out of 33). However, for both parameters, the relative variation rarely exceeded 10%. For all three parameters the relative variation due to harvest year was comparable to the biological variation. The acidity showed no common trend of variation across the cultivars as result of harvest year. In summary, the data of 2009 generally confirms the results found in 2008 regarding the quality parameters at harvest both for the range of variation and the distribution of the cultivars. In particular commercial cultivars were characterized by medium-sized fruits, lower firmness with the exception of Braeburn and Cripps Pink, higher TSS and lower acidity with the exception of Granny Smith compared to the old cultivars. Old cultivars were confirmed to be more diverse, frequently found at extreme values in the range of variation. The analysis was repeated once more for 11 cultivars in the harvest year 2010. Significant differences were found as effect of both cultivars and year but, again, a cultivar-dependent variation rather than a common trend was detected.

Content of Macroelements at Harvest and After Storage in 2008

Apples are generally regarded as a good source of dietary elements, the content and relative ratio of which have a direct impact on human nutrition, and are relevant for the overall quality of the fruits. Correlations have in fact been found between relative contents of dietary elements and quality parameters including storage potential (Bramlage 1993; de Jager and de Putter 1999).

In this study, the macroelements composition of 42 apple cultivars has been evaluated in 2008 both at harvest and after two months of cold storage. The contents of K, N, P, Ca, and Mg for each cultivar are reported in Figs. 2 and 3 and Table S4 (supplementary information). Table S7 (supplementary information) shows the relative change after storage. K was the most abundant dietary element in apples both at harvest and after storage, followed by nitrogen.

K was found in a range from 92.0 (Winter Bananenapfel) to 248 (Süßapfel) mg/100 g fresh weight (fw). After two months of cold storage, the range of K was from 103 (Winter Bananenapfel) to 210 (Tiroler Spitzlederer) mg/100 g fw and an overall increase in the content of K was registered. All commercial cultivars apart from Gala were found in the lower half of the total range at harvest. After storage, this tendency was maintained with the exception of Granny Smith, where the K content increased more than for other commercial cultivars.

N was the second most abundant macroelement with a content between 20.2 (Cripps Pink) and 55.5 (Baumanns Renette) mg/100 g fw. Gala was again the commercial cultivar containing the highest amount of N (53.7 mg/100 g fw), followed by Red Delicious (51.0 mg/100 g fw) and Jonagold (48.1 mg/100 g fw). Cripps Pink, instead, had the lowest content of N (20.2 mg/100 g fw) followed by Golden Delicious (27.6 mg/100 g fw), Braeburn, Fuji and Granny Smith (34.7 mg/100 g fw). After storage, the N content varied from 19.8 (Cripps Pink) to 58.4 (Gala) mg/100 g fw. Commercial cultivars were distributed over the whole range from the lowest content (Cripps Pink), to low-medium (Golden Delicious, Braeburn, Fuji, and Granny Smith) to high content (Jonagold, Red Delicious, and Gala), both at harvest and after storage. Old cultivars were spread as well over the range of variation, with some of them (Baumanns Renette and Schöner von Boskoop) containing peak amounts of N both at harvest and after storage. Notably, Tiroler Spitzlederer, Weisser Wintertaffet, Schöner von Boskoop, and Gala were in the higher part of the ranges for both K and N contents while the opposite was true for Winter Bananenapfel, Champagner Renette and Cripps Pink. However, Süßapfel, and Steinpepping, were characterized by high amounts of K, but contained medium-low amounts of N, and Baumanns Renette, which had the highest amount of N, was in the lower part of the range for K.

P was found in the apples in a range from 4.34 (Champagner Renette) to 15.0 (Süßapfel) mg/100 g fw. Tiroler Spitzlederer, Weisser Wintertaffet, Gala, and Süßapfel had the highest amounts with contents of 11.8, 12.4, 14.8, and 15.0 mg/100 g fw, respectively. Commercial cultivars, with the exception of Gala and Fuji, were found in the lower half of the range. After storage the range of variation was from 4.03 (Winter Bananenapfel) to 14.7 (Gala) mg/100 g fw and the tendency of commercial cultivars to contain lower amounts of P compared to old cultivars was less clear, because the mean content of this dietary element increased. For example, the P content of the commercial cultivars Fuji and Granny Smith increased by 65 and 50% after storage, respectively, passing from the central part of the range to the higher quartile.

It can be observed that the cultivars, characterized by higher amounts of P, were generally the ones containing also more K and vice versa, confirming a positive correlation between these two macroelements (Bramlage 1993). In particular, Süßapfel, Tiroler Spitzlederer, Weisser Wintertaffet, Steinpepping, and the commercial cultivar Gala had the highest contents of P and K both at harvest and after storage. Conversely, Winter Bananenapfel, Champagner Renette, and the commercial variety Cripps Pink were in the lower quartile of the range of variation for P and K.

Exceptions to such a similarity were detected in particular for the cultivars in the lower part of the range. Braeburn, for example, was at the very bottom of the boxplot for K

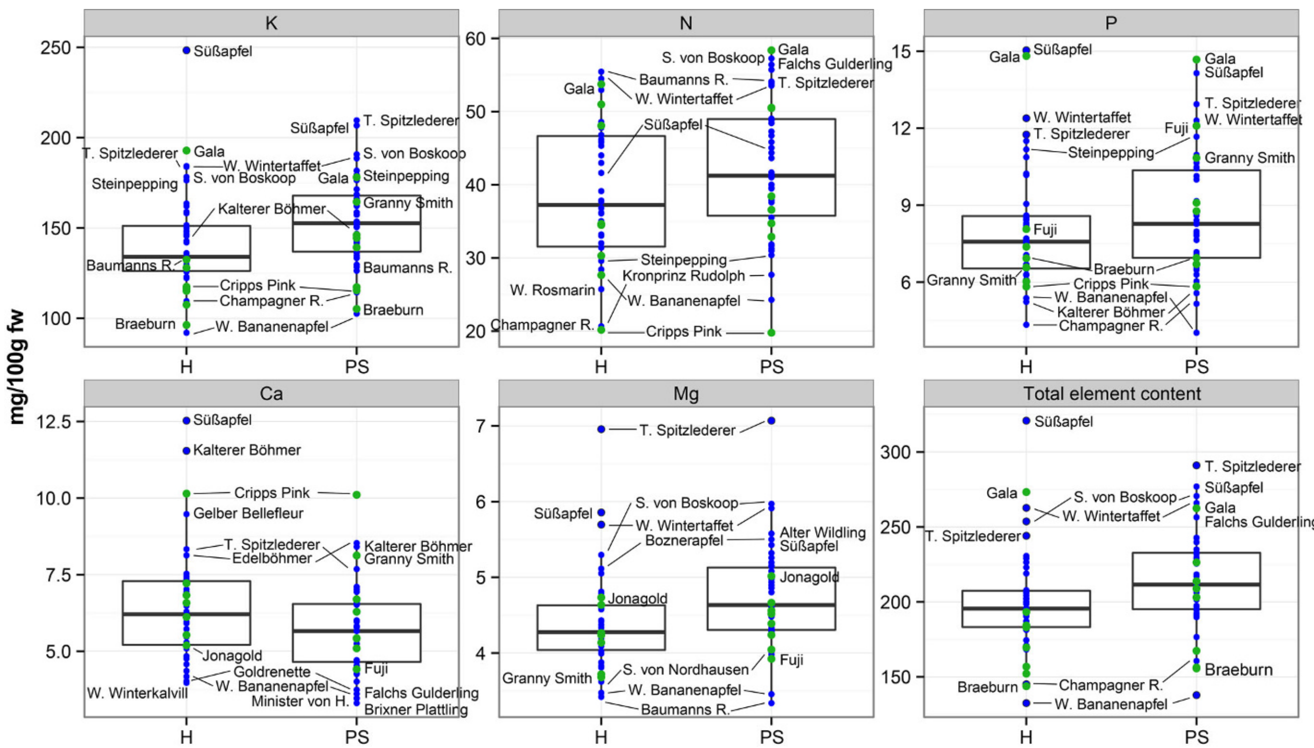

Fig. 2 Boxplots of the content of single macroelements and their total sum for 34 old (*blue dots*) and eight commercial (*green dots*) cultivars analyzed at harvest (*H*) and post-storage (*PS*, two months of cold storage) in 2008. Element contents are expressed as mg per 100 g of the apple pulp (fresh weight, fw); mean values are reported

but contained an average amount of P; the same was found for Kalterer Böhmer in the case of P.

The Ca content ranged from 3.97 (Weisser Winterkalvill) to 12.5 (Süßapfel) mg/100 g fw. After storage Ca was found from 3.31 (Brixner Plattling) to 10.1 (Cripps Pink) mg/100 g fw. Commercial cultivars were, in the case of Ca, and contrary to the tendencies found for the previous elements, distributed in the central part of the boxplot with the exception of Cripps Pink, containing the highest amount of Ca both at harvest and after storage, and Granny Smith found in the first upper quartile of the boxplot after storage. Süßapfel, which showed higher contents of K and P, respect to all other apple cultivars and Tiroler Spitzlederer, characterized by higher amounts of K, P, and N, were both also higher in Ca. Similarly, Winter Bananenapfel was confirmed to be lower also in Ca as well as in other macroelements compared to all the other cultivars considered. On the other hand, Cripps Pink which was lower in all previously discussed elements, had the highest Ca content. Minister von Hammerstein and Falchs Gulderling were characterized by low amounts of Ca, but high amounts of all other elements both at harvest and after storage. Kalterer Böhmer and Edelböhmer, both containing high amounts of Ca, contained medium to low amounts of the other chemical elements (data not shown).

Since the concentration of this element and its ratio with other nutrients have been associated with post-harvest disorders like superficial scald, bitter pit, and senescent breakdown, it is highly desirable for storage potential that apple fruits contain more Ca (de Jager and de Putter 1999; Perring and Pearson 1986). Commercial cultivars, which were selected, for example, for their improved storability, contained a medium-high level of Ca. Many old cultivars, instead, turned out to contain lower concentrations of this dietary element.

Mg was found in the lowest amounts among all macroelements, ranging from 3.42 (Baumanns Renette) to 6.96 (Tiroler Spitzlederer) mg/100 g fw. After storage the element was present in a range from 3.34 (Baumanns Renette) to 7.07 (Tiroler Spitzlederer) mg/100 g fw. Similarly to the other elements, Süßapfel, Tiroler Spitzlederer, Weisser Wintertaffet, and Schöner von Boskoop were found in the higher part of the range of variation, while in the lower end, Winter Bananenapfel was found consistently. Among commercial cultivars, Gala, together with Jonagold, were the highest in Mg; in general, however, old cultivars contained even more Mg.

Fig. 2 shows a tendency for all the considered elements apart Ca to increase after storage. This can be explained by the loss of water during storage (Ghafir et al. 2009). In the case of Ca an unexpected apparent decrease was

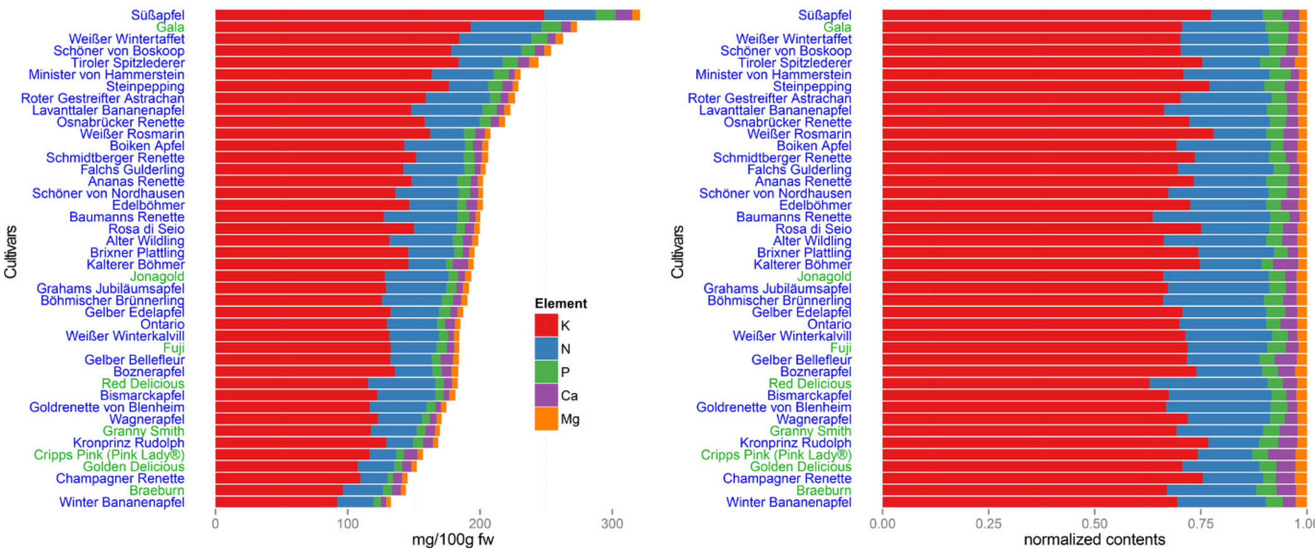

Fig. 3 Overview of the elemental composition of 42 different apple cultivars harvested in 2008. Mean values of ten apples per cultivar are shown. Absolute amounts (*left*) are reported along with normalized contents (*right*). Commercial cultivars are written in green while old cultivars are reported in blue

detected in the present dataset. We hypothesize that such a decrease may arise from a redistribution of this particular dietary element by migration in the flesh at the calyx to stalk ends of the fruit during storage. Since the core was removed before measurement, and only a portion of the equatorial disc was analyzed, this migration could account for the decrease. This tendency was reported by a series of studies by Perring et al. (Perring and Wilkinson 1965; Perring 1989).

Compared to the variation of the quality parameters, the relative changes of the macroelement contents during storage were, as expected, modest. Nevertheless, post-storage data are more relevant when assessing the dietary intake of nutrients or comparing apple cultivars as they are consumed, i. e. after storage, and the distinction should be considered in nutritional databases.

Changes of the individual elements during storage as well as differences between cultivars should be carefully evaluated keeping in account the intra-varietal biological variation. In 2008, ten biological replicates were used to create the mean values discussed above. Standard deviations and relative standard deviations are reported in Table S4 (supplementary information). For the considered chemical elements, biological variation ranged from 3 to 20%, only N and Ca displayed a higher biological variation, with RSD frequently exceeding 20% and reaching even 70 or 80% (Granny Smith for N and Süßapfel for Ca, respectively). Mean RSD for these two elements were 27 and 30%, respectively, while for the others the investigated mean RSD were 20% for P, and 14 and 15% for K and Mg, respectively. Some cultivars (Süßapfel and Steinpepping, and to a lesser extent Baumanns Renette) were characterized by a higher biological variation than others regardless of the element considered. It is important to note that the relative changes of the macroelement concentrations between harvest and storage (Table S7, supplementary information) were generally in the same order of magnitude as the biological variation (±20%). In fact, even if exceptions occurred, the relative order of the cultivars at harvest and after storage was conserved to a great extent.

ANOVA analysis revealed significant differences for all macroelements due to cultivars but also due to storage. However, only for N and Mg the effect of storage was independent from cultivars. For the other elements the effect of storage was also significant but differed among the cultivars.

In Fig. 2, also the total amount of all analyzed elements is reported at harvest and after storage. The total amount is obviously dominated by K and confirms the findings already discussed. A better overview of the total content of the five macroelements studied for each cultivar ordered from smallest to largest total amount is given in Fig. 3. Despite of an apparent variation in the total amount, the relative distribution of the single elements in apples was conserved in all cultivars to a great extent both at harvest and after storage (data not shown). K accounted for about 70% of the total content of macroelements analyzed in this study with a range from 63 to 78%. N accounted for 20% with a range from 12 to 28%, P for 4% with a range from 3 to 6%, Ca for 3% with a range from 1.4 to 6.5% and, finally, Mg for 2% with a range from 1.7 to 2.9%.

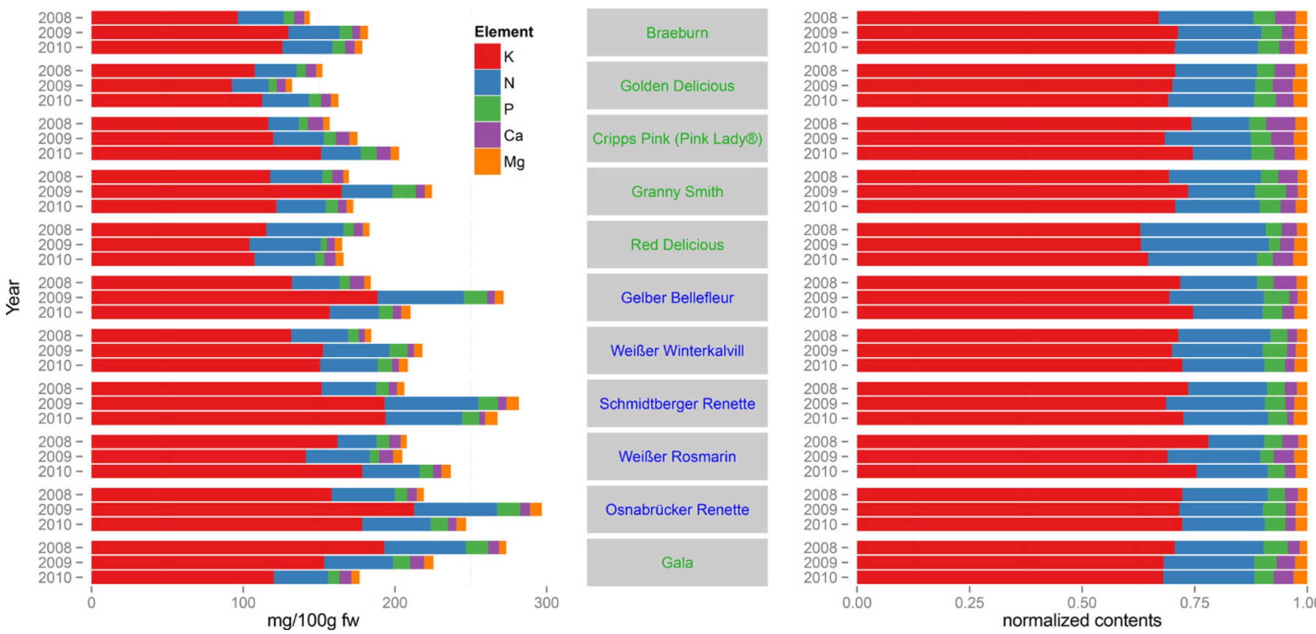

Fig. 4 Effect of harvest year on the elemental composition of 11 different cultivars harvested in 2008, 2009 and 2010. Absolute contents (*left*) are reported with normalized values (*right*). Commercial cultivars are written in green while old cultivars are reported in blue

Changes in Elemental Composition: Effect of Harvest Year

To evaluate the effect of the harvest year, the elemental composition was reanalyzed for 33 cultivars in 2009. The results at harvest are shown in Tables S5 and S8 (supplementary information), the latter reporting the relative changes of elemental composition in 2009 compared to 2008. In general, an overall increase in all elements except Ca was detected in the dataset. Mg was the dietary element with the most consistent trend: all cultivars showed an increased amount of Mg in 2009. For 28 out of 33 the increment was higher than 15%, which was the mean RSD for the biological variation of this element. Similarly, an overall increase was apparent for P and N; however while for many cultivars concentrations rose by more than 20%, being thus, comparable or higher than the mean biological variation (mean RSD was 20% and 27% for P and N, respectively), for some the variation was lower or even negative. The same was true for K, which showed an overall increase in 2009 that rarely exceeded 20% (7 cultivars out of 33) when the mean RSD for the biological variation of K was 14%. The mean content of Ca in 2009 was lower for 19 cultivars out of 33. The variation, however, was not much higher than the biological variation registered for this dietary element (mean RSD for Ca 30%) and, hence, a clear year effect could not be claimed.

ANOVA analysis was carried out on the dataset, and significant differences were found for all considered elements due to cultivars. Only for Mg, P, and K the variation due to harvest year was significant, while for N and Ca, characterized by higher biological variation, the year effect was not significant.

Regarding the relative contents of the single macroelements, some of the trends found in 2008 (Fig. 2) were not confirmed in 2009 (data not shown). Among the cultivars with the highest macroelement contents in 2008 (Tiroler Spitzlederer, Süßapfel, Weisser Wintertaffet, Schöner von Boskoop and Gala) only Tiroler Spitzlederer and Süßapfel were found to contain similarly high amounts of all macroelements also in 2009 (Table S5, supplementary information) Weisser Wintertaffet and the commercial cultivar Gala, were still above average in 2009, but not anymore in the highest part of the upper quartile of the range of variation (data not shown). The opposite occurred for Schmidtberger Renette and Osnabrücker Renette, containing higher amounts of K, P, N, and Mg in 2009 compared to 2008. Winter Bananenapfel, which was at the lower end of the range for all the elements in 2008 (Fig. 2), contained higher concentrations of elements in 2009 (Table S5, supplementary information). For Golden Delicious and Red Delicious, instead, a decrease was registered, and Cripps Pink was not anymore the cultivar containing the lowest amount of K, P and N like in 2008. Cripps Pink was still the commercial cultivar containing the highest level of Ca, and the tendency of many old cultivars to contain lower amounts of this element in comparison to commercial ones was confirmed.

For eleven cultivars, the considered elements were measured also in 2010 at harvest (Fig. 4). It is difficult to recognize a common trend of variation for the total amount of macroelements, but clearly the cultivars respond differently to the same environmental factors, as evident from the opposite trends shown, for example, by Cripps Pink and Gala or Golden Delicious and Schmidtberger Renette in the three harvest years. While Gala contained high concentrations in 2008, the opposite was true for Schmidtberger Renette, which contained high macroelement amounts in 2009 and 2010. On the other hand, the relative ratio of the single elements was conserved, as evident for the normalized contents in Fig. 4.

The contents of the single elements found in this project were compared to previous reports on the same cultivars. However, in all available studies the cultivars were grown in different places and harvested in different years (Table S9, supplementary information). Our results are generally in line with the values reported by other authors with the exception of the results reported by Skordas et al. 2013, which were much higher. Notably, the ranges of variation of the considered macroelements found in the present investigation are higher compared to previous studies, which could be due to the higher number of old cultivars considered.

Characterization of Cultivars by Quality Parameters and Elemental Composition

In order to evaluate patterns in the complete dataset, macroelement contents as well as quality parameters for all 42 cultivars at harvest were displayed in a heatmap (Fig. 5). The cultivars grouped in two major branches that to some extent can account for low (bottom, blue-colored squares) and medium to high (upper part, white to red-colored squares) amounts of Mg, K, P, and N. Each group was then further split in 2 subgroups based on the quality parameters weight, TSS, TA, firmness and the content of Ca.

The sub-group at the bottom of the heatmap was characterized by low amounts of K, N, P and Mg but low to medium-high amounts of Ca and low to medium values of TSS, firmness, acidity and weight. Braeburn was found in this group with a low amount of elements including Ca, low TSS value, high firmness, medium to medium-high acidity and an average weight. In the same group, but characterized by higher TSS than Braeburn and higher Ca contents, were Golden Delicious and Cripps Pink along with Gala. However, in 2008, Gala, due to the very high content of macroelements, was found in the upper part of the dendrogram. Even more intense was the effect of the harvest year on the compositional and physicochemical properties of Red Delicious, the three harvest years of which were distributed over three different subgroups of the dendrogram. Old cultivars in this group, mainly located together at the bottom of the sub-group and characterized by low amount of macroelements, including Ca, were Winter Bananenapfel, Gelber Edelapfel, Champagner Renette and Grahams Jubiläumsapfel. These cultivars showed generally low TSS and high acidity and firmness. Some other old cultivars in this cluster were characterized by different patterns of variation for the contents of chemical elements. For example, Baumanns Renette was low in all elements apart of N, Boiken Apfel contained medium amounts of Ca, and Boznerapfel was characterized by high contents of Mg and Ca.

Moving from bottom to top along the dendrogram clustering cultivars, another sub-group was defined by having in common low weight and high firmness, but varying pattern of variation for the other considered parameters. Tiroler Spitzlederer, Edelböhmer, Ananas Renette and Schmidtberger Renette were found here. Tiroler Spitzlederer and Schmidtberger Renette showed a high level of all considered elements, high firmness, TSS and acidity, and above all very little variation due to harvest year for both the quality parameters and the macroelement contents.

Higher weight and acidity were the main factor responsible for the differentiation of the first group from the second in the upper part of the heatmap. Osnabrücker Renette, Schöner von Boskoop, Weisser Winterkalvill, Falchs Gulderling, Minister von Hammerstein, Brixner Plattling, Böhmischer Brünnerling and Ontario were in this group. Osnabrücker Renette and Schöner von Boskoop were characterized by high amounts of all elements, but displayed different contents of Ca. The other cultivars showed different relative amounts of the single elements.

A higher amount of macroelements, in particular K and P, was the common feature of the second sub-group of cultivars in the upper part of the heatmap, containing Süßapfel, Lavanttaler Bananenapfel, but also the commercial cultivars Granny Smith and Fuji. Süßapfel, in particular, contained high amounts of all elements including Ca.

Regarding the variables, a correlation was observed between K and P, and, to a lesser extent to Mg and N. No consistent correlations were observed among the other considered variables. An inverse correlation between Ca and weight was previously reported (Perring and Jackson 1975) as well as a positive correlation with firmness (Thakur et al. 1997), but these correlations could not be observed in the current dataset.

Fig. 5 shows that commercial cultivars contained low amounts of Mg, K, P and N, but were generally higher in Ca compared to old cultivars. Exceptions to this were Fuji, Jonagold and Braeburn; the first two containing low to medium-high amounts of Mg, K, P and N, but low levels of Ca and the latter containing low concentrations of all elements including Ca. Old cultivars were characterized by a high diversity, being spread over different parts of the dendrogram; however, Fig. 5 points out several cultivars

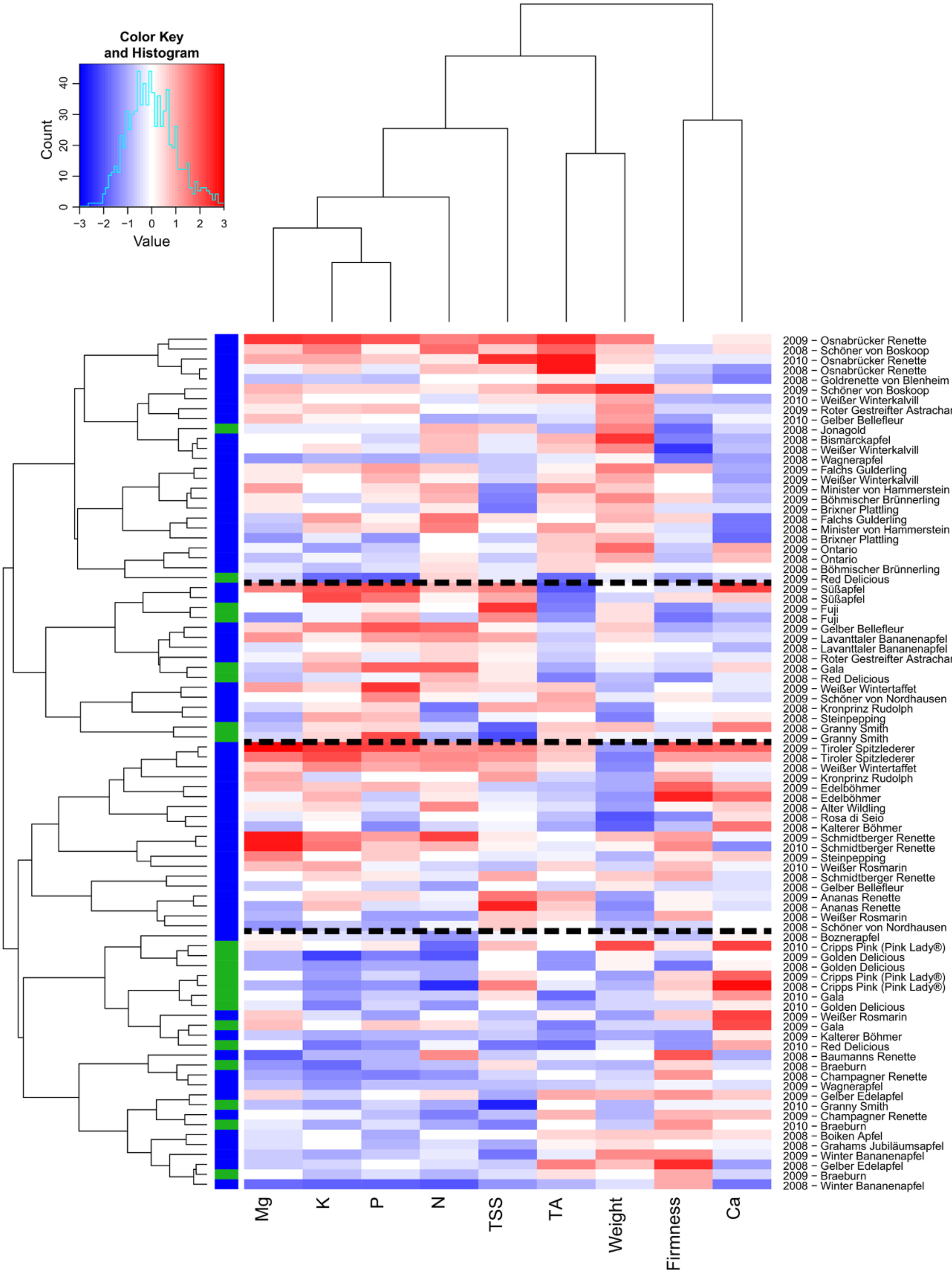

Fig. 5 Heatmap of quality parameters and elemental composition of 8 commercial (*green*) and 34 old (*blue*) cultivars in three harvest years (2008, 2009 and 2010). Data collected at harvest. Two separate dendrograms clusters cultivars (*vertical axis*) and variables (*horizontal axis*). The length of the lines connecting variables and cultivars are proportional to the distance (*correlation*). The major groups of cultivars are divided by a dotted black line

that would deserve to be rediscovered for human nutrition based on their high contents of macroelements, found in this study during different harvest years, both at harvest and after storage: Tiroler Spitzlederer, Süßapfel, Weisser Wintertaffet and Edelböhmer, Schmidtberger Renette and Osnabrücker Renette.

Conclusions

This study explored the content of five macroelements and the quality parameters of 34 old and eight commercial cultivars chosen to represent the apple fruits available for consumers on the market. The cultivars were analyzed in 2008 both at harvest and after storage, and their macroelement profile is reported for the first time. Furthermore, to assess the robustness of the obtained results, the elements and quality parameters were investigated again for 33 cultivars in 2009 and 11 cultivars in 2010.

In general, increased ranges of variation were found for all the measured elements and quality parameters when old cultivars were included, indicating a higher diversity for the latter respect to apples that are currently available on the market. Old cultivars containing high amounts of both dietary elements and nitrogen in different harvest years, were Tiroler Spitzlederer, Süßapfel, and with lower amounts of Ca, Weisser Wintertaffet, Edelböhmer, Schmidtberger Renette and Osnabrücker Renette. Based on our data, these cultivars would deserve to be rediscovered for human nutrition.

Despite other reports on the chemical profiles of different apple cultivars (Eisele and Drake 2005; Ekholm et al. 2007; Feliciano et al. 2010; Gorinstein et al. 2001; Grembecka and Szefer 2013; Guiné et al. 2009; Henríquez et al. 2010; Juranović Cindrić et al. 2012; Manzoor et al. 2012; Nour et al. 2010; Šavikin et al. 2014, Skordas et al. 2013; Todea et al. 2014; Wu et al. 2007) this is, to the best of our knowledge, the first study reporting quality parameters and contents of five macroelements of cultivars accurately identified by molecular genetic determination or even 'true to type' cultivars growing at the same site under the same conditions.

Nevertheless, the present work is surely not exhaustive, showing only a small excerpt of the remarkable undisclosed chemodiversity and nutritional value of certain old apple cultivars, and needs to be complemented with data for other important constituents.

Acknowledgements Irene Höller and Ines Ebner are acknowledged for their support during fruit harvesting and the acquisition of quality parameters.

Funding Financial support from the European Regional Development Fund Program (Project POMOSANO Nr. 5-1a-238 CUP: H21J12000060001 and Project APFELFIT Nr. 1-1a-56 CUP: H21J08000370006) and from the Autonomous Province of Bozen/Bolzano is gratefully acknowledged.

Conflict of interest S. Agnolet, F. Ciesa, E. Soini, A. Cassar, A. Matteazzi, W. Guerra, P. Robatscher, A. Storti, S. Baric, J. Dalla Via and M. Oberhuber declare that they have no competing interests.

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