A comparison of apple cultivars regarding ethylene production and physico-chemical changes during cold storage

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ABSTRACT: Measurements of titratable acidity, soluble solids, firmness, ethylene production and weight loss were made for five apple cultivars held in cold storage for 100 days. Carbosieve G in the traps of the enrichment column, which has only a moderate affinity for light hydrocarbons, was found to meet the requirements for the optimal thermal desorption of ethylene (130°C for 2 minutes) from the enrichment column to the analytical column. ANOVA showed significant differences in all these five parameters between the five cultivars Golden Delicious Reinders, Resista, Topaz, Meteor and Rubinstep, and also in the course of storage. In all cases, the changes in titratable acids measured during storage were especially significant, but the observed changes in sugar levels, as measured by refractometry, were too variable to be useful in this context. High rates of ethylene production impacted probably only indirectly on the loss of firmness and the other parameters which were measured. Discriminant analysis of the measurements of firmness, ethylene production and titratable acid provided the best means of differentiating the cultivars, although Golden Delicious Reinders and Resista still could not be completely separated. Other parameters (soluble solids and loss in weight) did not contribute to the discriminant resolution.

Keywords: apple cultivars; composition; ethylene production; firmness; headspace gas analysis

Large quantities of apples are kept in cold storage after harvest to preserve their quality. Temperature strongly affects the post-harvest life of apples, and the optimum temperature for slowing the deterioration in quality is usually 0–3°C, depending on the sensitivity of particular cultivars to chilling injury. Comparing the responses of different cultivars may allow a more complete understanding of how apple softening is influenced by temperature. Apples, like other climacteric fruits, display an autocatalytic increase in ethylene production during ripening, and this is responsible for the changes in texture, firmness, color, and other processes. Endogenous ethylene plays an important role in apple softening with the transition from initial to rapid fruit softening of several cultivars, and is associated with a rapid rise in ethylene production (Knee et al. 1983; Larrigaudiere et al. 1997; Stow et al. 2000; Johnston et al. 2001; Nilsson, Gustavsson 2007). Fruit infected by *Cleosporium album* Ostrw. displays lower ethylene production in cold store (3°C) than healthy fruit (Goliáš et al. 2006). The control of ethylene production in stored apples offers the prospect of preventing or delaying softening and improving the texture of the fruit available to consumers. The removal of ethylene from the storage environment of apples has been shown to improve quality, including firmness (Goliáš, Létal 1995).

Quality is a combination of many parameters, of which firmness is a very important one.

Indeed measurements of soluble solid content (SSC) and titratable acidity are often included in assessments of the postharvest quality of apples (Vangdal 1985; Jaeger et al. 1998). McClone et al. (2003) have shown that total sugars, measured as the SSC in post-storage apples, strongly correlate with dry matter, and low sugar fruit was unlikely to store for long without losing flavour and textural quality.

Firmness and soluble solids content are two important quality parameters in determining fruit maturity and harvest time, and they are also key parameters in assessing and grading the postharvest quality of apples. After harvest, fruit firmness declines over time, but SSC usually remains stable. At present, the destruc-

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tive Magness-Taylor firmness test is still the industry standard for measuring fruit firmness. In term of consumer acceptability appeared to be according to whether a sweet, hard apple or a juicy, but acidic apple was preferred (Daillant-Spinnler et al. 1996).

A wide range of factors influence the resistance of apple cultivars to storage diseases (Blažek et al. 2007), particularly the presence of phenolic compounds, skin thickness and its resistance to mechanical damage. This study used discriminant analysis to compare the performance of five different apple cultivars during cold storage, by measuring the production of ethylene and other, non-volatile compounds, changes in firmness of tissues and weight loss.

MATERIALS AND METHODS

Choice of adsorbent material for trapping ethylene

Detection of ethylene at trace levels (< 10 ppm) is necessary in order to be able to study the influence of this gas on plant development. Gas chromatography (GC) with FID combined with static headspace analysis is limited by the amount gas that can be reliably introduced into the GC inlet. Measurement by dynamic enrichment concentrates analytes to detectable levels and so increases the detection limits. As an adsorbent material for the traps, Carbosieve III has a high affinity for light hydrocarbons such as ethylene and this makes thermal desorption ineffective. However, the Carbosieve G adsorbent (Supelco Inc., Bellefonte, PA) provides optimal thermal desorption of analytes, at 130°C for 2 minutes, from the enrichment column to the analytical column.

Determination of ethylene in intact fruit

Prior to sampling the apples were placed in a cold storage room. To measure ethylene production, one intact apple was placed in a 0.5 l hermetically sealed jar, with five replicates. The jars were sealed with two gas ports. Purified air flowed into the jars at 50 ml/min at 20°C, prior to sampling. Gas samples were collected from the outlet of each jar and passed through the enrichment column containing 370 mg of the sorbent Carbosieve G for 6-minutes flow, so that the total volume was 300 ml. Under these suction conditions, ethylene was completely retained in the enrichment column (conservation version). Each sample was analyzed using a thermal desorption system model TD 2 operated in manual mode and an Agilent 4890D gas chromatograph with FID detection. An analytical column of 30 m, I.D. 0.32 mm and 20 µm film was chosen. The choice of column combined with careful temperature programming by thermal desorption allows samples to be injected without cryotrapping and provides excellent resolution of ethylene from water and other compounds present. Identities of ethylene from other compounds in the sample were confirmed in a separate analysis ($v_{(s)}$ the volume injected in the analytical column, $c_{(s)}$ the concentration of the standard in the calibration sample). The ethylene production from the intact fruit, released into the percolating gas (G – µl/kg/h), was calculated from the flow rate (F) of the percolating gas (50 ml/min), the volume (V) permeating through the enrichment column (3 l) and the weight (m) of the inserted fruit in the sampling chambers (kg).

Physico-chemical analysis

Flesh firmness was defined as the maximum force required to push the 11 mm Magness-Tylor probe into the fruit flesh (after skin removal) on two opposite sides of each fruit, to a depth of 8 mm, with a penetrometer (Toruni, Forli, Italy). The results were expressed in MPa.

The soluble solids content (SSC) was measured at 20°C using an Abbe refractometer (Carl-Zeiss Jena), using juice pressed from a sample of homogenized fruit slices. Titratable acidity (TA) was determined in a water extract of a weighed amount of homogenized apple tissue, by titrating to pH 8.1 with 0.1 NaOH. The results of TA were expressed as g/100 g apple tissue, calculated as malic acid.

Statistical analysis of data

ANOVA was performed on the results for the soluble solids, titratable acidity and flesh firmness, and Fisher's least significant difference (LSD) intervals, at 95% confidence limits, were computed for each parameter. Unistat 5.1 Statistics software was used.

RESULTS AND DISCUSSION

Entrapment of ethylene

Water is problematic with respect to normal thermal desorption analysis, because cryofocussing is generally used to immobilize analytes during the desorption step, thereby improving the chromatography performance dramatically. Here the problem can be circumvented by use of a chromatography column with a high loading capacity and a packing which enables resolution of ethylene from other light hydrocarbons and gases, as well as the water in the

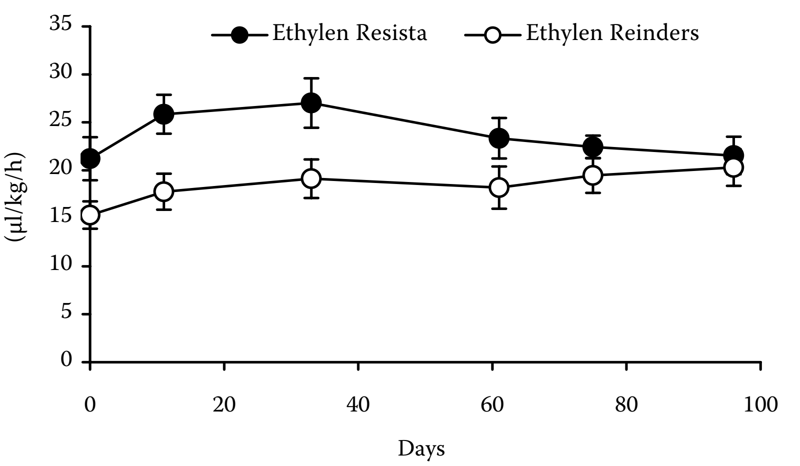

Fig. 1. Time pattern of ethylene production (µl/kg/h) for the cultivars Golden Delicious Reinders and Resista, stored in an ambient atmosphere. Each value represents 5 fruits and vertical bars indicate SE ($P < 0.05$)

sample. However, the gas-solids system with Porapak Q was unsatisfactory for determining ethylene concentrations lower than 1 ppm in the percolating gas. Therefore, a gas-solid system, with activated charcoals termed Carbosieve G and Carbosieve SIII as sampling tube packing, was tried. Carbosieve G was selected as the sorbent material for the traps for its relatively high affinity for light hydrocarbons and its better release of ethylene during the thermal desorption process.

Ethylene production

The ideal apple for long-term storage combines higher skin thickness and toughness with a lower production of ethylene, which slows the softening of fruit (Blažek et al. 2007). Apples are climacteric fruits, characterized by a burst of ethylene production during ripening. In our experiments the cultivars exhibited only small increases in ethylene production, with only Resista and Topaz showing the characteristic rise

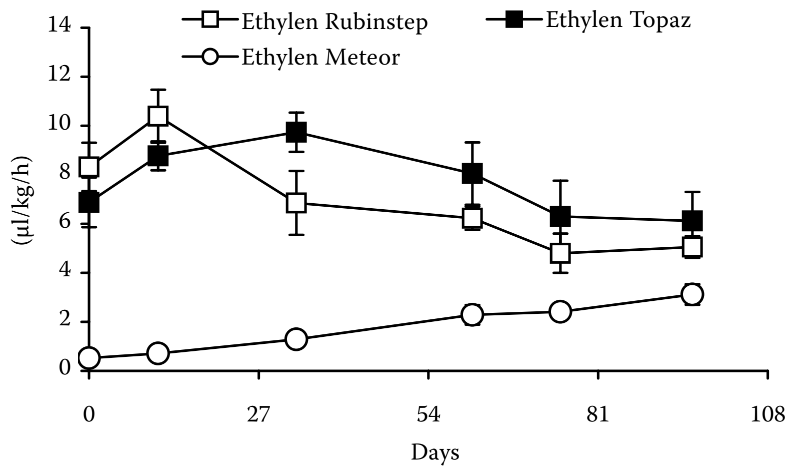

Fig. 2. Time pattern of ethylene production (µl/kg/h) for the cultivars Rubinstep, Topaz and Meteor, stored in an ambient atmosphere. Each value represents 5 fruits and vertical bars indicate SE ($P < 0.05$)

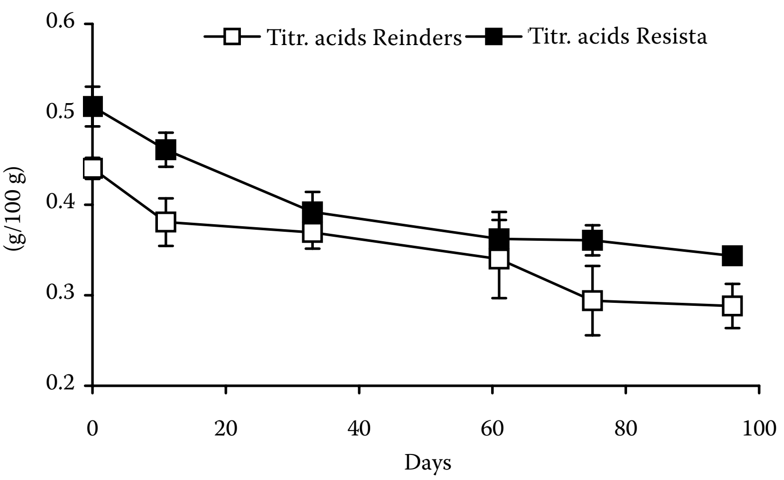

Fig. 3. Time pattern of titratable acidity for the cultivars Golden Delicious Reinders and Resista, stored in an ambient atmosphere. Each value represents 5 fruits and vertical bars indicate SE ($P < 0.05$)

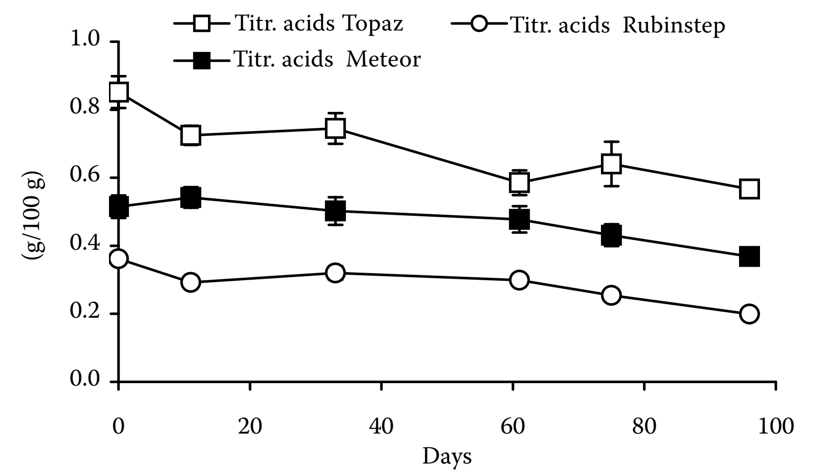

Fig. 4. Time pattern of titratable acidity for the cultivars Topaz, Rubinstep and Meteor, stored in an ambient atmosphere. Each value represents 5 fruits and vertical bars indicate SE ($P < 0.05$)

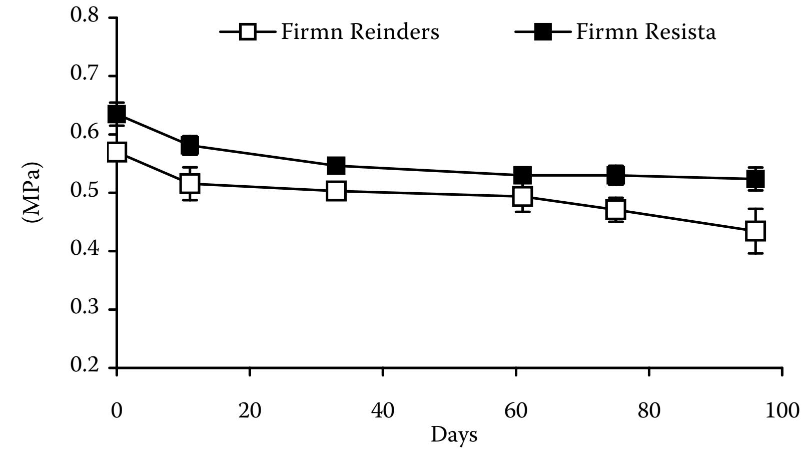

Fig. 5. Loss in firmness by cv. Golden Delicious Reinders and Resista, stored at 3°C

and fall over time (Figs. 1 and 2). The differences in ethylene production rates observed among the five apple cultivars cannot be explained by changes over time in the soluble solids and organic acids (Figs. 3 and 4). In elucidating how softening patterns differ among cultivars, Wakasa et al. (2003, 2006) distinguished four ripening-related genes, which influenced endogalacturonase enzymes in apple flesh.

Non-volatile compounds

The sugar:acids ratio is commonly used as an indicator of maturity in fruits such as grapes and citrus, but it is not a reliable indicator in apples. One of the best measures of maturity is the change in total soluble solids, measured using a refractometer. The Brix readings during the storage period remained

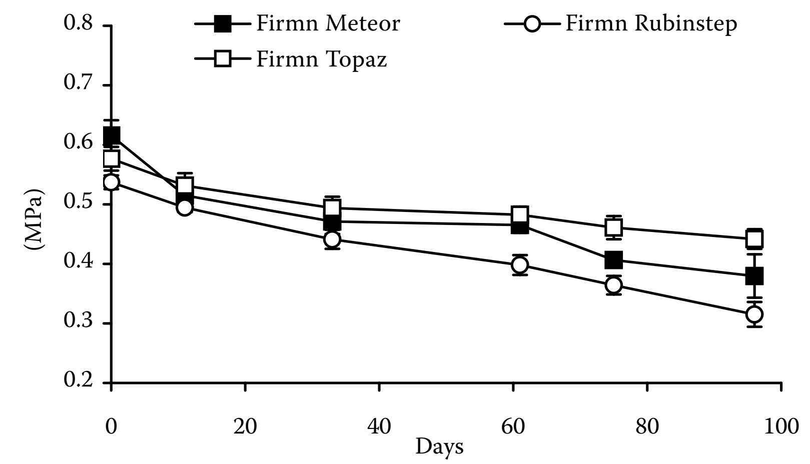

Fig. 6. Loss in firmness by cv. Meteor, Rubinstep and Topaz, stored at 3°C

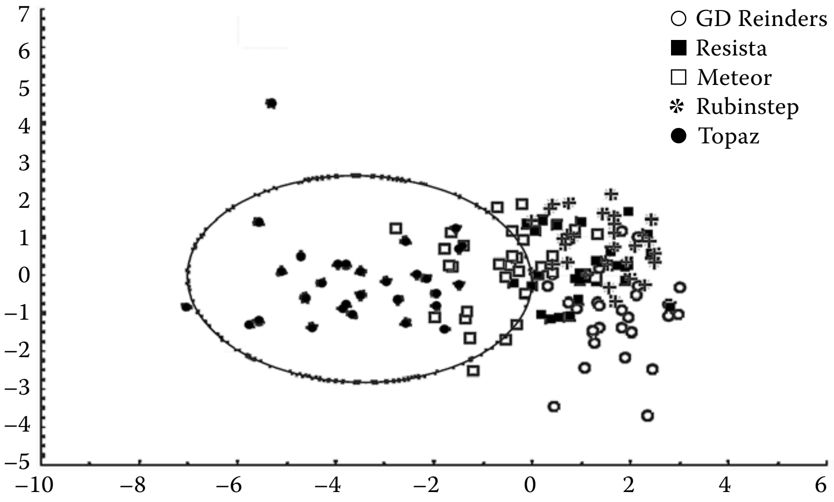

Fig. 7. Soluble solids, titratable acids and firmness of five apple cultivars held at 3°C for 96 days: Golden Delicious Reinders, Resista, Meteor, Rubinstep and Topaz. Only Topaz was discriminated from the other cultivars

unchanged in the range 12.8° to 14.6° Brix for all cultivars. The evaporation of water from the fruit surface and the consumption of sugars by respiration were perhaps in equilibrium. Organic acids, on the other hand, are an important source of respiratory energy in plant cells, and were depleted at roughly equal rates. The original differences in levels between cultivars remained unchanged over time (Figs. 3 and 4), although the varieties could be divided into two groups based on their rates of consumption of

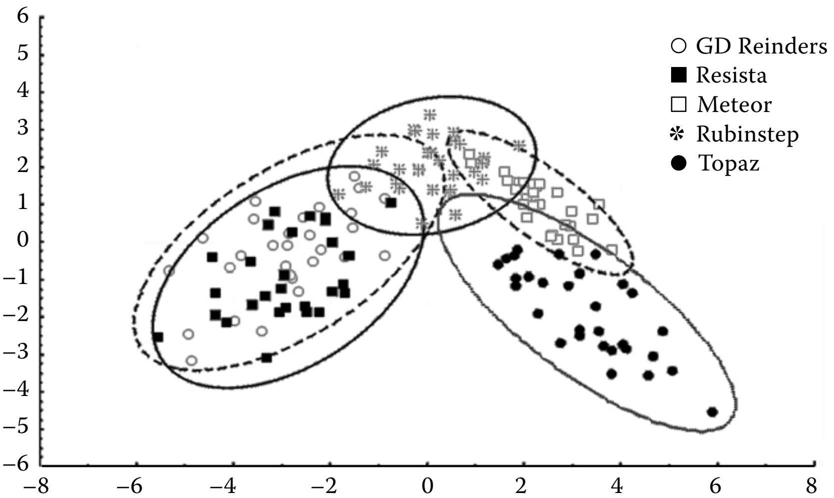

Fig. 8. Ethylene production, soluble solids, titratable acids, and firmness (without values for weight loss) for five cultivars held at 3°C for 96 days: Golden Delicious Reinders, Resista, Meteor, Rubinstep and Topaz, Resista and Golden Delicious Reinders were not discriminated from the other cultivars

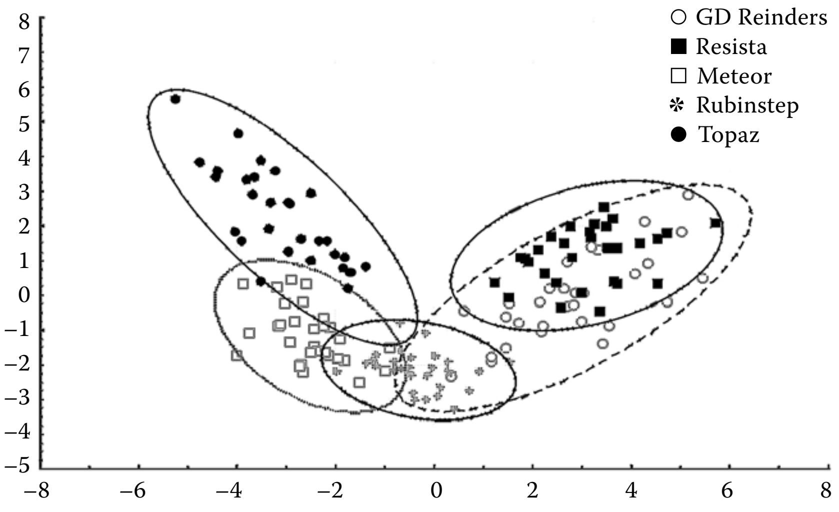

Fig. 9. Ethylene production, soluble solids, titratable acids and firmness (with weight loss) for Golden Delicious Reinders, Resista, Meteor, Rubinstep and Topaz. Golden Delicious Reinders and Resista were not discriminated

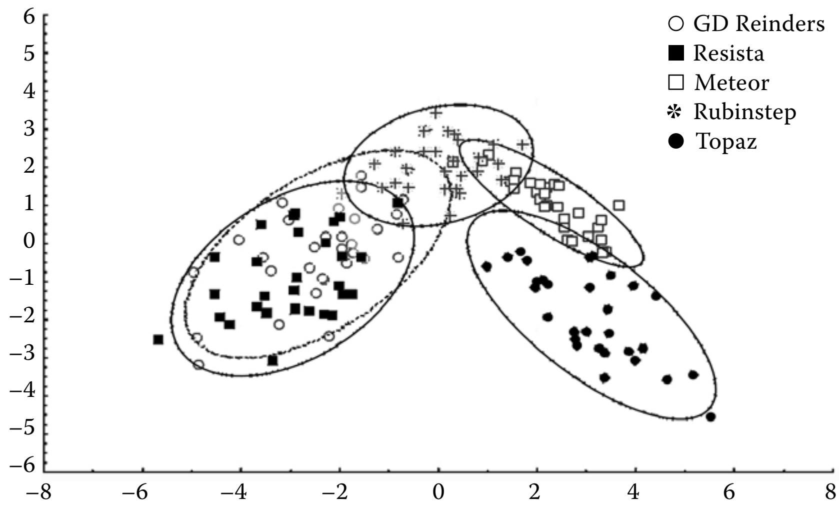

Fig. 10. Titratable acids, firmness and ethylene production for the discrimination of Golden Delicious Reinders and Resista held in similar storage conditions

organic acids (expressed as titratable acidity): cvs. Topaz, Resista and Rubinstep had losses of acidity of 2.76–2.96 mg/day, and Golden Delicious Reinders and Meteor had losses of 1.53–1.58 mg/day.

Evaporation

The skin protects fruit against uncontrolled evaporation and skin thickness should be greater than 60 µm. In addition, the skin also helps to protect against apple storage diseases (Homutová, Blažek 2006; Blažek et al. 2007). Water vapour presents about 90% of all weight losses, although the cultivars behaved differently: Golden Delicious Reinders, Resista and Rubinstep lost 93 to 114 mg/day of water, and Meteor and Topaz lost 60–65 mg/day.

Loss of firmness

The softening of fruit is the consequence of cell wall degrading enzymes, of which endopolygalacturonase causes the most prominent changes. Flesh firmness loss patterns were classified roughly into two categories: gradually decreasing (Meteor and Rubinstep) and maintained (Topaz, Resista and Golden Delicious Reinders). There was no apparent correlation between the rate of ethylene production and loss in flesh firmness (Figs. 1, 2, 5, 6). The production of very low levels of ethylene, such as in Meteor, should result in moderately high firmness when the fruit ripens. However, a high rate of ethylene production impacts probably only indirectly on the loss of firmness and other parameters of ripening which were measured. In conclusion, the MT instrument measured apple firmness precisely across a range of cultivars and storage conditions, with standard errors from 0.01 to 0.02, even though the punch is hand-operated.

Analysis of data

A discriminant analysis was performed to study whether the different parameters obtained were useful for discriminating between the cultivars under study. Discrimination among the five cultivars based on analysis of non volatile compounds, firmness, ethylene production and weight loss during cold storage is shown in Figs. 7 to 10. Fig. 7 shows that the cultivars did not divide clearly into groups, although Topaz was separated on the basis of soluble solids, titratable acid and firmness. In this way, it can be shown that the important parameters for distin-

Table 1. Statistically significant differences in five apple cultivars by measuring of ethylene production, non-volatile compounds and skin firmness

	Soluble solid	Firmness	Titratable acids	Ethylene production	Mass loss
Cultivar	**	**	**	**	**
Time	ns	**	**	ns	**
Cultivar × time	*	**	*	**	**

Two-way ANOVA: **$P < 0.01$, *$P < 0.05$, ns – not significant guishing these five apple cultivars are the titratable acids, ethylene production and firmness, although the cultivars Golden Delicious Reinders and Resista overlap closely and remain difficult to separate clearly. Differences in acid content were detected when apples differed by more than 800 mg/kg of titratable acidity. Thus measurements of titratable acidity may be a useful way to differentiate cultivars and predict the stability of apples in storage. This may also be important for assessing fruit quality, since consumers often have distinct preferences for acid levels (Figs. 8 and 9). For a higher level of discrimination between samples coming from the same growing area, it could be interesting to measure ethylene production, acids and firmness, which are more characteristic of the apple cultivars than the other parameters, and could provide complementary information for the resolution of apple cultivars (Fig. 10). All the observed parameters were useful in differentiating the cultivars held in cold storage (Table 1).

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Srovnání odrůd jablek podle produkce etylenu a fyzikálně chemických změn při chladírenském skladování

ABSTRAKT: Podle produkce etylenu, titrační kyselosti, rozpustné sušiny, pevnosti dužniny a hmotnostních ztrát při skladování byly hodnoceny vlastnosti odrůd jablek po dobu 100 dnů. Pro zachycení etylenu do obohacovací kolony je vhodný Carbosieve G, který má střední afinitu pro nízkomolekulární uhlovodíky, a v zařízení pro tepelnou desorpci se uvolňuje z obohacovací kolony při 130 °C za dobu dvou minut. Analýzou rozptylu byly zjištěny významné rozdíly mezi pěti odrůdami jablek (Golden Delicious Reinders, Resista, Topaz, Meteor a Rubinstep) a dobou skladování. Změny titračních kyselin měřené během skladování jsou statisticky významné, ale pozorované změny v obsahu rozpustné sušiny, měřené refraktometricky, byly značně variabilní pro použití v těchto souvislostech. Vysoká produkce etylenu ovlivňuje ztrátu pevnosti dužniny jen nepřímo. Diskriminační analýzou se prokázaly rozdíly na základě znalosti pevnosti dužniny, produkce etylenu a jejich obsahem titračních kyselin. Pouze pro odrůdy Golden Delicious Reinders a Resista nelze podle zadaných hodnot obě odrůdy od sebe rozlišit. Další zkoušené parametry, jako jsou rozpustná sušina a hmotnostní ztráty, nepřispívají ke zlepšení statistického rozlišení diskriminační analýzou.

Klíčová slova: odrůdy jablek; látkové složení; produkce etylenu; pevnost dužniny; headspace analýza

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