Accepted Manuscript

Effects of different organic and conventional fertilisers on flavour related quality attributes of cv Golden Delicious apples

A. Raffo, I. Baiamonte, R. Bucci, A. D'Aloise, M. Kelderer, A. Matteazzi, E. Moneta, N. Nardo, F. Paoletti, M. Peparaio

PII: S0023-6438(14)00398-3

DOI: 10.1016/j.lwt.2014.06.045

Reference: YFSTL 4009

To appear in: *LWT - Food Science and Technology*

Received Date: 16 December 2013

Revised Date: 17 June 2014

Accepted Date: 18 June 2014

Please cite this article as: Raffo, A., Baiamonte, I., Bucci, R., D'Aloise, A., Kelderer, M., Matteazzi, A., Moneta, E., Nardo, N., Paoletti, F., Peparaio, M., Effects of different organic and conventional fertilisers on flavour related quality attributes of cv Golden Delicious apples, *LWT - Food Science and Technology* (2014), doi: 10.1016/j.lwt.2014.06.045.

Effects of different organic and conventional fertilisers on flavour related quality attributes of cv Golden Delicious apples.

A. Raffo*[1], I. Baiamonte[1], R. Bucci[2], A. D'Aloise[2], M. Kelderer[3], A. Matteazzi[3], E. Moneta[1], N. Nardo[1], F. Paoletti[1], M. Peparaio[1].

[1] *Agricultural Research Council - Research Center on Food and Nutrition (CRA-NUT); Via Ardeatina, 546 -00178- Rome, Italy*
[2] *University of Rome La Sapienza - Department of Chemistry; P.le Aldo Moro, 5 -00185- Rome, Italy*
[3] *Research Centre for Agriculture and Forestry Laimburg; Laimburg, 6 -39040- Ora (BZ), Italy*

* Corresponding author
Phone: +39 0651494573;
Fax: +39 0651494550;
E-mail: antonio.raffo@entecra.it

Abstract

The effects of the application of different organic and conventional fertilisers on some flavour quality attributes (aroma volatiles, sugars and organic acids) of cv Golden Delicious apples were investigated by an experimental field trial in two harvest years (2010 and 2012). Through a balanced randomized block design, five organic fertilisation treatments (three different fertilisers at the same nitrogen dose, increase and fractionation of dose for one of the fertiliser) were compared to each other, to a conventional treatment based on a mineral fertiliser and to a non-fertilised control.

Fertilisation treatments significantly affected the level in fruits of several flavour related compounds, such as some aroma volatiles, sugars and organic acids, but few of these responses were consistent across the two harvest years and of remarkable size. Even when treatments gave place to marked differences in the soil mineral nitrogen level, this reflected in a limited impact on flavour related compounds in the fruit, the strongest effect being a 45% change in C6-aldehydes level. The different organic fertilisation treatments weakly affected the considered fruit quality attributes. Significant differences were observed for several sensory attributes between apples coming from different fertilisation treatments and characterised by a quite similar chemical profile.

KEYWORDS: fertilisation, organic farming, *Malus domestica*, volatile, organic acid, sugar.

1. Introduction

The potential of the organic farming system as an environmentally and economically sustainable option for apple production under appropriate cultivation conditions has been the subject of recent investigations (Alaphilippe, Simon, Brun, Hayer, Gaillard, 2013; Reganold, Glover, Andrews, Hinman, 2001). Alto Adige is an Italian geographic area that has proved to be well adapted for apple growing, having developed in last decades a strong territorial specialisation for apple cultivation and, in particular, for organic apple production, with a production share estimated at about 35% within the European Union. One of the main issues for organic apple cultivation in this area is linked to the insufficient plant nutrient supply due to the organic form of nitrogen fertilisers, which are characterised by a slower nutrient release when compared to the mineral conventional counterparts. In particular, a limited nitrogen supply tends to lower fruit yields and prevents an appropriate exploitation of available arable land. This is a general drawback for organic crops cultivation (Lester & Saftner, 2011; Seufert, Ramankutty, Foley, 2012), being exacerbated in this case by the relatively low soil average temperatures occurring in this geographic area at the beginning of spring. With a view to tackle this limitation, laboratory and field experimental trials have been undertaken in the last few years by comparing the performance of several organic fertilisers in terms of nitrogen mineralisation rates, in order to optimize the release of plant available mineral nitrogen from organic sources (Kelderer, Thalheimer, Andreaus, Topp, Burger, Schiatti, 2008). However, if the application of organic fertilisers of different origin and chemical composition is expected to produce differences in the whole supply of all the plant mineral nutrients, little is known about the effects of mineral soil nutrition on apple fruit quality and, in particular, on flavour quality at harvest (Ferguson & Boyd, 2002; Pelayo-Zaldivar, 2010).

Flavour quality, though not a primary driver of choices of organic food consumers, still has a significant role in determining their preferences (Oughton & Ritson, 2007). Fruit flavour is a complex trait formed by several sensory attributes, most notably aroma, taste, texture, appearance and chemesthetic sensations (Deibler & Delwiche, 2004). Considering, in particular, taste and

aroma of apple fruit, sweetness is mainly due to sugars, such as fructose, sucrose and glucose, sourness is mainly related to the presence of malic acid (Harker, Marsh, Young, Murray, Gunson, Walker, 2002), whereas olfactory sensations are evoked by a complex array of volatile substances (López Fructuoso, Echeverría Cortada, 2010). Even though more than 300 compounds have been identified in the volatile fraction of apple fruit (Nijssen, Ingen-Visscher, Donders, 2011), only a few dozens of them have been recognised to significantly contribute to the perceived fruit aroma (Aprea, Corollaro, Betta, Endrizzi, Demattè, Biasioli et al., 2012; López Fructuoso et al., 2010; Mehinagic, Royer, Symoneaux, Rique Jourjon, Prost, 2006; Plotto, McDaniel, Mattheis, 2000; Young, Gilbert, Murray, Ball, 1996).

Increased levels of nitrogen application usually enhance plant vigour and fruit yield, thus reducing fruit soluble solids concentration by dilution or shading effects (Ferguson & Boyd, 2002). In agreement with this general finding, reduced soluble solids levels have been observed in apple fruit in association with increased nitrogen leaf levels (Dris, Niskanen, Fallahi, 1999). A relationship between fruit potassium content and acidity has been found in some apple cultivars (Marcelle, 1995). While previous observations related poor apple flavour quality to reduced nitrogen fertilisation (Somogyi & Childers, 1964), a more recent study (Fellman, Miller, Mattinson, Mattheis, 2000) found little or no effect of nitrogen application on aroma production, even though an indirect effect on tree vigour and, consequently, on subsequent maturity stage at harvest was not ruled out as an influential factor. In particular, while branched-chain amino acids are precursors in the synthesis of branched-chain esters, which are important apple odorants, application of increasing levels of nitrogen did not affect amino acid precursors availability nor aroma production in apples (Fellmann et al., 2000). More recently, flavour quality of apples obtained under organic, conventional and integrated production systems has been compared (Peck, Andrews, Reganold, Fellman, 2006; Reganold et al., 2001). Significant differences appeared for some sensory attributes: even though they were not clearly related to differences in chemical composition, they suggested the potential impact of differences associated to these farm management systems on fruit flavour

quality, even when cultivar, soil, rootstock, plant age and all other conditions except management were kept constant. In addition, results from recent investigations have suggested that an insufficient nitrogen supply due to reduced release rates by organic fertilisers could stimulate plant stress responses, resulting in an enhanced accumulation of phenolic flavonoids in fruits of tomato, grapefruit and sweet pepper, thus potentially affecting both flavour and nutritional quality (Lester & Saftner, 2011).

The aim of this study was to evaluate the effect of the application of three different organic fertilisers on some flavour quality attributes of cv. Golden Delicious apples, in two non-consecutive harvest years. For one out of the three organic fertiliser a fractionation and an increase of the application dose was also tested. Fruits obtained by these fertilisation treatments were also compared with fruits from plants supplied with a conventional mineral fertiliser, and plants that did not receive any fertilisation treatment. To amplify all potential effects due to fertilisation treatments young trees were selected for the experimental field trial.

2. Materials and methods.

2.1 Experimental field trial

The trial was part of an experimental apple orchard at the Research Centre for Agriculture and Forestry Laimburg (Laces: lat. 46°62'N, long. 10°86'E, alt. 640 masl), in the province of Bolzano. In 2009, four replicate plots for each of the seven fertilisation treatments were planted with cv. Golden Delicious apples (*Malus* × *domestica* Borkh.) on M9 rootstock, complying with a balanced randomised complete block design. Well feathered trees were planted, according to a common practice in this geographic area, where trees generally go into production one year after planting. Each plot was formed by five trees (rows spaced by 3.2 m apart, trees spaced 0.8 m apart). Three different organic fertilisers were used. Fertiliser 1 (OF1) was a commercial nitrogen enriched organic fertiliser formed by vegetable oil cakes, feather meal and horn-hoof mixture. Fertiliser 2 (OF2) was an experimental fertiliser formed by a mixture of digested slurry from biogas plants and compost. Fertiliser 3 (OF3) was a commercial organic fungal biomass based fertiliser, obtained by

fermentation of a mixture formed by soya meal, sugar, syrups, cottonseed meal, trace elements and vitamins. Fertiliser 1 and 3 have been selected as products complying with organic production protocols adopted in this production area and on account of their relatively good mineralisation performance in laboratory test. Fertiliser 2 has been selected as a potential innovative product, also with a view to the optimisation of the use of local resources. In the first year, the three organic fertilisers were applied two weeks after planting, whereas in the following years they were given as a single dose early in the spring season. Two additional treatments were carried out by using fertiliser 1, the first by fractionating the same total dose in three one month-spaced applications (OF1 33%×3), and the second by fractionating a 50% increased dose in three applications (OF1 50%×3). A reference conventional treatment (CF) by application of ammonium sulphate as mineral fertiliser and a control non-fertilised treatment (NF) were also implemented. Details on number of applications and nitrogen doses used in the four years of the experiment for each treatment are reported in Table 1.

For all treatments common management practices (fruit thinning, weed control, pest and disease control) used in this area for production of organic apples were followed, with no marked differences between the two years 2010 and 2012.

2.2 Chemicals

Pure compounds of the volatile compounds, organic acids and sugars listed in Table 3, and of the internal standards (4-methyl-2-pentanol, allyl hexanoate), were purchased from Sigma-Aldrich Italia (Milan, Italy). Solvents and reagents were purchased from Carlo Erba Reagents (Milan, Italy).

2.3 Soil and leaves analyses

For soil analyses, five soil cores were collected from each plot at depths from 0 to 40 cm, as listed in Table 2. Plant available nitrogen in the soil was determined by the analysis of mineral nitrogen (VDLUFA, 2002). For leaves analyses, 25 leaves were collected from plants of each plot, selecting only fully grown leaves from the central part of the newborn shoots. Determination of total nitrogen content of the leaves was carried out by the dry combustion method (ISO, 2008). For the analysis of

potassium, leaves samples were dissolved in a microwave oven after addition of nitric acid and hydrogen peroxide; the analytical determination was performed by inductively coupled plasma-optical emission spectroscopy (EPA, 2007).

2.4 Chemical determinations on fruits

Apple quality was assessed on fruits harvested in October 2010 and September 2012, at commercial maturity stage. Fruit maturity stage at harvest was evaluated by determination of the starch hydrolysis index (Osterloh, Ebert, Held, Schulz, Urban, 1996) and flesh firmness (Zanella & Werth, 2004).

2.4.1 Aroma volatile compounds

Two hundred grams from peeled, sliced and cored apples (about 20 grams from each of 10 fruits, that were selected for each treatment from each block) were added with 400 mL of a NaCl 200 g L^{-1} water solution containing the internal standards (1.01 mg L^{-1} 4-methyl-2-pentanol and 1.11 mg L^{-1} allyl hexanoate methanolic solution) and then blended for one minute. The blended suspension was centrifuged for ten minutes at 22000 ×g and 4°C, then the supernatant was collected and filtered under vacuum with a Whatman filter paper n. 113. Isolation of volatile compounds from the obtained aqueous extract was carried out by the Stir Bar Sorptive Extraction technique (SBSE) (Prieto, Basauri, Rodil, Usobiaga, Fernández, Etxebarria et al., 2012). Application of this technique to the study of the impact of pre- and post-harvest factors on the formation of volatile compounds in fruit has been described in previous papers (Raffo, Nardo, Tabilio, Paoletti, 2008; Raffo, Kelderer, Paoletti, Zanella, 2009; Raffo, Nicoli, Nardo, Baiamonte, D'Aloise, Paoletti, 2012; Paoletti, Raffo, Kristensen, Thorup-Kristensen, Seljåsen, Torp et al., 2012). Conditions of SBSE isolation and GC-MS analysis were as previously described (Raffo et al., 2009), with the following modifications: duplicate SBSE extraction was performed on the above aqueous extract and a single GC-MS analysis on each SBSE isolate, the CIS-4 PTV injector was cooled at -50°C, the capillary GC column was a DB-1MS (Agilent Technologies Inc.) column (30 m x 0.25 mm i.d., 0.25 μm film thickness), the GC temperature program was from 40 °C (2 min) to 160 °C at 4 °C min^{-1}, and then

to 270 °C (5 min) at 20 °C min^{-1} (total run time of 42.50 min). Identification of compounds was carried out by comparing mass spectra obtained by the full scan mode (*m/z* range 40-400 amu) and Kovats linear retention indices determined on chromatograms of apple sample isolates, with spectra and retention indices obtained from authentic standards. For the semi quantitative determination of volatiles, spectrometric detection in the selected ion monitoring (SIM) mode was used: mass fragments (*m/z*) selected for each detected compound are reported in Table 1S. Levels of volatile compounds were expressed as the ratio of the analyte peak area to the peak area of one of the two internal standards, or to the sum of peak areas of the two internal standards. For some compounds, this latter option provided a better repeatability, presumably due to the fact that the combination of the structural features of the two internal standards reproduced the extraction and chromatographic behaviour of the target analyte better than those of each of the individual internal standards (Table 1S).

2.4.2 Organic acids and sugars

All HPLC analyses were performed on an Agilent system equipped with a 1100 Series quaternary pump, a diode array detector and a refractive index detector. Ten fruits, selected for each treatment from each block, were peeled, sliced, cored and then homogenised and the same homogenate was used for both organic acids and sugars analysis. The analytical procedures for organic acids and sugars described in a previous paper (Raffo, Baiamonte, Nardo, Paoletti, 2007) was followed, with the following modifications. For organic acids analysis, the obtained aqueous extract (Raffo et al., 2007) was subjected to chromatographic analysis on a Synergi Hydro-RP80A column (4 μm particle size, 250 × 4.6.mm), thermostated to 35 °C. Isocratic elution was carried out with a 0.02 mol L^{-1} aqueous solution of H_3PO_4 (pH 2.7) at a flow rate of 0.8 mL/min, and the eluate was monitored at 210 nm. For sugars determination, the obtained aqueous extract (Raffo et al., 2007) was subjected to chromatographic separation on a Luna NH2 column (3 μm particle size, 4.6 × 150 mm), thermostated to 35 °C. For both organic acids and sugars analyses, triplicate extraction was performed and a single HPLC analysis on each obtained extract.

193 *2.5 Sensory analyses*

194 On apple samples harvested in 2012, from two of the four replicate plots, sensory analyses were
195 carried out. Sensory evaluations were performed by a panel of ten assessors (ISO, 2012) with multi-
196 year experience in sensory analysis of fresh fruit. Assessors took part in two pre-testing sessions of
197 90 minutes each to develop a list of attributes appropriate for describing samples to be studied, to
198 reach a consensus on the definition of each attribute and to accomplish a calibration on the
199 evaluation scale. A list of 14 attributes were developed (Table 2S), which described taste, odour and
200 mouthfeel of apple fruit, according to the Descriptive Analysis method (Lawless & Heymann,
201 2010). Pre-testing sessions were also performed to test the homogeneity of panel outcomes by a
202 two-way ANOVA (considering samples and assessors as main effects), and the ability of each
203 assessor to discriminate samples and to provide repeatable results by a one-way ANOVA. Analyses
204 were performed during eight testing sessions, each of 90 minutes, in four days. Apple samples were
205 evaluated in a monadic sequence by triplicate analysis. Each assessor received one intact fruit at
206 room temperature in a plastic container, identified by a three digit code. All sensory attributes were
207 evaluated on peeled and cored apple slices. After each sample, assessors rinsed their mouth with
208 still bottled water, whereas the order of evaluation was randomized across panelists. Intensity was
209 measured by using an unstructured intensity scale of 150 mm, anchored at the extreme values 0 and
210 9 corresponding to “not detectable” and “high intensity”, respectively. Data were recorded by the
211 FIZZ software v.2.40 (Biosystemes, Couternon France).

212 *2.6 Statistical analyses*

213 Data obtained from chemical and sensory analyses were statistically tested for significance of the
214 effects of fertilisation treatments and blocks (plots) by multi-way analysis of variance (ANOVA),
215 without determination of the interaction treatments × blocks, as recommended for statistical
216 analysis of randomized complete block designs (Ireland, 2010). A mixed-effects model was
217 selected, fertilisation effects being introduced as fixed and block effects as random (van Es, Gomes,
218 Sellmann, van Es, 2007). Whenever a significant effect of a fertilisation treatment was observed, a

post-hoc Tukey-Kramer test was applied to seek significant differences between the compared means. Only on the volatile dataset, a preliminary Principal Component Analysis (PCA) was performed on mean values of the analytical replicate determinations, previously preprocessed by autoscaling. Pearson's correlation coefficients between fruit levels of flavour related compounds and leaf levels of nitrogen and potassium were also determined to investigate correlations between the formation of these compounds and the mineral status of the trees. All the previous statistical calculations were computed in MATLAB 2007b (The MathWorks, Inc., Natick, MA); PCA was performed by the PLS Toolbox 4.2 chemometric suite (Eigenvector Research, Inc., Wenatchee, WA), running in the same computational environment.

Partial Least Square Regression (PLSR) was used to investigate the relationships between the determined compounds and odour and flavour sensory perceptions. All chemical data constituted the independent X-block of variables, while the sensory attributes (only odour, taste and flavour attributes) represented the dependent Y-variables. Data were normalized using the 1/(standard deviation) transformation to remove scale effects. The calibration models were validated by full cross-validation. Weighted regression coefficients (BW) for the relationships between chemical and sensory variables with associated confidence limits were determined by jack-knifing (Martens & Martens, 2001). When the confidence distance did not exceed the value of the weighted regression coefficients they were significant at a level $p<0.05$. Data were processed (Unscrambler®, v. 10.2, CAMO Software A/S, Trondheim, Norway) using NIPALS algorithm.

3. Results and discussion.

3.1 Mineral nitrogen in soil and leaves

To provide a meaningful comparison between the performance of the different fertilisers in terms of nitrogen mineralisation, the doses of all fertilisation treatments, except for the non-fertilised treatment and OF1 50%×3, were set so that plants of all treatments were supplied with the same amount of total nitrogen per year (Table 1). For the treatment OF1 50%×3, the dose was deliberately increased to evaluate the effect of the amount of nitrogen supplied in the form of

organic fertiliser. Neither the different type of organic fertiliser nor the amount and fractionation of the application dose seemed to significantly influence plant available mineral nitrogen concentration in the soil, whereas organic treatments generally gave place to lower levels of mineral nitrogen when compared to the conventional treatment at the key time of fruit set (Table 2). As expected, the lowest mineral nitrogen levels were observed in the non-fertilised plots, but not in all cases these levels were significantly lower than those in the organic plots. These differences disappeared later in the growing season (data not shown). The observed differences in the levels of available mineral nitrogen in the soil tended to be reflected in differences in the nitrogen status of the tree, as monitored by measurement of total nitrogen levels in the leaves about one month after flowering (Table 2).

3.2 Fruit ripening stage

Ripening stage strongly influences fruit composition, and, in particular, concentrations of volatile compounds dramatically changes as ripening progresses (Fellmann et al., 2000; Mehinagic et al., 2006). So fruit ripening stage at harvest was assessed by determination of firmness and starch hydrolysis index (Table 3S). In the first harvest year apples from the non-fertilised treatment were more firm than those from the conventional treatment, whereas no significant differences in the ripening stage were detected by the starch index. In the second year no significant differences were observed for both the considered parameters. Apart from the slight difference of firmness in the first year, it is reasonable to assume that apples from all treatments were harvested at a quite similar stage of ripeness so that a significant contribute of ripening stage on the observed differences in flavour quality attributes between treatments could be ruled out.

3.3 Effects of fertilisation treatments on aroma volatiles

With reference to volatile production cv. Golden Delicious has been categorized among apple cultivars as an ester-type cultivar and, in particular, as an acetate ester-type, because acetates are the main constituents of fruit volatile fraction (Aprea et al., 2012; López Fructuoso et al., 2010; Mehinagic et al., 2006). In the present study twenty volatile compounds were identified and

quantified: nine straight-chain esters (acetates, propanoates and butanoates), four branched-chain esters (acetates and methylbutanoates), three alcohols, two C6-aldehydes and two other miscellaneous compounds. The detected profile showed the same major constituents previously observed in studies carried out on cv. Golden Delicious apples by different volatile isolation techniques, such as dynamic headspace on the intact fruit (López, Lavilla, Recasens, Graell, Vendrell, 2000; Song & Banghert, 1996), vacuum distillation (Mehinagic et al., 2006) or solid phase microextraction (Aprea et al., 2012) on sliced apples, whereas differences appeared with regard to the minor constituents of the volatile fraction. Interestingly, the profile determined in the present study contained many of the most potent odorants previously identified in cv. Golden Delicious apples by gas chromatography-olfactometry, such as 2-methylpropyl acetate, butyl acetate, 1-hexanal, 2-methylbutyl acetate, butyl propanoate, butyl butanoate, hexyl acetate and 1-hexanol (Mehinagic et al., 2006). Among miscellaneous compounds estragole and benzothiazole were both found in some apple cultivars (Nijssen et al., 2011), the former being recognized as an important odorant in cv. Gala apples (Plotto et al., 2000). Moreover, in our samples the potent odorant ethyl-2-methylbutanoate, previously detected at very low levels in Golden Delicious apples (Mehinagic et al., 2006), was not detected at all.

A first exploratory Principal Component Analysis on the whole dataset obtained from determinations of volatile compounds on apples from the seven fertilisation treatments in both harvest years, showed a marked season to season variability (Fig 1S), as expected on the basis of previous observations (López Fructuoso et al., 2010). For this reason, the following data analysis was performed separately on the two datasets from each year, looking later for consistent effects across the two harvest years. PCA on the two separate datasets showed that, in both years, apple field replicate samples obtained by the same fertilisation treatment from the different blocks generally did not group in the scores plot (Fig. 1, 2), suggesting that the effects of the considered fertilisation treatments on the global volatile profile did not overcome those due to non-uniformity between the blocks in the experimental field trial conditions. Only field replicates from the non-

297 fertilised treatment in 2010 tended to group and to separate from the other treatments (Fig. 1).
298 ANOVA of data on individual volatile levels indicated a significant effect of the fertilisation
299 treatments on 18 out of 20 volatile compounds in 2010, and on 8 compounds in 2012 (Table 4S). In
300 most of these cases, however, a significant effect of the blocks was also observed, showing again
301 that non-uniformity in the field experimental conditions significantly affected the formation of the
302 considered volatiles. As a whole, few of the effects of fertilisation treatments on individual
303 compounds were consistent across the two years and of remarkable size. Among these were the
304 effects on the formation of C6-aldehydes: in both years, apples from non-fertilised plots were
305 characterised by lower levels of C6-aldehydes when compared to all the other treatments (on
306 average by 30%). In both years, a reduction of about 45% was found in the C6-aldehydes level of
307 the non-fertilised treatment when compared to the treatment showing the maximum level. On the
308 contrary, apples from non-fertilised plots tended to contain increased levels of some straight-chain
309 esters, such as the important odorants butyl, pentyl and hexyl acetate, but only in 2010, and butyl
310 propanoate and hexyl butanoate, but only in 2012.
311 Considering only apples coming from the organic treatments, few significant effects were observed.
312 Moreover, even the marked differences observed in the levels of available nitrogen in the soil when
313 comparing the non-fertilised and the conventional treatment, and the corresponding significant
314 differences in the nitrogen status of the trees, had a limited impact on the global volatile profile.
315 This confirmed previous findings regarding the quite moderate influence of nitrogen nutrition on
316 the formation of aroma compounds, observed in a 2-years study on cv. Redspur Delicious apples
317 (Fellmann et al., 2000), and contradicted results from another rather dated study (Somogyi &
318 Childers, 1964). In particular, there were no consistent effects on the biosynthesis of branched-chain
319 esters, which was expected to be most sensitive to the nitrogen nutrition status of the tree, since it
320 requires branched-chain amino acids as precursors (Hansen & Poll, 1993). In a previous study, the
321 lack of an association between the nitrogen status of the tree and the level of branched-chain esters
322 in the fruit was related to the lack of a relationship between the availability of amino acids

precursors in the ripening fruit and the nitrogen fertilisation dose (Fellmann et al., 2000). However, a role of nitrogen nutrition on the formation of some groups of apple aroma compounds, such as C6-aldehydes (1-hexanal and (E)-2-hexenal) and straight-chain esters (hexyl acetate), could not be completely ruled out in the present experiment, since a significant, though not particularly strong, correlation (positive for C6-aldehydes and negative for hexyl acetate) between the nitrogen level in the leaves and the content of these volatiles in the fruit was observed in both years (Table 3).

3.4 Effects of fertilisation treatments on sugars and organic acids

ANOVA of sugars and organic acids data suggested a significant effect of the fertilisation treatments on the accumulation of sucrose, glucose, malic and oxalic acid (Table 4), resulting in a significant effect on the sum of sugars (only in 2010) and acids. In both years, apples from the non-fertilised plots tended to accumulate relatively high level of malic acid (on average 7.40 mg/g of fresh weight) and, consequently, of total organic acids (7.71 mg/g), whereas an opposite trend was observed for the organic treatment OF3, with an average malic and total acids content of 6.44 mg/g and 6.74 mg/g, respectively. Other effects of fertilisation treatments on organic acids and sugars were not consistent across the two harvest years. In addition, the effect of the blocks was significant for almost all the considered variables. The observed significant effects of fertilisation treatments on the content of sugars or organic acids were not associated to differences in nitrogen plant status between treatments (Table 3), thus not confirming previous observations of a correlation between fruit soluble solids and nitrogen leaf levels in apple trees (Dris et al., 1999) and suggesting a low impact of nitrogen uptake on the accumulation of these metabolites in the fruit. On the contrary, a slight, though significant, positive correlation ($R=0.434$ at $p=0.005$) was observed between total acids content and potassium concentration in leaves, partially confirming a previously observed association between acids content in the fruit and potassium plant status (Marcelle, 1995).

Restricting the analysis to the organic treatments, significant effects of fertilisation were observed on formation of both acids and sugars, even though in this latter case the effects were of limited size and significant only in the first harvest year. However, the observed effects were not consistent

349 across the two years, except for the tendency of OF3 apples to accumulate low levels of malic acid,
350 suggesting that the effects due to the fertilisation treatments were not strong enough to overcome
351 those associated to non-uniformity between the blocks of the field trial.

352 *3.5 Evaluation of fruit sensory attributes*

353 To evaluate the potential impact on fruit sensory properties of the observed effects on the chemical
354 profile a descriptive sensory analysis on samples from 2012 harvest was carried out. Results
355 showed that fruit samples characterised by a quite similar compositional profile could show
356 significant differences in several sensory attributes. Results from the ANOVA highlighted a
357 significant effect of fertilisation treatments on 10 of the 14 measured sensory attributes and a
358 significant effect of blocks on 11 sensory attributes (Table 5). In particular, fertilisation treatments
359 significantly affected green, citrus and floral odours, sweet and sour tastes, overall, fruity and green
360 flavours, hardness and mouthfeel. Having been obtained from analyses on only two of the four field
361 replicates, these results can not be considered as representative of the complete experimental design
362 and, hence, can not be generalised to evaluate the effects of the considered fertilisation treatments
363 on fruit sensory properties. However, data from this group of samples could be subjected to a
364 multivariate analysis to explore the relationships between chemical and sensory variables,
365 identifying the key drivers of the observed differences in the odour and flavour perceptions. To
366 achieve this, Partial Least Square (PLS) regression was applied to data of samples on which both
367 chemical and sensory data had been obtained. As reported in Table 6, the built PLS models could
368 explain more than 50% of the variance for all the sensory attributes as measured by the panel. The
369 high and significant weighted regression coefficients (BW) obtained from the estimated PLS
370 models identified the X-variables that mostly contributed to the differences observed in the
371 corresponding sensory attribute (Table 6). Concentration of organic acids was confirmed to be a
372 good predictor of sour taste in apples, as previously observed (Harker, Marsh, Young, Murray,
373 Gunson, Walker, 2002). The range of treatment mean values for the sum of acids found in the
374 second year samples, approximately 1.0 mg/g, exceeded the magnitude of concentration change that

was determined to be required to produce a perceptible sourness difference in apples, 0.8 mg/g (Harker et al., 2002). Several compounds seemed to be related to sweetness: sucrose, as expected, but also some volatiles. In agreement with this result, a significant contribution of some of these aroma compounds, such as, in particular, 2-methylbutyl acetate, hexyl acetate and 1-butanol, to the sweet flavour has been previously reported in Royal Gala apples (Young et al., 1996), suggesting a role of volatile substances in the perception of sweetness. In any case the presence of multiple chemical variables associated to this attribute seemed to confirm that in this case the identification of a simple predictor was not as straightforward as for sourness (Harker et al., 2002). Among volatiles that were associated to odour and flavour attributes some, such as 2-methylbutyl acetate, hexyl acetate, 2-methylpropyl acetate, butyl butanoate, propyl acetate and pentyl acetate, have been previously recognised as key odorants in cv Golden Delicious apples (Mehinagic et al., 2006).

As a whole, these results suggested that several significant perceptible sensory differences may emerge from apples samples characterized by a quite similar chemical profile, in line with conclusions from previous studies, which highlighted that sensory differences between apples samples could sometimes be revealed even when no differences in instrumental parameters were apparent (Harker et al., 2002).

4. Conclusions

It has been sharply suggested that future research on organic versus conventional farming systems should not be limited to the measurement of product quality differences but should hopefully focus, on both sides, on optimisation of cultivation practices and postharvest management, taking into account of the peculiarities of each system (Lester & Saftner, 2011). Important specific issues of organic crop production are those related to soil fertility/plant root interactions and to reduced release rates of mineral plant nutrients from organic fertilisers. However, even though plant mineral nutrition is recognised as a key input in the overall management of fruit crop production, this management has been generally directed in the past more to production outcomes than to postharvest and consumer-oriented fruit quality (Ferguson & Boyd, 2002; Pelayo-Zaldivar, 2002).

In this context, the present study aimed at providing information about the effects on fruit quality of different fertilisation strategies tested in order to optimise cultivation practices in organic apple production. Results obtained on cv. Golden Delicious apples grown in the geographic area of Alto Adige did not evidence a consistent and remarkable influence of the considered fertilisation approaches on the formation of flavour related compounds, even when they gave place to marked differences in soil available nitrogen levels. In this study, effects due to fertilisation treatments were evaluated on fruits from young trees and it is plausible that the quite moderate impact observed on the chemical profile in the present study would be even less noticeable in fruits from adult trees. However, a limited sensory test highlighted that significant differences for several sensory attributes could be detected between apples obtained by the different fertilisation treatments and characterised by a quite similar chemical profile. Further research is needed to establish whether the magnitude of these effects could produce significant changes in the quality as perceived by consumers.

Acknowledgements

Funding. This work was funded by the Italian Ministry of Agricultural, Food and Forestry Policies (Project: Qualità nutrizionale e organolettica e impatto ambientale di produzioni biologiche. Un caso studio: il melo).

Supplementary data

Tables reporting a list of determined volatile compounds (Table 1S), definition of sensory attributes (Table 2S), results of determination of fruit ripening indexes (Table 3S) and aroma volatile levels (Table 4S), and a figure reporting the scores plot of the PCA analysis on volatile levels determined in 2010 and 2012 (Figure 1S), are available free of charge via the Internet at http://www.sciencedirect.com.

References

Alaphilippe, A., Simon, S., Brun, L., Hayer, F., Gaillard, G. (2013). Life cycle analysis reveals higher agroecological benefits of organic and low-input apple production. *Agronomy for Sustainable Development, 33,* 581-592.

Aprea, E., Corollaro, M.L., Betta, E., Endrizzi, I., Demattè M.L., Biasioli, F., Gasperi, F. (2012). Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavor. *Food Research International, 49,* 677–686.

Deibler, K. D., Delwiche, J. (2004). *Handbook of flavor characterization: Sensory analysis, chemistry, and physiology*. New York: Marcel Dekker, Inc.

Dris, R., Niskanen, R., Fallahi, E. (1999). Relationship between leaf and fruit minerals and fruit quality attributes of apples grown under northern conditions. *Journal of Plant Nutrition, 22,* 1839-1851.

EPA (2007). Method 6010C. Inductively coupled plasma-atomic emission spectrometry. In: T*est Methods for Evaluating Solid Waste, Physical/Chemical Methods.* Environmental Protection Agency. Available online at: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6010c.pdf (last access on 2014-04-28).

Fellman, J.K., Miller, T.W., Mattinson, D.S., Mattheis, J.P. (2000). Factors That Influence Biosynthesis of Volatile Flavor Compounds in Apple Fruits. *HortScience, 35,* 1026-1033.

Ferguson, I.B., Boyd, L.M. (2002). Inorganic Nutrients and Fruit Quality. In M. Knee (Ed.), *Fruit Quality and its Biological Basis.* (pp. 17-45). Sheffield (UK): Sheffield Academic Press.

Kelderer, M., Thalheimer, M., Andreaus, O., Topp, A., Burger, R., Schiatti, P., (2008). The mineralisation of commercial organic fertilizers at 8°C temperature. In M. Boos (Ed.), *Proceedings of the 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing. (Weinsberg/Germany, 2008)* (pp.160-166). Weinsberg (Germany) Fördergemeinschaft Ökologischer Obstbau e. V.

Hansen, K., Poll, L. (1993). Conversion of L-Isoleucine into 2-Methylbut-2-enyl Esters in Apples. *LWT - Food Science and Technology, 26,* 178-180.

Harker, F.R., Marsh, K.B., Young, H., Murray, S.H., Gunson, F.A., Walker, S.B. (2010). Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. *Postharvest Biology and Technology, 24,* 241–250.

Ireland, C. (2010). *Experimental statistics for agriculture & horticulture.* (pp. 145-180) Wallingford (UK): CAB International, (Chapter 9).

ISO (2008). Food products - Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content - Part 1: Oilseeds and animal feeding stuffs (ISO 16634-1). Geneva (Switzerland): International Organization for Standardization.

ISO (2012). Sensory Analysis. General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors (ISO 8586). Geneva (Switzerland): International Organization for Standardization.

Lawless, H.T., Heymann, H. (2010). *Sensory evaluation of foods: Principles and practices*. New York: Springer.

Lester, G.E., Saftner, R.A. (2011). Organically versus Conventionally Grown Produce: Common Production Inputs, Nutritional Quality, and Nitrogen Delivery between the Two Systems. *Journal of Agricultural and Food Chemistry, 59,* 10401–10406.

López, M.L., Lavilla, M.T., Recasens, I., Graell, J., Vendrell, M. (2000). Changes in aroma quality of 'Golden Delicious' apples after storage at different oxygen and carbon dioxide concentrations. *Journal of the Science of Food and Agriculture, 80,* 311-324.

López Fructuoso, M.L., Echeverría Cortada, G., (2010). Apple (*Malus* × *domestica* Borkh.). In Y.H. Hui (Ed.), *Handbook of fruit and vegetable flavors* (pp. 247-264). Hoboken (NJ): John Wiley & Sons, Inc.

Marcelle, R.D. (1995). Mineral nutrition and fruit quality. *Acta Horticulturae, 383,* 219-226.

Martens, H., Martens, M. (2001). Modified jack-knife estimation of parameter uncertainty in bilinear modelling by partial least square regression (PLSR). *Food Quality and Preference, 11,* 5–16.

Mehinagic, E., Royer, G., Symoneaux, R., Jourjon, F., Prost, C. (2006). Characterization of Odor-Active Volatiles in Apples: Influence of Cultivars and Maturity Stage. *Journal of Agricultural and Food Chemistry, 54,* 2678-2687.

Nijssen, L.M., Ingen-Visscher, C.A. van, Donders, J.J.H. (2011). VCF Volatile Compounds in Food: database (Version 13.1.). Delft (The Netherlands): TNO.

Osterloh, A., Ebert, G., Held, W.H., Schulz, H., Urban, E. (1996). *Lagerung von Obst und Südfrüchten.* Stuttgart (Germany): Ulmer Verlag.

Oughton, E., Ritson, C. (2007). Food consumers and organic agriculture. In J. Cooper, U. Niggli, C. Leifert (Eds.), *Handbook of organic food safety and quality* (pp. 74-96). Cambridge: CRC Press.

Paoletti, F., Raffo, A., Kristensen, H., Thorup-Kristensen, K., Seljåsen, R., Torp, T., Busscher, N., Ploeger, A., Kahl, J. (2012). Multi method comparison of carrot quality from a conventional and three organic cropping systems with increasing levels of nutrient re-cycling. *Journal of the Science of Food and Agriculture, 92,* 2855-69.

Peck, G.M., Andrews, P.K., Reganold, J.P., Fellman, J.K. (2006). Apple Orchard Productivity and Fruit Quality, Under Organic, Conventional and Integrated Management. *HortScience, 41,* 99-107.

Pelayo-Zaldívar, C. (2010). Environmental Effects on Flavor Changes. In Y.H. Hui (Ed.), *Handbook of fruit and vegetable flavors* (pp. 73-92). Hoboken (NJ): John Wiley & Sons, Inc.

Plotto, A., McDaniel, M.R., Mattheis, J.P. (2000). Characterization of Changes in 'Gala' Apple Aroma during Storage Using Osme Analysis, a Gas Chromatography–Olfactometry Technique. *Journal of the American Society of Horticultural Science, 125,* 714–722.

Prieto, A., Basauri, O., Rodil, R., Usobiaga, A., Fernández, L. A., Etxebarria, N., Zuloaga, O. (2010). Stir-bar sorptive extraction: A view on method optimization, novel applications, limitations and potential solutions. *Journal of Chromatography A, 1217,* 2642−2666.

Raffo, A., Baiamonte, I., Nardo, N., Paoletti, F. (2007). Internal quality and antioxidants content of cold stored red sweet peppers as affected by polyethylene bag packaging and hot water treatment. *European Food Research and Technology, 225*, 395-405.

Raffo, A., Nardo, N., Tabilio, M.R., Paoletti, F. (2008). Effects of Cold Storage on Aroma Compounds of White- and Yellow-Fleshed Peaches. *European Food Research and Technology, 226*, 1503-1512.

Raffo, A., Kelderer, M., Paoletti, F., Zanella, A. (2009). Impact of Innovative Controlled Atmosphere Storage Technologies and Postharvest Treatments on Volatile Compound Production in Cv. Pinova Apples. *Journal of Agricultural and Food Chemistry, 57*, 915–923.

Raffo, A., Nicoli, S., Nardo, N., Baiamonte, I., D'Aloise, A., Paoletti, F. (2012). Impact of Different Distribution Scenarios and Recommended Storage Conditions on Flavor Related Quality Attributes in Ripening Fresh Tomatoes. *Journal of Agricultural and Food Chemistry, 60*, 10445−10455.

Reganold, J.P., Glover, J.D., Andrews, P.K., Hinman, H.R. (2001). Sustainability of three apple production systems. *Nature, 410*, 926-930.

Seufert, V., Ramankutty, N., Foley, J.A. (2012). Comparing the yields of organic and conventional agriculture. *Nature, 485*, 229–232.

Somogyi, L.P., Childers, N.F. (1964). Volatile constituents of apple fruits as influenced by fertilizer treatments. *Proceedings of the American Society of Horticultural Science, 84*, 51-58.

Song, J., Bangerth, F. (1996). The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. *Postharvest Biology and Technology, 8*, 259-269.

van Es, H.M., Gomes, C.P., Sellmann, M., van Es, C.L. (2007). Spatially-Balanced Complete Block designs for field experiments. *Geoderma, 140*, 346-352.

VDLUFA (2002). Methodenbuch Band 1 – Die Untersuchung von Böden. Darmstadt (Germany): Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten Verlag.

Young, H., Gilbert, J.M., Murray, S.H., Ball, R.D. (1996). Causal Effects of Aroma Compounds on Royal Gala Apple Flavours. *Journal of the Science of Food and Agriculture, 71*, 329-336.

Zanella, A., Werth, E. (2004). Comparison of the determination of chemico-physical apple quality parameters by means of an automated instrument ("Pimprenelle") with conventional analytics. *Laimburg Journal*, 1, 51-57.

Figure captions.

Figure 1.

Scores plot of PCA analysis on volatile compounds levels determined in cv. Golden Delicious apples grown under seven different fertilisation conditions in 2010. Four field replicates for each fertilisation treatments were analysed.

Note. Each symbol represents one of the seven fertilisation treatments: the non-fertilised treatment (NF), the conventional treatment (CF) and the organic treatments (OF1, OF1 33%×3, OF1 50%×3, OF2, OF3). Correspondence between fertilisation treatment and abbreviation used in the figure are given in the Material and Methods section. Numbers close to symbols denote the number of the block of the randomised block design. Percent variance explained by the first two principal components is reported on the corresponding axis header. The dotted line defines the confidence region (at a 95% level).

Figure 2.

Scores plot of PCA analysis on volatile compounds levels determined in cv. Golden Delicious apples grown under seven different fertilisation conditions in 2012. Four field replicates for each fertilisation treatments were analysed.

Note. Each symbol represents one of the seven fertilisation treatments: the non-fertilised treatment (NF), the conventional treatment (CF) and the organic treatments (OF1, OF1 33%×3, OF1 50%×3, OF2, OF3). Correspondence between fertilisation treatment and abbreviation used in the figure are given in the Material and Methods section. Numbers close to symbols denote the number of the block of the randomised block design. Percent variance explained by the first two principal components is reported on the corresponding axis header. The dotted line defines the confidence region (at a 95% level).

Table 1. Number of applications and nitrogen doses (g of N/plant /year) in the four years of the fertilisation experiment for each of the investigated treatments.

Fertilisation treatment	Number of applications	Application dose (g of N/plant /year)			
		1st year	2nd year	3rd year	4th year
Non-fertilised (NF)	-	-	-	-	-
Ammonium sulphate (CF)	1	16	20	24	24
Organic Fertliser 1 (OF1)	1	16	20	24	24
Organic Fertliser 1 (OF1 33% x 3)	3	16	20	24	24
Organic Fertliser 1 (OF1 50% x 3)	3	24	30	36	36
Organic Fertiliser 2 (OF2)	1	16	20	24	24
Organic Fertiliser 3 (OF3)	1	16	20	24	24

Table 2. Average mineral nitrogen level in the soil at the time of fruit set (mg/kg of dry matter), mineral nitrogen and potassium level in the leaves about one month after flowering (g/kg) for each fertilisation treatment. Mean values for each treatment, results of ANOVA and Tukey-Kramer test.

Fertilisation treatments	Measured parameter						
	Nitrogen level in the soil (mg/kg d.m.)			*Nitrogen level in the leaves (g/kg)*		*Potassium level in the leaves (g/kg)*	
	Growing year			*Growing year*		*Growing year*	
	2010		2012	2010	2012	2010	2012
	0-20 cm[a]	20-40 cm[a]	0-30 cm[a]				
NF	11.7 c[b]	6.2 c	9.0 b	21.3 c	29.0 b	20.4 a	22.6 bc
CF	147.2 a	56.7 a	188.0 a	28.4 a	32.9 a	17.9 ab	22.4 bc
OF1	40.7 ab	11.5 bc	59.0 b	24.8 abc	31.3 ab	18.7 ab	22.0 bc
OF1 33%×3	17.5 b	10.0 bc	64.5 b	25.3 ab	30.4 ab	18.8 ab	22.8 b
OF1 50%×3	20.7 b	9.2 bc	26.7 b	27.4 ab	30.3 ab	18.4 ab	23.4 ab
OF2	21.2 b	13.5 bc	65.2 b	24.0 bc	30.7 ab	19.1 ab	25.0 a
OF3	56.5 ab	14.5 bc	57.2 b	27.0 ab	31.2 ab	16.4 b	20.7 c
ANOVA							
Treatment sign.	< 0.01	< 0.001	< 0.001	< 0.001	< 0.05	< 0.05	< 0.001
Field replicate sign.	n.s	n.s.	n.s.	n.s.	< 0.05	n.s.	< 0.05
P.S.D.[c]	60.4	9.6	53.9	0.3		0.2	

Notes. [a]) Probing depth.
[b]) Mean values (from 4 field replicates) with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey-Kramer test.
[c]) Pooled Standard Deviation

Table 3. Pearson's correlation coefficients between nitrogen levels in leaves about one month after flowering and levels of flavor related compounds in apple fruits for the two harvest years**.**

Compound	Correlation coefficient	
	Harvest year	
	2010	2012
Alcohols		
1-butanol	0.229	-0.321
2-methyl butanol	0.213	-0.247
1-hexanol	0.305	-0.123
C6-Aldehydes		
1-hexanal	**0.520****	**0.511****
(E)-2-hexenal	**0.524****	**0.537****
Straight-chain esters		
propyl acetate	-0.206	**-0.484****
butyl acetate	**-0.600*****	-0.350
pentyl acetate	**-0.729*****	-0.347
hexyl acetate	**-0.618*****	**-0.389***
propyl propanoate	0.022	-0.343
butyl propanoate	-0.365	**-0.447***
propyl butanoate	-0.079	**-0.441***
butyl butanoate	-0.113	-0.296
hexyl butanoate	-0.032	-0.183
Branched-chain esters		
2-methylpropyl acetate	0.084	-0.080
2-methylbutyl acetate	**-0.500****	-0.359
butyl-2-methylbutanoate	**0.501****	-0.373
hexyl-2-methylbutanoate	0.144	-0.361
Other compounds		
estragole	-0.096	0.108
benzothiazole	0.036	0.082
Organic acids		
Malic acid	-0.213	0.155
Citric acid	0.365	0.190
Oxalic acid	0.263	**-0.405***
Sum of acids	-0.203	0.154
Sugars		
Fructose	-0.309	-0.350
Glucose	0.494	0.335
Sucrose	-0.532	0.120
Sorbitol	-0.418	0.106
Sum of sugars	0.008	0.060

Note. When reported in bold characters correlation coefficients were significant at a *p* level <0.05 (*), <0.01 (**), <0.001(***).

Table 4. Organic acid and sugar concentration (mg/g of fresh weight) in cv. Golden Delicious apples grown under different fertilisation conditions in two non-consecutive harvest years. Mean values for each fertilisation treatment, results of ANOVA and Tukey-Kramer test.

Fertilisation treatments	Measured parameter								
	Harvest year 2010								
	Organic acids				*Sugars*				
	Malic	Citric	Oxalic	Sum	Fructose	Glucose	Sucrose	Sorbitol	Sum
NF	7.34 a[a]	0.068	0.221 ab	7.63 a	70.2 abc	9.1 c	55.8 ab	6.4 ab	141.5 abc
CF	7.16 ab	0.081	0.231 a	7.47 ab	73.3 a	14.1 a	54.0 ab	6.6 a	147.9 a
OF1	7.47 a	0.075	0.218 bc	7.76 a	69.3 bc	10.8 abc	57.4 a	6.8 a	144.2 ab
OF1 33%×3	7.21 ab	0.076	0.211 bc	7.50 ab	64. 4 bc	11.1 abc	55.1 ab	5.9 ab	141.5 abc
OF1 50%×3	6.23 b	0.093	0.218 bc	6.54 b	70.1 abc	13.1 ab	49.8 b	4.9 ab	137.9 bc
OF2	6.68 ab	0.078	0.213 bc	6.97 ab	71.7 ab	9.9 bc	55.9 ab	5.6 ab	143.1 ab
OF3	6.49 ab	0.077	0.209 c	6.77 ab	68.1 c	11.5 abc	51.4 ab	4.6 b	135.6 c
ANOVA									
Treatment sign.	< 0.01	n.s.	< 0.001	< 0.01	< 0.01	< 0.001	< 0.01	< 0.01	< 0.001
Field replic. Sign.	< 0.001	< 0.01	n.s.	< 0.001	n.s.	n.s.	< 0.001	< 0.05	< 0.001
	Harvest year 2012								
NF	7.47 a	0.091	0.226 a	7.79 a	75.3	11.4 d	51.4 a	5.7	143.7
CF	7.36 a	0.101	0.215 c	7.68 a	73.2	14.5 a	50.6 ab	6.1	144.4
OF1	6.89 ab	0.102	0.219 abc	7.21 ab	74.6	12.4 cd	50.4 ab	5.1	142.5
OF1 33%×3	7.02 ab	0.101	0.224 ab	7.34 ab	75.3	14.5 a	49.6 ab	5.8	145.2
OF1 50%×3	7.06 ab	0.096	0.216 bc	7.38 ab	74.1	13.7 ab	50.2 ab	5.7	143.8
OF2	7.02 ab	0.094	0.226 a	7.34 ab	75.2	12.8 bc	45.9 b	5.1	139.0
OF3	6.39 b	0.111	0.220 abc	6.72 b	73.6	14.0 ab	47.2 ab	4.5	139.4
ANOVA									
Treatment sign.	< 0.001	n.s.	< 0.001	< 0.001	n.s.	< 0.001	< 0.05	n.s.	n.s.
Field replic. Sign.	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P.S.D.[b]	0.01	0.001	0.001	0.01	0.5	0.1	0.1	0.0	2.1

Note. [a)] Mean values (by averaging mean values obtained from 4 field replicates for each fertilization treatment; mean values for each field replicate were obtained by averaging results from 3 analytical replicates) with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey- Kramer test.
[b)] Pooled Standard Deviation.

Table 5. Sensory profile of cv. Golden Delicious apples grown under different fertilisation conditions in a single harvest year (2012). Mean values for each fertilisation treatment, results of ANOVA and Tukey-Kramer test.

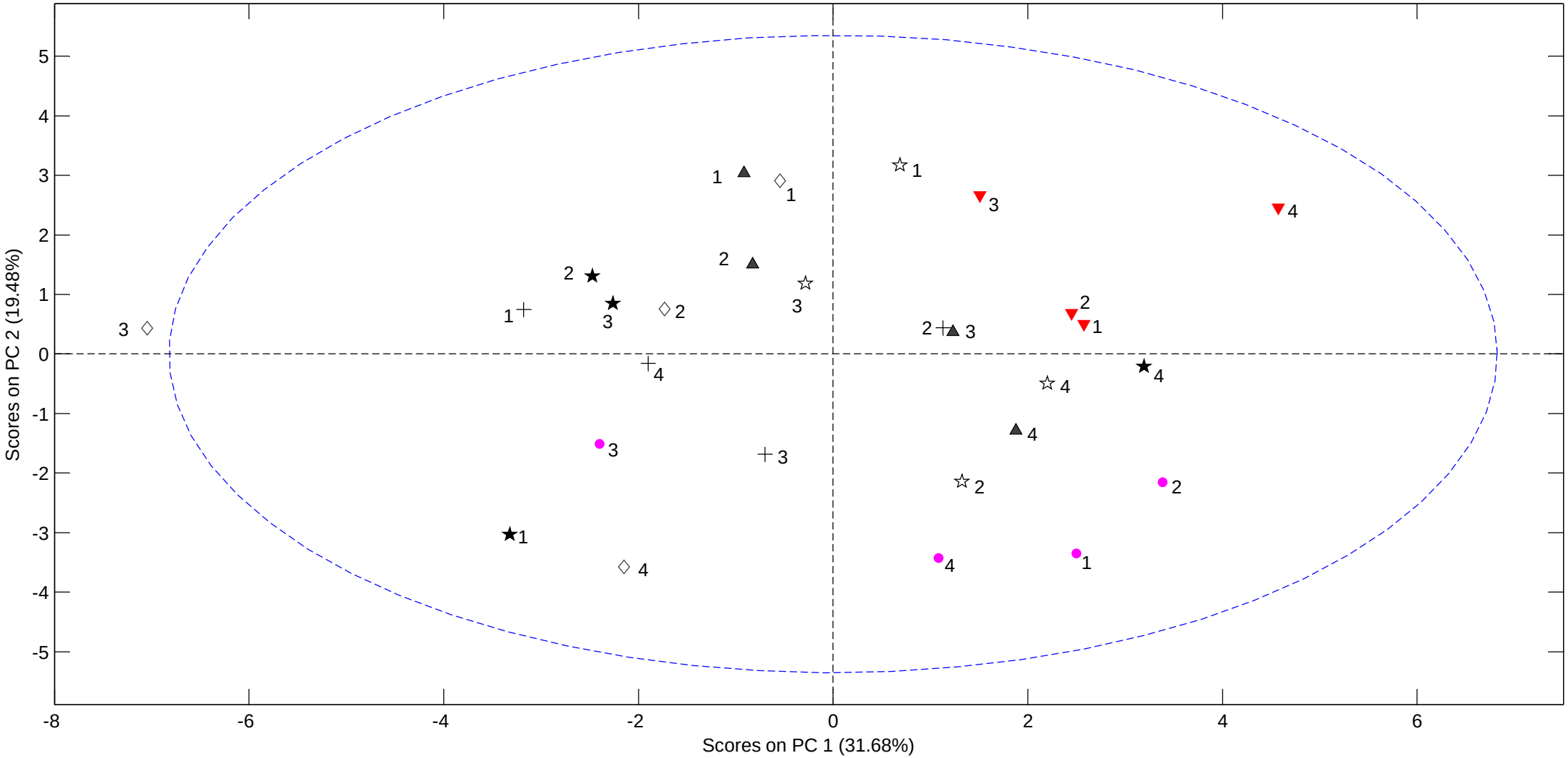
	Sensory attribute													
Fertilisation treatments	Overall Odour	Fruity (apple-like) odour	Green odour	Citrus odour	Floral odour	Sweet taste	Sour taste	Overall Flavour	Fruity (apple-like) flavour	Green flavour	Hardness	Crunchiness	Juiciness	Mouthfeel
NF	6.50[a]	6.10	3.13 bc	1.42 a	2.28 b	5.71 a	3.82 ab	6.89	6.22 ab	2.84 b	6.50 ab	6.34	6.72	1.21 ab
CF	6.63	6.00	3.88 a	0.93 ab	1.98 b	4.95 b	3.54 ab	6.72	6.07 ab	3.45 a	6.42 ab	6.10	6.65	1.35 ab
OF1	6.53	6.05	2.91 c	1.09 ab	2.21 b	5.60 a	3.60 ab	6.80	6.25 a	2.87 b	6.60 ab	6.11	6.89	1.19 ab
OF1 33%×3	6.54	5.81	3.26 bc	0.71 b	2.09 b	5.44 a	3.31 b	6.65	5.92 ab	2.71 b	6.64 a	6.23	6.79	1.60 a
OF1 50%×3	6.62	5.74	3.25 bc	1.05 ab	1.83 b	5.52 a	3.57 ab	6.52	5.83 b	2.65 b	6.54 ab	6.30	6.78	1.06 b
OF2	6.59	6.10	3.01 c	0.85 b	3.15 a	5.24 ab	3.95 a	6.77	6.06 ab	2.96 ab	6.18 b	5.96	6.77	1.54 ab
OF3	6.61	6.02	3.57 ab	1.36 a	2.31 b	4.86 b	3.84 ab	6.56	5.91 ab	3.35 a	6.48 ab	6.29	6.70	1.20 ab
ANOVA														
Treatment sign	n.s.	n.s.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.05	< 0.05	< 0.001	< 0.05	n.s.	n.s.	< 0.05
Field replic. sign.	n.s.	< 0.01	< 0.001	n.s.	< 0.001	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	n.s.	< 0.05
P.S.D.	0.37	0.45	0.69	0.53	0.60	0.54	0.65	0.35	0.42	0.64	0.48	0.57	0.40	0.66

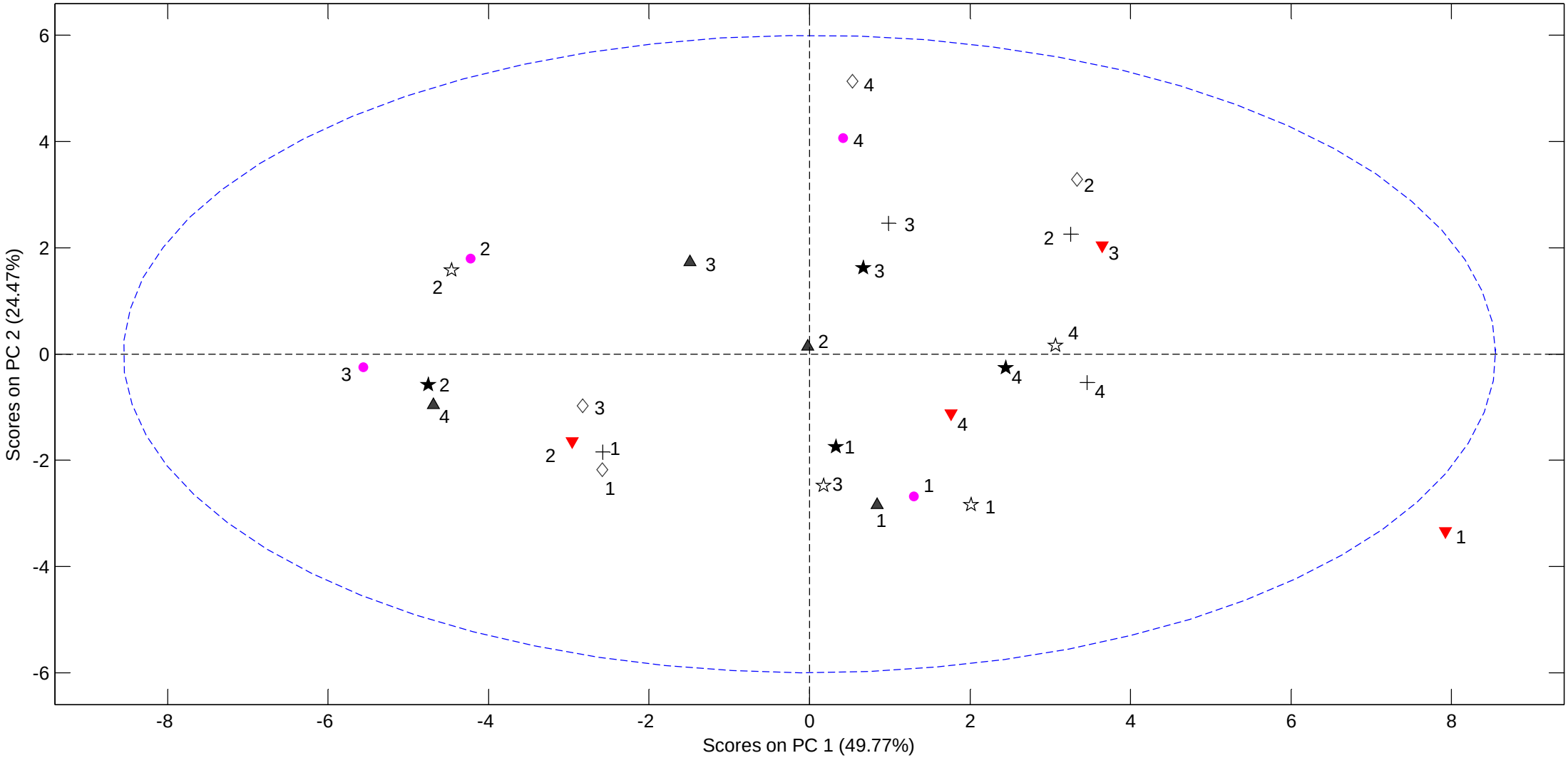
Note. [a)] Mean values with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey-Kramer test. Mean values were obtained by averaging scores obtained on 2 apple samples (2 field replicates), from each of 10 assessors, who carried out 3 analytical replicates on each apple sample.
[b)] Pooled Standard Deviation

Table 6. Partial Least Square (PLS) regression models to explore relationships between chemical and sensory variables. Parameters describing the model and list of chemical compounds with significant positive and negative weighted regression coefficients (BW) for each sensory attribute.

Sensory attribute	No. of components of the model	%Y Variance explained by the model	R2[a]	Compounds with significant positive weighted regression coefficients (BW)[b]	Compounds with significant negative weighted regression coefficients (BW)[b]
Overall odour	2	63	0.63	fructose; hexyl acetate; citric acid; (E)-2-hexenal.	hexyl butanoate; butyl propanoate; butyl butanoate.
Fruity odour	2	81	0.82	fructose; sucrose; sorbitol; malic acid; propyl acetate; citric acid; oxalic acid.	pentyl acetate; 1-hexanol; hexyl butanoate; 1-hexanal; (E)-2-hexenal.
Green odour	4	79	0.79	glucose; 1-butanol; benzothiazole.	2-methylpropyl acetate; (E)-2-hexenal; pentyl acetate; 2-methylbutyl acetate; sucrose; hexyl acetate.
Citrus odour	2	56	0.56	hexyl-2-methylbutanoate; butyl-2-methylbutanoate; propyl acetate.	(E)-2-hexenal; 1-hexanal.
Floral odour	3	72	0.72	2-methylpropyl acetate; oxalic acid; 2-methylbutyl acetate; benzothiazole; butyl-2-methylbutanoate; pentyl acetate.	hexyl butanoate; butyl butanoate; butyl propanoate; 1-hexanol.
Sweet	3	69	0.68	pentyl acetate; sorbitol; estragole; 2-methyl butanol; sucrose; hexyl acetate; propyl propanoate; 1-butanol; 2-methylbutyl acetate; butyl 2-methyl butanoate.	oxalic acid; citric acid; glucose; hexyl butanoate; butyl butanoate.
Sour	2	60	0.60	malic acid; citric acid; butyl butanoate; oxalic acid.	pentyl acetate.
Overall flavour	4	65	0.66	sorbitol; sucrose; propyl acetate; 2-methyl butanol; butyl-2-methylbutanoate; butyl butanoate; 2-methylbutyl acetate; estragole.	1-hexanol; glucose; oxalic acid; 2-methylpropyl acetate; pentyl acetate; hexyl butanoate.
Fruity flavour	4	78	0.78	sorbitol; sucrose; fructose; estragole; propyl acetate; malic acid; 1-butanol; propyl propanoate.	2-methylpropyl acetate; oxalic acid; hexyl butanoate; hexanol; (E)-2-hexenal; pentyl acetate; benzithiazole; 1-hexanal.
Green flavour	3	85	0.86	benzothiazole	sucrose

NOTES.
[a] The R2 value is the square of the Pearson correlation value and expresses correlation on a positive scale between 0 and 1.
[b] Compounds are listed according to descending order of the values of their weighted regression coefficients.





Highlights

- Effects of organic/conventional fertilisation on apple flavour were investigated
- Significant effects were observed on some aroma volatiles, acids and sugars
- However, few consistent effects were observed across two harvest years
- Different organic fertilisers weakly affected the considered flavour attributes
- Sensory differences emerged between apples with similar chemical profile

Table 1S. List of determined volatile compounds. For each volatile compound it is reported the internal standard used for semi quantitative determination, the *m/z* fragment used for GC-MS signal detection in SIM mode, and the average coefficient of variation (%) of replicate determinations (n=2 analytical replicates for each sample) across the whole dataset (56 apple samples).

compound	internal standard used for quantification[a]	m/z fragment	average coefficient of variation (%)
Alcohols			
1-butanol	a	56	4.0
2-methyl butanol	a	57	3.1
1-hexanol	a	55	1.7
Aldehydes			
1-hexanal	b	72	4.4
(E)-2-hexenal	a	69	3.8
Straight-chain esters			
propyl acetate	a	61	4.2
butyl acetate	b	56	3.4
pentyl acetate	b	70	1.5
hexyl acetate	c	56	1.1
propyl propanoate	b	57	4.3
butyl propanoate	b	57	2.1
propyl butanoate	b	71	2.7
butyl butanoate	c	71	1.3
hexyl butanoate	c	89	1.7
Branched-chain esters			
2-methylpropyl acetate	b	56	5.7
2-methylbutyl acetate	b	70	1.8
butyl-2-methylbutanoate	c	103	2.3
hexyl-2-methylbutanoate	c	103	2.9
Other compounds			
estragole	c	148	2.7
benzothiazole	b	135	9.0
Internal standards			
4-methyl-2-pentanol	-	45	-
Allyl hexanoate	-	99	-

Note: a) 4-methyl-2-pentanol;b) sum of 4-methyl-2-pentanol and allyl hexanoate; c) allyl hexanoate.

Table 2S. List of sensory attributes perceived in apple fruit samples.

Attribute	Definition	Scale
Overall Odour	overall orthonasal perception produced by volatile compounds	0 = weak; 9 = strong
Fruity (apple-like) odour	typical odour of a ripe apple, orthonasal perception	0 = weak; 9 = strong
Green odour	vegetable odour associated with cut leaf	0 = not present; 9 = strong
Citrus odour	odour associated with citrus fruit (lemon)	0 = not present; 9 = strong
Floral odour	odour sensation reminiscent of flower odour	0 = not present; 9 = strong
Sweet taste	basic taste produced by sugar	0 = not present; 9 = strong
Sour taste	basic taste produced by organic acids (typically citric and malic acids)	0 = weak; 9 = strong
Overall Flavour	overall impression of apple fruit, retronasal perception	0 = weak; 9 = strong
Fruity (apple-like) flavour	sensation associated with a ripe apple, retronasal perception	0 = not present; 9 = strong
Green flavour	sensation associated with cut leaf, retronasal perception	0 = not present; 9 = strong
Hardness	toughness perceived when biting with molar teeth	0 = soft; 9 = hard
Crunchiness	resistance to break into smaller pieces by crushing between molar teeth	0 = mealy; 9 = crunchy
Juiciness	ability to release juice on chewing	0 = dry; 9 = juicy
Mouthfeel	complex sensation, accompanied by shrinking, drawing or puckering of the skin or mucosal surface in the mouth, produced in the mouth by a dilute aqueous solution of products such kaki and sloe tannins	0 = not present; 9 = strong

Table 3S. Ripening stage indexes determined on apple fruits at harvest: firmness (kg/cm^2) and starch hydrolysis index (on a 1-5 scale).

Fertilisation treatment	Ripening indexes			
	Harvest year			
	2010		2012	
	firmness	starch index	firmness	starch index
Non-fertilised (NF)	7.91 b	3.20	8.15	2.50
Ammonium sulphate (CF)	7.20 a	3.18	7.96	2.25
Organic Fertliser 1 (OF1)	7.51 ab	3.20	7.97	2.00
Organic Fertliser 1 (OF1 33% x 3)	7.61 ab	3.25	7.92	2.35
Organic Fertliser 1 (OF1 50% x 3)	7.47 ab	3.33	8.14	2.25
Organic Fertiliser 2 (OF2)	7.41 ab	3.50	7.44	2.35
Organic Fertiliser 3 (OF3)	7.26 ab	3.33	7.95	2.25

Note. When significant effects of the fertilisation treatments on the ripening indexes were found, the Tukey-Kramer test was applied. Mean values with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey-Kramer test.

Table 4S. Volatile compounds levels (ratio of chromatographic peak area to internal standard peak area) in cv. Golden Delicious apples grown under different fertilisation conditions in two non-consecutive harvest years. Fertilisation treatments means, results of mixed-effects model ANOVA and Tukey-Kramer test.

Fertilisation treatments	Measured parameter										
	Harvest year 2010										
	C6-Aldehydes		*Straight-chain esters*								
	1-hexanal[b]	(E)-2-hexenal[a]	propyl acetate[a]	butyl acetate[b]	pentyl acetate[b]	hexyl acetate[c]	propyl propanoate[b]	butyl propanoate[b]	propyl butanoate[b]	butyl butanoate[c]	hexyl butanoate[c]
NF	0.0150 d	0.047 d	0.061 ab	1.009 a	0.042 a	0.688 a	0.0026 b	0.046 ab	0.0107 ab	0.120	0.039 ab
CF	0.0222 bc	0.067 ab	0.046 b	0.810 c	0.034 bc	0.556 c	0.0023 b	0.038 b	0.0099 b	0.115	0.043 a
OF1	0.0163 cd	0.053 cd	0.058 ab	0.889 bc	0.036 bc	0.593 bc	0.0028 b	0.043 ab	0.0117 ab	0.119	0.036 ab
OF1 33%×3	0.0215 bc	0.066 bc	0.061 ab	0.879 c	0.034 bc	0.578 bc	0.0030 ab	0.044 ab	0.0118 ab	0.119	0.037 ab
OF1 50%×3	0.0282 a	0.082 a	0.068 a	0.859 c	0.032 c	0.566 c	0.0042 a	0.047 a	0.0133 a	0.114	0.036 ab
OF2	0.0243 ab	0.075 ab	0.067 a	1.002 ab	0.038 ab	0.645 ab	0.0032 ab	0.046 a	0.0122 ab	0.123	0.035 b
OF3	0.0231 ab	0.067 ab	0.059 ab	0.891 bc	0.033 c	0.593 bc	0.0031 ab	0.042 ab	0.0114 ab	0.114	0.035 b
ANOVA											
Treatment sign	< 0.001	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001	< 0.01	< 0.05	< 0.05	n.s.	< 0.05
Field replicate sign.	< 0.01	< 0.001	< 0.001	< n.s.	n.s.	< 0.001	< 0.001	n.s.	< 0.05	< 0.001	< 0.01
	Harvest year 2012										
NF	0.0077 c	0.062 b	0.036	0.637	0.065 a	0.530	0.069	0.046 a	0.0049	0.105	0.058 a
CF	0.0170 a	0.112 a	0.022	0.555	0.052 b	0.395	0.060	0.024 b	0.0028	0.077	0.035 b
OF1	0.0110 bc	0.084 ab	0.028	0.633	0.061 ab	0.511	0.069	0.036 ab	0.0036	0.088	0.044 ab
OF1 33%×3	0.0114 b	0.085 ab	0.025	0.661	0.062 ab	0.529	0.071	0.036 ab	0.0041	0.093	0.040 ab
OF1 50%×3	0.0134 ab	0.085 ab	0.026	0.577	0.056 ab	0.499	0.062	0.028 ab	0.0040	0.077	0.030 b
OF2	0.0113 b	0.089 a	0.020	0.568	0.060 ab	0.473	0.060	0.028 ab	0.0027	0.084	0.045 ab
OF3	0.0119 b	0.089 a	0.026	0.604	0.056 ab	0.491	0.066	0.032 ab	0.0032	0.087	0.043 ab
ANOVA											
Treatment sign	< 0.001	< 0.001	n.s.	n.s.	< 0.05	n.s.	n.s.	< 0.05	n.s.	n.s.	< 0.001
Field replicate sign.	< 0.001	< 0.001	< 0.05	< 0.01	< 0.001	< 0.001	< 0.01	n.s.	< 0.05	< 0.001	< 0.01

Note. Mean values with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey-Kramer test. Levels are reported as ratio of substance peak area to 4-methyl-2-pentanol peak area ([a]), to the sum of peak area of 4-methyl-2-pentanol and allyl hexanoate ([b]), to allyl hexanoate peak area ([c]).

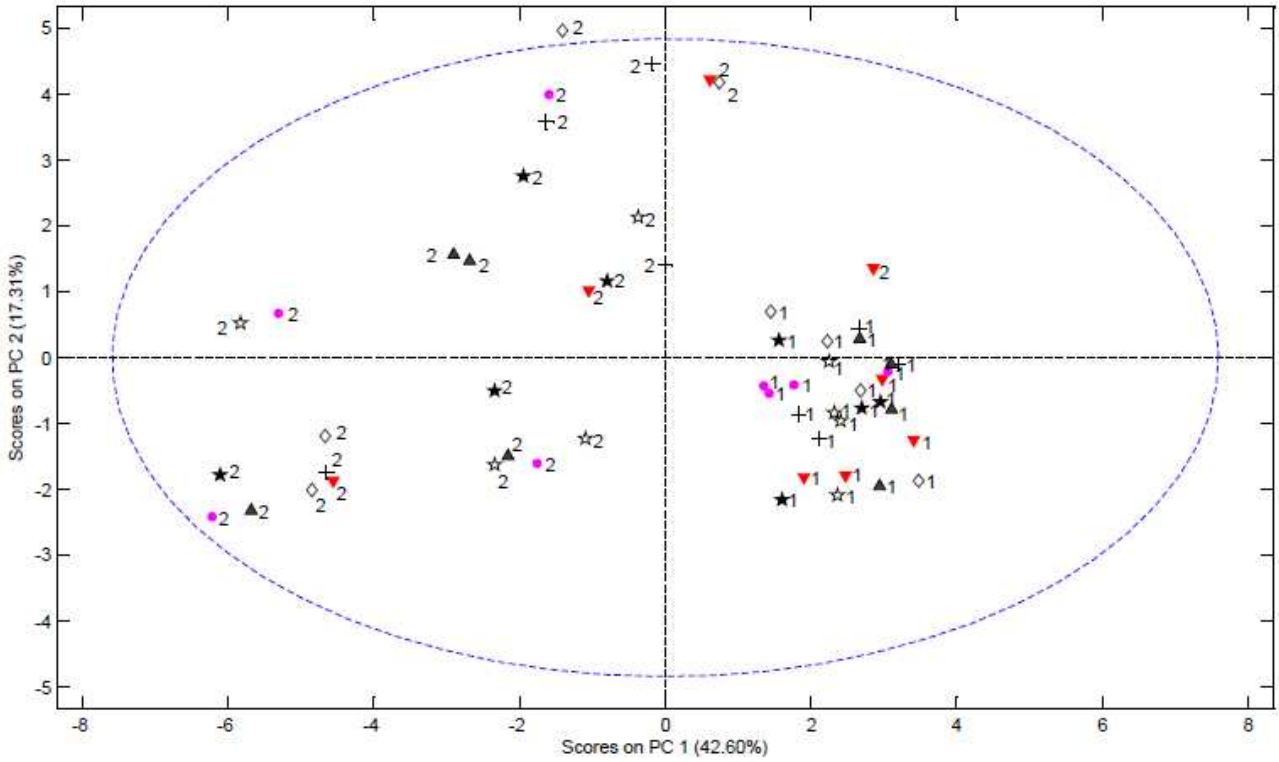
Table 4S (continued).

Fertilisation treatments	Measured parameter								
	Harvest year 2010								
	Branched-chain esters				*Alcohols*			*Other compounds*	
	2-methylpropyl acetate[b]	2-methylbutyl acetate[b]	butyl-2-methylbutanoate[c]	hexyl-2-methylbutanoate[c]	1-butanol[a]	2-methyl butanol[a]	1-hexanol[a]	estragole[c]	benzothiazole[b]
NF	0.0145	0.298 ab	0.0135 c	0.018 b	0.331 b	0.069 c	0.460 c	0.017 ab	0.0048 ab
CF	0.0155	0.270 b	0.0170 a	0.025 a	0.324 b	0.075 bc	0.501 abc	0.017 ab	0.0056 a
OF1	0.0140	0.308 a	0.0163 ab	0.021 ab	0.327 b	0.080 abc	0.466 bc	0.015 b	0.0044 ab
OF1 33%×3	0.0138	0.284 ab	0.0149 abc	0.019 ab	0.412 a	0.095 ab	0.582 a	0.019 ab	0.0037 b
OF1 50%×3	0.0133	0.277 ab	0.0151 abc	0.018 b	0.426 a	0.103 a	0.588 a	0.015 b	0.0048 ab
OF2	0.0146	0.305 a	0.0146 abc	0.019 b	0.384 a	0.087 abc	0.511 abc	0.022 a	0.0050 ab
OF3	0.0144	0.267 b	0.0140 bc	0.018 b	0.397 a	0.092 abc	0.547 ab	0.014 b	0.0044 ab
ANOVA									
Treatment sign	n.s.	< 0.001	< 0.01	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01	< 0.05
Field replicate sign.	n.s.	n.s.	n.s.	< 0.01	< 0.05	n.s.	n.s.	< 0.001	< 0.001
	Harvest year 2012								
NF	0.0124 ab	0.266	0.0192	0.025 a	0.283	0.077	0.596 ab	0.027	0.0052
CF	0.0122 ab	0.190	0.0106	0.013 b	0.250	0.065	0.592 ab	0.038	0.0050
OF1	0.0118 ab	0.206	0.0114	0.017 ab	0.253	0.060	0.577 ab	0.026	0.0047
OF1 33%×3	0.0129 a	0.260	0.0162	0.017 ab	0.302	0.085	0.677 a	0.049	0.0056
OF1 50%×3	0.0126 ab	0.237	0.0125	0.012 b	0.298	0.105	0.667 ab	0.044	0.0060
OF2	0.0133 a	0.199	0.0120	0.013 b	0.221	0.050	0.536 b	0.032	0.0064
OF3	0.0105 b	0.198	0.0131	0.015 ab	0.282	0.062	0.648 ab	0.028	0.0051
ANOVA									
Treatment sign	< 0.05	n.s.	n.s.	< 0.05	n.s.	n.s.	< 0.05	n.s.	n.s.
Field replicate sign.	< 0.05	n.s.	n.s.	n.s.	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001

Note. Mean values with different letters across columns are significantly different (at $p < 0.05$ level) according to the Tukey-Kramer test. Levels are reported as ratio of substance peak area to 4-methyl-2-pentanol peak area ([a]), to the sum of peak area of 4-methyl-2-pentanol and allyl hexanoate ([b]), to allyl hexanoate peak area ([c]).

Figure 1S.

Scores plot of PCA analysis on volatile compounds levels determined in cv. Golden Delicious apples grown under seven different fertilisation conditions in 2010 and 2012. Four field replicates for each fertilisation treatments were analysed.



Note. Each symbol represents one of the seven fertilisation treatments: the non-fertilised treatment (▼ NF), the conventional treatment (● CF) and the organic treatments (☆ OF1, + OF1 33%×3, ◇ OF1 50%×3, ▲ OF2, ★ OF3). Correspondence between fertilisation treatment and abbreviation used in the figure are given in the Material and Methods section. Numbers close to symbols denote the harvest year (1:2010; 2 :2012). Percent variance explained by the first two principal components is reported on the corresponding axis header. The dotted line defines the confidence region (at a 95% level).