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Sensory, GC-MS and PTR-ToF-MS profiling of strawberries varying in maturity at harvest with subsequent cold storage

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ABSTRACT

Harvesting strawberry fruit before they are fully ripe and allowing them to further ripen during postharvest cold storage is a common practice. The effect of these storage conditions on consumer liking is not well understood. The first aim of this study is to investigate the effects of maturity at harvest and subsequent cold storage on consumer liking, expressed as sweetness and aroma attributes, and volatile composition. The second aim of this study is to investigate whether volatile organic compounds (VOCs) can be used to predict consumer liking. Strawberries (*Fragaria* × *ananassa* cv. Lusa) were harvested either at the ¾ red stage or full red stage and stored at 4 °C for one, five or nine days. Strawberries were subjected to sensory profiling, colour-, firmness-, GC–MS- and PTR-ToF-MS- measurements. The sensory profile of strawberries harvested at ¾ red stage showed lower sweetness and aroma than full red harvested strawberries. VOC analysis of these strawberries showed lower presence of volatile fatty acids, furanones and most esters even after nine days of cold storage, compared to full red strawberries. Strawberries harvested at full red stage showed the highest value for aroma attributes after one day of cold storage. Surprisingly, peak intensities of most esters (except for methyl butanoate and methyl hexanoate) and furanones were low on the first day, compared to ripe harvested fruit after longer storage. Ripe harvested fruit stored for nine days showed the highest peak intensities for most VOCs, but this did not correspond to the highest sensory aroma attributes. These fruits were judged with the lowest values for aroma attributes, perhaps related to the production of volatiles with off-flavours (acetaldehyde, ethyl acetate). PLS modelling showed that VOCs exist that are characteristic for both sweet and aromatic sensory attributes of 'Lusa' strawberries, based either on GC–MS (mainly volatile fatty acids) or PTR-ToF-MS analysis (mainly alcohol/ester fragments). This could lead to fast, non-destructive, selection of strawberries with high consumer liking using PTR-ToF-MS.

1. Introduction

Strawberry is the most commonly consumed berry fruit crop worldwide and is valued for its unique flavour and nutritional quality. Its overall liking is most affected by the sensory attributes sweetness and flavour intensity (Schwieterman et al., 2014). Volatile organic compounds (VOCs) are essential components of strawberry flavour, even though they only account for less than 0.01 % of the fruit's weight (Yan et al., 2018). The volatilome of strawberry is one of the best studied of all fruit. Nevertheless, none of the identified VOCs were consistently present in all studies (Ulrich et al., 2018). The variation in volatile profiles is both complex and distinct due to genotype, growth conditions, ripeness at harvest, postharvest storage conditions and extraction techniques (Schwieterman et al., 2014; Li et al., 2015; Ulrich et al., 2018; Yan et al., 2018). In total, 979 volatile compounds have been identified in strawberry fruit. However, within strawberries of a single cultivar far fewer compounds are detectable (Ulrich et al., 2018). Less than twenty volatiles have a significant contribution to strawberry flavour based on concentration to sensory threshold ratio (Jetti et al., 2007). The furanones furaneol and mesifuran, due to their low sensory threshold values, provide the typical caramel-like, sweet, floral and fruity strawberry aroma (Zabetakis et al., 1999; El Hadi et al., 2013). Esters account

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for 25–90 % of all strawberry volatiles and provide fruity and floral flavour (Yan et al., 2018). The most frequently identified esters in strawberry fruit are methyl hexanoate, ethyl hexanoate, ethyl butyrate and methyl butanoate (Ulrich et al., 2018), derived from esterification by alcohol acyl-transferases (AATs) (Duan et al., 2018). Fatty acid derived alcohols and aldehydes such as hexanal, E-2-hexenal, and Z-3-hexenol, are responsible for green, fresh notes in strawberry (Jetti et al., 2007; Du et al., 2011). Fatty acid-derived volatile aldehydes are generally produced from linoleic- and linolenic acid through the action of lipoxygenase (LOX) or hydroperoxide lyase (HPL). These aldehydes may then be converted to alcohols by alcohol dehydrogenases (ADHs) (Yan et al., 2018). Volatile fatty acids generally only affect the perceived aroma slightly, except for butanoic, 2-methylbutanoic and hexanoic acid, which are important contributors to strawberry aroma (Ménager et al., 2004; Du et al., 2011). Volatiles from other volatile pathways, such as terpenoids, benzenoids and sulphur compounds are also frequently reported (Yan et al., 2018).

Harvesting fruit before they are fully ripe, and allowing them to further colour during postharvest storage, is a common practice for many fruits in the supply chain (Kader, 2008). This practice extends shelf-life, facilitates storage and transport and decreases the impact of harvesting and handling. Strawberry fruit of four cultivars reached the longest shelf-life when harvested at the white to pink colour stage (Rahman et al., 2016). Nunes et al. (2006) found that strawberries of three cultivars, harvested at the ¾ red stage developed the same level of soluble solids, ascorbic acid and total phenolics content during storage as strawberries harvested at the full red stage. However, Van de Poel et al. (2014) found lower sugar levels and lower overall volatile abundance for 'Portola' strawberries harvested at ¾ red stage compared to those harvested at the full red stage. Maturity at harvest is therefore likely an important factor affecting consumer liking of strawberries. How the liking of strawberries is affected by postharvest storage, during retail transport and home refrigeration, is currently mostly unknown. Total soluble sugars and total acidity, important factors determining sweetness (Magwaza and Opara, 2015) slowly decreased during storage (Li et al., 2015). It is clear though that the volatile composition changes during postharvest storage. During refrigerated storage aldehydes (E-2-hexenal, Z-3-hexenal, hexanal), hexanoic acid and the esters methyl butanoate and ethyl butyrate increased in red 'Sweet Charlie' strawberries (Ozcan and Barringer, 2011). Lower levels of total esters and total furanones, comparable levels of total acids, but higher levels of total terpenes were observed in cold stored red 'Akihime' strawberries compared to room temperature stored fruit (Li et al., 2015). Storage temperature and light conditions also interacted. Ester and furanone levels were comparable when white-pinkish 'Sweet Charlie' strawberries were stored for seven days either at 15 °C/dark, 25 °C/dark and 25 °C/light but lowest when stored at 15 °C in the light (Fu et al., 2017). This indicates complex and distinct behaviour of the volatile composition during postharvest storage.

The first aim is to investigate the changes in consumer liking and the volatile composition during postharvest storage of 'Lusa' strawberries, harvested at either the ¾ red stage or the full red stage. Liking was assessed by a strawberry expert panel and the volatile composition by gas chromatography–mass spectrometry (GC–MS), the most often used technique to detect strawberry volatile compounds (Ulrich et al., 2018). The second aim is to investigate whether the changes in the volatile composition as affected by initial maturity and postharvest storage can also be assessed by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS). PTR-ToF-MS enables fast and real-time monitoring of volatiles with high sensitivity without labour-intensive sample preparation (Majchrzak et al., 2018) but with the drawback that compound identification is more challenging and often not possible (Cappellin et al., 2012). We discuss the impact of harvesting strawberries either at the ¾ red stage or full red stage during cold storage on consumer liking and discuss the origin of the changes that appear in the volatile pathways as assessed by GC–MS. Finally, we discuss the possibility to use VOCs as markers to predict consumer liking.

2. Material and methods

2.1. Plant material and storage conditions

Strawberries (*Fragaria* × *ananassa* cv. Lusa) were harvested either at the ¾ red stage or full red stage (indicated as 'unripe' and 'ripe', respectively) from a grower in Prinsenbeek, the Netherlands on May 10th, 2017. In total 75 punnets per maturity stage were harvested with each punnet consisting of about 500 g of undamaged and uniformly sized strawberries. Punnets were transported to the lab in Wageningen within approximately one hour after harvest. At the start of the next day the punnets were labelled and randomly assigned to six treatments; two initial maturity stages (unripe and ripe) and three storage times (day 1, day 5 and day 9) with 25 punnets per treatment. These were randomly assigned to five crates, each containing five punnets, with punnets in one crate considered a replicate. Eighty percent of the punnets were used for PTR-ToF-MS measurements and sensory analysis, the other twenty percent for colour, firmness and GC–MS measurements. All punnets were stored in darkness at 4 °C and a relative humidity of 80 %. Prior to measurements, fruits were placed at room temperature (20 °C) for six hours.

2.2. Trained expert panel

Following PTR-ToF-MS measurements, fruits were evaluated by a trained expert panel (Greenhouse Horticulture, Bleiswijk, the Netherlands) after transport from Wageningen to Bleiswijk in approximately one hour. Strawberries were evaluated at room temperature by nineteen panellists in individual booths illuminated with red light to minimise the effect of strawberry colour differences. Evaluations took place during late afternoon and early evening sessions. Each panellist evaluated 21 attributes on a 1-to-100-point scale for six fruits per replicate for the unripe and ripe harvested fruits on day 1, day 5 and day 9 with data represented as average per replicate.

2.3. Colour and firmness measurements

Strawberry skin colour was measured using a LED colour matching cabinet (IPSS Engineering, Wageningen, the Netherlands) containing a RGB camera (MAKO G-192C POE, Allied Vision, Stadtroda, Germany). Calyxes were removed prior to acquiring the images. A set of images was recorded for 15 individual fruits per punnet, from five punnets per maturity stage and storage time. Two images were acquired, one from each side of 15 individual strawberries, randomly selected per punnet. Strawberries were placed on a blue holding tray and between images turned 180 ° over their proximal-distal axis. The acquired RGB images were calibrated using a 24-patch colour card (ColorChecker Classic, X-Rite Europe GmbH, Regensdorf, Switzerland). Image analysis was carried out by using multi-threshold colour image segmentation to remove the blue background and separate the individual strawberries in each image. Colour data were transformed from the RGB to the HSV colour model and expressed as the average Hue value of both sides of each strawberry. Due to the circular nature of the hue scale, hue values over 360 ° were expressed as negative values.

Firmness of the same strawberries that were selected for colour measurements were measured using a FirmTech FT7 (UP GmbH, Ibbenbüren, Germany). Firmness was expressed as the average force displacement (in g mm^{-1}) between 70 and 250 g of force applied on the strawberry shoulder.

2.4. VOC analysis by SPME/GC–MS and data processing

Extraction and detection of volatile metabolites were performed according to Tikunov et al. (2005) with slight modifications as follows.

Strawberries were cut in slices and immediately frozen in liquid nitrogen. The frozen slices were ground into powder with an analytical grinder (IKA A11, IKA, Staufen, Germany). Frozen strawberry powder (0.5 g) was added to 0.5 ml deionized water and incubated at 30 °C for 10 min. After adding 1 ml 100 mM, pH 7.5 EDTA/NaOH, 2.2 g solid $CaCl_2$ powder was mixed thoroughly to inhibit enzyme activity. One ml of the extract was transferred into a 10 ml crimp cap vial for headspace SPME/GC–MS detection. Individual vials were randomised to avoid systematic memory effects and placed into a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). A 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre (Supelco, Bellefonte, PA, USA) was exposed for 20 min to the vial headspace under continuous agitation and heating at 50 °C. The trapped compounds by SPME were desorbed into a Trace GC Ultra gas chromatograph (Thermofisher, Waltham, USA) injector for 1 min at 250 °C. Chromatographic separation was achieved on an Zebron ZB-5 (50 m ×0.32 mm ×1.05 µm) column (Phenomenex) with helium as the carrier gas (at a constant flow of 2 mL min^{-1}). The GC interface and MS source temperatures were 260 and 250 °C, respectively. The GC temperature program started at 45 °C for 2 min, was then increased to 250 °C at a rate of 5 °C min^{-1} and finally held at 250 °C for 5 min. Including oven cooling, the total run time was 60 min. Mass spectra in the 35–400 m/z range were recorded by an DSQII electron impact MS (Thermofisher) at a scanning speed of 2.8 scans s^{-1} and an ionization energy of 70 eV. The chromatography and spectral data were evaluated using Xcalibur software (Thermofisher). The raw data generated by SPME/GC–MS were processed by the MetAlign™ software package (http://www.metalign.nl) (Tikunov et al., 2012). VOCs were identified by matching mass spectra and the retention indices of the compounds extracted to the NIST mass spectral library using NIST MS Search software (http://www.nist.gov).

Per maturity stage and storage time five replicates were measured with each replicate consisting of pooled strawberry powder from one punnet per maturity stage and storage time. GC–MS analysis was carried out twice, three days apart from each other, on the same samples. Linear regression (using the lm package in R) was carried out to test whether a linear relationship existed between the intensities of all identified compounds from the first and second GC–MS analysis for all samples (five replicates per combination of maturity and storage time). Only the peak intensities of compounds showing a linear relation with a $R^2_{adj} > 0.85$ were selected for further analysis. This means that only compounds that were identified twice and at comparable peak intensities were included. Peak intensities of these compounds were averaged and normalised.

2.5. VOC analysis by PTR-ToF-MS and data processing

PTR-ToF-MS analysis was carried out by placing two punnets in an airtight high-density airtight polyethylene (HDPE) drum (Engels Logistiek B.V., Eindhoven, the Netherlands) with septa (Suba-Seal, Sigma-Aldrich) mounted on the lids. After thoroughly flushing the drums with clean air for 2 min, the punnets were placed in the drums, the drums were closed and incubated for 2 h at 20 °C for accumulation of volatiles. Thereafter headspace volatiles were measured using a PTR Qi-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) set with the following conditions: 110 °C drift tube temperature, 3.8 mbar drift pressure, 900 V drift voltage, leading to an E/N ratio of about 120 Townsend (Td; 1 Td = 10^{-17} V cm^{-2}), where E corresponds to the electric field strength and N to the gas number density. Mass spectrometric data were collected over a mass range of 20−512 *m/z* using a flow rate of 60 mL min^{-1}. PTR-ToF-MS data was extracted using PTRwid (Holzinger, 2015). Noise reduction was done by averaging 20 consecutive and stable ToF spectra with subsequent baseline correction. Per maturity stage and storage time five replicates were measured. Each replicate consisted of the averaged data from two identical samples (two drums, each containing two punnets). Peak identification was carried out by combining data from Aprea et al. (2009) for raspberry and Farneti et al. (2015) for apple. Peak intensities were corrected for fruit weight and normalised. No internal standard was added for both GC–MS and PTR-MS data processing purposes as many chemically diverse compounds were identified; an internal standard is only valid for quantification of one single class of compounds and using one would introduce errors for compounds of other chemicals classes.

2.6. Statistics

Statistical analyses were performed in R.4.0.2 (http://www.R-project.org/) with all tests conducted at a *P* value of 0.05. Colour and firmness data were compared applying two-way analysis of variance (ANOVA), sensory attributes with one-way ANOVA. Homogeneity and normality were tested using Bartlett's test and the Shapiro-Wilk test, respectively. Tukey HSD test was used as post-hoc test for the variables with a significant treatment effect. Heatmaps were created with the help of the packages heatmap.2, gplots and RColorBrewer. The Pairwise Wilcox test (pairwise.wilcox.test) was used to identify differences in intensities due to maturity or storage time applying the DH correction (Benjamini and Yekutieli, 2001) for multiple testing. PCA plots and hierarchical clustering were conducted with the help of packages prcomp, survival, nnet, MASS, splines, ellipse and car. PLS modelling was carried out using the pls and mdatools packages applying leave-one-out cross validation. Permutation testing, to avoid overparameterization, was carried out using the function randtest, applying 5000 permutations. The PLS models were calibrated applying a VIP (Variables Important for Projection) score lower than 1 (Galindo-Prieto et al., 2014).

3. Results

3.1. Colour and firmness development during storage

In unripe harvested fruit red colouration increased over storage time, as indicated by decreasing Hue values. Ripe harvested fruit did not change colour over time (Fig. 1A). Firmness decreased over time for unripe fruit whereas the firmness of ripe strawberries remained at the same level (Fig. 1B). Both colour and firmness values for the unripe fruits at day 9 did not reach those of ripe fruit at day 1.

3.2. Sensory profiling

Mean scores of individual sensory attributes of unripe and ripe harvested fruits are shown per storage time, expressed as spider web charts (Fig. 2). On day 1, unripe fruit showed significantly higher firmness, lower juiciness, lower sweetness, and higher sourness than ripe fruit (Fig. 2A). In addition, sensory attributes related to aroma showed significantly lower values for unripe fruit on day 1, for example aroma presence, aroma liking, fruity aroma and strawberry aroma. These differences between unripe and ripe fruit increased on day 5 compared to day 1 (Fig. 2B). In addition, significantly lower mealiness, higher firmness of the seeds, lower fruity (other) aroma and lower flower aroma were observed in unripe compared to ripe fruit at day 5. On day 9, unripe fruit showed significantly higher firmness, lower juiciness, lower sweetness, and higher sourness than ripe fruits, similar to day 1 and day 5 (Fig. 2C). Unripe compared to ripe fruit stored for nine days showed significant differences in flavour related attributes, such as lower aroma presence, higher green aroma, lower strawberry aroma and lower flower aroma. On day 9, differences between unripe and ripe fruit diminished compared to day 5 but were still larger than observed at day 1. For example, aroma liking, fruity aroma and fruity (other) aroma were similar in unripe and ripe fruit on day 9, but showed differences between unripe and ripe fruit on day 5. On day 9, significantly higher yeast and lower astringent attributes in ripe harvested fruit were observed.

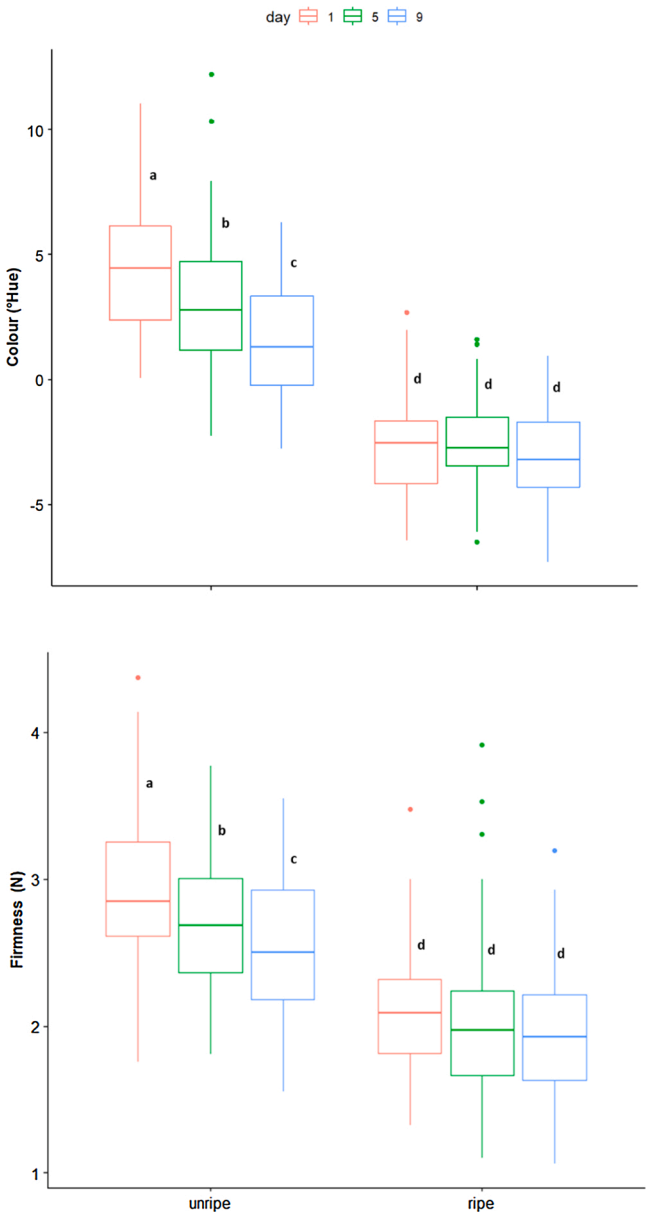

Fig. 1. Colour **(A)** and firmness **(B)** measurements for unripe and ripe harvested 'Lusa' strawberries after one, five and nine days of storage (4 °C) expressed as boxplots and analysed by 2-way ANOVA with *P*(ripeness) <0.001 and *P*(storage days) <0.001 for both colour and firmness measurements. Different letters indicate significant differences ($P < 0.05$). °Hue values over 360 ° were expressed as negative values. Data are means of five replicates (n = 5) from one punnet, consisting of 75 measurements per combination of ripeness stage and storage time.

3.3. Volatile profiling by GC–MS

Although 85 peaks were observed, only twenty-five passed the test of being present during two consecutive GC–MS runs with a similar peak intensity as detailed in the M&M section. Changes in relative peak intensities of these twenty-five compounds during storage in unripe and ripe harvested fruit are represented as a heatmap. Hierarchical clustering, indicating similar VOC behaviour, resulted in four groups of compounds (Fig. 3). The first group consisted mainly of volatile aldehydes which showed relatively small changes regardless of initial maturity and storage time. The second group consisted of volatiles fatty acids and alcohols. The volatile fatty acids showed lower peak intensities for the unripe compared to ripe fruits. The alcohols, similar to the volatile fatty acids, showed lower peak intensities for the unripe compared to ripe fruits. The VOCs in the third group, mainly volatile fatty acids, and esters had low peak intensities in unripe compared to ripe fruits, regardless of storage time. In the last group, group 4, the main constituents were esters, with low peak intensities in unripe fruits. Ripe harvested fruits on day 1 had comparable peak intensities to those of unripe fruits. During storage of ripe fruit, all peak intensities increased.

Typical strawberry volatiles, furaneol (group 4) and mesifuran (group 3), had low peak intensities in unripe fruits. At the first day of storage of ripe fruit, furaneol peak intensities were low, but increased during storage. The peak intensities of mesifuran, at the start of the storage of ripe harvested fruit, were higher than in unripe fruit and continued to increase during storage.The PCA plot of the GC–MS data indicated that the total variability explained by the first and second principal components is almost 70 % (Fig. 4A). The 95 % confidence ellipses indicate that there is a good separation between ripe and unripe fruit, and within the ripe harvested fruit, between storage days. Most volatiles present in the PC1 loading plot have positive loadings indicative of ripe fruit. The PC2 loading plot showed both positive and negative loadings, mostly indicative of the variation in storage days (Fig. 4B). In other words, a range of volatiles characteristic for variation in storage days was observed in ripe fruit, whereas only E-2-hexenal was characteristic for unripe fruit at day 1.

3.4. Volatile profiling by PTR-ToF-MS

Changes in peak intensities of twenty-six fragments and compounds as measured by PTR-ToF-MS in unripe and ripe harvested fruit over time are represented as a heatmap (Fig. 5). Hierarchical clustering indicates four main groups of compounds. Most groups consisted of a variety of fragment types. Fragments from alcohols, aldehydes (propanal and hexanal), and also esters are dominating in the first group. Most of the constituents of group 1 have lower peak intensities in unripe compared to ripe fruit, and hardly change over time. The second group consisted of fragments with peak intensities increasing over time. The third group consisted of fragments that, except for the C7H9+ fragment, did not show many changes as function of harvest maturity and storage time. The fourth group, consisting mostly of esters, and also acetaldehyde and ethanol, showed increasing peak intensities for unripe and ripe fruit over time.

The PCA plot of the PTR-ToF-MS data (Fig. 6A) had similarities with the PCA plot for the GC–MS data (Fig. 4A) with respect to the distribution of the 95 % confidence ellipses. The separation between ripe and unripe fruit was well established, and within the ripe fruit there is a clear separation between storage days. The total variability explained by the first and second principal components is high at 84.3 %. The PTR-ToF-MS PC1 loading plots showed mostly positive loadings, indicative of compounds and fragments characteristic for ripe fruits. The PC2 loading plot showed both positive and negative loadings, mostly indicative of the variation in storage days (Fig. 6B). Similar as for the GC–MS PCA plot (Fig. 4A), many compounds and fragments characteristic for variation in storage days in ripe fruit were observed, but not in unripe fruit. Only methanol was characteristic for unripe fruit.

3.5. Prediction of consumer liking by PLS modelling of VOCs

Partial Least Square (PLS) regression modelling was carried out to predict the sweetness and aroma attributes based on the volatile composition. Consumer liking is determined by sweetness and flavour intensity. Flavour intensity was described as the retronasal olfaction complimenting sourness and sweetness intensities' contribution to taste (Schwieterman et al., 2014). Here, sensory attributes related to flavour intensity were recorded such as aroma presence, aroma liking, fruity aroma, fruity (strawberry) aroma (Fig. 2). The average value of these attributes was regarded as aroma, describing flavour intensity. PLS

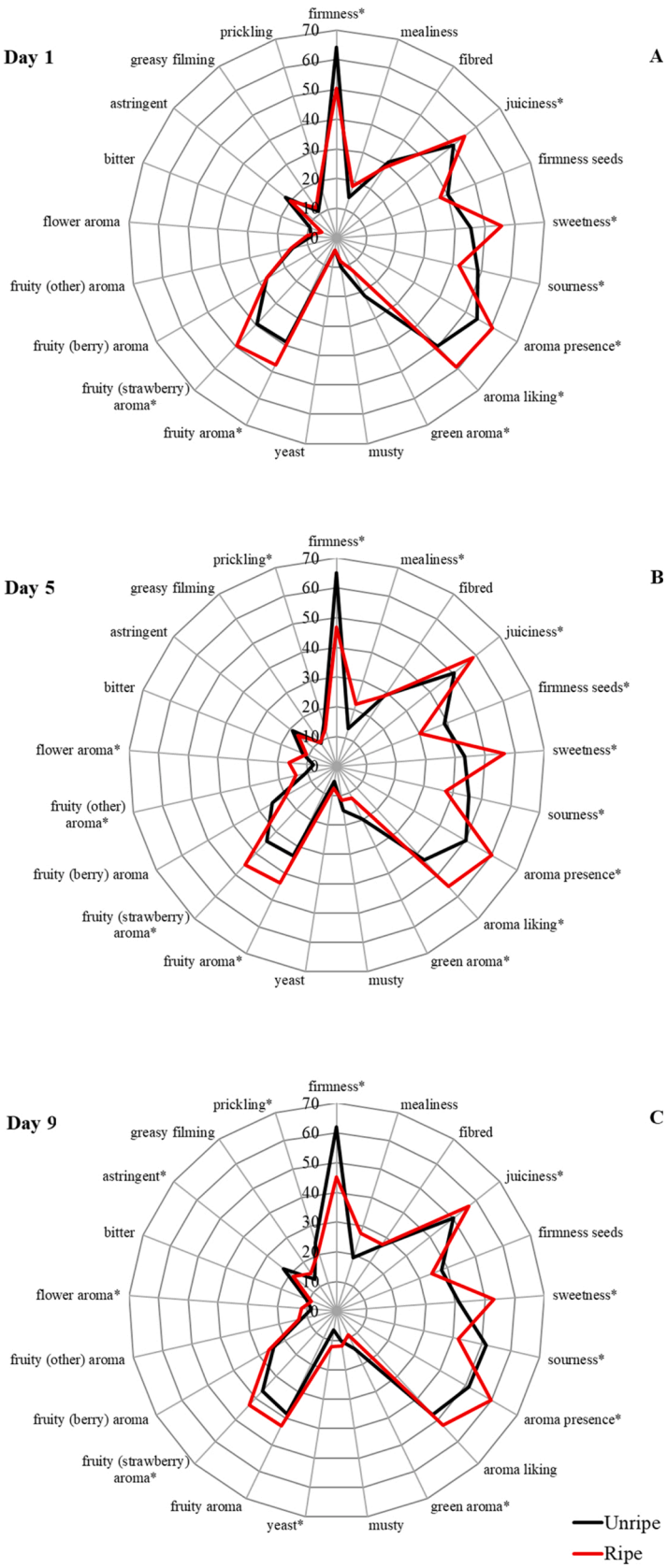

Fig. 2. Sensory evaluation for unripe and ripe harvested 'Lusa' strawberries after one **(A)**, five **(B)** and nine days **(C)** of storage (4 °C) expressed as spider plots. Asteriks indicate signifant differences as indicated by 1-way ANOVA ($P < 0.05$).

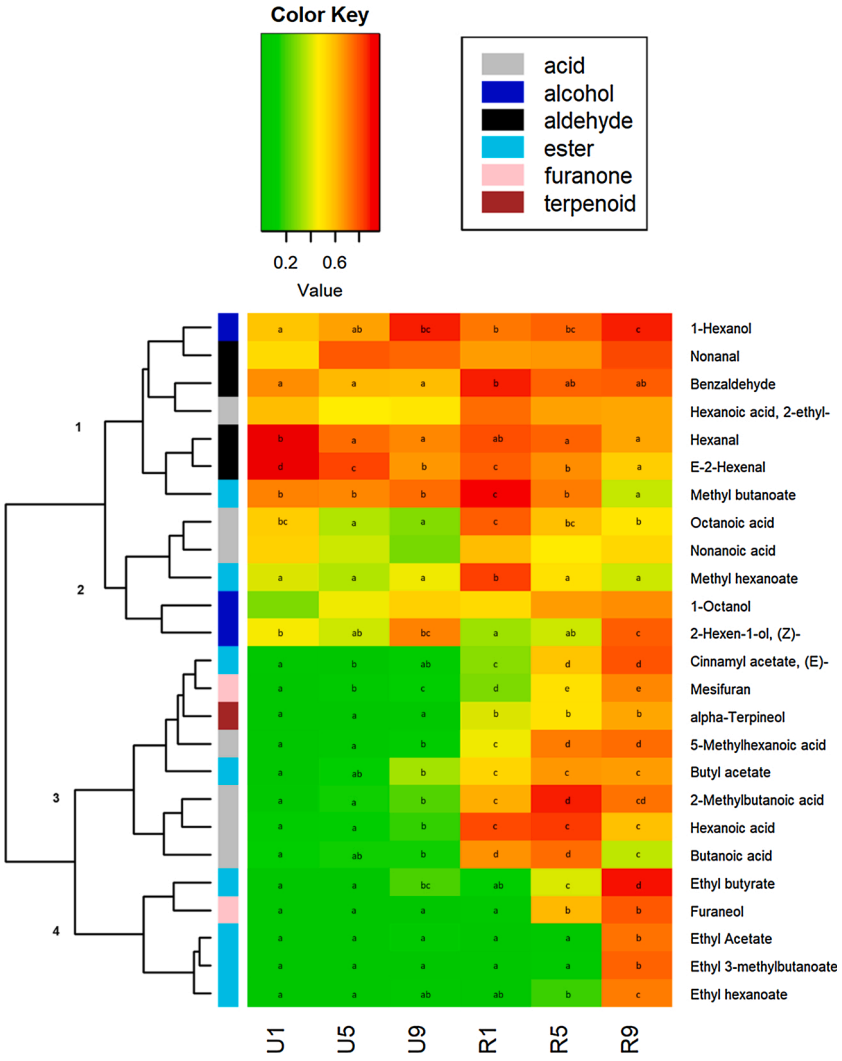

Fig. 3. Heatmap, including hierarchical clustering, of the untransformed and normalised GC–MS data for unripe (U) and ripe (R) harvested 'Lusa' strawberries after one (1), five (5) and nine (9) days of storage (4 °C). Distinct letters indicate statistical differences depicted by the pairwise Wilcox test ($P < 0.05$). Data are means of five replicates (n = 5) from one punnet.

models were built to predict sweetness and aroma based on peak intensities of volatiles gathered by either GC–MS or PTR-ToF-MS. The PTR-ToF-MS based PLS models showed higher explained variation of validation (R^2_p) and lower values for the root mean square error of prediction ($RMSE_P$) than GC–MS based PLS models (Table 1). Variables important for the prediction (VIP) of sweetness and aroma were often found to be in common (Table 2). Regression coefficients for sweetness and aroma, based on GC–MS data, showed a high correlation (R = 0.92), indicating high similarity between those PLS models.

4. Discussion

4.1. Volatile fatty acids

Volatile profiles between unripe and ripe harvested fruits as measured by GC–MS analysis were quite different, especially with regard to the volatile fatty acids. Many volatile fatty acids showed higher (group 2 and group 3, Fig. 3) peak intensities in ripe compared to unripe fruits, irrespective of storage duration. In a list of 54 strawberry volatiles, sorted on decreasing odour activity value (OAV), 2-methylbutanoic ('sour', 'cheesy', 'sweaty'), butanoic ('sour', 'cheesy') and hexanoic acid ('sweaty', 'cheesy') were placed on position 8, 15 and 18, respectively (Du et al., 2011). These volatile fatty acids are therefore not only important for perceived aroma, but also characteristic for differentiating unripe and ripe fruit. It is remarkable that these VOCs, with a generally negative perception, have higher peak intensities in ripe fruit, perhaps suggesting that they have a function in enhancing the characteristics of other fruity VOCs.

4.2. Esters

Esters observed in group 4 (Fig. 3) had low peak intensities in unripe fruits, whereas intensities increased during storage in ripe fruit. Methyl butanoate, ethyl butyrate and ethyl hexanoate are part of this group and have high OAV values (3, 7 and 30 respectively (Du et al., 2011)). The peak intensities of these esters, present in ripe fruit at the start of the storage (day 1), were comparable with those in unripe fruit, and increased up to day 5, except for ethyl butyrate. The relatively low peak intensities for most esters at the first day of storage in ripe fruit might be due to low substrate availability that governs ester biosynthesis, next to AAT specificity (Yan et al., 2018). Not all ester peak intensities were low at the first day of ripe fruit; exceptions are methyl butanoate and methyl hexanoate. Methyl butanoate (group 1) and methyl hexanoate (group 2) showed decreasing peak intensities during storage in ripe fruits. These esters might therefore be important for the fruity and floral aroma of freshly ripe strawberry fruit, as most other esters are not yet synthesized.

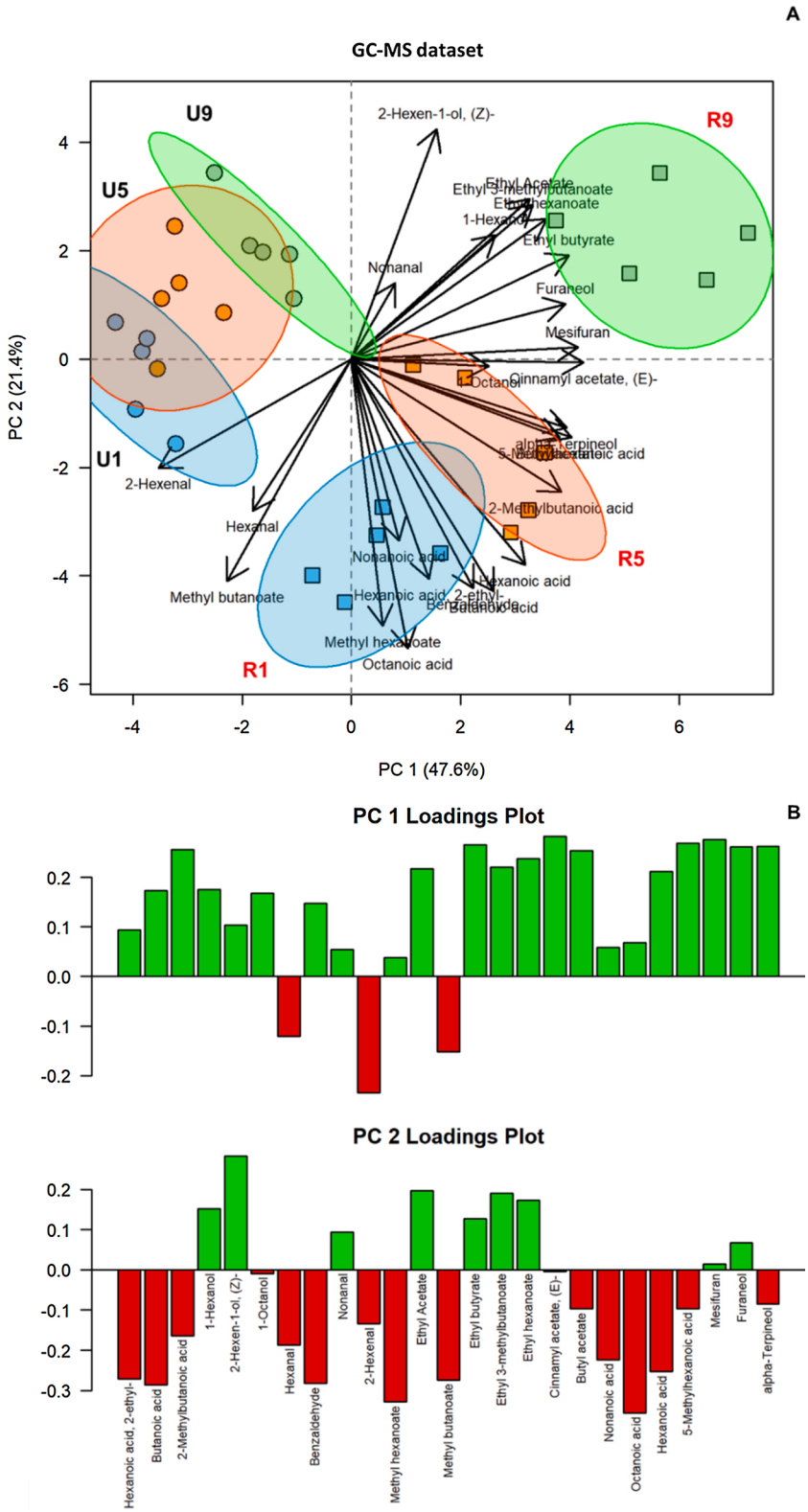

Fig. 4. PCA plot **(A)** and loading plot **(B)** for unripe (U) and ripe (R) harvested 'Lusa' strawberries after one (1), five (5) and nine (9) days of storage (4 °C) based on GC–MS data.

4.3. Aldehydes and alcohols

All identified aldehydes are part of group 1 (Fig. 3). Hexanal ('fresh', 'green') and E-2-hexenal ('grassy', 'pungent') are the aldehydes with the highest OAV (Du et al., 2011) and showed lower peak intensities over time, irrespective of harvest maturity (Fig. 3). Propanal, another aldehyde, was only registered during by PTR-ToF-MS analysis, and showed higher peak intensities in ripe compared to unripe fruit, irrespective of storage time (group 1, Fig. 5). This might indicate that propanal is not further metabolised. Benzaldehyde was the only compound identified

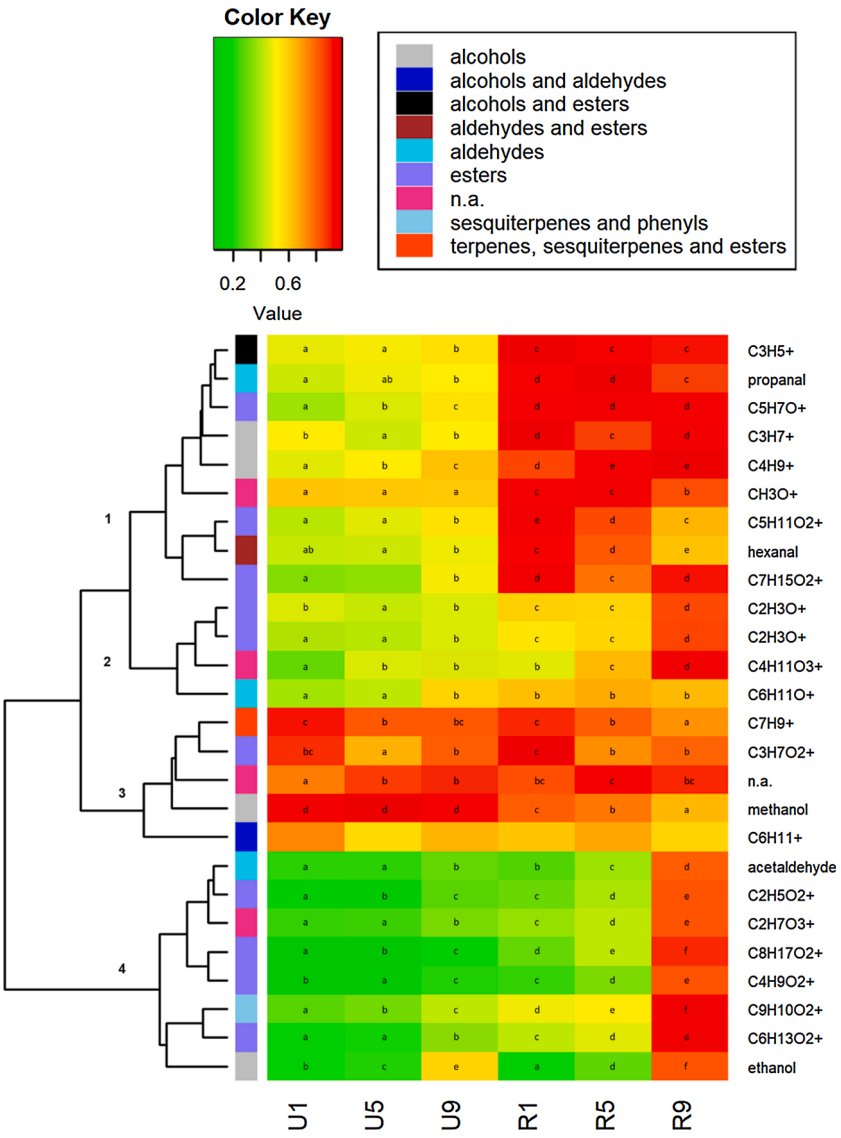

Fig. 5. Heatmap, including hierarchical clustering, of untransformed and normalised PTR-ToF-MS data for unripe (U) and ripe (R) harvested 'Lusa' strawberries after one (1), five (5) and nine (9) days of storage (4 °C). Distinct letters indicate statistical differences depicted by the pairwise Wilcox test ($P < 0.05$). Data are means of five replicates (n = 5) from four punnets.

that originates from the shikimate pathway (Fu et al., 2017). GC–MS analysis showed higher peak intensities of benzaldehyde in ripe compared to unripe fruits (group 1, Fig. 3). Currently, not much is known about the volatile benzenoid biosynthetic pathway in strawberries (Yan et al., 2018).

1-Hexanol (group 1, Fig. 3), 2-hexen-ol, and 1-octanol (group 2, Fig. 3) all increased for both unripe and ripe fruit during storage. These alcohols have high odour thresholds compared to their aldehyde homologues (Schwab et al., 2008). Therefore they probably do not contribute much to the strawberry aroma (Larsen and Watkins, 1995). Methanol peak intensities decreased for long-stored ripe fruit, as measured by PTR-ToF-MS analysis (group 3, Fig. 5). This might indicate that methanol is used for ester synthesis. In contrast, ethanol, and acetaldehyde ('green', 'apple', Du et al., 2011) levels increased during storage for both unripe and ripe fruit (group 4, Fig. 5). Ethanol and acetaldehyde are fermentation products that accumulate over time in strawberry (Ponce -Valadez and Watkins, 2008).

4.4. *α-Terpineol and furanones*

α-terpineol was the only identified terpenoid during GC–MS analysis (group 3, Fig. 3) with lower peak intensities observed in unripe strawberries, irrespective of storage time. Other terpenoids, like linalool and geraniol, often present in strawberry providing a fruity and floral aroma (Du et al., 2011) were not observed here.

Quinone oxidoreductase (FaQR) is the enzyme present in the last step of the furaneol biosynthesis (Yan et al., 2018). FaQR is strongly induced by ripening (Li et al., 2015). In the dark, increasing temperatures were accompanied by upregulated expression of *FaQR* (Fu et al. 2016). The initial lower furaneol peak intensity for freshly harvested ripe fruit (group 4, Fig. 3) might be due to the conversion of furaneol to mesifuran (group 3, Fig. 3) by an O-methyltransferase (Yan et al., 2018).

4.5. *The link between volatile profiles and consumer liking*

Soluble sugar accumulation is prevalent during the last phase of the maturation of strawberry fruit, roughly doubling the fructose, glucose, and sucrose levels from turning to full red (Tian et al., 2012). One of the

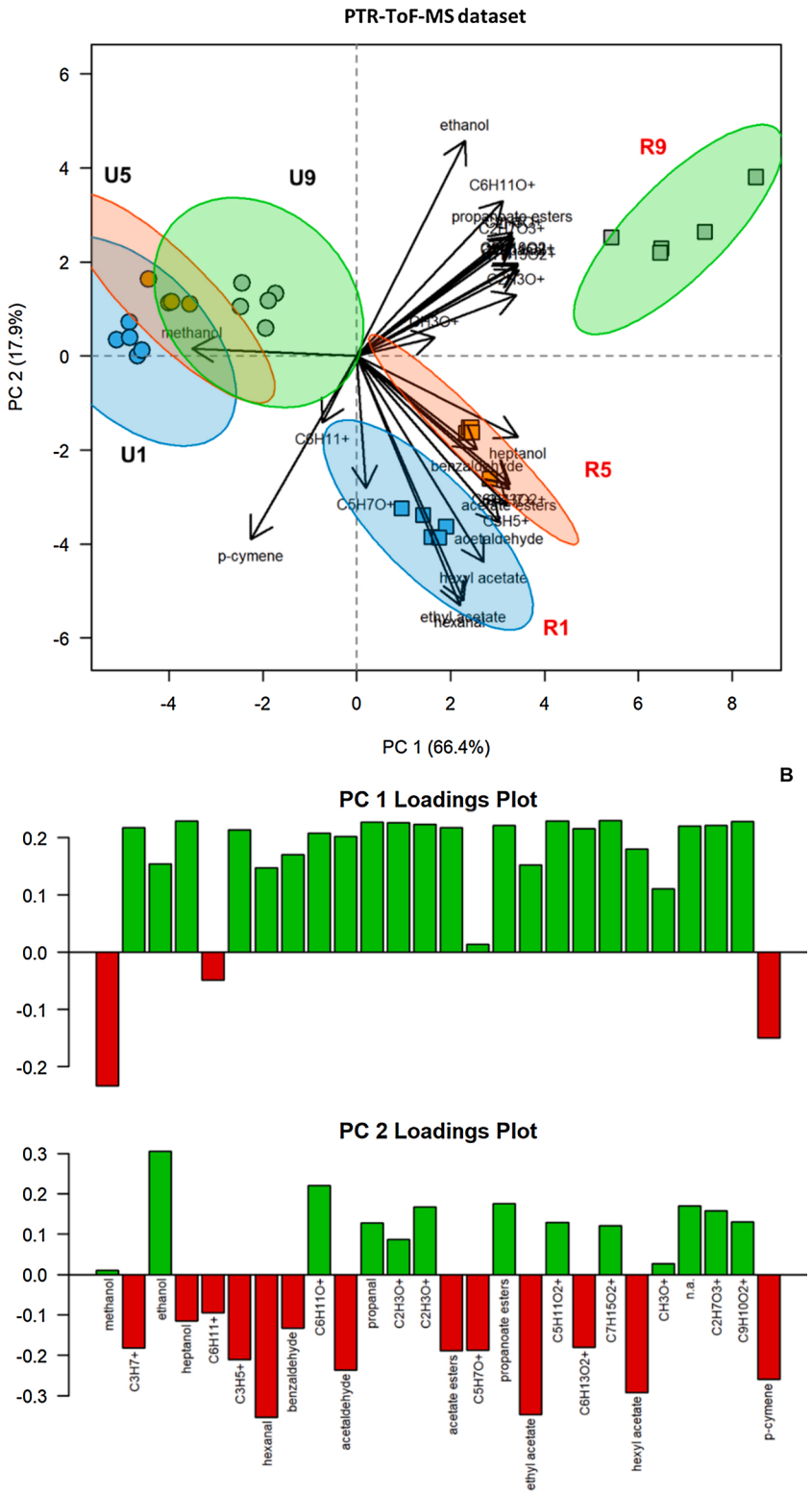

Fig. 6. PCA plot **(A)** and loading plot **(B)** for unripe (U) and ripe (R) harvested 'Lusa' strawberries after one (1), five (5) and nine (9) days of storage (4 °C) based on PTR-ToF-MS data.

main enzymes responsible for sucrose accumulation is FaSS1, a sucrose synthase. FaSS1 is proposed to have an important role in strawberry ripening (Zhao et al., 2017). It can be hypothesized that ripe harvested fruit, unlike unripe harvested fruit, has sufficient sucrose levels to jumpstart the production of a number of volatile fatty acids and esters during storage (Fig. 3).

Many esters, especially those present in group 4 (Fig. 3), showed increasing peak intensities over time in ripe fruit, including ethyl butyrate, ethyl hexanoate and ethyl 3-methylbutanoate ('fruity, 'apple', 'pineapple'). These compounds have high OAV values. This could indicate that ripe fruit stored for 9 days would have the best volatile related sensory scores. However, this doen not seem to be the case: flavour

Table 1
Performance of partial least square models using GC–MS and PTR-ToF-MS strawberry data of unripe and ripe harvested 'Lusa' strawberries after storage (4 °C). nLV, number of latent variables, root mean square error of calibration ($RMSE_C$) and validation ($RMSE_V$), root mean square error of prediction ($RMSE_P$), explained variation of calibration (R^2_c) and validation (R^2_p).

Sensory attribute	Analysis	Calibration		Prediction		nLV
		RMSEc	R^2_c	RMSEp	R^2_p	
Sweetness	GC-MS	2.6	0.84	3.1	0.78	2
Aroma	GC-MS	2.6	0.76	3.0	0.68	2
Sweetness	PTR-ToF-MS	2.4	0.87	2.8	0.81	3
Aroma	PTR-ToF-MS	2.0	0.86	2.6	0.75	4

attributes aroma liking, fruity aroma, and fruity (strawberry) aroma were highest on day 1, and lowest on day 9 for ripe fruit (Fig. 2AC). This might be due to methyl butanoate and methyl hexanoate. These compounds had high peak intensities for ripe fruit on day 1, that subsequently decreased during storage (Fig. 3). Aroma liking might be less dependent on the furanones, as especially furaneol showed comparable levels for unripe and ripe fruit on day 1 (Fig. 3). Lower flavour attributes for ripe fruit might also be related to increasing peak intensities for ethyl acetate over time for ripe fruit. Ethyl acetate has a pineapple-like aroma at lower, but a non-pleasant chemical odour at higher concentrations (Larsen and Watkins, 1995). The fruity ester perception of long stored ripe harvested fruit might be masked by acetaldehyde (group 4, Fig. 5) or due to the higher yeast score for long stored ripe fruit (Fig. 2C), although no signs of *Botrytis cinerea* infection were observed.

4.6. Is it possible to predict consumer liking?

Consumers are willing to pay a premium for strawberries with both great appearance and flavour (CBI, 2019). The PCA plots indicated that a range of volatiles (Fig. 4A) and fragments (Fig. 6A) are present that differentiate between ripe and unripe harvested fruits and between storage times of ripe fruits. The position and shape of the confidence ellipses in the PCA plots derived from both GC–MS and PTR-ToF-MS data are similar, despite the obvious differences in measurement protocols and principles. The high correlation (R = 0.92) between regression coefficients of the GC–MS based sweetness, and aroma PLS models indicated that consumer liking can be predicted (Table 2) with both PLS models. The three compounds with the highest VIP scores for both sweetness and aroma were all volatile fatty acids: butanoic, 2-methylbutanoic, and hexanoic acid. These volatile fatty acids have been mentioned as important contributors to aroma (Ménager et al., 2004; Du et al., 2011). Fan et al. (2021a) investigated whether volatile and non-volatile markers exist for sweetness and consumer liking for fully red harvested strawberries within set of diverse cultivar and breeding selections. Amongst a list of GC–MS measured volatile compounds important for both sweetness and liking were multiple medium-chain fatty acid esters, such as butanoic and hexanoic acid derived esters. In a sensory evolution study from the same authors sweetness and strawberry flavour were also linked to the presence of butanoic and hexanoic acid derived esters (Fan et al., 2021b). Butanoic and hexanoic acid, compounds with high VIP scores in our study, were not identified, but likely serve as precursor for these acid derived esters.

The PLS models for sweetness and aroma based on PTR-ToF-MS had only one VIP in common, the alcohol fragment C3H7+ (Table 2). Volatile profiling during ripening by PTR-ToF-MS was also carried out for avocado, banana, mango, and mangosteen (Taiti et al., 2015). It was indicated that PTR-ToF-MS might become a commercial standard tool to link volatile fingerprinting with consumer liking. Although PTR-ToF-MS is a non-destructive technique that is capable of fast, real-time monitoring, there are also drawbacks to incorporate PTR-ToF-MS devices in e.g., sorting and grading lines. One drawback is the current high equipment cost. Another, perhaps more troubling drawback is that the composition and abundance of strawberry volatiles is strongly cultivar dependant (Schwieterman et al., 2014). Volatile composition and abundance also depend on postharvest factors such as storage temperature and the use of coatings (Yan et al., 2018). Nevertheless, Ulrich et al. (2018) listed thirty strawberry volatiles that are mentioned at least twelve times in twenty-five studies. It might be of interest to scale up the approach proposed here for more cultivars and growing conditions to investigate whether there are common patterns in volatile production as measured by PTR-ToF-MS that can be linked universally to consumer liking.

5. Conclusions

Harvesting strawberries at the ¾ red stage lowered sweetness and aroma presence compared to harvesting full red 'Lusa' strawberries, irrespective of the storage time. Strawberries harvested at the ¾ red stage had lower presence of volatile fatty acids, furanones, and most esters, even after nine days of storage at 4 °C. Full red harvested strawberries received the highest consumer liking at day one of cold storage. The levels of many important esters and furaneol of ripe fruits harvested at day one were comparable with those of unripe harvested fruits, with the exception of methyl butanoate and methyl hexanoate. The lowest values for aroma attributes for full red strawberries were found at day nine, likely because volatiles with off flavours (acetaldehyde, ethyl acetate) were quickly increasing after day 5. PCA analysis of

Table 2
PLS analysis for the prediction of sweetness and aroma sensory attributes based on GC–MS and PTR-ToF-MS data for unripe and ripe harvested 'Lusa' strawberries during storage at 4 °C. Only variables there were significant ($P < 0.05$) are listed. VIP, variables important for projection.

Analysis	Attribute	Predictor	VIP	Regression coefficient		Attribute	Predictor	VIP	Regression coefficient	
				Value	Std.err.				Value	Std.err.
GC-MS	Sweetness	Hexanoic acid	1.60	0.15	0.03	Aroma	Hexanoic acid	1.62	0.14	0.03
		Butanoic acid	1.57	0.16	0.04		Butanoic acid	1.59	0.15	0.04
		2-Methylbutanoic acid	1.47	0.10	0.02		2-Methylbutanoic acid	1.38	0.08	0.02
		5-Methylhexanoic acid	1.38	0.09	0.02					
		α-Terpineol	1.29	0.07	0.03		α-Terpineol	1.19	0.04	0.04
		Butyl acetate	1.26	0.07	0.03					
		Benzaldehyde	1.21	0.11	0.04		Benzaldehyde	1.27	0.10	0.04
		Cinnamyl acetate, E-	1.18	0.04	0.02					
		Octanoic acid	1.09	0.14	0.03		Octanoic acid	1.30	0.15	0.03
		Methyl hexanoate	1.07	0.14	0.04		Methyl hexanoate	1.37	0.18	0.05
PTR-ToF-MS	Sweetness	CH3O+	1.42	0.22	0.07	Aroma	C5H11O2+	1.48	1.78	0.34
		propanal	1.33	0.16	0.05		C3H7+	1.36	2.99	0.56
		C3H5+	1.29	0.15	0.04		C7H15O2+	1.26	−2.02	0.62
		C3H7+	1.28	0.30	0.09		C5H7O+	1.25	−1.51	0.68
		ethanol	1.07	−0.38	0.08		C4H9+	1.15	1.04	0.47
		methanol	1.06	−0.32	0.15					

GC–MS and PTR-ToF-MS data indicated many compounds and fragments characteristic for variation in storage days in ripe fruit, but not in unripe fruit. PLS modelling identified VOCs exist that are characteristic for both sweet and highly aromatic 'Lusa' strawberries. Based on GC–MS data these VOCs are mainly volatile fatty acids, whereas based on PTR-ToF-MS data these are mainly alcohol/ester fragments. This opens up the possibility for fast, non-destructive, selection of strawberries with high consumer liking using PTR-ToF-MS.

Author contributions

HL, BB, NO, FPdS and YT contributed to this work in data acquisition. FPdS and BB performed the experiment design. RS and HL performed the data analysis. RS and HL drafted the manuscript. EW and JV contributed to data interpretation and discussion. FPdS, EW, BB and JV critically revised and approved the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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