LWT - Food Science and Technology 63 (2015) 21–28

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Dynamic volatile organic compound fingerprinting of apple fruit during processing

Brian Farneti [a, *], Iuliia Khomenko [b], Luca Cappellin [b], Valentina Ting [b], Guglielmo Costa [a], Franco Biasioli [b], Fabrizio Costa [b]

[a] Department of Agricultural Sciences, Bologna University, Via Fanin 46, 40127 Bologna, Italy
[b] Research and Innovation Centre, Fondazione Edmund Mach, Via Mach 1, San Michele all'Adige, 38010 Trento, Italy

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form
2 March 2015
Accepted 10 March 2015
Available online 18 March 2015

Keywords:
Malus x *domestica* Borkh
Esters
Alcohols
Aroma
PTR-ToF-MS

ABSTRACT

The aroma profile in apple was investigated during artificial processing operated with a device imitating the human consumption. The system, composed by a "chewing device" coupled with a Proton Transfer Reaction – Time of Flight-Mass Spectrometry (PTR-ToF-MS), allowed an accurate dynamic volatile organic compound (VOC) fingerprinting suitable to study the volatile kinetics of three apple cultivars ('Golden Delicious', 'Fuji', and 'Granny Smith') during shelf-life ripening.

The obtained results demonstrate the complementarity between the dynamic VOC assessment during "mastication" and the usual static headspace analysis. The great advantage of such analytical approach was the possibility to study the kinetics of the volatiles released during eating and the possibility to consider their concentration similar to *in vivo* condition resulting to an improved characterization of the aroma profile. Moreover, differences in textural properties of apple flesh revealed a possible direct role of the cell wall architectural structure in the regulation of VOC release during consumption. This strategy may be ideal for VOC assessment addressed to investigate fruit quality aspects impacting the consumer appreciation.

1. Introduction

The characteristic apple fruit flavor depends upon taste (balance between sweetness, acidity and low astringency perceived in the mouth) and aroma (concentrations of volatile organic compounds – VOCs-perceived by orthonasal and retronasal receptors). Although these two aspects contribute to the overall flavor, aroma is often considered the dominant component (Kader, 2008). In apple (*Malus* x *domestica* Borkh.) the aroma profile results from the interaction of more than 270 VOCs (Farneti et al., 2014; Nijssen, van Ingen-Visscher, & Donders, 2011; Ulrich & Dunemann, 2012) synthesized by intact fruit prior to consumption, as well as in response to cellular disruption caused by biting and mastication (Contreras & Beaudry, 2013). Among them, esters, alcohols and aldehydes are recognized as the most relevant volatiles responsible for aroma in apple (Cappellin et al. 2014; Dunemann et al. 2012; Holland et al., 2005; Ulrich & Dunemann, 2012).

The volatile emission behavior greatly depends by the fruit integrity. It has been demonstrated that VOC released from a whole intact fruit completely changes after cutting, due to the reaction of enzymes specifically activated during the fruit processing (Contreras & Beaudry, 2013; Farneti et al., 2014). For instance, during fruit disruption, C6 aldehydes (i.e. hexanal and cis-hex-3-enal) are immediately produced as a result of the action of LOX pathway enzymes, such as lipoxegenase, lipase, and fatty acid hydroperoxide lyase (HPL), on substrates released by cell disruption (Contreras & Beaudry, 2013).

The VOC release behavior will also be affected by the textural properties of the apple flesh matrix (Arvisenet et al., 2008; Charles et al., 2013) as well as by the volatile compounds rate of partitioning between air and liquid (De Roos, 2003). The mastication process, which is directly related to the textural and physico-chemical properties of the food matrix, has been reported as a substantial parameter affecting the *in vivo* aroma release (Charles et al. 2013; Foster et al., 2011; Mestres, Kieffer, & Buettner, 2006; Taylor, 2002). For a better understanding about the effect of the volatile compounds exerted on aroma perception, aromatic VOCs, beside their concentration, should be considered also for the interaction with human receptors (Farneti et al., 2013).

* Corresponding author. Tel.: +39 0512096443; fax: +39 0512096401.
E-mail address: brian.farneti@gmail.com (B. Farneti).

http://dx.doi.org/10.1016/j.lwt.2015.03.031
0023-6438/

Differences in VOC release behaviors may influence the human aroma perception during food consumption (Farneti et al., 2013) since VOCs are released from the matrix and then transported to mouth and nose receptors (Buettner et al., 2008). It is also supposed that the perception sensation is highly dependent to volatile emission. An abundant VOC production would allow, in fact, a more rapid and intense interaction with olfactory receptors. The perception of food flavor and aroma, indeed, is a complex process resulting from the concurrent chemical stimulation of orthonasal and retronasal receptors (Shepherd, 2006). To date, research about aroma release has been mainly focused on the VOCs present at levels exceeding the orthonasally measured odor threshold, ignoring the variation in the rate at which odor intensities increase above threshold (Tieman et al., 2012).

Considering such conditions, it seems not reasonable to compare human sensory perception with headspace volatile compounds quantified with traditional methodologies based on gas chromatographic techniques. These techniques, however, are limited by the use of a food matrix altered by chemical extraction (i.e. addition of salt and antioxidant reagents) and the need of a long incubation time before measurement (Farneti et al., 2013). The interaction of these methodological procedures may drastically alter the *in vitro* VOCs profile from the *in vivo* one. Therefore, the development of methods for rapid, repeatable and sensitive monitoring of VOCs emitted from food samples imitating the release in the human mouth during consumption resulted essential (Farneti et al., 2013).

In order to describe the release kinetics of VOCs during food matrix processing, Farneti et al. (2013) developed an analytical system based on an artificial chewing device coupled with a quadrupole proton transfer reaction mass spectrometry (PTR-MS). Among the various possibilities proposed and investigated for a rapid quantification and identification of VOCs by direct injection, PTR-ToF (Time of Flight)-MS is one of the most used, since it allows on-line measurement of a mixture of VOCs in a straightforward, fast and high sensitivity fashion (Biasioli, Gasperi, Yeretzian, & Märk, 2011). Furthermore PTR-ToF-MS has already been exploited in apple, validating the capacity of this technique to characterize the aroma profile in apple fruit (Cappellin et al. 2014; Farneti et al. 2014).

The aim of this work was the development of a fast and reliable system to study the volatile aroma profile of apple fruit, imitating the release of volatiles in the human mouth during fruit consumption without taking into account the several parameters varying between different consumers, such as number of bites, chewing speed, and liquid lubricant. The system, composed by a "chewing device" (Farneti et al., 2013) coupled with a PTR-ToF-MS, allowed to quantify the VOC production during apple fruit crushing as well as the study of the kinetics of the most significant VOC classes during ripening in three apple cultivars.

2. Materials and methods

2.1. Plant material

For the purpose of this investigation three apple cultivars, 'Golden Delicious', 'Fuji', and 'Granny Smith' were employed. Each plant, grown at the experimental orchard of the Fondazione Edmund Mach (FEM) in the Northern part of Italy (Province of Trento), was grown and maintained following standard agronomical management for pruning, thinning and pest disease control.

Fruit were harvested at commercial ripening stage, as defined by the Extension Service of FEM, and further analytically selected with a DA-Meter (TR, Forli, Italy), a portable VIS-spectrometer device able to detect chlorophyll variation in a non-destructive manner (Farneti et al., 2014). The detected index of absorbance (I_{AD}) ranges from 2.2 to 0, where higher values indicate a higher amount of chlorophyll, thus an unripe stage.

A homogenous apple batch for each cultivar was stored for 3 weeks at control temperature (20 °C with ~75% RH). Five apples were randomly selected from the batch every seven days (at day 0, 7, 14, and 21) and used for I_{AD} detection, texture, and VOC assessment.

2.2. Headspace PTR-ToF-MS analysis of intact apple fruit

Apple fruit were incubated in a sealed glass vessel (2000 ml) for 30 min at room temperature (~20 °C) before measurement. VOCs were then assessed by direct injection of the headspace mixture into a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria) set with the condition described in Farneti et al., 2014. Sampling measurement was performed in 60 cycles resulting in an analysis time of 60 s/sample. All apple cultivars were measured in five independent replicates for each measuring data point.

2.3. PTR-ToF-MS analysis of artificially chewed apple fruit

For volatile analysis after artificial chewing, apple fruit were assessed according to the modified method described by Farneti et al. (2013). The chewing device was composed of a cylindrical glass cuvette (800 ml) sealed with a cap and a manual notched plunger (Supplementary Fig. 1). All the device's elements deputed to the processing of the fruit samples are made of polytetrafluoroethylene. The main difference with the original method (developed for tomato fruit; Farneti et al., 2013) was the impracticality to start the measurement from a whole apple fruit. Therefore, the sample was represented by an apple flesh cylinder (1.7 cm diameter and 5 cm thickness) removed by the fruit previously assessed (Section 2.2). In detail, before crushing the headspace VOC concentration of the apple flesh cylinder was measured for 60 s. The chewing was performed by pressing the notched plunger 5 times within 10 s. VOC analysis continued for 120 s following mastication. This setting was optimized in preliminary trials in order to assure a variability lower than 5% on analysis repeated on the same fruit. The headspace was drawn from the chewing device to the PTR-ToF-MS at 2.4 L/h. PTR-ToF-MS settings and data analysis were the same used for the intact fruit headspace analysis (Section 2.2).

2.4. Texture analysis

Apple textural properties were analyzed with a Texture Analyzer (TAXTplus, Stable Microsystem Ltd, Godalming, UK), equipped with an Acoustic Envelop Device (AED), which simultaneously profiled the mechanical and acoustic signatures of apple. Samples (composed by five biological fruit samples and four technical replicates, for a total of twenty measurements per cultivar for each day of analysis) consisted of a flesh disc of 1.7 cm of diameter and 1 cm thick. The Texture Analyzer instrument setting and parameter were the same as reported in Costa et al. (2011 and 2012).

2.5. Data analysis

The analysis of PTR-ToF-MS spectral data – compound annotation, counting of correction of the spectra through Poisson statistics, internal calibration, noise reduction, baseline removal, and compound concentration quantification-proceeded according to Farneti et al. 2014.

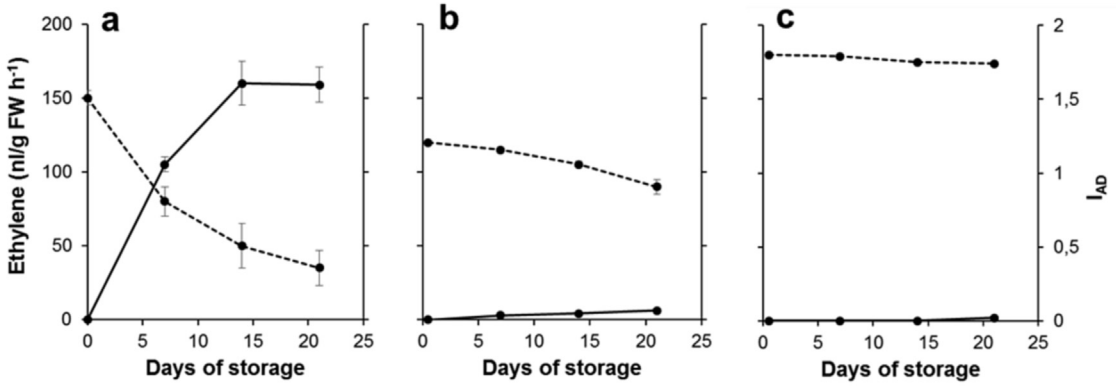

Fig. 1. Ethylene concentration (continuous line) an I_{AD} evaluation (dotted line) of 'Golden Delicious' (a), 'Fuji' (b), and 'Granny Smith' (c) apple cultivars during 21 days of shelf-life at 20 °C. Data are presented as average and standard deviation of 5 biological replicates.

Multivariate statistical analysis of PTR-ToF-MS data was performed with log transformed data using R 3.0.2 internal statistical functions and the external packages "PCA" and "gplots".

Data acquisition of the combined acoustic–mechanical profiles was processed by the software Exponent v.4 (Stable MicroSystem) provided with the TA-XT plus instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the force/sound curves (Costa et al., 2011).

3. Results and discussion

3.1. Fruit ripening progression

The main physiological modifications occurring during the apple fruit ripening were monitored over twenty-one days of shelf-life storage. For each sampling five apples per cultivar were used to assess the ripening progression by measuring the level of chlorophyll degradation (I_{AD}), with the DA-meter device, and the overall VOC production, including ethylene (ion signal at *m/z* 28.030), by PTR-ToF-MS analysis (Fig. 1).

The ethylene and I_{AD} variations during the three weeks of shelf-life at room temperature (~20 °C) depicted the different fruit physiological dynamics for the three apple cultivars here investigated. Fruit of 'Golden Delicious' showed the typical climacteric behavior characterized by a progressive increase of ethylene production (around 150 nl/g FW h^{-1}) in concomitance with a gradual decrease of chlorophyll content (I_{AD} reduction from 1.5 to 0.4; Fig. 1a). On the opposite, the ripening progression in 'Fuji' and 'Granny Smith' showed a different trend, since these two parameters changed with a lower magnitude respect 'Golden Delicious', following a more stable pattern over the time course (Fig. 1b and c). Fruits of 'Fuji', in fact, presented a low, but progressive, production of ethylene accompanied by a slight reduction of I_{AD} (from 1.2 to 0.9; Fig. 1b). 'Granny Smith', instead, did not reveal almost any relevant differences for both parameters (Fig. 1c). Since ethylene is considered the main triggering hormone in ripening related processes (Defilippi, Dandekar, & Kader, 2005; Johnston, Gunaseelan, Pikakala, Wang, & Schaffer, 2009), these differences in climacteric behavior may also influence other quality traits, such as texture and VOC composition.

Apple textural parameters, including both acoustic and mechanical properties, were assessed with a novel TAXT texture analyzer (Costa et al., 2011; 2012). The texture behavior of the three cultivars, processed at four dates over the time course (at harvest and after 7, 14, 21 days of shelf-life), is depicted by plotting the profiles of "mean force" and "number of acoustic peaks" (Fig. 2), which have been mostly related with the consumer perception of firmness and crispiness, respectively (Costa et al., 2011). In accordance with Costa et al. (2012), the textural behavior of the three apple cultivars differed during the ripening progression. The apple variety 'Golden Delicious' was characterized, indeed, by a rapid loss of firmness during ripening (almost 40%) together with a reduced acoustic response (around 60%), typical of a mealy fruit (Fig. 2a). Differently, 'Fuji' and 'Granny Smith' apples, appreciated by consumers for their enhanced crunchiness, showed a reduced loss of firmness (15% and 20%, respectively) and a favorable acoustic profile (increased of 70% and 50%, respectively; Fig. 2b and c). These textural variations are directly influenced by the physical feature of the cell wall architecture, and the adhesion between cells, influencing as consequence the fruit breaking during chewing.

3.2. VOC emission dynamics during artificial chewing

In order to evaluate the VOCs affecting the aroma perception during fruit processing in a situation closer to human consumption, the methodology proposed by Farneti et al. (2013) based on the real time analysis of volatiles emitted during "artificial mastication", was used. This analytical system, however, did not take into account the several parameters influencing the final aroma perception that greatly differ between consumers such as the number of bites, chewing speed, liquid lubricant, and the level of enzymes present in the saliva. The PTR-ToF-MS apparatus used in this investigation enabled, moreover, a full scan of the entire VOC profile in 1 s, allowing the real time monitoring for most of the volatiles emitted by fruit during chewing. This detailed characterization permitted the development of a dynamic VOC fingerprinting over 120 s after the beginning of chewing in 'Golden Delicious', 'Fuji', and 'Granny Smith, apples (Fig. 3). Masses were decreasingly ordered based on their initial level (thus before chewing) and anchored to 'Golden Delicious' used as reference. VOCs were finally grouped into three main classes, namely "esters", "alcohols", and "other VOCs". This last class consists of masses without a clear chemical identification (Farneti et al., 2014) since most of them were common fragment ions of esters, alcohols, or aldehydes (i.e. *m/z* 57.036, 81.070, and 83.086).

Differences between cultivars are in accordance with previous researches performed on apple aroma (Aprea et al., 2012; Farneti et al., 2014) demonstrating that 'Golden Delicious' fruit are distinguished by a more complex and intense VOC profile than 'Fuji' and 'Granny Smith', mainly due to higher esters and alcohols content. From the dynamic VOC profiling it is also worth noting the evident differences of the VOC patterns observed among the three apple

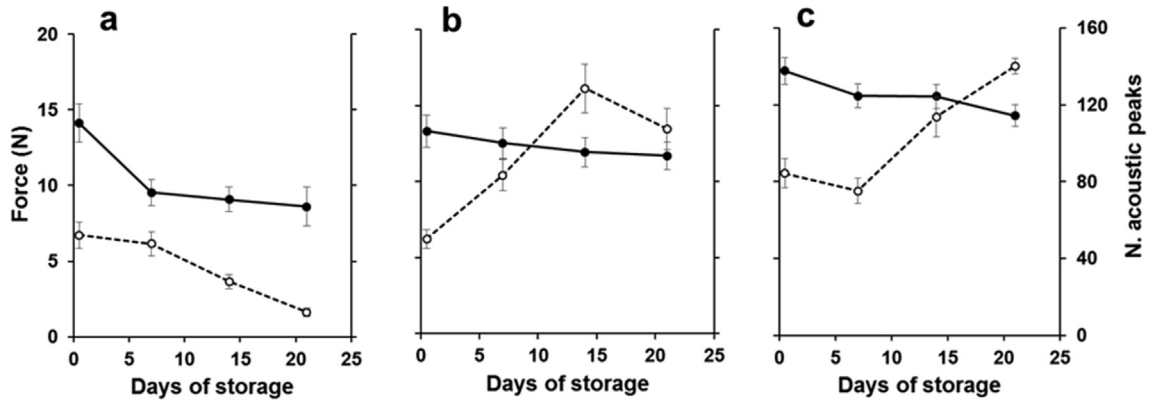

Fig. 2. Textural properties evaluation of 'Golden Delicious' (a), 'Fuji' (b), and 'Granny Smith' (c) apple cultivars during 21 days of storage at 20 °C. Data of Mean Force (continuous line) and of total number of Acoustic Peaks (dotted line) are average and standard deviation of 5 biological replicates.

cultivars. Similarly to the results presented by Farneti et al. (2013) and Boukobza, Dunphy, and Taylor (2001), volatile compounds are differently released from the food matrix according to their chemical nature. Overall, esters (i.e. *m/z* 43.018, 61.028, and 117.091) and several alcohols (i.e. *m/z* 43.054, 57.069, and 71.086) were rapidly released immediately after fruit crushing, while the release of other small alcohol molecules, such as methanol (*m/z* 33.033) and ethanol (*m/z* 47.049), was not directly influenced by the flesh disruption. Among the fragment ions categorized as "other VOCs", several compounds revealed a third type of emission, characterized by a constant linear release of VOCs delayed of about 30 s from the initial tissue disruption. In agreement with Farneti et al. (2013) most of these compounds, presumably produced enzymatically as a consequence of fruit crashing, are aldehydes

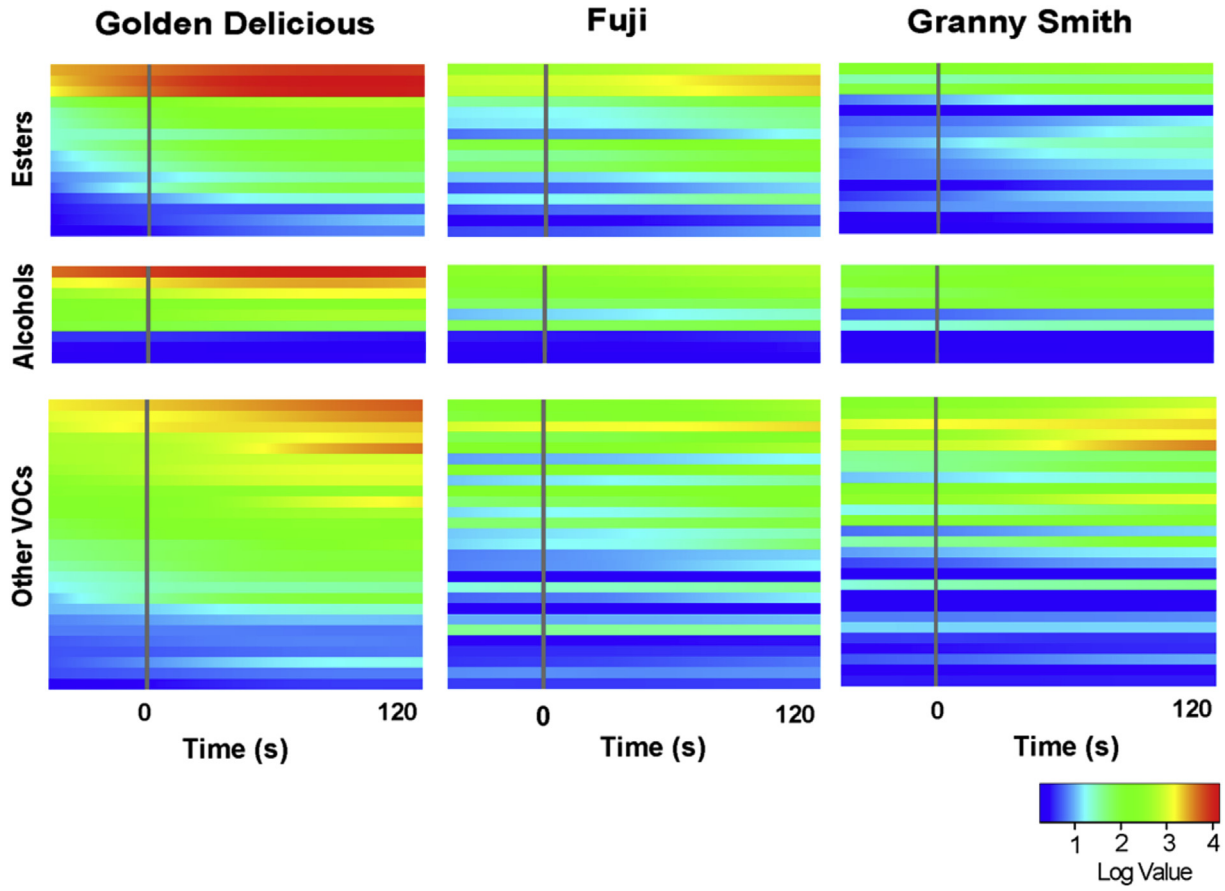

Fig. 3. Heat map of the dynamic VOC fingerprinting of apple fruit assessed by PTR-ToF-MS coupled with the "artificial chewing" device. Each graph is divided by a line, at time 0, in two phases, respectively before and after the "chewing moment": i) headspace analysis of the intact apple flesh cylinder, and ii) headspace analysis of "chewed" apple flesh (between 0 and 120 s). For graphically purpose in this example only 55 selected mass peaks (based on results of Farneti et al. 2014) were reported. Masses were decreasingly ordered based on their initial level (before chewing) and, using 'Golden Delicious' as a reference cultivar, VOCs were grouped into three classes according to their chemical affinity, namely "esters", "alcohols", and "other VOCs".

such as hexanal (*m/z* 83.086 and 101.097) and trans-hex-2-enal (*m/z* 81.070 and 99.081).

These indications may explain some of the inconsistencies often observed in studies where sensory analysis were related with analytical VOC measurements. In order to relate analytical measurements of volatile and consumer perception, the description of the release kinetics of volatile compounds during fruit chewing can be important as other parameters generally used in sensory science, such odor perception thresholds and log odor unit values.

To better elucidate and quantify the VOC release changes during fruit crushing and related to cultivars and ripening stages, the total amount of volatiles (expressed in ppb_v) emitted by the fruit during the artificial chewing was considered (Fig. 4). Despite the oversimplification and univariate explanation of the VOC profile changes, this data representation may give a direct idea of the amount of volatiles released during chewing, which can potentially interact with human flavor receptors. In order to simplify the evaluation of the apple cultivars VOC profiles emitted during storage (at 0, 7, 14, and 21 days after harvest) we arbitrarily decided to compare only three time points (*time 0*, *30*, and *120*) of the entire VOCs fingerprinting dynamic, which correspond to the three most relevant points of the overall profile. The analysis of the apple VOCs after 30 s from the mastication point derived by the evidences reported by Farneti et al. (2013) which elucidated that the measurements carried out 30 s after the artificial chewing provide the best representation of the volatile pattern during consumption.

In accordance with the profile showed in Fig. 3, fruit of 'Golden Delicious' revealed a release patterns different from 'Fuji' and 'Granny Smith'. Beyond a higher initial concentration level (*time 0*), all 'Golden Delicious' apples during storage showed a similar trend with a sudden increase in the total VOC content immediately after chewing (till around 30 s) followed by a constant released of volatiles at a significant lower rate (between 30 and 120 s after chewing). On the contrary, 'Fuji' and 'Granny Smith' apples revealed a lower release of volatiles immediately after chewing (between 0 and 30 s) and an enhanced production between 30 and 120 s. Differences in the VOC emission rate in the first 30 s (Supplementary Fig. 2) additionally underline the differences related to the cultivars as well as the day of storage. While the emission rate was stable at low levels for 'Fuji' and 'Granny Smith', for 'Golden Delicious' apples it increased during storage (maximum at day 14) till a rate around 2.7 μl/s. Interestingly, the decrease in the emission rate recorded in 'Golden Delicious' at day 21 (1.5 μl/s) may be more related to the fruit textural changes than to the actual internal VOC content. Indeed, the VOC level detected from the fruit cylinder (before being chewed) was the same for fruit assessed both at 14 and 21 days of storage, while, according to textural analysis (Fig. 2), 'Golden Delicious' apples, at day 21, had a significantly lower value of both mechanical and acoustic parameter typical of a more soft and mealy apple fruit (Costa et al., 2011; Szczesniak, 2002). Studies have related apple texture to cell wall structure and the relative areas cell-to-cell contact (Brummell, 2006; Brummell & Harpster, 2001; Dražeta, Lang, Hall, Volz, & Jameson, 2004). In case of mealy apple, the fruit tissue breaks into small fragments containing undamaged cells causing a reduced release of juice and presumably also volatiles.

3.3. VOC pattern variation during apple ripening

The effect of shelf-life ripening on VOC production is clearly depicted by the results presented in the Principal Component Analysis (PCA) plot (Fig. 5). The first two principal components (PC1 and PC2) explained more than 83% of the total variability of the apple VOC profiles (Fig. 5a). According to the variable projection plot (Fig. 5b) the first principle component (PC1), describing the 63.1% of the total variability, mainly correlates with VOC concentration magnitude. The second principal component (PC2, 20.2%), instead, resulted mainly related with VOC chemical affinity, represented by ester, alcohol and "other VOCs" (aldehydes and common fragmentation masses) groups. Positive values of PC2 indicate a higher concentration mainly of alcohols (i.e. *m/z* 33.033) and "other VOCs" (i.e. *m/z* 81.07, 93.07, and 149.098), while negative values are instead more associated to a greater abundance of esters (i.e. *m/z* 75.044, 103.07, or 117.091).

Beyond a clear separation of the samples according to the cultivars and the days of storage, it is interesting to note the allocation of the samples on the PCA plot according to the time of VOC assessment after chewing (0, 30, 120 s). In accordance to the results shown in Fig. 4, fruit of 'Golden Delicious' showed a considerably more clear differentiation of the profile assessed before chewing (*time 0*) and after 30 and 120 s (*time 30* and *120*) with respect to 'Granny Smith' and 'Fuji'. In these two cultivars, indeed, there were almost no significant differences between fruit assessed at *time 0* and *30*, unlike the marked difference between 30 and 120 s after "chewing", when the production of VOCs gradually increased. These differences in VOC emission may be caused by a different volatile composition as well as fruit flesh matrix structure. 'Golden Delicious' apples, in fact, greatly differ in texture with regards to 'Fuji' and 'Granny Smith', as it is reported by the analysis showed in Fig. 2. Besides these differences in VOC release kinetics during fruit

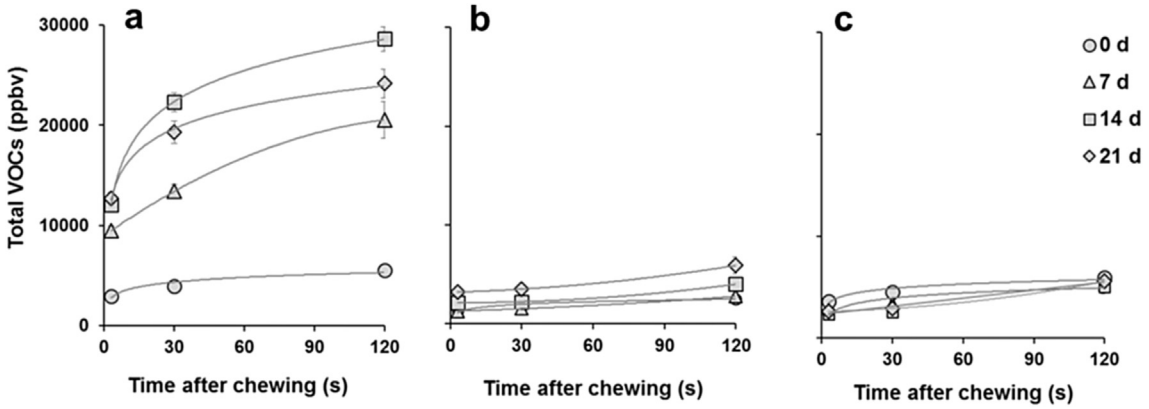

Fig. 4. Comparison of the time course of the total VOC release of three apple cultivars ('Golden Delicious' (a), 'Fuji' (b), and 'Granny Smith' (c)) after chewing, measured by PTR-ToF-MS. Data are averages and standard deviation of 5 biological replicates assessed during 21 days of storage at 20 °C on days 0, 7, 14, and 21. Lines indicates the smoothed data (assessed every second) using the function 'smooth.spline' (library "stats", R 3.0.2).

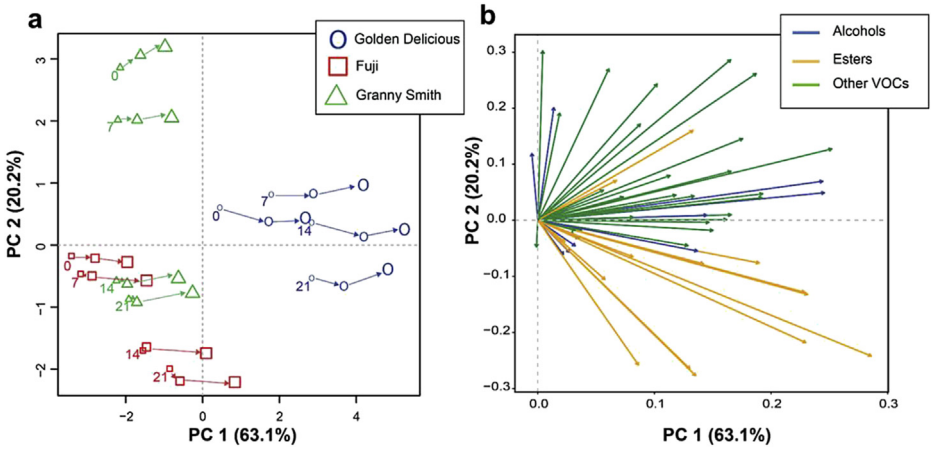

Fig. 5. PCA plot (a) and loading projection (b) of the VOC distribution assessed by PTR-ToF-MS during the "artificial chewing". The plot "a" depicts the apple profile distribution over the PCA score plot defined by the first two principle components. The three cultivars ('Golden Delicious', 'Fuji', and 'Granny Smith') are visualized by different colors and symbols, as indicated in the *top right box*; the increasing dimension of the symbol indicated the time of assessment during the "artificial chewing" (*time 0*, *30*, and *120*). Each data is the average of 5 biological replicates assessed at 0, 7, 14, and 21 days of storage (indicated by the respective number). The plot "b" shows the projection of significant VOCs (based on Farneti et al. 2014). Detection masses are reported using different colors according to the chemical family, as indicated in the *top right box*.

crushing, also the variation over the progression of fruit ripening (days of storage) resulted to be evident. During storage, VOC profiles in both 'Golden Delicious' and 'Fuji' apples revealed an increased volatile concentration, mainly in esters and alcohols, resulting in a lower values of PC1 and PC2. 'Granny Smith' profiles, instead, changed mainly according to the PC2, suggesting a not significant increase of VOCs, especially ester compounds.

3.4. Comparison of VOCs profiling of intact and processed apple fruit

The majority of the investigations carried out on VOC composition in fruit, and related with physiological ad genetic studies, are based on static headspace analysis of intact fruit (i.e. Costa et al., 2013; Ferenczi, Dilley, & Beaudry, 2006; Rowan et al., 2009; Schaffer et al., 2007; Soukoulis et al., 2013), since the correlation between VOC emitted by an intact fruit and its internal content is still commonly accepted. The results presented in this survey highlighted the importance of dynamic measurement during fruit processing and the limits of the static headspace analysis of intact fruit. These observation are consistent with results presented for tomato (Farneti, Cristescu, Costa, Harren, & Woltering, 2012), where the discrepancies in VOC profiles between intact and cut fruit were for the most attributed to the variation in skin and fruit calyx composition. Differences between apple VOC profiles assessed on whole and "chewed" apples (30 s after the chewing) are emphasized in the PCA plot of Fig. 6, where the first two principal components (PC1 and PC2) explained almost the 88% of the total variability of the apple VOC profiles (Fig. 6a). Similarly with the PCA output of Fig. 5, the first principle component (PC1), describing the 75.2% of the total variability, mainly correlates with VOC concentration magnitude, while the second one (PC2, 12.5%) was mainly related with the VOC chemical affinity (ester, alcohol and "other VOCs" groups; Fig. 6b). Negative values of PC2 indicate a higher concentration mainly of alcohols (i.e. *m/z* 33.033) and "other VOCs" (i.e. *m/z* 93.070, 83.085, 45.033), while positive values are associated to a greater abundance of esters (i.e. *m/z* 61.027, 103.075, or 117.090). As previously reported (Fig. 5), the VOCs analysis assessed after the apple flesh crushing (30 s after the chewing) allowed a clear discrimination of both cultivars and ripening stages, whereas results of intact fruit measurements were less discriminating, especially for 'Fuji' and 'Granny Smith' apples.

The lack of correlation between the results obtained from the assessment of headspace of intact and processed fruit (cortex sample) is more evident when each chemical class of compound was analyzed separately (Fig. 7 and Supplementary Figs. 3, 4, and 5). In 'Golden Delicious' both esters and alcohols, determined after fruit chewing, were highly correlated with the outcome of the intact fruit headspace analysis (Supplementary Figs. 3 and 4). This correlation was much lower in 'Granny Smith' and turned to be negative for 'Fuji'. No correlation was instead revealed for the "other VOCs" class, presumably because most of these compounds, mainly C6 aldehydes, are formed just after the fruit cutting and smashing (Supplementary Fig. 5; Contreras & Beaudry, 2013). Discrepancies between the VOC profiles obtained from the intact and processed fruit may be related to differences in skin composition for both physical (i.e. thickness) and chemical (i.e. wax content) proprieties.

Based on these results, the ratio between VOC levels detected in intact and chewed fruit is related to both cultivar and stage of ripening. Therefore, the proposed method seems an interesting and necessary complement to non-destructive analysis of apple headspace especially for quality related investigation. For instance, the quality parameters "A/E index" (identifying the total alcohols over the total esters content), recently introduced to discriminate the apple aroma (Farneti et al., 2014; Ulrich & Dunemann, 2012), was not totally comparable in the three cultivars tested here when derived by the analysis of the intact or mashed fruit at different ripening stages (Fig. 7).

4. Conclusions

The aim of this work was to develop an analytical system, based on direct inject VOCs analysis, to study the aroma profile of apple fruit and mimic the human perception during consumption. The great advance of such analytical system, composed by and "artificial chewing" device coupled with a PTR-ToF-MS, is the possibility to study the release kinetics of volatiles during fruit chewing and to develop an accurate dynamic VOC fingerprinting. Moreover, the use of a solvent and incubation free methodology excludes the possible alteration of the VOC profile, giving a more reliable outcome about the true aroma of a fruit.

This analytical approach resulted to be ideal for fast and truthful screening of large apple collection, such as breeding programs, as

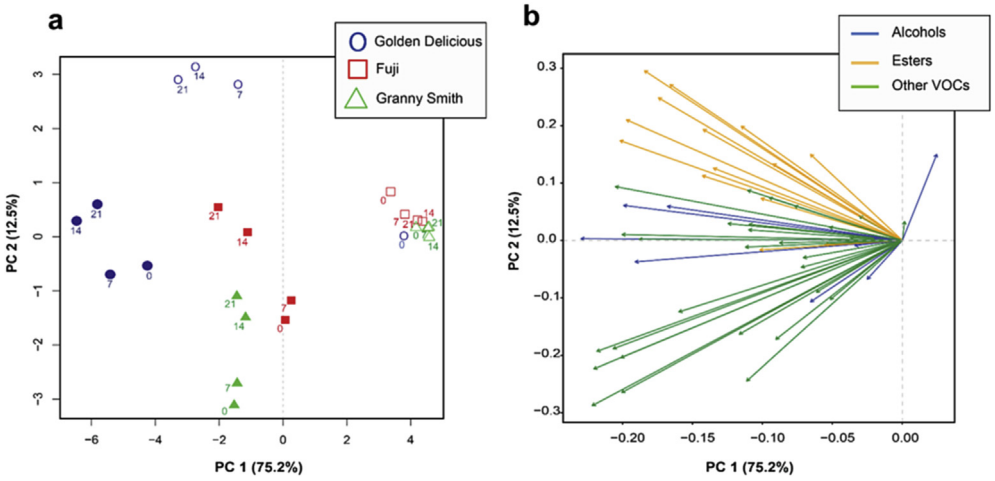

Fig. 6. PCA plot (a) and loading projection (b) of the VOC distribution assessed by PTR-ToF-MS on the headspace of intact fruit (empty symbols) and 30 s after the "artificial chewing" (filled symbols). The plot "a" depicts the apple profile distribution over the PCA score plot defined by the first two principle components. The three cultivars ('Golden Delicious', 'Fuji', and 'Granny Smith') are visualized by different colors and symbols, as indicated in the *top right box*. Each data is the average of 5 biological replicates assessed at 0, 7, 14, and 21 days of storage (indicated by the respective number). The plot "b" shows the projection of significant VOCs (based on Farneti et al. 2014). Detection masses are reported using different colors according to the chemical family, as indicated in the *top right box*.

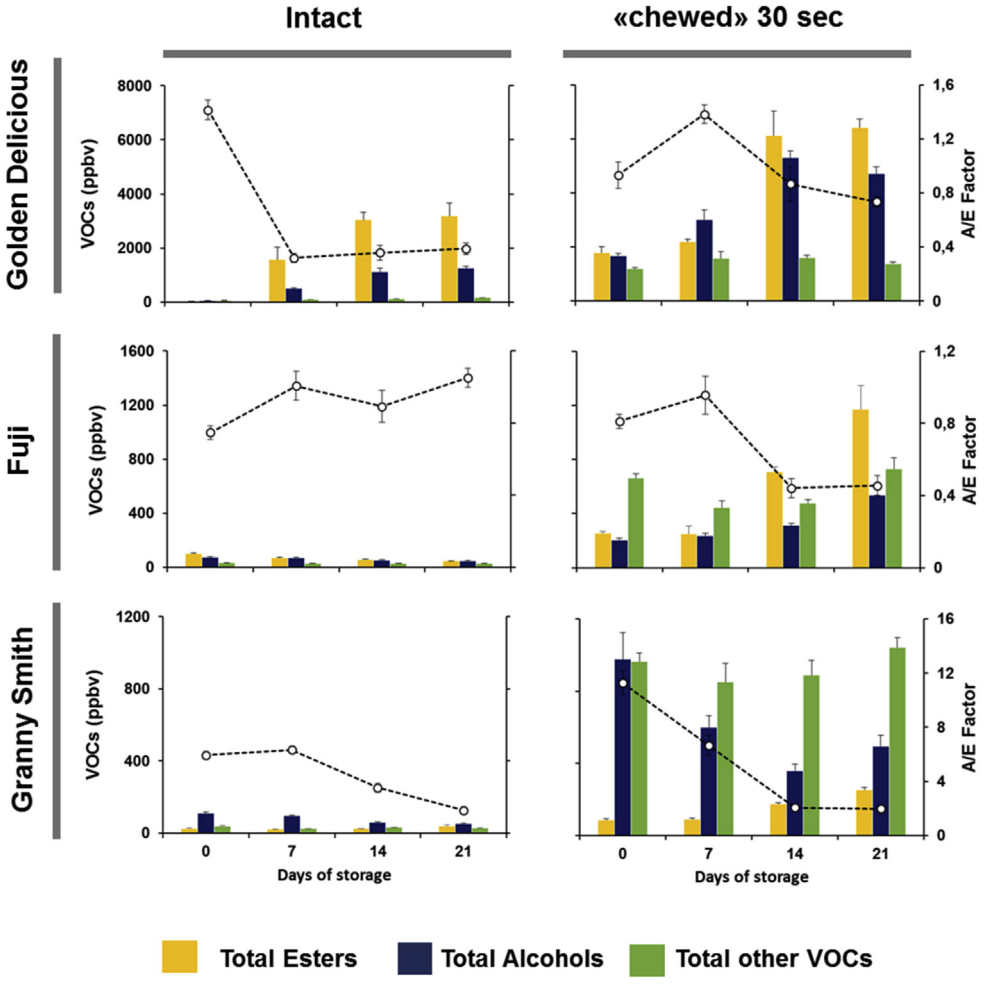

Fig. 7. VOC evaluation of 'Golden Delicious' (a), 'Fuji' (b), and 'Granny Smith' (c) apple cultivars during 21 days of storage at 20 °C assessed by PTR-ToF-MS on the headspace of intact fruit (charts of left column) and 30 s after the "artificial chewing" (charts of the right column). VOCs were sorted into three main classes, namely "total esters", "total alcohols", and "total other VOCs" according to Farneti et al. 2014. Data are averages and standard deviation of 5 biological replicates.

well as for physiological investigation concerning fruit ripening/senescence processes. The results of this investigation clearly showed how imprecise, for fruit quality studies, can be the apple VOC assessment based on intact fruit with regards to processed ones. Evident differences observed between apple cultivars and ripening stages depicted how complicate can be a meaningful comprehension of the fruit VOC profile especially in relation with the consumer perception. Differences in textural properties of apple flesh, for instance, revealed a possible direct role in the regulation of volatile release during chewing.

Acknowledgments

This work was supported by the Agroalimentare e Ricerca project (AGER Grant No. 2010–2119).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2015.03.031.

References

Aprea, E., Corollaro, M. L., Betta, E., Endrizzi, I., Demattè, M. L., Biasioli, F., et al. (2012). Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. *Food Research International, 49*(2), 677–686.

Arvisenet, G., Billy, L., Poinot, P., Vigneau, E., Bertrand, D., & Prost, C. (2008). Effect of apple particle state on the release of volatile compounds in a new artificial mouth device. *Journal of Agricultural and Food Chemistry, 56*(9), 3245–3253.

Biasioli, F., Gasperi, F., Yeretzian, C., & Märk, T. D. (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. *Trends in Analytical Chemistry, 30*(7), 968–977.

Boukobza, F., Dunphy, P. J., & Taylor, A. J. (2001). Measurement of lipid oxidation-derived volatiles in fresh tomatoes. *Postharvest Biology and Technology, 23*(2), 117–131.

Brummell, D. A. (2006). Cell wall disassembly in ripening fruit. *Functional Plant Biology, 33*, 103–119.

Brummell, D. A., & Harpster, M. H. (2001). Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology, 47*, 311–340.

Buettner, A., Otto, S., Beer, A., Mestres, M., Schieberle, P., & Hummel, T. (2008). Dynamics of retronasal aroma perception during consumption: cross-linking on-line breath analysis with medico-analytical tools to elucidate a complex process. *Food Chemistry, 108*(4), 1234–1246.

Cappellin, L., Farneti, B., Di Guardo, M., Busatto, N., Khomenko, I., Romano, A., et al. (2014). QTL analysis coupled with PTR-ToF-MS and candidate gene-based association Mapping validate the role of Md-AAT1 as a major gene in the control of flavor in apple fruit. *Plant Molecular Biology Reporter*. http://dx.doi.org/10.1007/s11105-014-0744-y.

Charles, M., Poinot, P., Texier, F., Arvisenet, G., Vigneau, E., Mehinagic, E., et al. (2013). The 'Mouth to Nose Merging System': a novel approach to study the impact of odour on other sensory perceptions. *Food Quality and Preference, 28*(1), 264–270.

Contreras, C., & Beaudry, R. (2013). Lipoxygenase-associated apple volatiles and their relationship with aroma perception during ripening. *Postharvest Biology and Technology, 82*, 28–38.

Costa, F., Cappellin, L., Fontanari, M., Longhi, S., Guerra, W., Magnago, P., et al. (2012). Texture dynamics during postharvest cold storage ripening in apple (Malus x domestica Borkh.). *Postharvest Biology and Technology, 69*, 54–63.

Costa, F., Cappellin, L., Longhi, S., Guerra, W., Magnano, P., Porro, D., et al. (2011). Assessment of apple (Malus × domestica Borkh.) fruit texture by a combined acoustic-mechanical profiling strategy. *Postharvest Biology and Technology, 6*(1), 21–28.

Costa, F., Cappellin, L., Zini, E., Patocchi, A., Kellerhals, M., Komjanc, M., et al. (2013). QTL validation and stability for volatile organic compounds (VOCs) in apple. *Plant Science, 211*, 1–7.

De Roos, K. B. (2003). Effect of texture and microstructure on flavour retention and release. *International Dairy Journal, 13*(8), 593–605.

Defilippi, B., Dandekar, A., & Kader, A. (2005). Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissues. *Journal of Agricultural and Food Chemistry, 53*, 3133–3141.

Dražeta, L., Lang, A., Hall, A. J., Volz, R. V., & Jameson, P. E. (2004). Air volume measurements of 'Braeburn' apple fruit. *Journal of Experimental Botany, 55*(399), 1061–1069.

Dunemann, F., Ulrich, D., Malysheva-Otto, L., Weber, W. E., Longhi, S., Velascom, R., et al. (2012). Functional allelic diversity of the apple alcohol acyl-transferase gene MdAAT1 associated with fruit ester volatile contents in apple cultivars. *Molecular Breeding, 29*(3), 609–625.

Farneti, B., Algarra-Alarcón, A., Cristescu, S. M., Costa, G., Harren, F. J. M., Holthuysen, N. T. E., et al. (2013). Aroma volatile release kinetics of tomato genotypes measured by PTR-MS following artificial chewing. *Food Research International, 54*(2), 1579–1588.

Farneti, B., Cristescu, S. M., Costa, G., Harren, F. J. M., & Woltering, E. J. (2012). Rapid tomato volatile profiling by using proton-transfer reaction mass spectrometry (PTR-MS). *Journal of Food Science, 77*(5), 551–557.

Farneti, B., Khomenko, I., Cappellin, L., Ting, V., Romano, A., Biasioli, F., et al. (2014). Comprehensive VOC profiling of an apple germplasm collection by PTR-ToF-MS. *Metabolomics*. http://dx.doi.org/10.1007/s11306-014-0744-9.

Ferenczi, A., Dilley, D., & Beaudry, R. (2006). Volatile ester suppression and recovery following 1-Methylcyclopropene application to apple fruit. *Journal of the American Society for Horticultural Science, 131*(5), 691–701.

Foster, K. D., Grigor, J. M., Cheong, J. N., Yoo, M. J., Bronlund, J. E., & Morgenstern, M. P. (2011). The role of oral processing in dynamic sensory perception. *Journal of Food Science, 76*(2), 49–61.

Holland, D., Larkov, O., Bar-Yaákov, I., Bar, E., Zax, A., & Brandeis, E. (2005). Developmental and varietal differences in volatiles ester formation and acetyl-CoA: alcohol acetyl transferase activities in apple (Malus domestica Borkh.) fruit. *Journal of Agricultural and Food Chemistry, 53*(18), 7198–7203.

Johnston, J. W., Gunaseelan, K., Pikakala, P., Wang, M., & Schaffer, R. J. (2009). Co-ordination of early and late ripening events in apples is regulated through differential sensitivities to ethylene. *Journal of Experimental Botany, 60*(9), 2689–2699.

Kader, A. A. (2008). Perspective. Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture, 88*(11), 1863–1868.

Mestres, M., Kieffer, R., & Buettner, A. (2006). Release and perception of ethyl butanoate during and after consumption of whey protein gels: relation between textural and physiological parameters. *Journal of Agricultural and Food Chemistry, 54*(5), 1814–1821.

Nijssen, L. M., van Ingen-Visscher, C. A., & Donders, J. J. H. (2011). *VCF volatile compounds in food: Database (Version 13.1). Zeist (The Netherlands)*.

Rowan, D. D., Hunt, M. B., Dimouro, A., Alspach, P. A., Weskett, R., Volz, R. K., et al. (2009). Profiling fruit volatiles in the progeny of a "Royal Gala" x "Granny Smith" apple (Malus x domestica) cross. *Journal of Agricultural and Food Chemistry, 57*(17), 7953–7961.

Schaffer, R. J., Friel, E. N., Souleyre, E. J. F., Bolitho, K., Thodey, K., Ledger, S., et al. (2007). A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiology, 144*(4), 1899–1912.

Shepherd, G. (2006). Smell images and the flavour system in the human brain. *Nature, 444*, 316–321.

Soukoulis, C., Cappellin, L., Aprea, E., Costa, F., Viola, R., Märk, T. D., et al. (2013). PTR-ToF-MS, a novel, rapid, high sensitivity and non-invasive tool to monitor volatile compound release during fruit post-harvest storage: the case study of apple ripening. *Food and Bioprocess Technology, 6*(10), 2831–2843.

Szczesniak, A. S. (2002). Texture is a sensory property. *Food Quality and Preference, 13*(4), 215–225.

Taylor, A. J. (2002). Release and transport of flavors in vivo: physicochemical, physiological, and perceptual considerations. *Comprehensive Reviews in Food Science and Food Safety, 1*(2), 45–57.

Tieman, D., Bliss, P., McIntyre, L. M., Blandon-Ubeda, A., Bies, D., Odabasi, A. Z., et al. (2012). The chemical interactions underlying tomato flavor preferences. *Current Biology, 22*(11), 1035–1039.

Ulrich, D., & Dunemann, F. (2012). Towards the development of molecular markers for apple volatiles. *Flavour and Fragrance Journal, 27*(4), 286–289.