Does bruising influence the volatile profile of pears?

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Abstract

Purpose – Volatile flavor plays a main role in defining the fruit quality by consumers. Bruising leads often to dark spots on fruits and its amount could highly affect product quality. This paper aims to study the effect of bruising on the volatilome released by pears by using proton transfer reaction – mass spectrometry (PTR-MS).

Design/methodology/approach – Fingerprints of non-bruised and bruised pear samples were collected through PTR-MS for 28 days, and discriminant analysis was used to discriminate the fruit products. The CIELAB color changes were also measured during the entire ripening period.

Findings – Bruised pears released a higher intensity of volatile organic compounds (VOCs) compared to non-bruised pears ($p_{16days} = 0.049$, $p_{22days} = 0.012$, $p_{28days} = 0.006$). In particular, the release of m/z 45 and m/z 47 were significantly ($p_{m/z\ 45} = 0.076$, $p_{m/z\ 47} = 0.095$.) higher in bruised samples, suggesting that the bruising event accelerated the natural ripening process. CIELAB color coordinates were also recorded. The coordinate a* showed a linear increase during the whole 28 days because of the loss of the green component. The CIELAB ΔE* was higher in the bruised pears than the non-bruised pears ($p = 0.022$).

Originality/value – Bruising can affect food quality and taste. Bruise susceptibility has been largely studied on apples, tomatoes and peaches, but rarely on pears. Very little is known about the effect of bruising on the volatilome of pears. Moreover, bruising research usually involved the study of physical properties; on the contrary, PTR-MS, applied to bruising research, has never been used before. Besides the analysis of volatilome, the changes in color were also recorded for the whole 28 days of analysis. The proposed method could be applied for the monitoring of pears quality in the food industry.

Keywords Food industry, Color, VOCs, Ripening, Fruit quality

Paper type Research paper

1. Introduction

Aroma is one of the most valued fruit features, and volatile flavor plays a key role in determining the perception of fruit's quality by consumers (Chen *et al.*, 2018). For a fruit like pear (*Pyrus communis*), volatile organic compounds' (VOCs) emissions are particularly relevant: its aroma is rich and easily recognized by consumers. Flavor was considered the most important quality feature of pears by 54% of consumer, whereas only 41% of them rated texture as the most important feature (Vangdal, 1982).

Bruising is the most common mechanical damage which can occur in fruit (Hussein *et al.*, 2020). Bruising event causes dark spots on fruit, and its extent is closely related to the product quality and taste (Blahovec and Paprštein, 2005). Dark spots are a direct results of impact, and the bruising occurs in every post-harvesting step (Mattus *et al.*, 1959). Thus,

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bruising is the main factor limiting the mechanization in harvesting and postharvest activities, leading to longer and more expensive manual processes (Blahovec *et al.*, 2002). Fruit bruising causes loss of millions dollars to the food industry, because the bruising event stimulates the production of ethylene, a ripening agent. Accordingly, bruised fruits ripen at a higher rate than non-bruised fruits (Mazzola, 1992). The ripening process leads to the production of acetaldehyde and ethanol (Chervin *et al.*, 1999). These two molecules are easily detectable by proton transfer reaction – mass spectrometry (PTR-MS).

PTR-MS allows fast VOCs detection, in particular in regard with fingerprint (Granitto *et al.*, 2007). One of the advantages of this device is the possibility of measuring the headspace of whole samples directly without any preparation (Bodner *et al.*, 2019). PTR-MS has been previously applied to the study of strawberry (Granitto *et al.*, 2007), blueberry (Farneti *et al.*, 2017), apples (Ciesa *et al.*, 2015) and tomato (Farneti *et al.*, 2012) cultivars; however, very little is known about the potential use of PTR-MS to study the effect of bruising on the volatilome of pears.

CIELAB L*, a* and b* color measure is the established method for the detection of color changes in the food industry. The lightness value (L*) indicates the darkness/lightness of the sample (0 is total darkness and 100 is total lightness). The variable a* is a measurement of the greenness/redness of the sample (negative values are related to green color and positive ones the red color), and b* is a measurement of blueness/yellowness (negative values are related to blue color and positive ones the yellow color) (Bhookya *et al.*, 2020).

Here, we collected the fingerprint of non-bruised and bruised pears for 28 days and used the discriminant analysis (DA) to classify the fruit products. Color changes were also monitored for the same period. The objective of this study aimed to investigate the suitability of CIELAB color measurement and PTR-MS volatilome spectra in the analysis of the effect of bruising on pears' quality and taste.

2. Materials and methods

2.1 Plant material

A total of 8 pears (*Pyrus communis*, variety Anjou) were purchased at local market in Bolzano (Italy). The pears were as much similar as possible in terms of color, shape, weight (100.0 ± 10.0 g), height (7.0 ± 0.5 cm) and diameter (3.5 ± 0.5 cm). Immediately after the purchase (0 days), the initial headspace and the color were measured. Then, half of the pear samples (four) were let to fall for three times from a height of 10.0 ± 1.0 cm. The headspace of these pears ("bruised samples") was then analyze and the color was measured. The headspace and the color of non-bruised (NB) samples and bruised (N) samples were analyzed at 2, 3, 4, 7, 14, 16, 22 and 28 days. Samples were let to ripe naturally at room temperature (22.0 ± 2.0°C) for 28 days, to simulate the consumers' daily experience.

2.2 Proton transfer reaction mass spectrometry

The headspace of pear samples was measured by PTR-QMS 500 (Ionicon Analytik GmbH, Innsbruck, Austria). Each pear was placed to equilibrate in a 700-mL glass vial at 30°C for 20 min under ambient air. Three replicates for each sample were analyzed. The measurement order was randomized to avoid possible memory effect. Ambient air passed through a carbon filter (Supelpure HC, Supelco, Sigma-Aldrich, Steinheim, Germany) was used as carrier gas. The instrumental conditions were the following: drift voltage 600 V, drift temperature 70°C, inlet temperature 70°C and drift pressure 2.20 mbar, affording an E/N value of 141 Townsend ($1\,\mathrm{Td} = 10^{-17}\mathrm{cm}^2\,\mathrm{V}^{-1}\,\mathrm{s}^{-1}$). The signal intensities were corrected and normalized as previously described (Beauchamp *et al.*, 2013), with the following equation:

$$\text{ncps}(RH^+) = \frac{\text{cps}(RH^+) \cdot 10^7 \cdot \text{trasmission rate}}{500 \cdot \text{cps}\left(H_3^{18}O^{+3}\right) + \text{cps}(H_2O \cdot H_3O^+)} \quad (1)$$

where ncps(RH^+) was the normalized count rate for each ion intensity, cps(RH^+) was the count per second of each ionized molecule, cps $\left(H_3^{18}O^{+3}\right)$ was related to the primary ion (m/z 21) and cps (H_2O H_3O^+) to water cluster (m/z 37). Compounds were tentatively identified based on the reports of previous literature about fragmentation patterns and isotopic ratios (Capozzi *et al.*, 2016; Ciesa *et al.*, 2015; Crespo *et al.*, 2012; Farneti *et al.*, 2012; Farneti *et al.*, 2017; Granitto *et al.*, 2007; Materić *et al.*, 2017; Mikoviny *et al.*, 2010; Schwarz *et al.*, 2009).

The total VOCs were calculated by summing the intensity of each of the 181 ion fragments, except m/z 21 and 37, measured by the PTR-QMS.

2.3 Color measurement

Color of the pear samples was measured by using a colorimeter (Minolta Chroma Meter II Reflectance CR-300, Milano, Italy). To understand the color change of all samples (non-bruised and bruised), CIELAB L*, a* and b* color coordinates were recorded. The lightness value (L*) indicates the darkness/lightness of the sample, where 0 represents total darkness and 100 total lightness; a* is a measurement of the greenness/redness of the sample, where negative values indicate green and positive ones red color, and b* is the extent of blueness/yellowness, where blue color is related to negative values and yellow to positive ones. Color measurements were performed in triplicates, and the results expressed as mean values and standard deviations, as previously described by Alam *et al.* (2019). ΔE* describes the difference between two colors in L*a*b* color space. ΔE* varies between 0 and 100. When the value of ΔE* is 0, the two colors are identical, but when the value of ΔE* is 100, the two colors are least similar. A ΔE* value of 2.30 is indicated as just noticeable difference (JND) (Bhookya *et al.*, 2020).

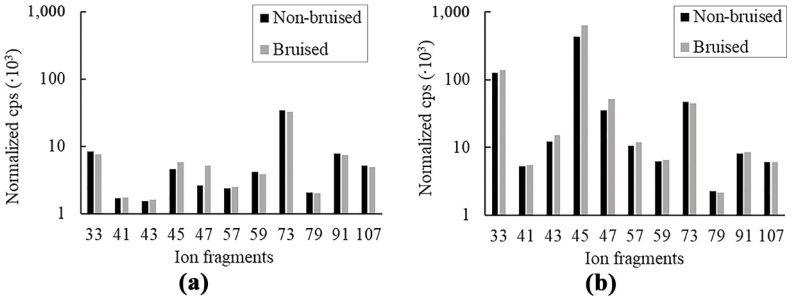

Figure 1.
Mean (n = 4) mass spectra of non-bruised (black) and bruised (grey) pear samples after 2 days (a) and 22 days (b) from the bruising event. The intensity of the ion fragments is expressed as normalized count pro second (cps) · 10^3 and shown on a logarithmic scale. Among the 180 ion fragments measured by the PTR-MS, only the most intense and characteristic ion fragments are indicated. The tentative identification of the 11 ion fragments is the following: m/z 33 (methanol), 41 (monoterpene fragment), 43 (propanol), 45 (acetaldehyde), 47 (ethanol), 57 (pentanol), 59 (acetone and propanal), 73 (butanal), 79 (benzene), 91 (oxalic acid) and 107 (ethylbenzene and/or xylene)

2.4 Statistical analysis

All determinations (both PTR-MS spectra and color measurements) were carried out in triplicate, and the data analysis was performed by using XLSTAT software version 2016.02.28014 (Addinsoft, New York, USA). Student's *t*-test was applied to the changes in the volatilome, the changes of intensity of m/z 45 and m/z 47 and in the changes of the total color differences (ΔE*). DA was performed on eleven ion fragments (m/z 33, 41, 43, 45, 47, 57, 59, 73, 79, 91 and 107). A p value of <0.05 was used to designate the statistical significance in all analyses.

3. Results

3.1 Proton transfer reaction mass spectrometry spectrum

Mean ($n = 4$) mass spectra of non-bruised and bruised pear samples after 2 days (Figure 1a) and after 22 days (Figure 1b) from the bruising event are shown in Figure 1. The most intense ion fragments for both types of sample and their tentative identification were m/z 33 (methanol), 41 (monoterpene fragment), 43 (propanol), 45 (acetaldehyde), 47 (ethanol), 57 (pentanol), 59 (acetone and propanal), 73 (butanal), 79 (benzene), 91 (oxalic acid) and 107 (ethylbenzene and/or xylene). All these ion fragments belong to compound classes, typically found in fruit aroma: alcohols, ketones and aldehydes and esters (Ciesa *et al.*, 2015). The ions which change the most between non-bruised and bruised samples were m/z 45 and 47. A similar pattern was reported in other works involving strawberry, blueberry and apples ripening (Ciesa *et al.*, 2015; Granitto *et al.*, 2007; Farneti *et al.*, 2017).

3.2 Changes in the volatilome

Changes in the volatilome, calculated as total VOCs (expressed as normalized cps 10^3), are reported in Figure 2. The VOCs released by both non-bruised and bruised pears constantly raised during the 28 days of analysis, following an order two polynomial progression ($R^2_{\text{non-bruised}}$: 0.96, R^2_{bruised}: 0.96). Differences in the total VOCs were measurable since Day 3, although it became statistically significant after 16 days from the bruising event ($p = 0.049$ at 16 days, 0.012 at 22 days and 0.006 at 28 days). The increase in the VOCs released during ripening was consistent with what observed by Farneti in tomatoes (2012). As the increase of the total VOCs was higher in the bruised pear samples than the non-bruised ones, it can be inferred that the bruising event played a role in the intensity of VOCs release.

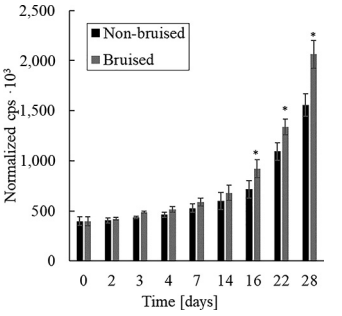

Figure 2.
Total VOCs (expressed as normalized cps · 10^3) of non-bruised (black) and bruised (grey) pear samples during the 28 days of analysis. * = $p < 0.05$. Statistical difference was calculated applying Student's *t*-test. $p_{16days} = 0.049$, $p_{22days} = 0.012$, $p_{28days} = 0.006$. The total VOCs released by both non-bruised and bruised pears constantly raised during the 28 days of analysis, following an order two polynomial progression ($R^2_{\text{non-bruised}}$: 0.96, R^2_{bruised}: 0.96). The total VOCs of bruised pears raised at an increased rate compared to the non-bruised pears, suggesting a role of the bruising event in the release of volatile organic compounds

3.3 Changes in the intensity of m/z 45 and m/z 47

Acetaldehyde and ethanol are produced during the ripening process (Chervin *et al.*, 1999). Changes in the intensity of m/z 45 (tentatively identified as acetaldehyde) and m/z 47 (tentatively identified as ethanol) are reported in Table 1. The intensity of m/z 45 released by both non-bruised and bruised pears constantly raised during the 28 days of analysis, following an exponential progression ($R^2_{\text{non-bruised}}$: 0.92, R^2_{bruised}: 0.92). For the first 7 days, no significant difference was detected; however, starting from day 14, the ion fragment m/z 45 released by bruised pear samples increased more rapidly than by non-bruised samples.

As for m/z 45, similarly the intensity of m/z 47 released by both non-bruised and bruised pears constantly raised with an exponential progression during the 28 days of analysis ($R^2_{\text{non-bruised}}$: 0.98, R^2_{bruised}: 0.98). The difference in intensity became significant after 22 days. These results suggested that the bruising event accelerated the natural ripening process in which ripening is governed by the release of ethylene, which induces the production of acetaldehyde and ethanol (Chervin *et al.*, 1999; Lang and Hübert, 2012).

Ion fragments m/z 45 and m/z 47, in combination with m/z 33 (tentatively identified as methanol) and m/z (tentatively identified as acetone and propanal) represented almost 90% of the VOCs released by non-bruised and bruised pears. Similar composition was detected by Farneti and collaborators in tomatoes (2012). These ion fragments are not considered essential as aroma volatiles but could interact with other molecules and have a role in the final overall aroma. In tomatoes, ethanol has been associated with the sweetness perception (Farneti *et. al.*, 2012).

3.4 Discriminant analysis

The eleven previously selected ion fragments (m/z 33, 41, 43, 45, 47, 57, 59, 73, 79, 91 and 107) were used as predictor in the DA (Figure 3). The DA was performed to test the ability of the model to recognize non-bruised samples from bruised ones. Samples were grouped according to the date of the headspace analysis: 0 days (samples measured right after the purchase), 2-7 days (samples measured between 2 and 7 days from the bruising event), 14-16 days (samples measured between 14 and 16 days from the bruising event) and 22-28 (samples measured between 22 and 28 days from the bruising event). Samples measured during the first 7 days were quite similar (blue and black dots and triangles). Samples measured between 14 and 16 days were separated from the other samples, and bruised and non-bruised samples were not overlapping (red dots and triangles), indicating differences in the intensity of VOCs release. The difference of non-bruised and bruised samples increased greatly after 22 days (green dots and triangles).

Table 1. m/z 45 and 47 intensity (expressed as normalized cps 10^3) of non-bruised and bruised pear samples

	m/z 45		m/z 47	
Days	Non-bruised	Bruised	Non-bruised	Bruised
0	4.99 ± 0.63	5.65 ± 0.21	1.13 ± 0.92	2.24 ± 0.99
2	4.62 ± 0.23	5.83 ± 0.75	1.94 ± 0.73	2.45 ± 0.49
3	4.78 ± 0.18	5.98 ± 0.59	2.15 ± 0.45	2.82 ± 0.46
4	5.00 ± 0.27	6.21 ± 0.60	2.25 ± 0.12	3.98 ± 0.24
7	5.50 ± 0.53	6.84 ± 0.61	2.45 ± 0.19	7.11 ± 0.23
14	154 ± 6.08	196 ± 5.36	5.96 ± 3.60	16.69 ± 3.36
16	190 ± 7.64	290 ± 19.13	9.09 ± 3.46	25.86 ± 4.46
22	432 ± 33.42	631 ± 35.83	25.44 ± 4.4	86.17 ± 9.84
28	631 ± 44.77	899 ± 23.49	122 ± 10.25	219 ± 15.64

Notes: Values are the mean of three replicates of four samples ± standard deviation. Statistical difference was calculated applying student's *t*-test. $p_{\text{m/z 45}} = 0.076$, $p_{\text{m/z 47}} = 0.095$

3.5 Color measurement

CIELAB L*, a* and b* color coordinates were recorded. The lightness value (L*) indicates the darkness/lightness of the sample, a* is a measurement of the greenness/redness of the sample and b* is the extent of blueness/yellowness. During the 28 days, the trend of the three coordinates is similar in both sets of samples. L* and b* values decreased for the first 7 days because pears' color tend to become darker and then they increased for the following 9 days, owing to the fact that pears changed from greenish color to a more yellowish (and thus lighter) one. Finally, they decreased again for the natural ripening of the fruits. The color changed from a more yellow one to a bluer one, and this was explained by Lang and Hübert (2012) as an effect of the partial reduction of the peroxymolybdate by ethylene. CIELAB coordinate a*, on the contrary, had a more linear trend. It increased constantly during the whole 28 days ($R^2_{\text{non-bruised}}$: 0.95, R^2_{bruised}: 0.95) because of the loss of the green component. Similar trends were reported in the case of apple ripening (Lang and Hübert, 2012).

CIELAB ΔE* was then calculated to visualize the changes of total color differences (Table 2). The ΔE* value was higher than the JND (2.30) for both bruised and non-bruised pear samples even on Day 2. This result indicated that the natural browning induced color changes from the very first hours. ΔE* value for bruised pear samples was always higher than that of non-bruised pear samples, suggesting a role of bruising in the rapidity of ripening.

4. Discussion

Bruising is the mechanical damage which can occur in fruit (Hussein *et al.*, 2020), and the consequent discoloration can affect the product quality and taste (Blahovec and Paprštein, 2005). As bruising causes loss of millions dollars to fruit industry, it has been largely studied by food scientists. A great deal of attention was given to sweet cherries (Crisosto *et al.*, 1993), apples (Lewis *et al.*, 2007; Lu *et al.*, 2010), tomatoes (Moretti *et al.*, 2002), peaches (Ahmasi *et al.*, 2010), bananas (Banks and Joseph, 1991) and

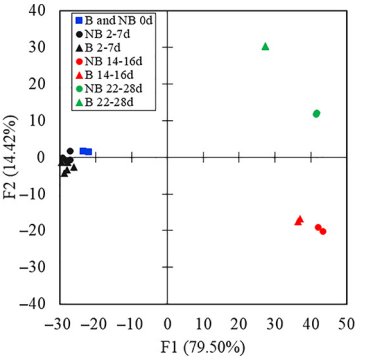

Figure 3.
Discriminant analysis (DA) performed on the 10 selected ion fragments. Each dot represent the mean of four replicates. B = bruised samples, NB = non bruised samples, 0d = at 0 days, after purchase, 2-7d: samples measured between 2 and 7 days after the bruising event, 14-16d: samples measured between 14 and 16 days after the bruising event, 22-28d: samples measured between 22 and 28 days after the bruising event. Wilks' Lambda test (Rao's approximation): Lambda = 0.000, F (Observed value) = 11.354, F (Critical value) = 2.090, *p*-value < 0,0001. Bartlett's test for eigenvalue significancy: F1 *p*-value = 0.000, F2 *p*-value = 0.000

pomegranates (Shafie *et al.*, 2015) Although the research on bruising is extensive, very little is known on the effect of bruising on pears quality and taste. Moreover, to the authors' knowledge, bruising research usually involved the study of physical properties, such as texture and bulk density, and almost no research on the degree of changes in terms of taste and flavor has been published. In particular, PTR-MS, applied to bruising research, has never been used before. Studies of Ciesa, Granitto and Farneti focused their attention to the investigation of volatiles release during the ripening process of strawberry, blueberry and apples (Ciesa *et al.*, 2015; Granitto *et al.*, 2007; Farneti *et al.*, 2017).

Here, bruised and non-bruised pears have been analyzed for 28 days, recording changes in color and volatiles release. As far as the volatilome was concerned, the ion fragments that changed the most were m/z 45 (tentatively identified as acetaldehyde) and m/z 47 (tentatively identified as ethanol). These ion fragments were also the ones which changed the most during ripening in other fruits (Ciesa *et al.*, 2015; Granitto *et al.*, 2007; Farneti *et al.*, 2017), as acetaldehyde and ethanol are produced during the ripening process (Chervin *et al.*, 1999). The bruising process is the leading cause of accelerated ripening, which induces the release of ethylene and, as a consequence, the synthesis of acetaldehyde and ethanol (Chervin *et al.*, 1999; Lang and Hübert, 2012). Although the volatilome intensity was higher in bruised samples than in non-bruised ones, and the intensity of both m/z 45 and m/z 47 increased in case of bruising, these aspects had probably little effect on the sensory properties of pears. When a fruit is bought by consumers, it is usually consumed within the first 7–10 days, thus limiting the effect of bruising on the aroma of pears would probably not be perceived by consumers.

As far as the color is concerned, CIELAB variables L* and b* followed a similar trend in both non-bruised and bruised pear samples: they decreased in the first week of analysis because pears darkened, then they increased since Day 16 because the color changed from a greenish one to a more yellowish, and therefore, lighter, one. Eventually the color coordinates decreased again because the pears tend to become more bluish. The color coordinate a* increased in both samples following a linear trend, owing to the constant loss of the green component. Similar results were obtained by Lang and Hübert (2012), who explained these colors change as an effect of the partial reduction of the peroxymolybdate by ethylene, a ripening hormone. The changes of total color differences (CIELAB ΔE*) were then calculated. When the ΔE* value is higher than 2.30, the human eye is able to see the difference in color. In case of both non-bruised and

	ΔE*	
Days	Non-bruised	Bruised
2	7.65	8.86
3	16.57	17.48
4	20.29	24.69
7	8.30	14.85
14	6.83	9.45
16	11.34	13.05
22	20.26	22.51
28	28.47	40.95

Notes: Values are the mean of three replicates of four samples ± standard deviation. Statistical difference was calculated applying student's *t*-test, $p = 0.022$

Table 2. Changes of the total color differences (ΔE*) of bruised and non-bruised pears samples in a period of 28 days

bruised pear samples, the ΔE* value was higher than 2.30 from the very first days of the analysis. This indicated that the natural browning and ripening caused color changes immediately. What was interesting to notice is that the bruised samples were characterized by higher ΔE* value than the non-bruised ones, confirming the hypothesis that the bruising event is the leading cause for accelerated ripening.

5. Conclusion

In conclusion, we applied PTR-MS to analyze differences in the volatilome released by non-bruised and bruised pears when were let to ripen naturally at room temperature. The CIELAB L*a*b* color changes in the pear samples were also measured. During the ripening process, the intensity of total VOCs increased in both samples, in particular the release of m/z 45 and m/z 47 was higher in bruised samples than in non-bruised ones. This result is in accordance with previous studies, which suggested that ripening induces the release of ethylene and, therefore, the production of acetaldehyde and ethanol (Chervin *et al.*, 1999; Lang and Hübert, 2012). The differences in the volatilome became significant after more than two weeks from the bruising event, suggesting that it has little effect on the overall aroma. As the number of samples was limited, additional studies to correlate these results with sensory analysis are appropriate. On the contrary, differences in the color was detectable since the very first days, suggesting that the use of CIELAB L*a*b* can be considered the most reliable method to evaluate food quality by the food industry.

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