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# Metabolomic study of volatile compounds in the pigmented fruit from Mexico *Crataegus* genotypes

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## Summary

*Crataegus* is distributed worldwide and presents a phenotypic diversity in size, shape, color and aroma of the fruit. The objective of this study was to identify genotypes of *Crataegus* with a similar profile of volatile compounds by means of a metabolomic study. In addition, the content of pigment was evaluated to contribute to the agronomic, medicinal and chemotaxonomic value. Color determination, total carotenoids (TC) and total anthocyanins (TA) were determined in the exocarp and mesocarp of fresh fruits by means of spectrophotometry. The volatile compounds were determined by Low Temperature Plasma coupled to Mass Spectrometry (LTP-MS). A total of 75 volatile compounds were detected, according to abundance and mass-to-charge ratio, which by means of Principal Component Analysis (PCA) and selection of variables; genotypes were grouped according to size and origin. The pigment content was related to the physical color of the fruit. The highest concentration of carotenoids was 42.35 $\mu g \cdot g^{-1}$ FW. in the genotype PO5, and 992.34 $\mu g \cdot g^{-1}$ FW for anthocyanins in the genotype CH18, concentrations of both compounds found in the exocarp of the fruit.

**Keywords:** Anthocyanins, carotenoids, *Crataegus* sp., volatile compounds.

## Introduction

The genus *Crataegus* (Family Rosaceae) displays wide phenotypic and genetic diversity grouping approximately 150 species, of which 55 are located in the Euro-Asian continent (Europe, the Middle East, East Asia), and 95 in the American continent (Phipps, 1997; Phipps et al., 2003). In Mexico there are 15 species reported (Eggleston, 1909; Phipps, 1997; Núñez-Colín et al., 2011), which are called "*tejocote*" (Mexican hawthorn), a word that derives from Nahuatl "*te-xocotl*" in reference to the hardness and acidity of the fruit (Cabrera, 1992). The phenotypic diversity of this genus is appreciated in the different organs of the tree, among them the fruit, which vary in shape, size and color of the exocarp (skin) (yellow, orange, red and black), with different tonality and intensity. The mesocarp (pulp) goes from yellow, orange, greenish white, reddish white to non-homogeneous diffuse red (Nieto-Angel and Borys, 1992; Phipps et al., 2003). The fruit is also characterized by its flavor (flavor and taste) particular property of this species; in China, it has an intense and unique aroma that influences the acceptability and taste of food products, such as juices and jams due to the presence of some volatile compounds (Zhao et al., 2015).

Attributes such as color, aroma, taste, texture, appearance, food safety and the nutritional value of fresh fruit are factors critical to consumer (Barret et al., 2010). In tejocote, specifically the color and aroma influence the quality of the fruit and importance of its agro-industrial use (preparation of beverages with and without liquor, fruit paste and syrups); as well as, its consumption as fresh fruit. In Mexico, since prehispanic times, different parts of the Mexican hawthorn tree have been used in traditional medicine (Edwards et al., 2012), currently, this fruit is used preferably in traditional offerings, religious ceremonies and Christmas (Borys and Leszczyńska-Borys, 1994). Morphological and molecular studies (Nieto, 2007; Betancourt-Olvera et al., 2017) of some genotypes and species of the genus *Crataegus* have contributed to the taxonomic characterization and conservation of this under-utilized resource.

Many studies of some species located in China, Turkey, Europe and the United States report the presence of bioactive compounds (simple phenols, polyphenols, carotenoids, anthocyanins and flavonoids, among others) (Caliskan et al., 2012; Kostić et al., 2012; Veberic et al., 2015; Liu et al., 2016); which justify its medicinal properties (hypertension, angina pectoris, atherosclerosis, indigestion and abdominal distension) (Chang et al., 2002) and pharmacological properties of the cardiovascular system, as well as its antioxidant activity (Cui et al.,2006).

Despite the great genetic and phenotypic diversity, few metabolites are identified in Mexican species (Banderas-Tarabay et al., 2015; García et al., 2012). The variation of volatile compounds and pigment content (anthocyanins and carotenoids) among these species is hitherto unknown; the profile of these elements could be considered the chemical fingerprint of each species, keys for the understanding of their genetic variation. On the other hand, metabolomic studies of bioactive compounds have been used to describe the phenotypic variation of a wide range of phytochemicals or changes in plant chemical composition. Therefore, the objective of this study was to identify genotypes of *Crataegus* that presented a similar profile of volatile compounds by means of a metabolomic study, and to evaluate the pigment content to contribute to the agronomic, medicinal and mainly chemotaxonomic value.

## Materials and methods

### Plant material

Hawthorn fruit was randomly collected at a commercial maturity stage in September-October 2016 and 2017 from the *ex situ* germplasm bank at the University of Chapingo (UACH), Estado de Mexico, Mexico; located at 19° 29'N and 98° 53' W, at 2249 m height. The climate is classified as C (Wo) (w)b (i') g, corresponds to temperate sub-humid, with a mean annual precipitation of 645 mm and an average annual temperature of 15 ± 2 °C (García, 1988).

The material was placed in paper bags previously labeled, in a cooler at 4 °C. The samples harvested in 2016 were stored at -4 °C until analysis. The material harvested in 2017 was stored at -20 ± 2 °C until study. Tab. 1 shows the site of origin of the genotypes studied, identified and grouped according to their morphological characteristics (Nieto, 2007; Betancourt-Olvera et al., 2017). Before the analysis of metabolites, the equatorial diameter of 10 fruits was

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measured using a vernier, an interval was obtained from the measurements; based on that fruits were grouped by size as follows: small (12.60 - 18.40 mm), medium (18.50 - 28.00 mm) and large (29.00 - 34.00 mm). Fig. 1 shows the size and color of each of the genotypes studied.

**Preparation of the sample**

Three fruits fruit of each genotype (EU) were analyzed separately, without pre-treatment, each fruit was cut longitudinally, immediately the surface of the cut was exposed directly to the mass spectrometry team for analysis. For this study we used the methodology proposed by Martínez-Jarquín and Winkler (2013), with a Low-Temperature Plasma ionization source coupled to Mass Spectrometer (LTP-MS) LCQ Fleet (Thermo Scientific, Waltham, Massachusetts, USA). The advantage of this technique allows to analyze the volatile compounds of a sample without the previous extraction, avoiding low yields and losses of some compounds during the extraction, as well as the detection of these metabolites present in low concentrations (Martínez-Jarquín and Winkler, 2017). Recently, the method has been applied for the analysis of volatile compounds in coffee, tequila and mezcal (Gamboa-Becerra et al., 2017; Martínez-Jarquín et al., 2017).

**Detection of volatile compounds**

The fresh fruit cut was exposed directly to the plasma beam coupled to the mass spectrometer until obtaining 20 mass spectra in a range of 50 to 500 *m/z* per cut in a fruit. Three fruits were analyzed separately, finally 60 spectra were obtained by genotype. Helium was used as discharge gas, the capillary temperature was 80 ± 2 °C, voltage of 10 Kv and a frequency of 10 Khz. The equipment's previous calibration was previously performed as reported by Martínez-Jarquín and Winkler (2013).

**Measurement of color parameters**

For the color analysis (physical color measurement), 23 genotypes were used because the genotype CH69 was not fruitful in the year of collection (2017). The color of the exocarp and mesocarp of fresh fruit was determined by means of the evaluation of *L** (luminosity), *Hue angle* (*Hue*) and color purity or chromaticity index (*Chroma*) with a Miniscan® EZ 4500L spectrophotometer (Hunterlab, Virginia, USA). The readings of *a** and *b** were obtained to identify the color differences between tissue and genotypes in numerical form. Variables were estimated with the following equations: $Hue = tan^{-1}(a/b)$; $Chroma = (a^2 + b^2)^{1/2}$ (Mc. Guire, 1992).

Three fruits of each genotype with similar commercial maturity stage were selected; for each fruit three different points of the exocarp were studied. Subsequently, for the measurement of pulp color, the fruit was cut into three portions longitudinally, each measurement was made in triplicate.

**Measurement of total carotenoids**

The total quantification of carotenoids (TC) was carried out separately in the exocarp and mesocarp of the fresh fruit as indicated by Méndez-Iturbide et al. (2013) with minor modifications. To obtain the sample, between three and twenty fruits were used per genotype, depending on the size of the fruit. The tissue (2 g)

**Tab. 1:** Geographical characteristics of 24 genotypes of the Mexican hawthorn (*Crataegus* sp.).

| Genotype | Species* | State[a] | Origin[b] | Lat | Long | H(m) | MD[c] |
|---|---|---|---|---|---|---|---|
| CH04 | *sulfurea* | Chiapas | S C de las Casas | 16.75 | 92.67 | 2300 | Oct-Dec |
| CH08 | *tracyi* | Chiapas | S C de las Casas | 16.75 | 92.67 | 2300 | Oct-Nov |
| CH10 | *tracyi* | Chiapas | S C de las Casas | 16.75 | 92.67 | 2300 | Sept-Nov |
| CH13 | *tracyi* | Chiapas | Rancho Nuevo | 16.67 | 92.57 | 2400 | Dec-Jan |
| CH15 | *aurescens* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Jan-Feb |
| CH16 | *tracyi* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Nov-Jan |
| CH18 | *tracyi* | Chiapas | Rancho Robelo | 16.67 | 92.45 | 2250 | Nov-Feb |
| CH19 | *baroussana* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Dec-Jan |
| CH22 | *rosei* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Oct-Nov |
| CH27 | *gracillior* | Chiapas | Rancho Robelo | 16.67 | 92.45 | 2250 | Jan-Feb |
| CH43 | *greggiana* | Chiapas | Rancho Robelo | 16.67 | 92.45 | 2250 | Jan-Feb |
| CH44 | *gracillior* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Oct-Nov |
| CH51 | *mexicana* | Chiapas | Mitzitan | 16.65 | 92.55 | 2380 | Nov-Feb |
| CH69 | *cuprina* | Chiapas | Candelaria | 16.70 | 92.53 | 2320 | Oct-Nov |
| CH72 | *baroussana* | Chiapas | S J Yashitinin | 16.65 | 92.45 | 2350 | Nov-Dec |
| CH83 | *tracyi* | Chiapas | S C de las Casas | 16.75 | 92.67 | 2300 | Sept-Nov |
| P02 | *tracyi* | Puebla | Rancho Nuevo | 19.14 | 98.57 | 2620 | Sept-Oct |
| P05 | *aurescens* | Puebla | Mitzitan | 19.14 | 98.50 | 2628 | Sept-Oct |
| P06 | *tracyi* | Puebla | Mitzitan | 19.14 | 98.50 | 2625 | Sept-Oct |
| P25 | *cuprina* | Puebla | Origen | 19.10 | 98.47 | 2420 | Dec-Feb |
| P55 | *sulfurea* | Puebla | S C de las Casas | 19.17 | 98.40 | 2280 | Dec-Feb |
| P86 | *mexicana* | Puebla | Huejotzingo | 19.17 | 98.40 | 2280 | Nov-Dec |
| P100 | *mexicana* | Puebla | Huejotzingo | 19.17 | 98.40 | 2280 | Oct-Dec |
| EM66 | *tracyi* | Edo. Mex. | S C de las Casas | 19.48 | 98.77 | 2700 | Sept-Oct |

[a] S C de las Casas = San Cristóbal de las Casas; S J Yashitinin = San José Yashitinin; S C del Monte = Santa Catarina del Monte. [b] Edo. Mex. = Estado de México. [c] MD = Commercial maturity date (Nieto, 2007).

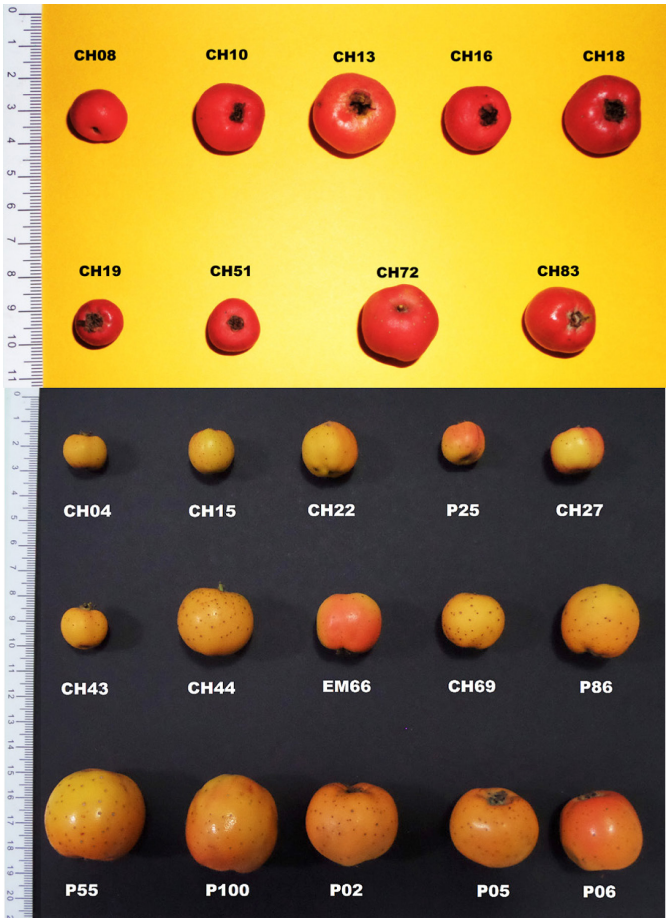


**Fig. 1:** Fruit image of 24 hawthorn (*Crataegus* sp.) genotypes.

was ground in a mortar with 20 mL of hexane, acetone and ethanol (50:25:25 v/v), the solution was kept at 4 °C in the dark for 30 min, then it was filtered. Each extraction was made in triplicate. The absorbance of the organic phase was measured at 450 nm in a Multiscan® GO spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). Hexane was used as a blank solution. To calculate the concentration (µg) of total carotenoids the following equation was used: $\mu g = A \times V\ (mL) \times 10^6 / \varepsilon \times 100$; where ε = extinction coefficient of β-carotenoid (2505); V = volume of sample; A = Absorbance obtained (Méndez-Iturbide et al., 2013). Results are expressed in $\mu g \cdot g^{-1}$ of fresh weight (FW): $\mu g \cdot g^{-1}$ = µg / weight of sample (g).

### Measurement of total anthocyanins

The concentration of total anthocyanins (TA) was determined by the differential pH method (Giusti and Wrolstad, 2001). The extraction of anthocyanins was carried out in the exocarp and mesocarp of the fresh fruit, in triplicate. For each replication, 2.0 g of fresh tissue was ground in a mortar with 20 mL of methanol acidified with HCL at 0.01 (v/v %) in a 10:1 ratio. Then the extract was allowed to rest for 24 h in the dark at 4 °C. Two test tubes were prepared with 0.5 mL of the extract, for each sample; then 25 mL of KCL and 25 mL of $CH_3COONa{\cdot}3H_2O$ solution were added to one tube. The tubes were allowed to rest for 15 min in the dark to stabilize the reactions.

The absorbance of each sample was measured at 520 and 700 nm in a Multiscan® GO spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). The blank solution used was acidified methanol. The concentration of anthocyanins was expressed as cyanidin-3-glucoside with the equation: TA $(mg \cdot L^{-1}) = A \times MW \times DF \times 1000\ \varepsilon^{-1}$; where TA $(mg \cdot L^{-1})$ is the concentration of total anthocyanins, MW = molecular weight of cyanidin-3-glucoside (445.2), DF = dilution factor, ε = extinction coefficient (26 900) and A = absorbance obtained with the following equation: $Abs = (A_{520}\text{-}A_{700})_{pH1}\text{-}(A_{520}\text{-}A_{700})_{pH4.5}$ (Wrolstad et al., 2005).

### Statistic analysis

The R Program Version 3.4.3 (http://www.rproject.org) with the Rstudio interface (http://www.rstudio.com) was used for the data analysis (volatile compounds and physical-chemical color variables). The mass spectra obtained in the analysis of volatile compounds in a range of 50 to 500 *m/z* in format .raw were changed to the numerical format .mzML using the Program MSConver. The package *MALDIquant* was used to process the mass data of the 60 spectra obtained by genotype, to obtain the final mass spectrum. Subsequently, the package *pheatmap* generated the heat map with the abundance of the volatile compounds and the principal components analysis (PCA) was obtained by means of the *prcomp*, to integrate the metabolomic analysis (Grace and Hudson, 2016).

The color variables (*L*, *hue*, *chroma*, TA and TC) studied in the exocarp and mesocarp of the fruit were subjected to a one-way ANOVA and to the analysis of the ANOVA assumptions and a comparison of means ($P < 0.05$). Due to the fact that not all the variables fulfilled the data normality test, for the variable *hue* in the exocarp, and TA and TC in the mesocarp, a data transformation was carried out using the logarithm method. The TC variables in the exocarp and *hue* in the mesocarp were analyzed by means of the Kruskal-Wallis test ($P < 0.05$). The packages used in this study were: *agricolae*, to obtain Tukey's mean comparison ($P < 0.05$); *car*, to perform the Levene test in the analysis of assumptions and *pgirmes* to carry out the Kruskal-Wallis test.

## Results and discussion

### Volatile compounds

The analysis of volatile compounds by ionization with low-Ttemperature plasma coupled to mass sSpectrometry (LTP-MS) allowed to detect 75 compounds with increased relative intensity (%). Their low molecular weight, 50 to 500 *m/z*, suggests that those volatile compounds are associated with the aroma of the fruit. Fig. 2 shows only the mass spectra of two genotypes (P86, spectrum **A** and CH44, spectrum **B**) due to reasons of space.

A Principal Components Analysis (PCA) was carried out with the 75 metabolites detected (compounds of the fruit aroma). The results were interpreted based on their eigenvalues and eigenvectors. The eigenvalues and the variance explained for each of the compounds are shown in Tab. 2, where it is observed that five compounds justify 81.96% of the total variance. It was also found that the volatile compounds that provided 34.89% of the total variance in the first component (PC1) were those with *m/z* ratio: 144.98, 173.01, 270.8, 344.74, 117.03, 187.01, 200.99, 284.8, 159.0, 298.77, 256.79, 278.97, 358.74, 330.73, 235.1, 218.48, 103.06, 345.67, 204.12, 223.04 and 400.76. The volatile compounds of *m/z*: 288.74, 260.78, 98.99, 274.77, 117.03, 232,86, 302.68, 56.95, 81.01, 246.76, 153.12, 92.92, 127.06 and 250.91 provided 23.61% of the total variance of the second component (PC2). In the third component (PC3) the volatile compounds 228.87, 214.90, 219.0, 262.87, 258.86 and 230.79 provided 22.18% of the total variance.

From the PCA, the dispersion of the study genotypes in the first two principal components was obtained graphically (Fig. 3), where a cluster was observed with the genotypes CH08, CH10 and CH51, from the state of Chiapas. The second group corresponded to the genotypes PA06, PA05, PA02 and P100 from Atexcac and Huejotz-

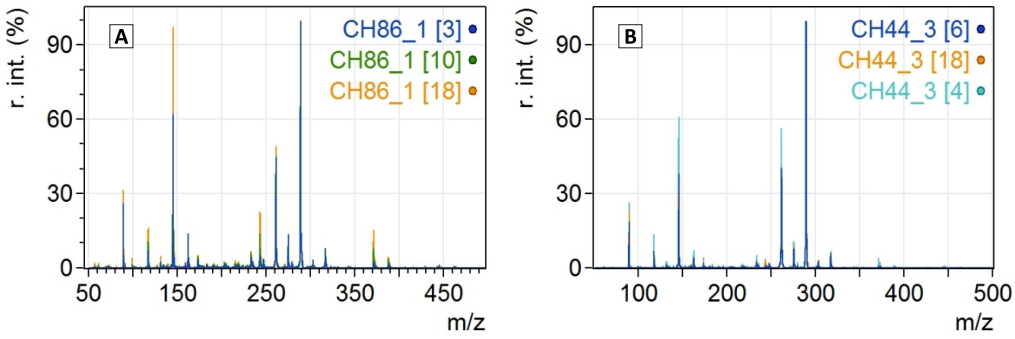


**Fig. 2:** Metabolomic profile of the volatile compounds obtained by LTP-MS. **A**) Genotype P86 (*C. mexicana*); **B**) genotype CH44 (*C. gracillior*).

**Tab. 2:** Eigenvalues and variance proportion explained by the principal component analysis of volatile compounds from the genotypes of *Crataegus*.

| Compound | Eigen values | VPC (%) | CV (%) |
|---|---|---|---|
| 1 | 26.170 | 34.89 | 34.89 |
| 2 | 17.707 | 23.61 | 58.50 |
| 3 | 8.385 | 22.18 | 69.68 |
| 4 | 5.930 | 7.91 | 77.59 |
| 5 | 3.279 | 4.37 | 81.96 |

VPC: Variance per compound. CV: Cumulative variance.

ingo, Puebla. In this same group, we found the genotypes CH83 and CH18 from two different regions of the state of Chiapas (Fig. 3).

In the dendrogram located in the upper part of Fig. 4 there are two groups (I and II) with the 24 genotypes evaluated; in the lower part, the heat map is shown containing the abundance (relative intensity %) and the presence of the 37 volatile compounds (*m/z* ratio) selected by the principal component analysis.

The color row of the dendrogram represents the color of each genotype, e.g. the red boxes are associated with the red coloration of the exocarp of some genotypes. Group I grouped the genotypes of small size (12.60 - 18.40 mm), more than 50% with red exocarp and 92% are from Chiapas, with the exception of the genotype P25 (yellow exocarp from Puebla). Group II has genotypes from three states (Chiapas, Puebla and Estado de Mexico) that were characterized by medium to large fruits (18.50 - 34.00 mm), with yellow, yellow-orange and red exocarp. The variation in the size and in some cases the origin explain the differences between groups, the coloration of the exocarp of the fruit, as the species were not determining factors. The clusters showing high similarity (P100-P02, CH08-CH10, CH44-P86, CH13-CH72, CH15-CH27) in their volatile profile had in common the origin, species and/or color of the fruit, the exception was the cluster with the genotypes CH83 and P05.

On the other hand, the volatile compounds of greater abundance are shown on the heat map (Fig. 4), this is represented by the color scale where yellow corresponds to the lowest abundance up to red which corresponds to greater abundance. The five most abundant volatiles were only present in some genotypes (Tab. 3) of the two groups of the dendrogram, e. g. the compound 260.78 *m/z* was found only in the genotypes of Group II, with different abundance. In contrast, the compound 144.98 *m/z* with different abundance was identified in all genotypes of Groups I and II. In this regard, two structural metabolites (hexyl acetate and butyl butanoate) with the same *m/z* (144,21) have been reported in different species (*C. aestivalis*, *C. opaca* and

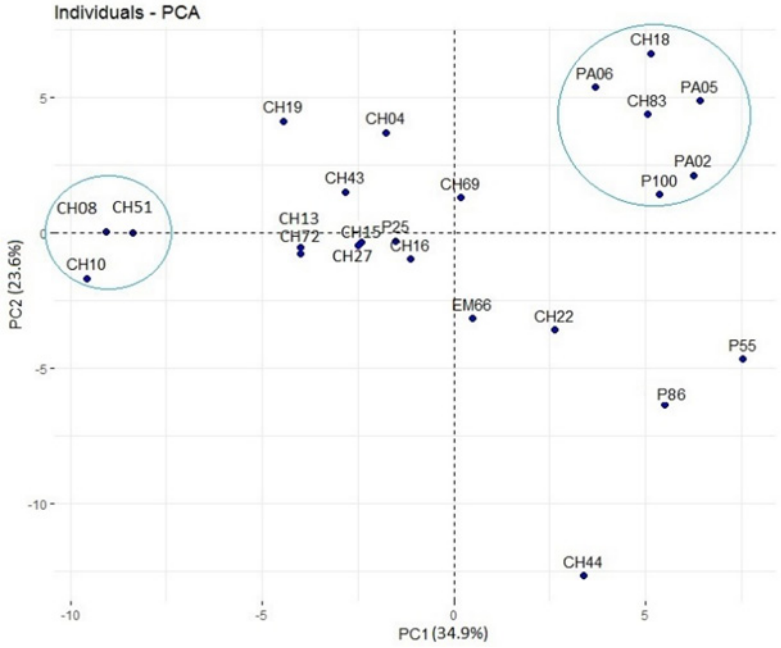


**Fig. 3:** Principal components of *Crataegus* sp. genotypes by volatile compounds.

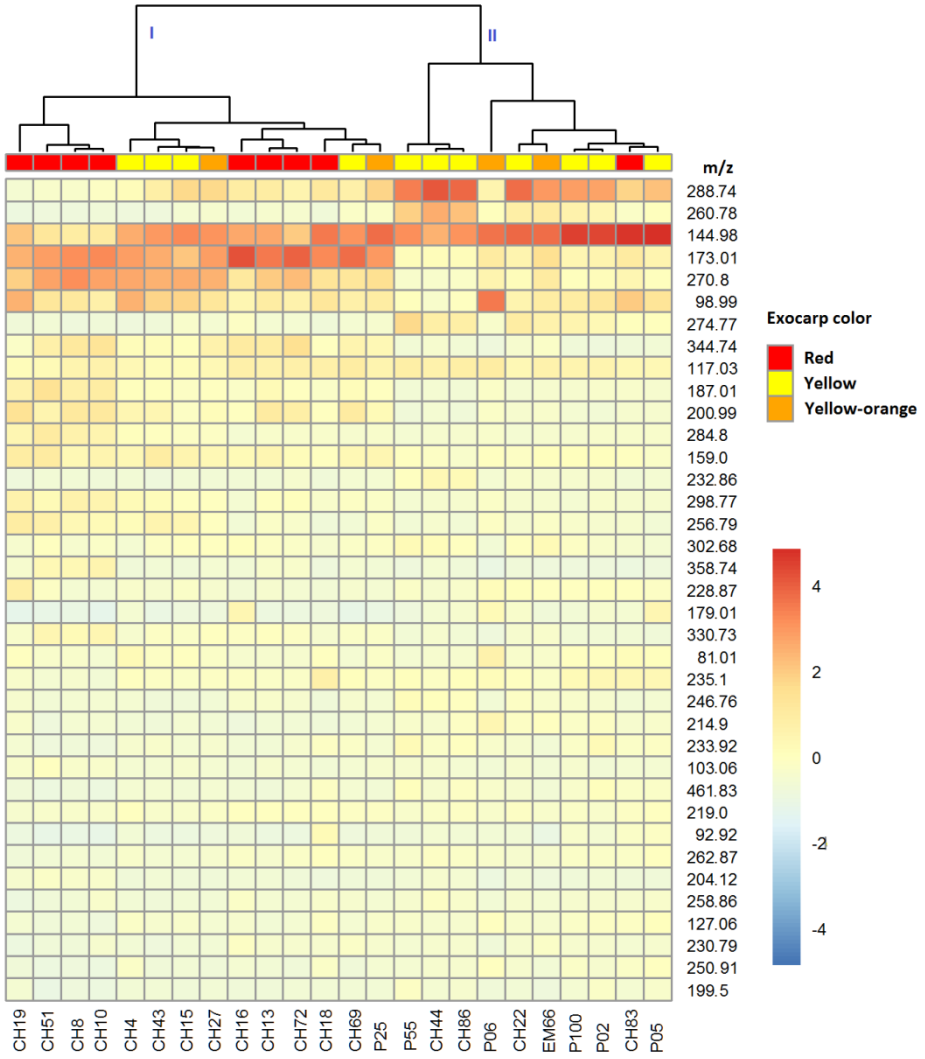


**Fig. 4:** The heat map shows the abundance (color intensity) of each of the volatile compounds listed in the right column (*m/z* ratio). The dendrogram (upper part) shows the clusters of the genotypes by coloration of the hawthorn (*Crataegus* sp.) fruit.

*C. rufula*) of *Crataegus* from the United States by Horvat and Chapman (1991) and Cha et al. (2011), however the last metabolite was found in the aroma of a different species (*C. pinnatifida*) cultivated in China (Lingyun and Bijun, 1997). Both compounds have also been identified in the aroma of apple (Espino-Díaz et al., 2016). There are few studies that report the volatile profile in the *Crataegus* fruit; of the 37 compounds reported in this study only seven coincide with those reported in the fruit (Tab. 4), however volatile compounds have been reported in flowers (Kovaleva et al., 2009) and leaves (Lakache et al., 2014).

In this study, even though the detection of volatiles with the LTP-MS technique did not allow the structural identification of the compounds identified by their *m/z* ratio, the heat map allowed to prove a different chemical profile for each genotype.

## Color parameters

The color parameters evaluated provide quality attributes that are important in consumer preferences. The lower values (< 52) of *chroma* (purity of color) obtained in the exocarp of the red fruits showed great variability in the intensity of color in comparison with yellow fruits which showed higher values (> 66) (Tab. 4). The fruits with yellow exocarp (P55 and P05) and mesocarp (CH72 and CH83) had the highest values (> 61 and > 71, respectively) of luminosity (tissues with brighter colors) and *hue* (yellow exocarp and mesocarp), compared to red exocarp fruits with a luminosity of 32.46 to 47. 49 h 26 to 32 ° (Tab. 5).

It is important to mention that yellow and larger fruits (P55, P02 and P100), grown in Puebla (first producer state at a national level with a production of 3500 t per year), are the most demanded in Mexico due to their agroindustrial and cultural use (SAGARPA, 2015).

## Content of carotenoids

Carotenoids are natural pigments metabolized by plants, are responsible for the colors yellow, orange and red in some fruits and vegetables. The apocarotenoids (derivatives) are the result of the breaking of the carotenoids present in the aroma of flowers and fruits (Namitha and Negi, 2010). The exocarp of the genotypes P55 and P05 showed the highest concentrations of carotenoids (42.09 and 42.35 $\mu g \cdot g^{-1}$ FW, respectively), which coincides with the higher values found for *hue* in the present study. However, despite of having small fruits, the mesocarp of genotype P25 had the highest concentration of carotenoids (20.92 $\mu g \cdot g^{-1}$ FW), but also a considerable concentration in the exocarp (38.17 $\mu g \cdot g^{-1}$ de FW), this non-commercial genotype from Puebla was the only one in Group I, where most of the

**Tab. 3:** The five volatile compounds with increased relative intensity (%) by genotype.

| Genotype | m/z (% Abundance) | | | | |
|---|---|---|---|---|---|
| CH4 | 173.01 (4.31) | 270.80 (4.11) | 144.98 (4.01) | 242.81 (3.99) | 98.99 (3.94) |
| CH8 | 270.80 (5.18) | 173.01 (5.12) | 344.74 (2.74) | 98.99 (2.87) | 144.98 (2.47) |
| CH10 | 173.01 (5.02) | 270.80 (4.39) | 344.74 (2.87) | 144.98 (2.57) | 187.01 (2.51) |
| CH13 | 173.01 (5.03) | 144.98 (4.14) | 270.80 (3.44) | 200.99 (2.37) | 288.74 (2.34) |
| CH15 | 144.98 (4.95) | 270.8 0 (4.22) | 173.01 (3.70) | 98.99 (3.34) | 288.74 (3.21) |
| CH16 | 173.01 (6.53) | 144.98 (4.53) | 270.80 (2.73) | 344.74 (2.63) | 288.74 (2.36) |
| CH18 | 144.98 (4.11) | 173.01 (3.79) | 270.80 (2.16) | 98.99 (2.11) | 288.74 (2.02) |
| CH19 | 98.99 (3.19) | 173.01 (3.15) | 144.98 (2.87) | 270.80 (2.83) | 200.99 ( 2.44) |
| CH22 | 144.98 (6.91) | 288.74 (6.82) | 274.77 (2.68) | 260.78 (2.54) | 173.01 (2.13) |
| P25 | 144.98 (5.45) | 173.01 (4.62) | 288.74 (3.32) | 270.80 (2.99) | 98.99 (2.44) |
| CH27 | 144.98 (4.62) | 173.01 (4.35) | 270.80 (4.01) | 288.74 (3.08) | 98.99 (2.56) |
| CH43 | 144.98 (4.28) | 173.01 (3.87) | 270.80 (3.75) | 98.99 (3.14) | 288.74 (2.11) |
| CH44 | 288.74 (12.25) | 260.78 (8.12) | 144.98 (7.85) | 274.77 (3.72) | 117.03 (3.10) |
| CH51 | 173.01 (4.46) | 270.80 (4.42) | 187.01 (2.97) | 98.99 (2.81) | 144.98 (2.75) |
| P55 | 288.74 (6.45) | 144.98 (6.07) | 260.78 (4.11) | 274.77 (3.82) | 117.03 (2.46) |
| EM66 | 144.98 (5.62) | 288.74 (4.70) | 173.01 (2.99) | 270.80 (2.52) | 260.78 (2.46) |
| CH69 | 173.01 (4.56) | 144.98 (4.02) | 270.80 (2.29) | 200.99 (2.24) | 117.03 (2.07) |
| CH72 | 173.01 (5.95) | 270.80 (3.98) | 144.98 (3.72) | 344.74 (3.11) | 200.99 (2.30) |
| CH83 | 144.98 (5.95) | 98.99 (3.19) | 28.84 (3.02) | 173.01 (2.19) | 270.80 (1.56) |
| P86 | 288.74 (8.03) | 144.98 (6.74) | 260.78 (5.21) | 117.03 (2.73) | 274.77 (2.65) |
| P100 | 144.98 (6.55) | 288.74 (4.56) | 98.99 (2.30) | 260.78 (1.85) | 117.03 (1.77) |
| P02 | 144.98 (5.52) | 288.74 (3.81) | 98.99 (2.25) | 173.01 (1.62) | 117.03 (1.61) |
| P05 | 144.98 (6.46) | 288.74 (3.52) | 98.99 (2.59) | 173.01 (1.69) | 117.03 (1.45) |
| P06 | 144.98 (4.58) | 98.99 (4.52) | 173.01 (2.06) | 117.03 (2.00) | 270.8 (1.72) |

**Tab. 4:** Volatile compounds identified by GC-MS in the *Crataegus* fruit

| Name | Formula | *m/z* | M W | Species | Source |
|---|---|---|---|---|---|
| 2-Hexenal | $C_6H_{10}O$ | 98.99 | 98.145 | *C. aestivalis*, *C. opaca*, *C. rufula y C. piinnatifida* | Lingyun and Bijun, 1997 Horvat and Chapman, 1991 |
| Benzaldehyde | $C_7H_6O$ | 106.99 | 106.124 | *C. aestivalis*, *C. opaca*, *C. rufula y C. piinnatifida* | Lingyun and Bijun, 1997 Robertson et al., 1993 |
| Hexyl acetate | $C_8H_{16}O_2$ | 144.98 | 144.214 | *C. aestivalis*, *C. opaca*, *C. rufula y C. piinnatifida* | Lingyun and Bijun, 1997 Horvat and Chapman, 1991 |
| Butyl butanoate | $C_8H_{16}O_2$ | 144.98 | 144.214 | *C. aestivalis y C. opaca* | Cha et al., 2011 |
| Citral | $C_{10}H_{16}O$ | 152.06 | 152.237 | *C. piinnatifida* | Lingyun and Bijun, 1997 |
| Hexyl hexanoate | $C_{12}H_{24}O_2$ | 200.99 | 200.322 | *C. aestivalis*, *C. opaca y C. rufula* | Horvat and Chapman, 1991 |
| β-caryophyllene | $C_{15}H_{24}$ | 204.12 | 204.357 | *C. monogyna* | Robertson et al., 1993 |

*m/z*: Mass-to-charge ratio; MW: Molecular weight.

genotypes from Chiapas were grouped. Concentrations in the exocarp of the two genotypes (P100 and P02) from *C. mexicana* were higher (38.68 and 33.61 $\mu g \cdot g^{-1}$ FW) than that reported (26.4 $\mu g \cdot g^{-1}$ FW) by Méndez-Iturbide et al. (2013), but in lyophilized exocarp of the same species from the state of Tlaxcala, Mexico (Tab. 5). The differences in the contents of both studies could be explained mainly by environmental factors, different stage of fruit ripening and post-harvest management (Reilly, 2013), as has been reported in other fruits (López-Vidal et al., 2014; Mesejo et al., 2011). The content of carotenes in hawthorn was lower than that reported for melon

(185.0 μg·g$^{-1}$ FW) by Martínez-Valdivieso et al. (2014), but higher than that found in peach (12.0 μg·g$^{-1}$FW) (Cambell and Padilla, 2013). Therefore, the consumption of hawthorn fruits represent a source of carotenoids in the human diet, important in the prevention of vitamin A deficiencies, cancer, cardiovascular diseases, age-related macular degeneration and cataract formation; beside this fruit has an important antioxidant activity (Arathi et al., 2015).

**Anthocyanin content**

The anthocyanins comprise another type of pigments responsible for the coloring of fruits from red to purple, are phenolic with antioxidant properties, which justify their nutraceutical properties (Delgado-Vargas et al., 2000). It was found that the genotypes with intense and dark red exocarp (CH18, CH51 and CH19) had the highest concentrations (992.34, 844.69 and 747.69 μg·g$^{-1}$FW, respectively) (Tab. 4) which corresponds to the smallest values of *hue* (< 28 °) and c*hroma* (< 52), concentrations higher than those reported by Froehlicher et al. (2009) (58.0 mg·100 g$^{-1}$ FW) in the exocarp of *C. monogyna*; however, lower than those reported for cranberries (206.0 mg·100 g FW) (Ribera et al., 2010) and raspberry (28.7 - 55.6 mg·100 g$^{-1}$ FW) (Peña-Varela et al., 2006). In contrast, yellow exocarp fruits showed very small concentrations (5.56 - 30.3 μg·g$^{-1}$ FW). In the mesocarp of all genotypes a lower concentration of these pigments was observed (6.95 - 29.22 μg·g$^{-1}$ FW), the exceptions were the genotypes CH10 and CH18, which had the highest anthocyanin values (29.22 and 28.38 μg·g$^{-1}$) and higher *hue* values (> 51°) (Tab. 5). It should be noted that in most of the genotypes the highest concentration of both pigments predominated in the exocarp, the exception was the genotypes P25 and CH18, where the concentrations in the fruit (exocarp + mesocarp) were important from the nutraceutical and medicinal point of view, because their consumption could provide high content of antioxidant pigments, for health benefits.

## Conclusions

The profiles of chemical volatile compounds were different among the 24 genotypes of the Mexican *Crataegus*. The metabolomic study allowed to cluster the genotypes according to their chemical profile. The differences in the volatile profile were related to the size of the fruit, and in some genotypes to their origin (state of Chiapas). No relationship of volatile compounds by color or by species was found. The highest concentration of carotenoids was observed in the exocarp in comparison with the mesocarp of yellow fruits, in contrast the red fruits showed the highest concentrations in the exocarp. The LTP-MS technique allowed to detect volatile compounds of low abundance when analyzing the sample without pretreatment. The results of the present research could be used for the development of products derived from hawthorn with high levels of bioactive compounds that would justify their medicinal properties.

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**Tab. 5:** Color parameters, total carotenoid content (TC) and total anthocyanins (TA) in the exocarp and mesocarp of 23 genotypes of the genus *Crataegus*.

| Genotype | Exocarp | | | | | Mesocarp | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Luminosity** | ***Hue*** | ***Chroma*** | **TC* (µg·g$^{-1}$)** | **TA* (µg·g$^{-1}$)** | **Luminosity** | ***Hue*** | ***Chroma*** | **TC* (µg·g$^{-1}$)** | **TA* (µg·g$^{-1}$)** |
| CH4 | 60.73 a | 64.14 ab | 66.21 abc | 23.80 ab | 5.56 e | 65.43 defg | 65.75 ab | 59.02 a | 15.61 ab | 22.26 abc |
| CH8 | 35.95 ef | 29.91 efg | 54.88 ghj | 16.41 ab | 563.59 c | 64.07 efg | 62.42 ab | 49.17 bcd | 9.85 bcd | 16.70 abc |
| CH10 | 37.10 def | 28.73 fg | 54.60 hi | 11.84 ab | 368.77 d | 61.15 fg | 51.08 bc | 51.83 abc | 4.51 fg | 29.22 a |
| CH13 | 47.49 bc | 37.31 d | 62.15 bcdef | 28.70 ab | 239.35 d | 68.47 bcdef | 66.88 ab | 50.14 bc | 7.39 cdef | 16.70 abc |
| CH15 | 64.35 a | 67.25 a | 68.27 a | 13.77 ab | 6.95 e | 65.87 cdefg | 68.54 ab | 54.30 ab | 9.78 bcd | 10.43 abc |
| CH16 | 42.80 cd | 32.41 e | 57.51 fghi | 11.99 ab | 285.27 d | 67.91 bcdef | 67.60 ab | 46.51 bcd | 10.57 bcd | 14.61 abc |
| CH18 | 32.46 f | 26.99 g | 45.05 j | 19.54 ab | 992.34 a | 70.08 abcde | 71.93 ab | 48.15 bcd | 5.04 efg | 28.38 ab |
| CH19 | 34.68 ef | 28.06 fg | 51.94 i | 5.71 b | 747.69 b | 67.74 bcdef | 70.22 ab | 47.54 bcd | 3.95 g | 18.17 abc |
| CH22 | 50.73 b | 55.31 c | 57.78 efgh | 22.26 ab | 16.19 e | 61.49 fg | 69.95 ab | 45.69 bcd | 7.28 def | 12.01 abc |
| P25 | 52.48 b | 59.38 bc | 60.94 abc | 38.17 ab | 23.66 e | 52.98 h | 64.69 ab | 52.02 abc | 20.92 a | 16.70 abc |
| CH27 | 52.13 b | 61.47 abc | 60.56 cdefg | 14.41 ab | 15.83 e | 60.00 gh | 66.97 ab | 49.20 bcd | 7.77 cde | 14.96 abc |
| CH43 | 59.97 a | 63.09 ab | 63.76 abcd | 10.71 ab | 23.66 e | 66.21 cdefg | 70.91 ab | 52.86 ab | 9.55 bcd | 9.73 abc |
| CH44 | 64.42 a | 63.47 ab | 66.16 abc | 16.92 ab | 10.43 e | 66.72 cdefg | 70.21 ab | 45.60 bcd | 6.67 defg | 8.35 abc |
| CH51 | 34.83 ef | 28.77 fg | 52.59 hi | 15.37 ab | 844.69 ab | 60.13 gh | 57.35 b | 43.93 cd | 9.6 bcd | 26.44 abc |
| P55 | 64.93 a | 63.08 ab | 67.36 ab | 42.09 a | 12.51 e | 71.94 abcd | 69.58 ab | 47.62 bcd | 8.98 bcd | 6.95 c |
| EM66 | 60.64 a | 62.75 ab | 65.11 abc | 13.38 ab | 6.96 e | 68.39 bcdef | 65.81 ab | 53.60 ab | 9.11 bcd | 16.70 abc |
| CH72 | 40.93 de | 32.62 e | 58.00 defgh | 38.10 ab | 315.89 d | 71.60 abcd | 72.65 ab | 52.43 abc | 7.37 def | 15.30 abc |
| CH83 | 40.31 de | 31.38 ef | 55.76 ghi | 22.06 ab | 528.80 c | 73.22 abc | 73.31 ab | 46.94 bcd | 7.87 cde | 13.92 abc |
| P86 | 62.23 a | 60.75 abc | 65.52 abc | 13.53 ab | 12.52 e | 69.66 bcdef | 72.48 ab | 46.12 bcd | 4.74 efg | 23.66 abc |
| P100 | 61.79 a | 62.23 ab | 62.86 abcdef | 33.61 ab | 25.04 e | 71.71 abcd | 69.34 ab | 53.85 ab | 12.84 abc | 8.35 bc |
| P02 | 62.44 a | 61.94 abc | 67.28 ab | 38.68 ab | 18.09 e | 70.18 abcde | 69.32 ab | 53.93 ab | 5.22 efg | 20.87 abc |
| P05 | 61.26 a | 59.26 bc | 67.44 ab | 42.35 a | 20.87 e | 75.99 a | 79.80 a | 41.23 d | 10.34 bcd | 11.13 abc |
| P06 | 60.89 a | 62.05 abc | 63.42 abcde | 22.06 ab | 30.30 e | 74.74 ab | 79.71 a | 40.41 d | 9.83 bcd | 9.67 abc |
| **HSD** | 6.26 | 5.81 | 5.79 | 8.09 | 158.92 | 7.42 | 8.89 | 8.89 | 5.57 | 24.04 |

* µg·g$^{-1}$ of fresh tissue. Means with the same letters are not statistically different ($P < 0.05$). HSD: Honestly significant difference.

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