

Effect of conventional and organic fertilizers on volatile compounds of raspberry fruit

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Abstract

Raspberries are widely consumed; the taste of these is determined by the interaction between sugars, organic acids and a set of volatile compounds. Meanwhile, organic agriculture has developed rapidly as an alternative to conventional system, and has been driven by the demand for pesticide-free food that brings greater benefits to human health. However, this system can alter the integral quality of the fruit, including the aroma, which has been little studied. Therefore, the objective of this study was to evaluate the effect of the application of organic and conventional fertilizers, in the presence of volatile compounds synthesized in the cv. 'Heritage' raspberry, during two crop cycles. The qualitative profile of volatile compounds was analysed by mass gas chromatography, obtaining as a result that fertilization treatments significantly affected the emission of volatile compounds in the most abundant functional group (C13 norisoprenoids) 48.5 vs. 25.8% and 56.9 vs. 29.1% in conventional and organic, in 2017 and 2018, respectively ($p \leq 0.05$). The crop cycle affected the concentration of aldehydes (11.2 vs. 30.6% in organic, in 2017 and 2018, respectively ($p \leq 0.05$)). Impact compounds such as α -ionone (13.92 vs. 9.08% and 25.34 vs. 9.17% in conventional and organic in 2017 and 2018, respectively) and β -ionone (24.93 vs. 14.10% and 22.66 vs. 15.94% in conventional and organic in 2017 and 2018, respectively), presented greater abundance in conventional fertilization ($p \leq 0.05$). Since there were changes in the volatile compounds, it is recommended to study whether the consumer perceives these changes.

Keywords: agricultural; quality; *Rubus idaeus* L.; season; taste

Introduction

Raspberry (*Rubus idaeus* L.) is a perennial crop, a member of the *Rosaceae* family that produces a red fruit with a sweet and sour taste at the same time (Aprea *et al.*, 2015). Raspberry production in Mexico for 2018

exceeded 130,000 tons. The main producing states are Jalisco (71%), Baja California (10%), Michoacán (18%) (SIAP, 2018). The most important raspberry varieties in México are 'Autumn Bliss', 'Summit' and 'Heritage', which is originally from the United States (Guzman *et al.*, 2004).

These types of raspberries are widely consumed due to their benefits in human health; since they contain a wide variety of phytochemicals that help to prevent chronic diseases (Rao and Snyder, 2010), although they are also appreciated for their characteristic flavour. The taste of raspberry is determined by the interaction between sugars, organic acids and a set of approximately 300 volatile compounds (Aprea *et al.*, 2015).

Volatile compounds participate in the development of aroma, which is often used by the consumer as an indicator of quality, maturity and freshness. Likewise, the lacks of aroma or unpleasant aromas are indicators of deterioration, decomposition, lack of maturity and, in general, poor quality (Forney, 2001).

Volatile compounds reported in raspberries include esters, aldehydes, alcohols, ketones, monoterpenes, C-13 norisoprenoids (Aprea *et al.*, 2009; Aprea *et al.*, 2015). Highlighting the compounds: α - and β -ionones, α -ionol, β -damascenone, linalool, geraniol, (Z)-3-hexenol, benzyl alcohol, acetoin, raspberry ketone, acetic acid and hexanoic acid (Larsen *et al.*, 1991). The synthesis of these compounds is affected by several factors, including the genetic composition of the fruit, its maturity, and environmental conditions during its cultivation, handling in the harvest and storage (Forney, 2001).

In raspberry, as in other crops, there are two production alternatives: conventional and organic, the first guarantees high yield and quality, but requires extensive use of chemical fertilizers, which are important, but create environmental problems; therefore, there has been a resurgence of interest in agricultural practices that are environmentally friendly and sustainable, such as organic production (Esitken *et al.*, 2005). Organic agriculture has developed rapidly as an alternative to conventional system, and has been driven by the demand for pesticide-free foods that provide greater benefits for human health (Gomiero, 2017). In addition to the above, organic farming systems have proven been capable of producing food with high quality standards (Lairon, 2010) and higher phytochemical content (Frias *et al.*, 2019). It has been proved that the type of fertilization used has an impact on the nutritional components of fruits, these being greater in fruits fertilized with organic fertilizers (Benbrook, 2009). However, to date, little or no importance has been given to the impact of type fertilization on the concentration of volatile compounds in fruits, specifically on raspberry fruit. In a study conducted on the effects of the application of different fertilizers, organic and conventional, on the volatile compounds of 'Golden Delicious' apples were investigated, finding that the type of fertilization significantly affected the level of compounds related to flavour, although few of these responses were consistent during the two years of harvest in which the study was carried out. Even when the treatments resulted in marked differences in the level of mineral nitrogen in the soil, this was reflected in a limited impact on the flavour-related compounds in the fruit, the strongest effect being a 45% change in the level of C6 aldehydes (Raffo *et al.*, 2014).

Despite, the economic and nutritional importance of raspberry, there is limited information previously published related to the effect of conventional and organic fertilizers on volatile compounds present on this fruit. Therefore, the objective of this study was to evaluate the effect of the application of organic and conventional fertilization on emissions of volatile compounds in raspberry cv 'Heritage', during two consecutive agronomic cycles.

Materials and Methods

Biological material

The work was carried out at the Faculty of Agrotechnological Sciences of the Autonomous University of Chihuahua, Cuauhtémoc campus, located at 28° 24' 45.1" N, 106° 52' 54.9" W and at 2060 meters above sea level. The climate is mild with minimum temperatures of -16 °C in winter and maximum temperatures in

summer of 38 °C, with an annual rainfall of 499.3 mm (Table 1). The soil is clay-loam texture. The raspberry plantation was established with the autumn-producing 'Heritage' variety, in February 2015 with bare roots.

Table 1. Weather conditions

Month	2017				2018			
	Min	Max	Pres	Rad solar	Min	Max	Pres	Rad solar
	(°C)	(°C)	(mm)	Kw m ⁻²	(°C)	(°C)	(mm)	Kw m ⁻²
January	-0.9	17.0	2.5	0.16	-2.6	16.7	0.0	0.17
February	0.3	19.8	0.4	0.20	2.9	18.2	3.6	0.17
March	4.0	23.1	0.6	0.25	3.5	22.1	1.7	0.24
April	6.3	25.6	1.9	0.31	6.8	25.1	0.4	0.31
May	7.7	27.4	4.5	0.35	8.8	28.9	5.9	0.33
June	12.5	31.0	11.1	0.32	13.6	30.4	20.5	0.30
July	13.6	25.6	288.0	0.23	13.8	27.3	125.9	0.26
August	13.2	24.5	184.8	0.22	12.9	26.8	125.4	0.25
September	10.3	25.1	38.7	0.23	10.8	24.3	89.5	0.21
October	7.5	24.6	7.0	0.21	7.4	21.1	57.2	0.17
November	4.5	24.3	0.0	0.16	0.0	18.5	3.90	0.17
December	2.3	20.3	0.0	0.12	-1.80	15.62	20.3	0.14
Total and average	6.78	24.01	44.96	0.24	7.08	23.58	40.95	0.23

Experimental

The experimental garden had 6 rows of 20 linear meters, with experimental units (UE) of 4 linear meters, with a distance between rows of 2 m and 1 m between each UE, with a density of 30 and 35 rods per linear m. The distribution of the treatments was carried out at random, considering two treatments, one of conventional management and another organic, assigning the experimental units by agronomic management.

Crop management

The organic management consisted of applying vermicompost, leaching of vermicompost and commercial organic fertilizer. The physicochemical properties of vermicompost, leaching of vermicompost and commercial fertilizer are indicated in Table 2. The compost was applied at a rate of 15 t ha⁻¹ for the two years of study (2017 and 2018). The vermicompost leaching was applied in the order of a rate of 24 m³ ha⁻¹. Before applying the vermicompost leaching, its pH was lowered from 8.2 to 7, with organic apple vinegar. Finally, a commercial organic fertilizer 7.5 t ha⁻¹ was also applied (Table 3). Fertilizer applications started in May in both crop cycles and continued weekly until September (16 and 9 applications, respectively).

In conventional crops, commercial (synthetic) fertilizers of nitrogen, phosphorus and potassium (NPK) were applied, which were based on ammonium nitrate, triple 17 and potassium sulfate. The nitrogen inorganic fertilizer was applied at a rate of 150 kg ha⁻¹ from May to October for 2017 and 2018. The nitrogen rates remained the same during the two years to promote reproductive growth. Inorganic fertilizers of P were also added at a rate of 40 kg ha⁻¹ and 190 kg ha⁻¹ of K, according to the recommendation of Parra *et al.* (2008) (Table 4).

The irrigation system was drip with an expense of 2 l hr⁻¹ per dropper. With this system, it was watered three times a week, lasting five hours. Weed control was carried manually. The weather condition to which the raspberry crop was exposed in the two years of study is shown in Table 1.

Harvest

The harvest was done manually, two partial crops per week during the 2017 production cycle, beginning and mid-June. For 2018, start in the first week of July. The raspberries were harvested once they could be

detached from the receptacle and had an intense red colour (ripening grade 3) (Peña *et al.*, 2006). The fruits were randomly selected within the experimental unit and subsequently taken to the laboratory for analysis.

Table 2. Chemical and physical characteristics of vermicompost, leaching of vermicompost and commercial fertilizer

Parameter	Vermicompost	Leaching of vermicompost	Commercial organic fertilizer
pH	6	8.2	6.7
Organic material (%)	85	40	45
Total organic carbon (%)	18.57	-	-
N total (%)	2.24	2.3	3
P total (%)	0.12	0.3	4
K total (%)	0.01	0.37	4
Ca total (%)	1.33	0.01	16
Mg total (%)	1.21	1	1.6

Table 3. Organic fertilizers applied by production season

Organic fertilizers	2017	2018
Vermicompost	15 t ha ⁻¹	15 t ha ⁻¹
Leaching of vermicompost	24 m ³ ha ⁻¹	24 m ³ ha ⁻¹
Commercial organic fertilizer	7.5 t ha ⁻¹	7.5 t ha ⁻¹

Table 4. Chemical fertilizers applied by production season

Conventional fertilizers	2017	2018
(NH ₄) ₂ HPO ₄	315 kg ha ⁻¹	315 kg ha ⁻¹
K ₂ SO ₄	40 kg ha ⁻¹	40 kg ha ⁻¹
(NH ₄) ₂ SO ₄	284 kg ha ⁻¹	284 kg ha ⁻¹

Sample preparation

Fifteen raspberry fruits per treatment were macerated manually, in a porcelain mortar, until a homogeneous mixture was obtained. 20 ml of juice were taken and placed in 20 ml PTFE vials and stored at -80 °C until analysis.

Volatile compounds

The concentration of volatile compounds was determined by gas chromatography coupled to mass spectrometry, using the solid phase microextraction technique (SPME). A 2 ml aliquot of thawed juice was taken in a thermobath (Felisa), it was placed in 4 ml vials with 0.65 g of sodium chloride and kept under stirring while the SPME fiber (65 µm, PDMS-DVB, Agilent USA) was exposed to the sample head space, for 60 min, at room temperature. The fibre was desorbed in the injection port for 5 min at 200 °C within the GC-MS (Agilent) system, equipped with a DB-WAX column (60m × 0.25mm, 0.25 µm, Agilent USA). The chromatographic conditions were as follows: injector temperature of 200 °C, initial oven temperature of 33 °C sustained for 5 min, increasing to 50 °C at 2 °C min⁻¹, increasing the temperature again to 250 °C at a rate of 5 °C min⁻¹, holding it for 6.5 min. Helium was used as a carrier gas with a linear velocity of 30 cm s⁻¹. Mass spectra were obtained by electronic ionization at 70 eV. The temperatures of the transfer line and the ion trap were 250 and 180 °C, respectively. The identification of volatile compounds was made by comparing the mass spectra obtained from the samples against those reported in the NIST MS 2014 library. All values represent the average of triplicated sampled. The results were expressed in relative concentration as the ratio of the area of the base peak of the compound to the total area of all the compounds.

Statistical procedures

Likewise, an analysis of variance (one-way ANOVA) and multiple comparison of Tukey means ($p \leq 0.05$) was performed using the statistical program Minitab version 16 (Statistical Software, USA).

Results and Discussion*Volatile compounds*

Volatile compounds identified in raspberry fruits grown with conventional and organic management during two years of study are shown in Table 5. Seven alcohols, five C13-norisoprenoids, five monoterpenes, three aldehydes, three ketones, three acids and two esters were identified. It was observed that the determinations of most volatile compounds in raspberries were influenced by fertilization and / or seasonal variation.

Table 5. Relative concentrations of volatile compounds in raspberry fruit (*Rubus idaeus* L.)

Parameter			2017		2018	
Rt (min)	Compound	Diagnostic ions (m/z)	Fertilizer			
			Conventional	Organic	Conventional	Organic
7.81	Ethyl acetate	(43) 999 (61) 153 (45) 146 (29) 124 (70) 118	2.24 ± 0.13aA	11.93 ± 5.76aA	1.64 ± 0.19aA	5.48 ± 0.10bB
10.05	Ethanol	(31) 999 (45) 514 (29) 298 (27) 224 (46) 216	1.70 ± 1.07aA	2.05 ± 0.18aA	1.16 ± 0.35aA	4.58 ± 0.82bB
15.18	α -Pinene	(93) 999 (91) 454 (92) 440 (77) 358 (41) 325	3.28 ± 0.80aA	4.11 ± 0.31a	1.15 ± 0.36A	n.d.
17.83	Hexanal	(56) 999 (44) 889 (41) 810 (57) 668 (43) 569	2.49 ± 1.25aA	0.78 ± 0.24aA	2.67 ± 0.30aA	11.99 ± 1.25bB
20.44	3-Hexenal	(41) 999 (69) 516 (55) 398 (39) 352 (42) 190	2.59 ± 1.31aA	8.06 ± 2.28aA	3.54 ± 1.48aA	7.14 ± 1.75bB
21.84	α -Phellandrene	(93) 999 (91) 319 (77) 307 (92) 252 (136) 173	2.00 ± 0.71aA	2.68 ± 0.29a	1.34 ± 0.16A	n.d.
22.41	2-Heptanone	(43) 999 (58) 601 (71) 136 (41) 110 (27) 106	3.61 ± 1.68aA	7.22 ± 0.09aA	1.30 ± 0.91aA	1.83 ± 0.22aA
23.27	(E)-2-Hexenal	(41) 999 (55) 942 (69) 782 (83) 668 (39) 639	1.19 ± 0.58aA	2.60 ± 0.02aA	0.69 ± 0.02aA	11.83 ± 0.13bB
23.55	β -Phellandrene	(93) 999 (77) 274 (91) 274 (136) 177 (79) 168	1.47 ± 0.60A	n.d.	0.68 ± 0.02A	n.d.
25.73	Acetoin	(45) 999 (43) 556 (88) 99 (27) 95 (29) 55	2.98 ± 0.90a	1.90 ± 0.20aA	n.d.	0.26 ± 0.01B
26.40	3-Hexenyl acetate	(43) 999 (67) 648 (82) 374 (41) 192 (39) 130	2.74 ± 1.05aA	1.84 ± 0.26aA	0.86 ± 0.14aA	0.30 ± 0.04bB
27.41	2-Heptanol	(45) 999 (55) 292 (41) 235 (43) 224 (44) 142	6.96 ± 1.69aA	6.01 ± 0.36aA	2.41 ± 0.32aA	2.34 ± 0.13aA
28.42	1-Hexanol	(56) 999 (43) 762 (31) 571 (55) 571 (41) 570	n.d.	n.d.	1.43 ± 0.12a	0.53 ± 0.05b
28.73	3-Hexen-1-ol	(41) 999 (67) 599 (39) 390 (55) 343 (82) 282	2.62 ± 0.64A	n.d.	2.60 ± 0.05aA	1.08 ± 0.22b
29.35	3-Hexen-1-ol, (Z)	(41) 999 (67) 700 (55) 351 (39) 314 (82) 303	n.d.	3.79 ± 0.21	n.d.	n.d.

30.68	Acetic acid	(43) 999 (45) 874 (60) 570 (15) 416 (42) 137	2.55 ± 1.60	n.d.	n.d.	n.d.
31.54	6-Methyl-5-hepten-2-ol	(95) 999 (41) 778 (45) 411 (69) 408 (67) 372	4.17 ± 2.45aA	2.18 ± 1.80aA	1.69 ± 0.55aA	0.31 ± 0.05aA
33.73	Linalool	(71) 999 (93) 760 (55) 637 (43) 598 (41) 564	1.68 ± 0.72aA	2.09 ± 0.58aA	0.60 ± 0.07aA	0.51 ± 0.31aA
36.62	Acetophenone	(105) 999 (77) 858 (51) 406 (120) 327 (43) 208	1.83 ± 0.13aA	1.84 ± 0.59aA	1.18 ± 0.18aB	0.60 ± 0.06bA
36.95	Ionone	(177) 999 (43) 424 (91) 176 (135) 153 (178) 133	24.93 ± 1.34aA	14.10 ± 0.41bA	22.66 ± 3.21aA	15.94 ± 1.01aA
38.46	Dihydro- α -ionone	(43) 999 (95) 690 (136) 690 (121) 600 (41) 400	1.53 ± 0.21aA	0.70 ± 0.40aA	1.04 ± 0.09aA	0.51 ± 0.04bB
39.80	Dihydro- β -ionone	(121) 999 (43) 630 (161) 432 (123) 363 (136) 363	3.09 ± 0.21aA	n.d.	3.79 ± 0.73aA	2.22 ± 0.11a
40.87	Hexanoic acid	(60) 999 (73) 444 (41) 422 (27) 375 (43) 237	n.d.	7.99 ± 4.27	2.82 ± 0.92	n.d.
40.96	α -Ionone	(121) 999 (93) 770 (43) 760 (136) 620 (77) 333	13.92 ± 3.11aA	9.08 ± 1.91aA	25.34 ± 1.36aA	9.17 ± 0.11bA
41.30	Propanoic acid	(71) 999 (56) 793 (89) 722 (43) 600 (41) 221	n.d.	n.d.	1.54 ± 0.03a	1.52 ± 0.37a
41.45	Benzyl alcohol	(79) 999 (108) 884 (77) 640 (107) 616 (51) 290	2.41 ± 0.59aA	1.58 ± 0.33aA	1.56 ± 0.25aA	0.35 ± 0.07bB
41.92	α -Ionol	(95) 999 (43) 656 (138) 400 (41) 158 (96) 135	2.63 ± 0.53aA	1.73 ± 0.52aA	4.25 ± 1.19aA	1.61 ± 0.04aA
42.07	(E)- β -Ionone	(177) 999 (43) 408 (91) 173 (135) 153 (178) 134	5.38 ± 3.83aA	5.75 ± 1.76aA	12.05 ± 4.98aA	19.89 ± 0.43aA

Rt Retention time

¹Identification by comparison of mass spectra, retention times and mass spectra from the NIST Mass Spectral Library and based on rules of ion fragmentation in the mass spectrometer.

²Calculated with the abundance of the identified compound divided to the total abundances of all the compounds ± standard deviation.

n.d. not detected.

Means within the same crop cycle followed by different small letters are significantly different at $p \leq 0.05$ (LS means test).

Means within the different crop cycle followed by different capital letters are significantly different at $p \leq 0.05$ (LS means test).

Volatile compounds by functional group

Figure 1 shows the relative abundance by functional group, the C13-norisoprenoids being the most abundant group during the two crop cycles and fertilization systems. These compounds are generated by the oxidative breakdown of carotenoids (Hampel *et al.*, 2007) and have been reported as the most abundant group in other varieties (Aprea *et al.*, 2010; Morales *et al.*, 2014). Unlike other authors who detected ketones as the most abundant group (Kafkas *et al.*, 2019). This functional group was significantly affected ($p \leq 0.05$) by the fertilization system, presenting higher relative abundance percentages in conventionally fertilized raspberry, in both years of study. As can be seen in Table I, the percentages in the macronutrient content were higher in conventional fertilization ($p \leq 0.05$); this may have influenced these differences presented by the C-13 norisoprenoids related to the fertilization system. On the other hand, other groups such as aldehydes and esters were more abundant only in the second cycle (2018) ($p \leq 0.05$), knowing that fertilization was constant in the two crop cycles (Tables 2 and 3) the change in these two functional groups, more than to the fertilization

system, it could be attributed to seasonal variation, with the 2017 cycle having greater rainfall in the months of July and August (Table 4). Ketones were influenced only by the crop cycle having a higher percentage of abundance in the first crop cycle (2017) ($p \leq 0.05$).

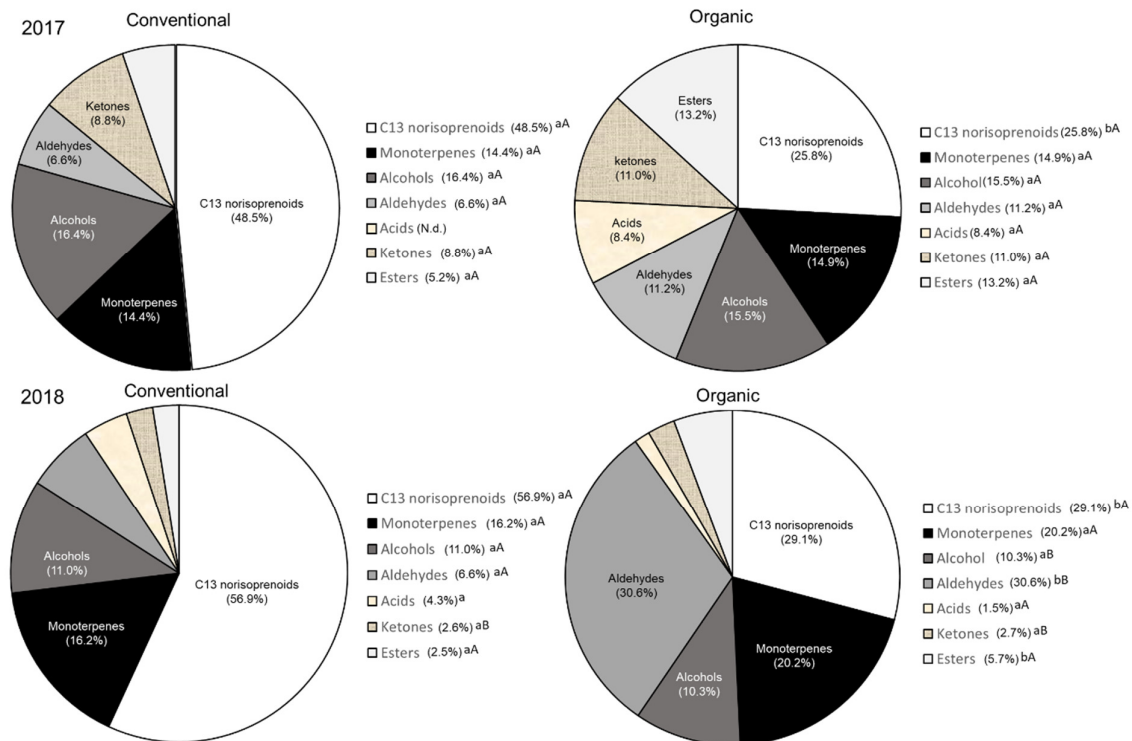


Figure 1. Volatile compounds in raspberry fruit (*Rubus idaeus* L.) according to chemical class
Means within the same crop cycle followed by different small letters are significantly different at $p \leq 0.05$ (LS means test)
Means within the different crop cycle followed by different capital letters are significantly different at $p \leq 0.05$ (LS means test)

Volatile impact compounds

Within the norisoprenoid group, α - and β -ionone are recognized as impact compounds in raspberries (Larsen *et al.*, 1991) since α -ionone produces fruity, floral and raspberry odour and β -ionone has an aromatic odour floral (Jaeger *et al.*, 2013; Aprea *et al.*, 2015). Even β -ionone is considered one of the most relevant compounds of red raspberries aroma (Larsen *et al.*, 1991; Klesk *et al.*, 2004). In this study, both compounds were detected as the most abundant, Kafkas *et al.* (2019) reporting similar results with other raspberry varieties ('Tulameen', 'Kweli' and 'Imara'). Although these compounds were significantly affected by the fertilization system, presenting a higher percentage of abundance in conventional management, this difference was not consistent by crop cycles, since β -ionone was more abundant in the 2017 cycle and α -ionone in the 2018 cycle ($p \leq 0.05$). Other of the identified compounds, β -phellandrene, which are characteristic of the raspberry aroma, was significantly affected by type of fertilization ($p \leq 0.05$). This belongs to the terpenes group (Aprea *et al.*, 2015) and it's responsible for floral and herbaceous aromas (Boylston, 2010). Meanwhile, acetoin, belonging to the ketone group, was mainly affected by the cycle of cultivation ($p \leq 0.05$).

On the other hand, some compounds considered to have an impact in raspberries such as linalool, from the terpenes group, which provides floral essence in fruits (El Hadi *et al.*, 2013) and α -ionol (C-13 norisoprenoid) that gives sweet aroma to lemon and hot tea (Larsen *et al.*, 1991; Robertson *et al.*, 1995), were not significantly influenced by fertilization systems or seasonal variation ($p \geq 0.05$) (Table 5).

Conclusions

The effect of the crop cycle as well as the fertilizer system (organic and conventional) of the cv. 'Heritage' raspberry, modified the qualitative profile of volatile compounds related to flavour. The raspberry grown under conventional fertilization showed a greater abundance of volatile compounds, especially from the C-13 norisoprenoid group. While ketones had less abundance in the second crop cycle. It is advisable to conduct studies that establish the relationship of the effect of nutrients absorbed by plants from organic and conventional fertilizers, as well as investigate whether the changes that occur modify the aromatic perception of these fruits by consumers.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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