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Estimating CO_2 and VOCs production of *Colletotrichum fragariae* and *Rhizopus stolonifer* grown in cold stored strawberry fruit

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ABSTRACT

The aim of this work was to investigate the early detection of anthracnose and soft rot diseases in cold stored strawberry fruit by evaluating the CO_2 and volatile organic compounds (VOCs) released by the fungi *Colletotrichum fragariae* and *Rhizopus stolonifer*. Strawberries were stored at 5, 10 and 21 °C (control group) and the VOCs and CO_2 production of inoculated and non-inoculated strawberries were followed by gas chromatography. To evaluate and estimate the growth of both fungi, the CO_2 data were fitted to the Gompertz model. Data of the VOCs released at the end of the fungal growth were analyzed using principal components analysis (PCA) to discriminate between infected and non-infected strawberries. The results showed that fungal growth was affected by temperature and *C. fragariae* had a maximum growth after 14.6 h at 5 °C and *R. stolonifer* at 21 °C after 45.2 h. On the other hand, through VOCs released by *C. fragariae* and *R. stolonifer* and PCA, four groups were obtained: a) strawberry infected with *C. fragariae*, stored at 10 °C, b) strawberry infected with *R. stolonifer*, stored at 21 °C, c) control group kept at 10 °C and, d) strawberry infected with *C. fragariae* and control group (5 and 21 °C), and strawberry infected with *R. stolonifer* at 5 and 10 °C. In conclusion, CO_2 and VOCs released by *C. fragariae* and *R. stolonifer* on strawberries could infer the presence of anthracnose and soft rot during storage of the fruit at low temperature.

1. Introduction

Anthracnose is one of the most important diseases affecting strawberries. It is caused by different *Colletotrichum* fungal species including *C. fragariae*, *C. acutatum* and *C. gloeosporioides* (Feliziani and Romanazzi, 2016; Jayawardena et al., 2016). Alternatively, soft rot disease is caused by the fungus *Rhizopus stolonifer* and is considered one of the most destructive postharvest phytopathogens due to its ability to rapidly grow on the fruit, causing a total loss of quality within 3–4 days after being infected (Bautista-Baños et al., 2014). To reduce the presence of these microorganisms, the use of fungicides has become a common practice, including the use of technologies such as modified atmosphere packaging, edible coatings, and smart packaging (Atala and Avara, 2007; Barikloo and Ahmadi, 2018; Ventura-Aguilar et al., 2018). However, to date, there is a lack of information about the development of portable technologies that carry out faster and more efficient detection of phytopathogen fungi on fresh fruit. These technologies detect biomarkers that allow recognizing a sample. Kai et al. (2009) and Ramírez and Rodríguez (2010) have shown that CO_2 and volatile metabolites are released during the anabolic and catabolic metabolisms of all aerobic organisms. Therefore, they could act as indicators of fungal growth. Consequently, the detection and quantification of these products could allow early detection of fungal infection of fruit as the strawberries.

On the one hand, CO_2 is released during fruit ripening, and its concentration can be increased by the presence of fungi in the course of the infection process (fungi-host) (Ramírez and Rodríguez, 2010). In this respect, Setälä and McLean (2004) undertook an *in vitro* assessment on fungal growth, measured as CO_2 release, from a humus layer of a spruce forest (*Picea abies*) isolate. They found that the CO_2 release was positively related to the number of fungal taxa, irrespectively of the sampling time. Also, Borjesson et al., 1992 found a high positive

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correlation between the production of CO_2 and VOCs, and the fungal growth of *Penicillium brevicompactum*, *P. glabrum*, *P. roqueforti*, *Aspergillus candidus*, *A. flavus*, and *A. versicolor* on wheat and oats as substrate. Because CO_2 production is associated with fungal growth, mathematical models can be used to predict the evolution of the infection. Such information can be used 1) to find the appropriate temperature during the storage of strawberries as a method of controlling the fungi growth, 2) to allow the establishment of biomarkers to facilitate the detection of selected pathogens and 3) to establish the optimal time for the application of technologies that facilitate fungal control. In particular, the mathematical model proposed by Gompertz in 1825 has been frequently used to describe the growth of an organism. For instance, through the use of this model, Eifert et al. (1996) predicted the growth of *Staphylococcus aureus* 196E at different incubation conditions of pH, NaCl, and temperature. Similarly, Zhang et al. (2006) used the Gompertz model to study the effect of gamma irradiation on the microbial growth of fresh-cut lettuce, and they reported a notable bacterial inhibition at 4 °C after 9 days.

Additionally, VOCs are important metabolites that can act as indicators of the presence of pathogenic fungi during the infection process in time for it to be controlled, and before the appearance of symptoms (Lavine et al., 2012; Heddergott et al., 2014). For example, Morath et al. (2012) found that VOCs production is biologically dynamic. Consequently, it depends on the substrate, species or strain, growth conditions (incubation time, type of nutrients, temperature), and other environmental factors. In a recent study, Rojas-Flores et al. (2018) reported the presence of 102 VOCs for *C. fragariae* and 86 VOCs for *R. stolonifer* incubated at 10 and 20 °C in potato dextrose broth. Additionally, they observed that α-terpineol was detected in the whole growing period of *C. fragariae* and *R. stolonifer*, whereas γ-terpinene was emitted only by *R. stolonifer* incubated at 20 °C.

On the other hand, although the VOCs profile of fungi provides specific information, its analysis may be complicated because it includes a large number of chemical compounds. Consequently, the data obtained from this analysis may be explained through multivariate statistics to facilitate the interpretation, as long as it is adjusted to the objectives of the expected response. Perhaps the most commonly-used is Principal Component Analysis (PCA) that is a statistical procedure used to reduce and group data. Pan et al. (2014) evaluated the VOCs of strawberry fruit infected by three common postharvest fungal pathogens in the early storage stage at 5 ± 1 °C. After using a PCA score plot, it was clearly distinguished the fungal infection types caused by *Botrytis* sp., *Penicillium* sp. or *Rhizopus* sp. on strawberries during 4 days storage. In accordance with the aforementioned, this study aimed to evaluate if CO_2 and VOCs released by *C. fragariae* and *R. stolonifer* on strawberries at 5, 10 and 21 °C can serve to detect anthracnose and soft rot diseases in their early storage stage of the fruit.

2. Materials and methods

2.1. Fungal strains and preparation of conidial stocks

Colletotrichum fragariae and *R. stolonifer* were isolated from strawberry fruit showing disease symptoms and subsequently, they sent to the Phytosanitary Diagnostic Laboratory (LADIFIT) for their molecular identification (*C. fragariae* access number AJ30912, and *R. stolonifer* access number AM933546). They were grown and maintained on Petri dishes containing potato dextrose agar medium (Becton Dickinson Inc., France) and stored at 20 ± 1 °C for approximately 5 and 10 days for *R. stolonifer* and *C. fragariae*, respectively. Later, the spores of each fungus were suspended by adding 20 mL of sterile water to the Petri dishes and rubbing the agar surface with a glass rod. The spore suspensions were collected, filtered through a cotton cloth and adjusted to 10^5 spores·mL^{-1} using a Neubauer counting chamber and an optical microscope (Nikon, Japan).

2.2. Plant material characteristics

Strawberry fruit (*Fragaria* x *ananassa*) cv. Camarosa were obtained from an orchard located in Tepoztlan Morelos, Mexico (18°59′07″N 99°05′59″W). They were harvested when 75% of the fruit surface was red according to the official Mexican norm NMX-FF-062-SCFI-2002 (Secretaría de Economía, 2002).

2.3. Experimental conditions and treatments

The glass containers (350 mL) were sterilized (121 °C for 15 min). The strawberries were rinsed with 400 ppm NaClO for 3 min and left to dry and two strawberries (20–25 g) were placed inside the glass containers and an airstream coupled to an activated carbon filter was recirculated inside it for 5 min. Then, inoculation was carried out by spraying the spore suspension (10^5 spores·mL^{-1}) on the fruit and the glass containers were hermetically sealed (static system).

The treatments applied were the following: strawberries inoculated with 1 mL of *C. fragariae*, strawberries inoculated with 1 mL of *R. stolonifer,* and non-inoculated strawberries (control). A total of twenty-seven containers were used of which nine belonged to each treatment with three repetitions. They were then stored at 5, 10 and 21 °C (control group) for 144 h, respectively. The released CO_2 of the samples were evaluated after 1, 24, 48, 72, 96 and 144 h. The VOCs were evaluated after 144 h at 5 and 10 °C, and after 96 h at 21 °C, which represented the maximum period of storage of the strawberries.

2.4. Anthracnose and soft rot severity on strawberry fruit

Severity was evaluated by the following scale: 0 = 0% 1 = 1–25% 2 = 26–50%; 3 = 51–75% and 4 = 76–100% of the fruit surface rotten.

2.5. CO_2 production

250 µL sample of the gas composition inside the glass containers was taken with a gas-tight syringe after 1, 24, 48, 72, 96 and 144 h. The sample was injected into a GOW-MAC series 550 gas chromatograph, equipped with an HP CTR1 column (Alltech, USA). Helium was the carrier gas at a flow rate of 90 mL·min^{-1}, the oven temperature was held at 40 °C, and the injector and the thermal conductivity detector temperatures were 50 °C and 115 °C, respectively. The retention time was 0.82 min for CO_2. Data were reported as mg·m^{-3} (Ishikawa et al., 1997).

2.6. CO_2 data fitting to the Gompertz mathematical model

Experimental data of the CO_2 release over storage and incubation time were adjusted to the Gompertz mathematical model, according to the following equation:

$$N(t) = \propto * e^{-\beta * e^{(-\gamma * time)}}$$

where: N(t) represents the size of the population at the time "t" expressed as CO_2 content (mg·m^{-3}); α is the maximum value of fungi growth expressed as mg·m^{-3} (when t→ ∝); β is the slope of the curve that was used to obtain the time of maximum CO_2 release rate from $t_{max} = (\ln \beta)/\gamma$, and γ (h^{-1}) is the intrinsic rate of growth. The data estimated by the model were used to make the response surface graphs.

2.7. Identification of volatile compounds using the Head Space Solid Phase Micro-Extraction (HS-SPME) technique

One fiber of carboxen/polydimethylsiloxane (CAR-PDMS, 75 µm, Supelco, Bellefonte, PA, USA) was conditioned in the injector of a gas chromatograph (GC, Agilent 780B) at 200 °C for 1 h. For volatile extractions through HS-SPME, the fiber was exposed during 30 min inside

Table 1
Development of anthracnose and soft rot diseases on strawberries cv. Camarosa stored at 5, 10 and 21 °C during given sampling times.

	Sampling time (h)																				
	1			24			48			72			96			144			312		
	Temperature (°C)																				
	5	10	21	5	10	21	5	10	21	5	10	21	5	10	21	5	10	21	5	10	21
Anthracnose	0	0	0	0	0	0	0	0	1	0	1	2	0	1	3	1	1	4	1	2	4
Soft rot	0	0	0	0	0	2	0	0	3	0	0	4	0	0	4	0	1	4	1	1	4
Control	0	0	0	0	0	0	0	0	1	0	1	1	0	1	2	1	1	3	1	2	4

Disease severity: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of the fruit surface rotten.

Table 2
Evolution of the CO_2 production ($mg{\cdot}m^{-3}$) during given sampling times, in strawberries inoculated with *C. fragariae* and *R. stolonifer*, and non-inoculated fruit, stored at different temperatures.

	Sampling time (h)					
	1	24	48	72	96	144
Inoculated with *C. fragariae*						
5 °C	$438.6 \pm 80^{aB*}$	715.8 ± 142^{aC}	1143.9 ± 272^{bBC}	1150.6 ± 200^{bBC}	1263 ± 50^{cA}	1673.1 ± 171^{cA}
10 °C	169.2 ± 32^{aA}	310.2 ± 46^{aA}	423.8 ± 41^{bA}	503.9 ± 133^{bA}	1428 ± 181^{cA}	564 ± 47^{bB}
21 °C	91.3 ± 22^{aA}	944.1 ± 117^{bBC}	1606.6 ± 358^{cC}	1928.4 ± 291^{cD}	2125.5 ± 230^{cB}	–
Inoculated with *R. stolonifer*						
5 °C	382.7 ± 65^{aB}	967.5 ± 312^{abBC}	1279.3 ± 331^{bC}	1218.6 ± 212^{bC}	919 ± 50^{abA}	440.6 ± 97^{aB}
10 °C	153.7 ± 39^{aA}	275.5 ± 70^{aA}	$516.8 \pm 139b^{AB}$	592.1 ± 179^{cAB}	826.9 ± 50^{cA}	440.8 ± 97^{bB}
21 °C	107.3 ± 24^{aA}	703.9 ± 109^{aB}	1415.5 ± 201^{bC}	2406.7 ± 373^{cD}	3026.6 ± 483^{cB}	–
Non-inoculated (control group)						
5 °C	431.5 ± 73^{aB}	892.8 ± 187^{aC}	1283.2 ± 144^{bC}	1349.5 ± 126^{bC}	2078 ± 50^{cA}	2163 ± 377^{cA}
10 °C	185.6 ± 44^{bA}	249.4 ± 33^{bA}	610 ± 50^{aAB}	1037.7 ± 249^{aBC}	2264.4 ± 230^{aB}	179.8 ± 17^{bB}
21 °C	96.5 ± 16^{aA}	1246 ± 225^{bC}	1036.6 ± 169^{bABC}	1228.3 ± 227^{bC}	1508 ± 400^{cA}	–

(———) data not available. Each data point is the mean of three replicates ± standard deviation. (*) = Values followed by the same lowercase and capital letter in each row and column, respectively, are not significantly different ($P \leq 0.05$).

the glass containers. Later, the fiber was retracted and exposed to the GC–MS injector for 3 min at 200 °C, where the thermal desorption of the VOCs was carried out (Saucedo-Lucero and Revah, 2018).

An Agilent 6850 N/VLMSD model 597B gas chromatograph coupled to a mass spectrometer (MS) (Agilent Technologies, USA) was used to identify the VOCs. This system used the NIST-05 software. Only the species with a data match quality with the mass spectral library over 85% were considered as correctly identified. The MS was operated in the scan mode, ranging from 50 to 450 mass/charge. The GC oven was fitted with an HP5 capillary column of 30 m length, 0.30 mm internal diameter and 1 mm film thickness (Agilent Technologies, USA). The oven temperature was 30 °C for 20 min, then increased to 100 °C at 25 °C min^{-1} and finally held for 10 min. The injector and the detector were kept at 200 and 220 °C, respectively. Helium was used as a gas carrier at a flow rate of 90–100 $mL{\cdot}min^{-1}$.

2.8. Statistical analysis

The CO_2 production was analyzed using a completely randomized design, and the means were compared using the Tukey test ($P \leq 0.05$). The CO_2 content was adjusted to the Gompertz model, and later the estimated CO_2 release data were used to create a response surface plot. Finally, VOCs were analyzed through PCA, using the InfoStat software (Systat Sofware Inc., CA, USA).

3. Results and discussion

3.1. Anthracnose and soft rot severity on strawberry fruit

In the study, the temperatures and time of storage affected *C. fragariae* and *R. stolonifer* development on strawberry fruit (Table 1). At 96 h (4 days) and 5 °C storage temperature, the strawberries did not show any typical symptoms of the inoculated *C. fragariae*, but, when the temperature and storage time increased, the anthracnose covered the whole fruit as it was seen after 144 h (6 days) and 312 h (13 days), while for the soft rot disease, the symptoms were visible from 24 h (1 day) storage onwards at 21 °C.

In previous studies, it was reported that *C. fragariae* development on the surface of strawberry fruit was in a range of 1–25% on day 13th while for *R. stolonifer* it was from 51 to 75% at day 3th (Ventura-Aguilar et al., 2018). On the same subject, Romanazzi et al. (2013) reported a soft rot development of 61–80% on strawberry cv. Camarosa stored for 7 days at 0 °C, followed by 3 days of shelf life at 20 °C. These results apparently differ from those found in the present study, and an explanation for this could be attributed to the different storage temperatures used.

3.2. Fungal growth measured as CO_2

In this study, the evolution of the fungal growth was evaluated through the CO_2 production and it differed depending on the inoculated fungus, the storage temperature, and the evolved time. Overall, as it can be observed in Table 2, the values of CO_2 significantly ($P \leq 0.05$) increased during incubation period at all temperatures. Specifically, after 96 h incubation, the quantification of CO_2 in fruit inoculated with *C. fragariae* was twice as higher at 5 °C than at 10 °C and 21 °C. Conversely, strawberries inoculated with *R. stolonifer* showed the CO_2 maximum release at 21 °C after 72 h with corresponding values of 2406.7 mg m^{-3}. This CO_2 production was 49% higher than at 5 °C (1218.6 mg m^{-3}) and 76% higher than at 10 °C (592.1 mg m^{-3}). By means of the CO_2 released during the infection process, it was possible to differentiate the inoculated strawberries from the non-inoculated.

Table 3
Estimated parameters from the Gompertz model to predict the production of CO_2 by *C. fragariae* and *R. stolonifer* during the infection process on strawberry fruit and stored at 5, 10 and 21 °C.

Fungi	T[a] (°C)	CO_{2max}[b] ($mg \cdot m^{-3}$)	β[c]	γ (h^{-1})	t_{max}[d] (h)	R^2
Colletotrichum fragariae	5	1723	1.3	0.02	15	0.96
	10	1810	2.3	0.01	83	0.85
	21	6137	2.2	0.01	80	0.73
Rhizopus stolonifer	5	12639	2.5	0.0017	532	0.86
	10	2159	2.1	0.01	75	0.92
	21	4289	3.9	0.03	45	0.99

R^2 = Coefficient of determination.
[a] T = storage temperature of the fruit.
[b] $CO_{2\ max}$ = the maximum release estimated of CO_2.
[c] β = the slope of the curve used to obtain the time of maximum CO_2 release rate from $t_{max} = (\ln\beta)/\gamma$, and γ ($h^{-1}$).
[d] t_{max} = time of maximum CO_2 release represents a CO_2 production rate constant.

After 48 h at 21 °C, it was observed that the strawberries inoculated with *C. fragariae* and *R. stolonifer* released higher CO_2 (35 and 27%, respectively) than the non-inoculated fruit. Conversely, at 5 and 10 °C, the inoculated strawberries had lower CO_2 production than the control group, but only after 72 h of evaluation. Specifically, the presence of *C. fragariae* in strawberry at 5 and 10 °C caused a diminishing of CO_2 level by 15 and 51% in comparison with the non-inoculated strawberries, respectively. While the strawberries inoculated with *R. stolonifer* reduced by 97 and 43% the CO_2 levels at 5 and 10 °C, respectively; compared to the fruit of the control group.

These results confirmed that the presence of fungi on strawberry modified the production of CO_2 of the sample, and therefore, it could be used as a sensitive response to the fungal activities during the emerging phase as it was reported by Zhou et al. (2016). Furthermore, Hall et al. (2010) indicated that the microorganisms themselves generate CO_2 into their microenvironment, which has the potential to impact on the organism's virulence. They observed that CO_2 released by *Candida albicans* grown in blood agar plates activated adenylyl cyclase protein, which is crucial for activating its pathogenicity. In the same way, Neethirajan et al. (2010) indicated that during the spoilage of grains by fungi an excessive respiration was quantified; since CO_2 is produced by the metabolism of both.

3.3. Modeling the CO_2 production

The Gompertz model was used to represent the CO_2 evolution obtained during the infection process. This asymmetric logistic model allows estimating growth rates and saturation parameters which can be useful in predicting storage time and hence, exerting control measures. The results showed that for *C. fragariae* the maximum growth, expressed as CO_2 production, was after 14.6 h at 5 °C, while at 10 °C and 21 °C, it was at 83 and 80 h, respectively. For *R. stolonifer* incubated at 5 °C, the calculated time was of 532 h, followed by 75 h at 10 °C and 42 h at 21 °C (Table 3).

From the Gompertz model, a set of data were generated over the range of the given incubation temperatures (5, 10 and 21 °C) and the sampling periods (1, 24, 48, 72, 96 y 144 h), and two response surface plots were obtained. The response surface plots enabled us to describe the effect of influencing factors such as the temperature and time of incubation on fungal growth that was quantified as CO_2 production. Fig. 1 (A–B) showed a different behavior between the CO_2 production of both fungi, while *C. fragariae* grew faster at low temperature, *R. stolonifer* did so at warmer conditions. However, both fungi had a similar growth at 10 °C. Therefore, at this temperature, a competition might occur for the substrate if both infect the fruit at the same time. The information obtained is important to optimize the storage and processing conditions, not only in strawberries, but also in other commodities.

Some aspects can be highlighted from the results obtained. Firstly, CO_2 was a rapid and reliable detection method of the typical *C. fragariae* and *R. stolonifer* growth on strawberry fruit during the early postharvest storage, because CO_2 is produced through the tricarboxylic acid cycle in which sugars contained in the substrates turn into energy. This process allows the fungi to carry out metabolic processes such as growth. Secondly, the use of mathematical tools such as the Gompertz

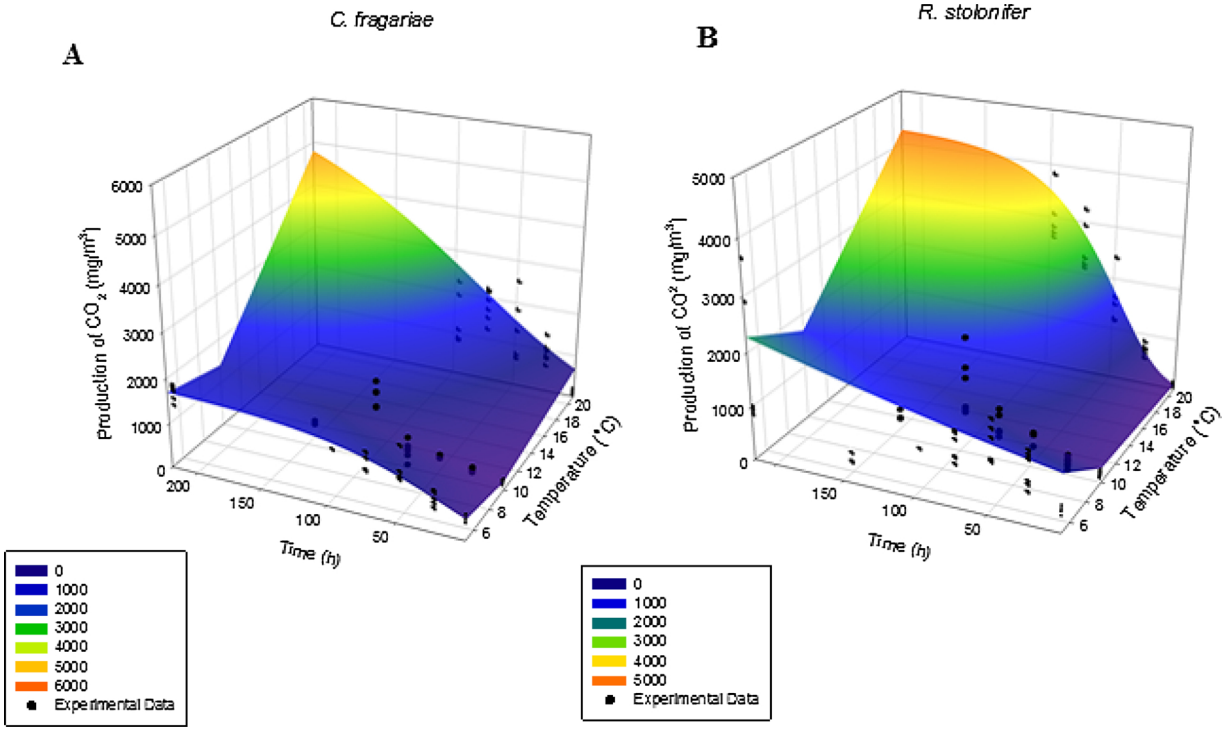

Fig. 1. Surface plot of the effect of storage temperature and incubation time on the CO_2 production of *C. fragariae* (A) and *R. stolonifer* (B).

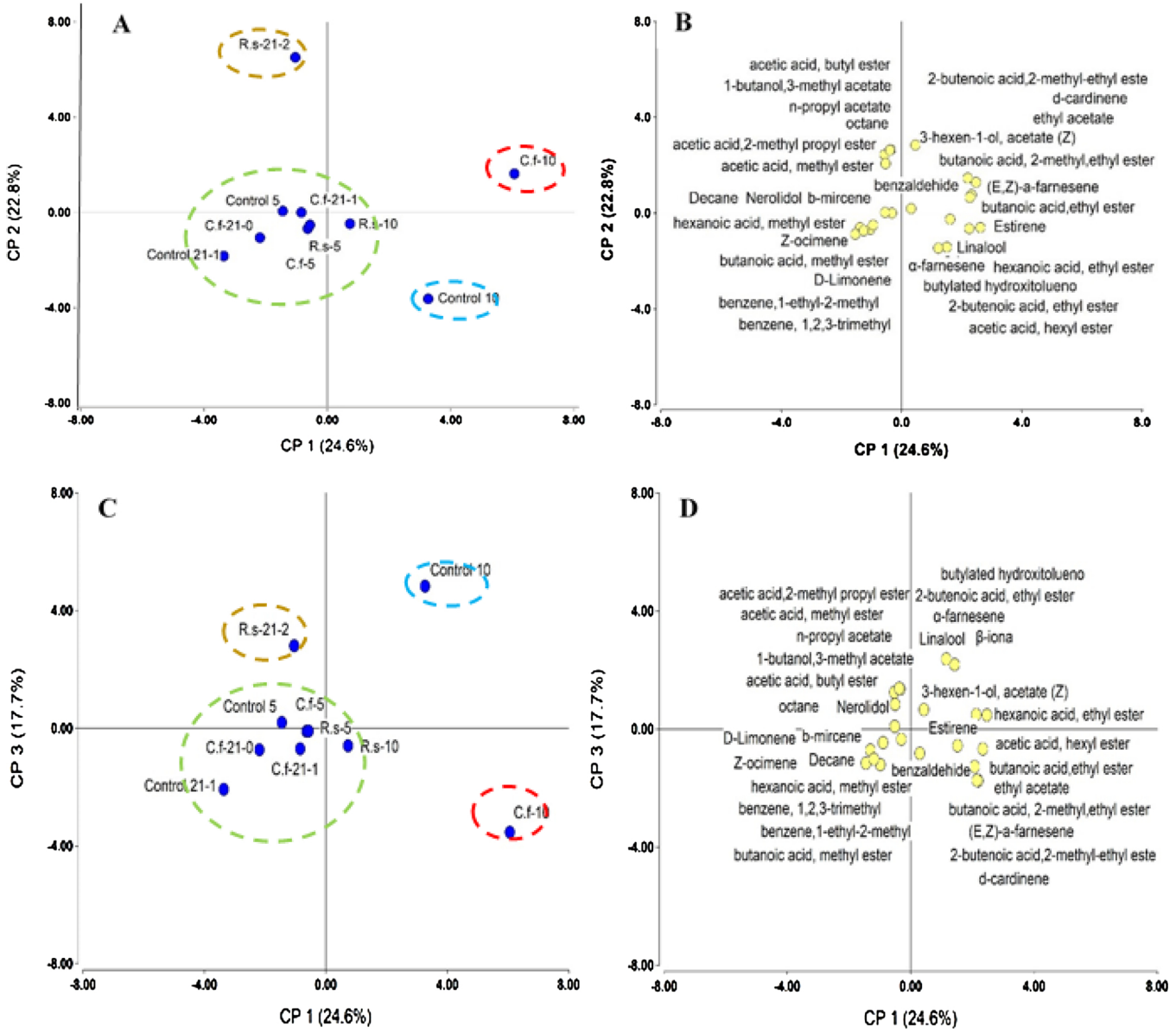

Fig. 2. Distribution of VOCs emitted by strawberries inoculated with *C. fragariae* and *R. stolonifer* and stored at 5, 10 and 21 °C (A-D), according to eigenvectors from PCA calculated for PCA 1, PCA 2 and PCA 3 (65% variance).
Cf-5, CF10, CF 21, Rs-5, Rs-10, and Rs-21, represent *C. fragariae* and *R. stolonifer* grown on strawberry at 5, 10 and 21 °C, respectively, and control group represent strawberries without inoculating. VOC was evaluated after 144 h at 5 and 10 °C, and after 96 h at 21 °C.

model has been used to explain the disease progress such as that reported by Ntahimpera et al. (1996) who estimated from this model, the development of anthracnose in different cultivars of bean, such as Ruddy, Redkloud, and Sacramento during 3 years. Finally, the Gompertz model has also been employed for modeling the sporulation dynamics of fungi as reported by Declerck et al. (2001) for *Glomus* strains grown in a well-defined nutrient agar medium. In addition, the Gompertz model has been used to estimate the content of ergosterol and the diameters of fungal colonies as growth indicators of various *Fusarium* species, *Cladosporium cladosporioides*, *A. alternata* and *A. carbonarius* (Marín et al., 2008).

3.4. PCA of VOCs in strawberries inoculated and non-inoculated with C. fragariae and R. stolonifer

From the graphical representation of the PCA (Fig. 2 A–D), it can be seen that the emission of VOCs by *C. fragariae* and *R. stolonifer* allowed the formation of different groups. Specifically, the first group of VOCs corresponded to those emitted by the strawberries inoculated with *R. stolonifer* and stored at 5 and 10 °C, and by *C. fragariae* and the control group stored at 5 and 21 °C. The second group corresponded to the strawberries stored at 10 °C. The third was formed by the VOCs of strawberries infected by *C. fragariae* at 10 °C, while in the fourth, the VOCs emitted were of the inoculated fruit with *R. stolonifer* at 21 °C. This last group represented the optimal conditions of growth for *R. stolonifer* in accordance with the results obtained in the CO_2 release (real data) and the Gompertz model. Based on the results above, the strawberries of the control group were no completely different from those infected by *C. fragariae* and *R. stolonifer*. Especially at 5 °C the host and the interaction host-fungi had a similar volatile profile. It suggests that although the fungi produced VOCs, these are not necessarily different from those produced by the fruit. Then, one of the contributions of the present investigation was that through VOCs emitted, it could be possible to suspect that any of the evaluated pathogens has infected the

Table 4
Importance of the analysis of principal component factors obtained from data of the VOCs produced by strawberry fruit inoculated and non-inoculated, and stored at 5, 10 and 21 °C.

	Eigenvalues	Proportion (%)	Cumulative (%)
PC1	7.62	25	25
PC2	7.07	23	47
PC3	5.49	18	65
PC4	4.04	13	78
PC5	3.05	10	88
PC6	1.89	6	94
PC7	1.32	4	98
PC8	0.50	2	100

strawberry fruit.

This multivariate analysis has also been used to explain the behavior of different fungi, and the quality of strawberries. For example, Khan et al. (2010) determined the geographic origin of strawberries through its VOCs production by PCA. They observed that the groups formed by the PCA were able to be correlated in terms of their content of mineral nutrients, such as potassium, iron, and calcium. Dong et al. (2013) informed that is possible to classify strawberries into different spoilage conditions using a combination of techniques such as FTIR spectra, VOCs and PCA. Similarly, Pan et al. (2014) investigated the performance of E-nose in the detection and discrimination of strawberry fruit infected by *Botrytis* sp., *Penicillium* sp., and *Rhizopus* sp. at the early storage stage. The differences in VOCs observed in the infected fruit and in the control were further analyzed by GC–MS.

On the other hand, in this present study, the four groups obtained by PCA are explained through three main components that represent at least 65% of the experimental variability (Table 4). Each of the main components correlate positively or negatively with a set of VOCs. In this sense, the principal component PC1 was positively correlated with various compounds, including among others, butanoic acid ethyl ester, ethyl butanoate, and estirene, PC2 described VOCs such as 3-hexen-1-ol acetate, acetic acid butyl ester, 1-butanol 3-methyl acetate, propyl acetate, and isoamil acetate, and PC3, included the linalool, β-iona, and farmesene (Fig. 2).

In conclusion, although the VOCs emitted by the inoculated and non-inoculated strawberries were mainly esters and terpenes, there was a difference between the compounds for each group. Strobel et al. (2008) indicated that esters of propanoic acid, 2-methyl- and butanoic acid, 2-methyl- and butanoic acid, 3-methyl- provide fruity smells. Also, 1-butanol, 3-methyl-acetate was able to inhibit completely the growth of fungi such as *Rhizoctonia solani* and *Tapesia yallundae* and oomycetes such as *Pythium ultimum*. However, it is noteworthy that in the present work, 1-butanol, 3-methyl-acetate was released by the strawberries infected with *R. stolonifer* at 21 °C. Conversely, this fungus showed the highest rate of CO_2 release in the shortest incubation time, compared to the remaining treatments. An explanation for this is that *R. stolonifer* released 1-butanol, 3-methyl-acetate that prevented the growth of others fungi. Additionally, some other VOCs such as propanoic or isobutyric can also act as fungicides (Strobel et al., 2001). Alternatively, VOCs are not only produced to inhibit fungal development, but they are also a part of the sensory quality of strawberries as it was indicated by Jetti et al. (2007). They found that the presence of esters such as benzaldehyde, nerolidol, linalool, hexanal, hexyl acetate, terpineol, 3-hexen-1-ol contribute to the fruity, fresh, and green notes of the strawberry aroma. At the same time, the terpenoids are responsible for the fruity and citrus aromas.

4. Conclusions

The CO_2 content and the VOCs released by *C. fragariae* and *R. stolonifer* on strawberry stored in the early stages at low temperature could be used to verify the sanitary conditions of the fruit i.e. the incidence of anthracnose and soft rot diseases. By adjusting the CO_2 data to the Gompertz model the growth of both fungi at different storage times can be estimated. The only tea infected strawberries with *C. fragariae* at 10 °C and *R. stolonifer* at 21 °C and the non-inoculated ones at 10 °C could be differentiated through their emitted VOCs at the end of storage.

Declaration of Competing Interest

The authors declare no potential conflict of interest.

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