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ORIGINAL ARTICLE

Changes in phenolic compounds and antioxidant capacity of Andean raspberries in response to *Peronospora sparsa*

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Abstract

In Colombia, the Andean raspberry (*Rubus glaucus* Benth) is of large economic significance because of its use in industry and widespread consumption as a fresh fruit. However, this crop is highly susceptible to disease by *Peronospora sparsa*, a fungus that causes between 50 % and 70 % production loss. Plants respond to pathogen-induced damage by increasing the production of specific secondary metabolites, such as phenolic compounds, which have broad industrial applications. This work estimated the antioxidant capacity and phenolic content of healthy and *Peronospora sparsa*-infected Andean raspberry fruits. Antioxidant capacity was analyzed by DPPH and FRAP methods, while phenolic compounds were analyzed by high performance liquid chromatography coupled to diode array detection (HPLC-DAD). According to the DPPH method, antioxidant capacity increased from 45.9 ± 1.61 μmol TE g^{-1} fresh sample in healthy fruits to 67.02 ± 0.58 μmol TE g^{-1} fresh sample in affected fruits. The FRAP method revealed an antioxidant response difference from 5.19 ± 0.8 mmol TE 100 g^{-1} fresh sample in healthy fruits vs. 10.97 ± 0.27 mmol TE 100 g^{-1} fresh sample in affected fruits. The phenolic compound content was observed in a range of 4.14 ± 1.16 to 72.03 ± 26.68 mg GAE L^{-1} for healthy fruits and from 4.48 ± 1.76 to 221.89 ± 1.18 mg GAE L^{-1} for affected fruits. Phenolic acids were the main phenols detected, encompassing derivatives of gallic acid, chlorogenic acid, ferulic acid, ellagic acid, and p-coumaric acid. This work confirmed that the *Peronospora sparsa*-infected berries contained relatively more antioxidants and phenolic acid compounds than their healthy counterparts, and that this difference was likely due to a defense mechanism to cope with pathogen-induced damage.

Keywords: Antioxidants; liquid chromatograph; pathogens; phenolic acids; *Rubus glaucus* Benth.

Introduction

Over the past four years, keen attention has been paid to the use and consumption of metabolites, antioxidants in particular, available in fruits and vegetables and their nutraceutical properties [1]. Fruit shrubs of the genus *Rubus* are a rich source of such compounds. These are synthesized in small quantities based on the phenological stage of the plant, ecosystem services, and the biotic or abiotic stress it experiences. In general, when pathogenic microorganisms are present, the defense mechanism of the plants is triggered, and the physiological and biochemical process (related to pathogenesis) may lead to protein synthesis. Production of phenolic compounds, deposition of lignin, generation of signaling compounds, and liberation of the reactive species of oxygen are also used to cope with induced stress [2-3].

The Andean raspberry, *Rubus glaucus* Benth, displays remarkable organoleptic features and is of ample economic significance in Colombian domestic and international markets. It bears diverse phytochemicals including carbohydrates, phenolic compounds, dietary fibers, vitamins, minerals, and volatile compounds [4–6]. Prominent among the Andean raspberry's phenolic compounds are the phenolic acids, e.g., gallic acid and its derivatives, caffeic acid, coumaric acid, ferulic acid derivatives, epicatechin, and ellagic acid derivatives [7]. Flavonols and anthocyanins have also been described in Andean raspberry; the former include quercetin derivatives, glucosides, and glucuronides of quercetin [7-8], and the latter include cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-xylosisutinoside, pelargonidin-3 glucoside, and pelargonidin-3-rutinoside [8-9].

In Colombia, Andean raspberry is grown in 19 departments and is considered a competitive fruit. In 2016, an 110 453-ton yield was recorded [10] indicating its economic significance. It is a sought-after fruit because of its attractive color, taste, and the alleged therapeutic value it possesses due to its high concentration of chemical compounds [11]. However, this plant is susceptible to diseases, such as hairy mildew, induced by the *Peronospora* sp.; grey mold, caused by *Botrytis cinereal*; powdery mildew, caused by the *Oidium* sp.; and anthracnose, caused by *Colletotrichum* sp. [12]. These diseases compromise fruit quality and lead to consumption decline.

In the Colombian departments of Antioquia, Caldas, Risaralda, Santander, Cauca, and Cundinamarca Andean raspberry crops are plagued by downy mildew, which causes a 50 % to 70 % production loss [13]. The affected fruits are regarded as by-products, as this microorganism halts fruit ripening, induces dehydration in the developing fruits, and causes intense

malformations or dullness of color [12]. Therefore, this study evaluated the chemical response in the fruits, particularly the antioxidant capacity and phenolic compounds synthesized in response to the biotic stress, to determine its potential as a natural source of biologically active compounds with a variety of applications.

Materials and Methods

Andean raspberry stems and fruits

The sampling was carried out in the municipalities of Santuario and Santa Rosa de Cabal, in the department of Risaralda (Colombia); the coordinates of the sampling locations are provided in **Table 1**. Fruits were collected twice per municipality using a fully randomized block design between February and May of 2017. Both healthy and *Peronospora sparsa*-affected fruits of the same maturity state were sampled. Approximately 500 g of each plant material were taken by crop.

The characteristic downy mildew symptoms were identified in the field and a morphological characterization of the fungus was subsequently conducted in the laboratory. Approximately 10 stems per crop were sampled and transferred to the laboratory for further analysis. Portions of berry stems with signs of downy mildew were inspected under a light microscope. Next, the samples were dried with CO_2 in a critical point desiccator (Sandri-780A, USA) for 40 min and placed in a copper sample holder and coated with gold in an ionizer (Ion Sputter JFC-1100, JEOL, Japan) for 1 min. Finally, the preparations were observed and photographed in a scanning electron microscope, FEI model Quanta 250 (Thermo Fisher Scientific, Japan) as described by Cardona-Hurtado *et al.* [14], and the structures observed were compared with those reported by Fierro-Corrales *et al.* [15].

Healthy Andean raspberry fruits and those affected by *Peronospora sparsa*, at maturity stages 5 and 6 (based on the NTC 4106 classification; ICONTEC, 1997) were collected in polyethylene bags. These were then packed in coolers at 4°C and transported from the sampling site to the Technological University of Pereira. They were then stored until further analysis at -70°C.

Extract collection from ripe berries

First, the harvested berries were passed through a mill; subsequently, the plant material (sorted by healthy and affected fruits) was weighed by collection site and in triplicate. The extracts were collected using acid hydrolysis with 80 % ethanol. The pH was adjusted to 2.6 by adding

Table 1. Location of the sampled Andean raspberry (*Rubus glaucus* Benth) fields.

Municipality	Sampling site	Coordinates	Altitude (m)
Santa Rosa de Cabal	1	N 04° 52' 37.2" W 075° 32' 31.1"	2244 ± 4
	2	N 04° 53' 24.1" W 075° 33' 44"	2085 ± 3
Santuario	3	N 05° 07' 25.2" W 076° 00' 01.2"	2114 ± 3
	4	N 05° 06' 45.0" W 075° 59' 54.9"	2120 ± 3

citric acid, according to Guzmán-Nieves [17]. Each sample was mixed with the solvent in a ratio of 1:8 via mechanical orbital shaking (Thermo Fisher Scientific MaxQ 4450, Canada) at 250 rpm for 2 h at room temperature, following Velićanski *et al.* [18] and Abu-Bakar *et al.* [19].

The derived extracts were filtered to remove any solid fruit particles and their pH was adjusted by adding ethanol to 10 mL. Next, the phenolic acids were extracted three times, using ethyl acetate in a ratio of 1:1 of crude extract to solvent extract at a working pressure and temperature of 84.9 kPa and 21 °C [20]. Finally, the samples were concentrated under vacuum (Heidolph Laborota 4001, Germany), and the residual solvent was removed via nitrogen flow. The extracts were then stored at 4°C until further analysis.

Evaluation of antioxidant capacity

The spectrophotometric method with DPPH (2, 2-diphenyl-1-picrilhydrazyl) was used for antioxidant capacity evaluation implementing the modified methodology by Ortiz *et al.* [4]. Using a microplate spectrophotometer (Thermo Scientific, Multiskan GO, Japan), 10 μL of the extract (diluted earlier in the ratio of DF: 20) were mixed with 200 μL of the ethanolic DPPH solution at 50.7 μM (20 mg L^{-1}). After incubating this mixture for 30 min, its

absorbance was measured at 517 nm. Then a calibration curve was made with Trolox as the reference standard. The results were expressed as micromoles of the Trolox Equivalents per gram of fresh sample weight (μMol TE g^{-1} fresh sample).

The FRAP (Ferric Reducing Antioxidant Power Assay) analysis was conducted following Calderón-Oliver *et al.* [21]. The FRAP reagent was prepared mixing a 300 mM acetate buffer solution (pH 3.6) with 10 mM TPTZ, 40 mM HCl, and 20 mM $FeCl_3$ solution (10:1:1). Using a microplate spectrophotometer, 150 μL of the FRAP were mixed with 20 μL of extract (diluted earlier in a ratio of DF: 20). After incubating this mixture for 30 min, the absorbance was measured at 593 nm. Then, a calibration curve was used with Trolox as the reference standard. Results were expressed in milligrams of Trolox Equivalents per 100 g of extract (mmol TE 100 g^{-1} fresh sample).

Evaluation of phenolic compounds by HPLC-DAD

The presence of phenolic compounds was assessed via High Performance Liquid Chromatography with diode array (HPLC-DAD), following the modified protocol by Estupiñan *et al.* [22]. This involved using a high efficiency liquid chromatography system (Jasco HPLC 2000 Plus, Japan), which possessed a quaternary gradient pump (Jasco PU-2089 Plus, Japan), autosampler (Jasco AS-2059 Plus, Japan), column oven, and a diode array detector (Jasco MD-2015 Plus, Japan). The system was controlled by the EZChrom Elite software.

The separation was done employing an ODS2 column (Spherisil 250 mm × 4.6 mm ID × 5 μm), a precolumn (Spherisil 5 mm × 4.6 mm ID × 5 μm), and an elution system either using 5 % formic acid in water (Solvent A) or 5 % formic acid in acetonitrile (Solvent B). A linear gradient was implemented, beginning with 5 % Solvent B, and increasing its concentration to 15 %, 19 %, and 20 % at 15, 17 and 25 min, respectively. The analysis run was performed with solvent B at 20 % for 30 minutes. The column was maintained at 25 °C, at a flow rate of 1 mL min^{-1}. An injection volume of 20 μL was utilized, and data were recorded between 200 and 700 nm.

Qualitative analysis of phenolic acid

Preliminary phenolic acid identification was performed contrasting the retention times of the following reference standards: 100 % gallic acid, 99.63 % protocatechuic acid, 95 % chlorogenic acid, 95 % syringic acid, 98 % p-coumaric acid, and 100 % ferulic acid, Sigma-Aldrich. These standards were assessed under conditions identical to those of the HPLC-DAD analysis

(see preceding section). Furthermore, the absorbance maxima of the peaks were determined based on the corresponding ultraviolet spectrum of the standards and the samples evaluated. To identify the compounds that were not determined by their retention times, the absorbance maxima of the samples were compared with those reported for phenolic compounds [23]. The amount of the identified compounds was inferred relative to that of gallic acid ($\geq$ 95 %), as an external reference compound. The quantities were expressed in milligrams of gallic acid equivalents per liter of sample (mg GAE L^{-1}).

Statistical analysis

The results were expressed as mean $\pm$ standard deviation and were obtained in triplicate for each of the samples from the two municipalities. The triplicates corresponded to independent extractions from each of the samples. An analysis of variance (ANOVA) was conducted using IBM SPSS Statistics Version 22 software, followed by the Tukey test. P values of < 0.05 were indicative of statistically significant differences between the amount the phenolic compounds and antioxidant capacity of healthy and affected berries. The relation between phenolic acids and the antioxidant capacity determined using the DPPH and FRAP methods were analyzed using Pearson's correlation.

Results

The antioxidant capacities of the extracts from ripe healthy and infected Andean raspberry fruits grown in the department of Risaralda, measured by DPPH and FRAP methods, are shown in **Table 2**.

The statistical analysis on antioxidant capacities revealed significant differences ($p < 0.05$) between healthy and *Peronospora sparsa*-infected berries. However, no difference was noted between sampling sites, implying that the antioxidant capacities of Andean raspberries cultivated throughout the department of Risaralda are chiefly dependent on their *Peronospora sparsa* infection status; namely, more antioxidant content was detected in affected fruits than in healthy ones. The fruits affected by *Peronospora sparsa* had antioxidant capacities surpassing those of healthy fruits by up to 50 %; 45.9 $\pm$ 1.61 vs 67.02 $\pm$ 0.58 μmol TE g^{-1} of fresh sample, in healthy and affected fruits, respectively, and 5.19 $\pm$ 0.8 vs. 10.97 $\pm$ 0.27 mmol TE 100 g^{-1} of fresh sample, in healthy and affected fruits, respectively, according to the DPPH and FRAP methods (Table 2).

Table 2. Antioxidant capacity of the Andean raspberry (*Rubus glaucus* Benth) extracts, cultivated in the department of Risaralda, measured by the DPPH and FRAP methods. The different letters of a, b, c, d, and e indicate significant differences for each method ($p < 0.05$) TE: Trolox Equivalents, N=3.

	ANTIOXIDANT CAPACITY			
Sampling site	DPPH (µmol TE g^{-1} fresh sample)		FRAP (mmol TE 100 g^{-1} fresh sample)	
	Healthy fruits[b]	Affected fruits[c]	Healthy fruits[d]	Affected fruits[e]
1[a]	53.70 ± 1.47	64.07 ± 0.97	3.76 ± 0.05	5.19 ± 0.80
2[a]	40.41 ± 3.38	63.94 ± 1.79	7.40 ± 0.02	8.64 ± 0.30
3[a]	52.73 ± 2.53	67.02 ± 0.58	8.74 ± 0.15	10.97 ± 0.27
4[a]	35.53 ± 0.49	45.94 ± 1.61	3.49 ± 0.03	5.24 ± 0.10

The chromatographic profiles shown in **Fig. 1** revealed seven peaks that were likely to correspond to phenolic compounds. The absorbance maxima of these compounds were between 255 nm to 312 nm (**Table 3**); however, it was not possible determine the identity of these compounds because their retention time did not coincide with the retention time of the standards used. Only compounds four, five, six, and seven were q uantifiable. The concentrations of phenolic acids (Table 3) were found in a range between 4.14 ± 1.16 to 72.03 ± 26.68 mg GAE L^{-1} in healthy fruits and between 4.48 ± 1.76 to 221.89 ± 1.18 mg GAE L^{-1} in affected fruits.

Correlations between antioxidant capacities and phenolic acid content were performed to gain insight into the oxidative mechanisms in the evaluated samples. The antioxidant capacity measured by DPPH and phenolic acid content in healthy and affected ripe fruits were of 29 % and 46 %, respectively. Antioxidant capacity, measured by FRAP, exhibited correlations with phenolic content in healthy and affected fruits of 90 % and 94 %, respectively (**Fig. 2**).

Discussion

One of the main characteristics of the Rubus genus is its antioxidant activity. This is elicited by the action phenolic compounds, and by an ability to remove free radicals. Concerning phenolic acids, antioxidant activity is dependent upon the number of hydroxyl groups and their locations in relation to the functional carbonyl group [24].

Table 3. Maximum absorbance, quantification, and retention times of the compounds (peaks) present in Andean raspberry (*Rubus glaucus* Benth) extracts. The maximum absorbance, retention times, and names of the standard compounds are also provided. -: Not quantifiable 1, 2, 3 and 4: Sampling sites in the department of Risaralda.

Peak		1	2	3	4	5	6	7
Retention time (t_R)		4.05	4.32	6.19	17.39	22.75	23.1	24.36
Maximum absorbance (nm)		271	227-260	252	255	354	352	355
Healthy fruits concentration (mg GAE L^{-1})	**1**	-	-	-	17.68 ± 7.60	-	4.90 ± 3.25	6.75 ± 5.03
	2	-	-	-	46.75 ± 1.09	-	4.14 ± 1.16	-
	3	-	-	-	72.03 ± 26.68	-	5.17 ± 1.14	10.27 ± 2.46
	4	-	-	-	12.73 ± 3.15	-	-	-
Affected fruits concentration (mg GAE L^{-1})	**1**	-	-	-	46.10 ± 15.39	4.87 ± 1.26	6.21 ± 1.18	7.48 ± 0.82
	2	-	-	-	116.13 ± 1.43	8.10 ± 0.20	11.92 ± 0.63	10.36 ± 0.40
	3	-	-	-	221.89 ± 1.18	19.64 ± 0.80	25.76 ± 0.84	32.91 ± 1.81
	4	-	-	-	14.86 ± 4.64	4.48 ± 1.76	6.14 ± 1.88	8.42 ± 4.42
Standard Mix								
Peak		**1**	**2**	**3**	**4**	**5**	**6**	
Retention time (tR)		4.04	6.64	12.29	15.81	19.76	22.61	
Maximum absorbance (nm)		270	257-289	248-323	273	306	247-320	
Compound name		Gallic acid	Protocatechuic acid	Chlorogenic acid	Syringic acid	p-coumaric acid	Ferulic acid	

To assess the antioxidant activity in Andean raspberry fruits, two methods (DPPH and FRAP) were employed. The DPPH method involves the reduction of the DPPH radical; this provides a rate to estimate the ability of a compound to capture radicals [25], and is based on an electron transfer reaction or hydrogen-atom abstraction as a marginal reaction pathway [26]. The FRAP method measures the ability of the compounds to reduce ferric 2,4,6-tripyridyl-s-triazine (TPTZ) by electron transfer [26-27].

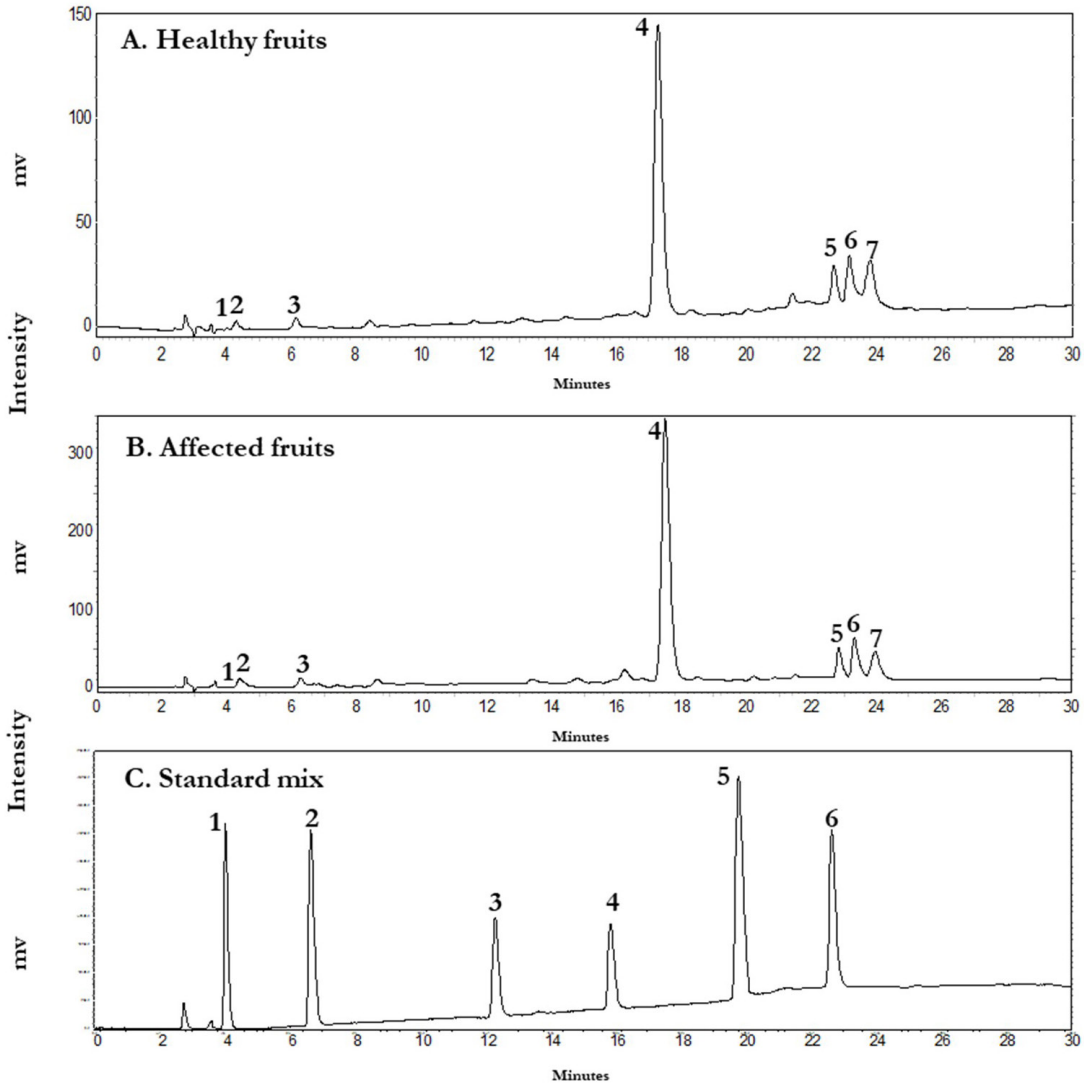

Figure 1. Chromatographic separation of the extracts drawn from the samples of ripe fruits of Andean raspberry (*Rubus glaucus* Benth) fruits affected and unaffected by *Peronospora sparsa*. Only one profile is displayed for each plant sample, as no significant differences ($p > 0.05$) were observed among sampling locations.

The antioxidant capacities of healthy Andean raspberry fruits assessed by the DPPH and FRAP methods was found in a range between 35.53 ± 0.49 to 53.70 ± 1.47 μmol TE g^{-1} and 3.49 ± 0.03 and 8.74 ± 0.15 mmol TE 100 g^{-1} fresh samples, respectively. Compared to other studies carried for the same species using the DPPH method, the values were within ranges reported by Vasco *et al.* [28] and Bernal *et al.* [29], with values around to 33.29 ± 5.56 and 41 ± 16 μmol TE g^{-1} for the fresh samples, respectively. However, our values surpass those reported by Wang and Lin [30] and Sellappan *et al.* [31] for other raspberry species, such as *Rubus fructicosus* and *Rubus idaeus*.

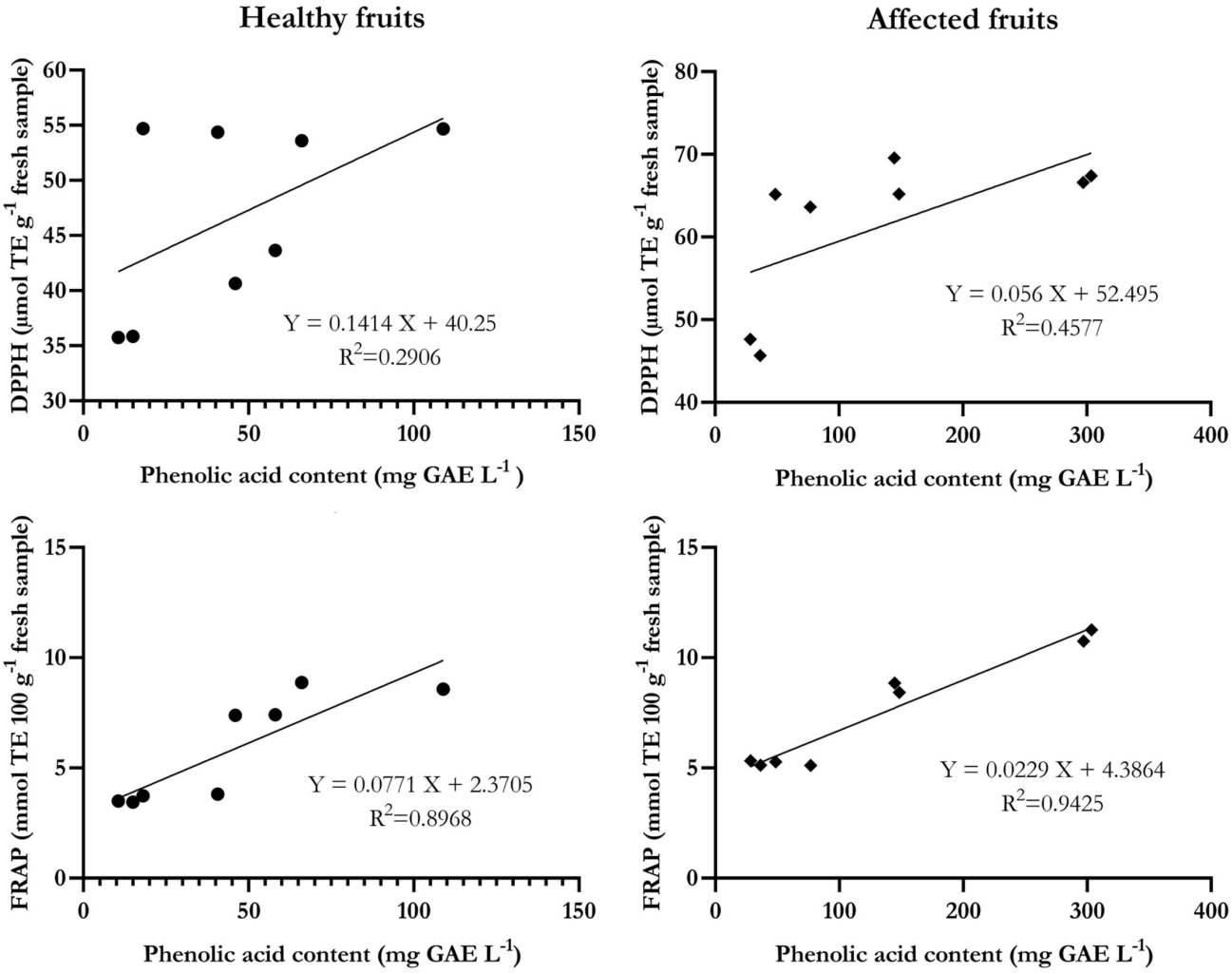

Figure 2. Correlation between phenolic acid content and antioxidant capacity of healthy and affected fruits measured by DPPH and FRAP methods.

Concerning the results obtained by the FRAP method, the antioxidant capacities for healthy Andean raspberry fruits were similar to those reported by Garzón *et al.* [32] and Vasco *et al.* [28], with values around to 4.5 and 6 mmol TE 100 g^{-1} for the fresh samples. Furthermore, this method also revealed a higher antioxidant capacity of Andean raspberry fruits affected by *Peronospora sparsa*.

This higher antioxidant capacity of infected berried could be due to phenolic compounds being accumulated by the plant as a defense mechanism against *Peronospora sparsa* [33]. These compounds offer strong protection against hostile factors. Based on the above facts, healthy plants contain these compounds in low concentrations. However, in infected plants these levels

show substantial increases to cope with the pathogenic invasion, as revealed in the synthesis of chlorogenic, caffeic, and ferulic acids, which are the types of phenolic compounds the plants accumulate as defense substances against fungal infections [34]. Mikulic-Petkovsek *et al.* [35] reported a higher concentration of phenolic content in *Rubus idaeus* infected tissue, observing values of 8 566 ± 721 mg kg^{-1} fresh weight for healthy tissue and of 21 699 ± 1 310 mg kg^{-1}fresh weight for infected tissue.

A maximum of seven compounds were present in healthy and affected Andean raspberry fruits (Fig. 1). These compounds were found in higher concentration in extracts from berries affected by *Peronospora sparsa*. According to their UV-Vis spectra, these compounds are likely phenolic acids with 255 nm to 312 nm absorbance maxima (Table 3). The comparison of the observed retention times and ultraviolet profiles of these compounds in the literature facilitated the recognition of their phenolic acid nature, which from prior studies conducted in Andean raspberry are likely derivatives of gallic acid, such as methyl gallate, ethyl gallate, propyl gallate [36]; chlorogenic acid; ferulic acid and its derivatives, ellagic acid and p-coumaric acid; as well as its derivatives such as p-cumaroyldopamine [7].

In the assessed Andean raspberry fruits, the phenolic acid concentration, estimated by HPLC, showed a strong correlation with the antioxidant capacity, assessed by the FRAP method. In contrast, the antioxidant capacity of the same berries, estimated by the DPPH method, showed little correlation with their phenolic acid content. Many studies have shown a high correlation between phenols and FRAP-based antioxidant assessments, given the hydrophilic nature of phenolic compounds. But there was a relatively low correlation with the elimination of radicals [37]. The correlation between FRAP and phenolic acids describes approximately 90 % of the oxidative phenomenon. Thus, it can be established that these compounds present in Andean raspberry are good antioxidant, performing their roles as reducing agents that react primarily through an electron transfer mechanism [38].

The antioxidant capacity of the Rubus species is mainly attributed to phenolic compounds which exhibit higher antioxidant power than carotenoids and E and C vitamins [39]. Chiefly phenolic acids were present in ripe Andean raspberry fruits affected by *Peronospora sparsa*. Finally, this work confirmed that the *Peronospora sparsa*-infected berries contained a relatively higher percentage of phenolic acid compounds than their healthy counterparts and that this difference was likely due to a defense mechanism to cope with pathogen-induced damage. Furthermore, these compounds possess valuable pharmacological, medicinal, and cosmetic properties [39].

Conclusion

This study demonstrated that the amount of the phenolic acids in Andean raspberry (*Rubus glaucus* Benth) fruits was affected by *Peronospora sparsa*, showing an increase from 72.03 ± 26.68 mg AGE L^{-1} in healthy fruits to 221.89 ± 1.18 mg GAE L^{-1} in affected fruits. This high phenolic content is directly correlated with the antioxidant capacity of the berries, evaluated by the DPPH and FRAP methods. Antioxidant capacity presented an increase from 45.9 ± 1.61 μmol TE g^{-1} fresh sample for healthy fruits to 67.02 ± 0.58 μmol TE g^{-1} fresh sample for affected fruits, according to the DPPH method, and from 5.19 ± 0.8 mmol TE 100 g^{-1} fresh sample for healthy fruits to 10.97 ± 0.27 mmol TE 100 g^{-1} fresh sample for affected fruits, with the FRAP method. Andean raspberry (*Rubus glaucus* Benth) crops are highly susceptible to attack by *Peronospora sparsa*, causing between 50 % and 70 % of production loss, with affected fruits being discarded as by-products. However, our results provide a new usage possibility of downy mildew-affected berries because of their high antioxidant capacity.

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Conflict of interest

The authors declare having no conflict of interest.

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Cambios en los compuestos fenólicos y capacidad antioxidante de las moras andinas en repuesta a *Peronospora sparsa*

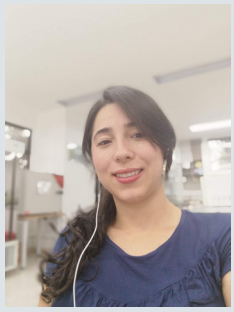
Resumen: En Colombia, la mora andina (*Rubus glaucus Benth*) es de gran importancia económica por su uso en la industria y amplio consumo como fruta fresca. Sin embargo, el cultivo es altamente susceptible a la enfermedad producida por *Peronospora sparsa*, un hongo que causa entre 50 y 70 % de pérdidas en la producción. Las plantas responden al daño inducido por el patógeno incrementando la producción de metabolitos secundarios específicos, como compuestos fenólicos, que tienen amplias aplicaciones industriales. Este trabajo estimó la capacidad antioxidante y el contenido fenólico de moras andinas sanas e infectadas por *Peronospora sparsa*. La capacidad antioxidante fue analizada por los métodos DPPH y FRAP, mientras que los compuestos fenólicos se analizaron con cromatografía liquida de alta resolución acoplada con detección de arreglo de diodos (HPLC-DAD). Según el método DPPH, la capacidad antioxidante aumentó de 45.9 ± 1.61 µmol TE g^{-1} de muestra fresca en frutos sanos a 67.02 ± 0.58 µmol TE g^{-1} en muestra fresca de frutos afectados. El método FRAP reveló una diferencia en la respuesta antioxidante de 5.19 ± 0.8 mmol TE 100 g^{-1} de muestra fresca en frutos sanos vs. 10.97 ± 0.27 mmol TE 100 g^{-1} de muestra fresca en frutos afectados. El contenido de compuestos fenólicos se observó en un rango de 4.14 ± 1.16 a 72.03 ± 26.68 mg GAE L^{-1} para frutos sanos y de 4.48 ± 1.76 a 221.89 ± 1.18 mg GAE L^{-1} para frutos afectados. Los ácidos fenólicos fueron los principales fenoles detectados, junto con derivados del ácido gálico, ácido clorogénico, ácido ferúlico, ácido elágico y ácido *p*-cumárico. Este trabajo confirmó que las moras infectadas con *Peronospora sparsa* contenían relativamente más antioxidantes y compuestos ácidos fenólicos que sus contrapartes sanas, y que esta diferencia se debió probablemente a un mecanismo de defensa para hacer frente al daño inducido por el patógeno.

Palabras clave: antioxidantes; cromatografía líquida; patógenos; ácidos fenólicos; *Rubus glaucus* Benth.

Mudancas nos compostos fenólicos e capacidade antioxidante das amoras andinas em resposta a *Peronospora sparsa*

Resumo: A amora andina (*Rubus glaucus* Benth) possui grande importância econômica em Colômbia pelo seu uso na indústria e amplo consumo como fruta fresca. Entretanto, o cultivo é altamente suscetível a doença produzida por *Peronospora sparsa*, um fungo que causa entre 50 e 70 % de perdas na produção. As plantas respondem ao dano induzido pelo patógeno aumentando a produção de metabólitos secundários específicos, como compostos fenólicos, que tem amplia aplicações industriais. Este trabalho determinou a capacidade antioxidante e o teor de compostos fenólicos de amoras andinas sadias e infectadas por *Peronospora sparsa*. A capacidade antioxidante foi analisada pelos métodos de DPPH e FRAP, e os compostos fenólicos se analisaram por Cromatografia Líquida de Alta Eficiência acoplada a detector de arranjo de diodos (HPLC-DAD). De acordo com os resultados de DPPH, a capacidade antioxidante aumentou de 45,9 ± 1,61 μmol TE g^{-1} de amostra fresca em frutos sadios a 67,02 ± 0,58 μmol TE g^{-1} em amostra fresca de frutos afetados. O método FRAP revelou uma diferença na resposta antioxidante de 5,19 ± 0,8 mmol TE 100 g^{-1} de amostra fresca em fruto sadio contra 10,97 ± 0,27 mmol TE 100 g^{-1} de amostra fresca em frutos afetados. O teor de compostos fenólicos se observou em uma faixa de 4,14 ± 1,16 a 72,03 ± 26,68 mg GAE L^{-1} para frutos sadios e de 4,48 ± 1,76 a 221,89 ± 1,18 mg GAE L^{-1} para frutos afetados. Os ácidos fenólicos foram os principais fenóis detectados, juntamente com derivados de ácido gálico, ácido clorogénico, ácido ferúlico, ácido elágico e ácido *p*-cumárico. Este trabalho confirmou que as amosras infectadas com *Peronospora sparsa* continham relativamente mais antioxidantes e compostos tipo ácidos fenólicos que suas contrapartes sadias, e que essa diferença foi devida provavelmente a um mecanismo de defesa em contra ao dano induzido pelo patógeno.

Palavras-chave: Antioxidante; cromatografia líquida; patógenos; ácidos fenólicos; Rubus glucus Benth.



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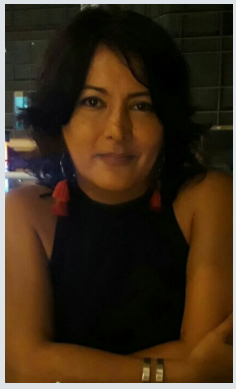

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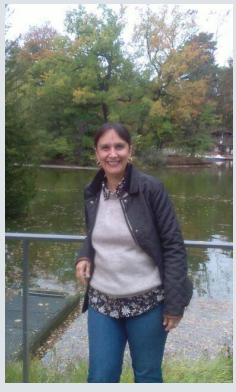

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