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RESEARCH ARTICLE

Phytochemical profile of *Capsicum* spp. fruits related to ripeness level, shading and harvest season in the Southeast of Mexico

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ABSTRACT

Secondary metabolites of *Capsicum* spp. have biological activity, which can be modified by external factors as the amount of incident light and soil water availability during crop growing, and by internal factors as fruit ripeness level. The study aim was to determine by HPLC method the phytochemical profile of *C. annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill (AMA and GAR genotypes) and *C. frutescens* L. (PIP genotype) grown under open sky and 70% shade during dry and rainy harvest season. Phytochemicals were affected by genotype, light level, harvest season, and fruit ripeness level. Phytochemicals number changed among genotypes: PIP > AMA> GAR. In immature fruits AMA (4.74 mg g^{-1}) and GAR (3.83 mg g^{-1}) had highest capsaicin content; and PIP (0.43 μg g^{-1}), AMA (0.18 μg g^{-1}) and GAR (0.14 μg g^{-1}) in lutein content in all harvest seasons and light level conditions studied. In mature fruits, PIP had the highest capsaicin (5.77 mg g^{-1}) and β-carotene (0.45 μg g^{-1}) content. Gallic and syringic acids were major constituents of phenolics acids, and quercetin and rutin for flavonoids. Mature and immature fruits from 70% shade showed the quercetin highest content (108.4-160.02 μg g^{-1}), increasing during dry season (180.9-1368.6 μg g^{-1}). Gallic acid (789.3-1076.7 μg g^{-1}) and rutin (114.0 μg g^{-1}) increased in AMA immature fruits when grown under open sky. Ferulic acid was not detected in GAR under any of the conditions studied. In AMA, ferulic, protocatechuic and *p*-hydroxybenzoic acids were detected only in rainy season fruits in both light levels. The harvest season and shading level of these *Capsicum* spp. should be considered when evaluating the biological activity of chili peppers fruits extracts in tropical crops.

Key words: Chili peppers, fruit maturity, harvest time, metabolites, shade.

INTRODUCTION

The most common *Capsicum* species used for human consumption worldwide is *C. annuum* (Kraft et al., 2014). Mesoamerica, the region to which Mexico belongs, is considered the center of origin of this species due to the large number of types of chilies found throughout the region, including *C. annuum* var. *glabriusculum* (Dunal) Heiser & Pickersgill, and *C. frutescens* L. Local populations of these species are adapted to the various environmental conditions of the region, ranging from very humid to semidesert. In Mexico, these species have been found along the entire coast of the Gulf of Mexico, from Tamaulipas to Tabasco (Ramírez-Meraz et al., 2018; Gutiérrez-Burón et al., 2020), on the shores of the Pacific Ocean and in the center of the country (Ramírez-Novoa et al., 2018).

In the humid tropical region of the Gulf of Mexico, *C. anuumm* var. *glabriusculum* and *C. frutescens* grow spontaneously in different agroecosystems, such as cocoa, coconut and banana plantations, in home gardens, and in lowland forest relics known as acahuales or mountains, where sunlight does not directly affect the chili plants and they receive less solar radiation. These species can also be found in grasslands or on the edge of rural roads, where they receive direct sunlight (Gutiérrez-Burón et al., 2020). The fruits of both species are of nutritional importance in regional gastronomy (Velázquez-Ventura et al., 2018), and contain phytochemicals (De la Cruz-Ricardez et al., 2020a), which exhibit biological activity against microbial pathogens (De la Cruz-Ricardez et al., 2020b), so they could be part of sustainable alternatives for the control of pests and diseases.

Diverse studies have shown that variations in incident solar radiation affect the composition and levels of phytochemical compounds, such as terpenes, phenolic compounds and those that contain N and sulfur (Darko et al., 2014). Such variations in the concentration of secondary metabolites in response to incident solar radiation are related to the protective function that these compounds confer on plants against exposure to said abiotic factor (Anjali et al., 2023; Chen et al., 2023). In *C. annuum* grown under conditions of high incident radiation, the content of total carotenoids in the fruits increases (Alkalai-Tuvia et al., 2014), as does that of polyphenols, flavonoids and total soluble solids (Díaz-Pérez et al., 2020). However, just like the amount of incident light influences growth (Jiménez-Leyva et al., 2022) and the content of secondary metabolites in *Capsicum*; the spectral composition also affects the concentration of these compounds. Blue light increases the content of phenolic acids and flavonoids and decreases the content of capsaicin (CAP) (Darko et al., 2022). Recently, De la Cruz-Ricardez et al. (2023) observed that the total contents of phenolic compounds, flavonoids and carotenoids in the fruits of *C. annuum* var. *glabriusculum* and *C. frutescens* increased when grown under open sky conditions.

The increase in the total content of secondary metabolites in these chili peppers grown under conditions with a greater incidence of sunlight can also modify the profile of these metabolites, as has been observed in spectral composition studies (Darko et al., 2022; Jiménez-Viveros et al., 2023). Since the fruits of *C. annuum* var. *glabriusculum* and *C. frutescens* are an important source of secondary metabolites, which can be used as alternative control of diseases due to the antimicrobial activity presented by its extracts, it is needed to elucidate whether the profile of metabolites is modified when the plants are grown under different conditions of light availability, to improve the growing conditions and to maintain or increase metabolites concentration with biological activity. Therefore, the aim of this study was to investigate the phytochemical composition of mature and immature fruits of *C. annuum* var. *glabriusculum* and *C. frutescens* grown under open sky and shade during the period of fruit production.

MATERIALS AND METHODS

Plant material, experimental site and light conditions

Ripe (red) fruits of the AMA (amashito) and GAR (garbanzo) genotypes of *Capsicum annuum* L. var. *glabriusculum* (Dunal) Heiser & Pickersgill, and the PIP (Pico paloma) genotype of *C. frutescens* L. were harvested in the morning from a chili pepper plot in the municipality of Huimanguillo (17°43'18.2" N, 93°23'10.7" W), Tabasco, Mexico. The fruits were dried at room temperature in the shade. The seeds were extracted and treated with a solution of 500 mg L^{-1} gibberellic acid (GA) for 24 h to break dormancy and promote germination. In December 2019, after the treatment with GA, the seeds were sown in germination trays using the soil of the harvest site as a substrate. In January 2020, 40-d-old seedlings were transplanted to the experimental plot located near the collection site under shade (70%) and in the open sky. To control the amount of shade, a black monofilament shade mesh with high-density polyethylene, pigmentation and UV additives, was used according to the specifications of the manufacturer (Hydro Environment, Tlalnepantla, México). To approximate the amount of solar radiation received by *Capsicum* plants that grow in agricultural plantations and/or backyard orchards, a shade mesh of 70% was chosen. Chili plants were also grown under open sky to simulate the conditions of plants that grow on roadsides and in paddocks where they receive direct solar radiation. The distance between seedlings was 1 m.

Harvesting and processing of fruits

Ripe and immature fruits were randomly harvested of the plants by treatment (16 plants grown in the shade and 16 plants under open sky) at two time points during the year: In the dry season (April to June) and in the rainy season (July to October). Whole fruits were dried at room temperature and ground in a coffee bean mill (GX410011, KRUPS, Ciudad de Mexico) until a fine powder was obtained, and later, the material was stored in the dark at 5 °C until analysis.

Phenolic acids and flavonoids extraction and quantification

For the extraction, 1 g sample with 2 mL 80% ethanol was placed in an ultrasound bath for 10 min with rest intervals of 5 min. The process was repeated three times. This was followed by centrifugation at 5000 rpm for 5 min. The supernatant was filtered on a filter (Acrodisc, Pall Corporation, New York, USA) and stored in amber vials for detection and quantification by high-performance liquid chromatography (HPLC) following Aguiñiga-Sánchez et al. (2017). For quantification, a Nucleosil 100 A° (125 × 4.0 mm) Hewlett Packard column was used in a binary system A: H_2O pH 2.5 with trifluoroacetic acid (TFA), B: acetonitrile (ACN). The flow was 1 mL min^{-1}, temperature was 30 °C, and 20 µL extract was injected. Phenolic acids were read at 254, 280 and 330 nm. The compounds used as standards were acids: Chlorogenic (ACL), ferulic (AFE), rosmarinic (ARO), protocatechuic (APR), vanillic/caffeic (AVC), 3.5-dihydroxybenzoic (A3.5DHB), gallic (AGA), *p*-cumaric (APC), sinapic (ASN), β-resorcylic (ABR), syringic (ASR) and *p*-hydroxybenzoic (APH). The flavonoids were read at 254, 280, 330, and 365 nm; standards used were rutin (RUT), morine (MOR), quercetin (QUE), catechin (CAT), hesperidin (HES), floridzin or phlorizin (FLO), naringenin (NAR), apigenin (API), kaempferol (KAE), isorhamnetin (ISO) and myricetin (MIR). The analysis time was 25 min. All standards were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The results were expressed in µg g^{-1} dry weight.

Capsaicinoids extraction and quantification

Samples (500 mg) were extracted with 5 mL HPLC grade acetonitrile and incubated in a water bath at 60 °C with shaking every 30 min for 5 h. The supernatant was filtered through an Acrodisc filter and stored in amber vials until quantification. For the quantification in HPLC (Morales-Fernández et al., 2020), a Hypersil ODS brand column of 4.0 × 125 mm with a diode array detector was used. The eluents were A (65% acetonitrile) and B (35 mM monobasic potassium phosphate). Readings were obtained at 202 and 246 nm, 28 °C, flow rate was 1.7 mL min^{-1} and the volume of injected sample was 20 µL; the analysis time was 5 min. Capsaicin (CAP) and dihydrocapsaicin (DHC) standards were purchased from Sigma-Aldrich. The results were expressed in mg g^{-1} dry weight.

Carotenoids extraction and quantification

A sample (100 mg) was homogenized in 1 mL petroleum ether:toluene (1:1). The mixture was stirred for 12 min in a shaker (Multi Reax, Heidolph, Schwabach, Germany), and then the sample was centrifuged at 5000 rpm for 5 min. Finally, the sample was decanted and stored in amber vials until use. For the HPLC analysis, a 150 × 4.6 mm 100-5 C-18 Nucleosil column was used with a diode array detector. The mobile phase consisted of A: acetonitrile:water (9:1) with 0.25% triethylamine and B: ethyl acetate with 0.25% triethylamine. The flow was 1 mL min^{-1} at 25 °C and 43 bars, and the analysis was performed by gradient at 450 nm. The injection volume of the extract was 20 µL. Lutein (LUT) and β-carotene (BCA) were used as standards and were purchased from Sigma-Aldrich.

For the quantification of each compound, the absorption spectrum and the retention time were used. Calibration curves of at least six points in the range from 0.01 to 0.25 mg mL^{-1} were generated for each compound. The results were expressed in µg g^{-1} dry weight.

Climatic data

The information on precipitation (mm) and maximum and minimum temperature (°C) as of December 2019 and 2020 was obtained from the website of the National Meteorological Service based on data from the automatic station of Paredón located 9.5 km from the experimental site. The photosynthetically active radiation (PAR, µmoles photons m^{-2} s^{-1}) incident on the canopy of the plants of the three genotypes of chili peppers grown in the shade and under open sky was measured at 10:00 h every week throughout the period of growth and production of chili plants using a PAR sensor (BQM model, Apogee Instruments, Logan, Utah, USA). The leaf temperature was also measured using an infrared thermometer (TRUPER, ciudad de México).

Statistical analysis

A randomized design with a split plot arrangement was used for the field experiment. The main plot was the light level (open sky *vs*. 70% shade), and the small plot was the chili genotypes (AMA, GAR and PIP), with four replicates. Each replicate consisted of four chili plants. The phytochemical data were analyzed by harvest season (dry or rainy). Prior to the statistical analyses, Shapiro-Wilks and Levene's test were performed to verify the assumptions of normality and homogeneity of variances, respectively. Tukey's multiple range test (α = 0.05) was used when significant differences were found. The concentrations of phenolic acids and flavonoids obtained were reported as the mean ± standard deviation by genotype, light level and maturity of the fruit. An exploratory analysis was performed using principal component analysis. All statistical analyses were performed in the software RStudio version 1.2.5033 (2009-2019) (RStudio, PBC, Boston, Massachusetts, USA).

RESULTS

Climatic conditions during growth

Table 1 shows the precipitation data (mm) and temperature (°C) from sowing (December 2019) to the last fruit harvest of the three *Capsicum* genotypes (November 2020); the lowest rainfall was recorded during the vegetative phase and the first reproductive phase (flowering and fruiting) in January to April (< 80 mm mo^{-1}); the fruits reached maturity in May, initiating the first red fruit harvest. In June, there was a new flowering (second reproductive phase), and in the following months of high rainfall, the fruiting and ripening of fruits occurred. The time of greatest rainfall was from September to November (> 450 mm mo^{-1}).

The highest temperature (36.9 °C) occurred in April, just when the first reproductive stage began and some plants presented flower abortion. During the entire crop cycle, the minimum and maximum temperatures ranged from 19.4 and 24.7 °C and from 27.9 and 36.9 °C, respectively. In open sky, the PAR incident on the plants of the three genotypes of chili peppers averaged 1738 µmol m^{-2} s^{-1}, while in 70% shade, the average was 562 µmol m^{-2} s^{-1}. In all three *Capsicum* genotypes, there was the lower leaf temperatures in January (19-22 °C) and February (17-21 °C). In March, leaf temperature started to increasing (21-27 °C), and the PIP and AMA genotypes had the highest leaf temperature in June (30-32 °C). In general, the genotypes of *C. annuum* var. *glabriusculum* under 70% shade had the lowest leaf temperature (18-30 °C) during the entire phenological cycle.

Capsaicinoids composition

The genotype effect was significant ($P < 0.05$) for both capsaicinoids and carotenoids in both harvest seasons. The Light level × Genotype (LL×G) interaction was significant ($P < 0.05$) for both capsaicinoids and carotenoids (Table 2). The CAP content was always higher than that of DHC in all the fruits analyzed (Figure 1). The AMA and GAR genotypes showed a higher content of capsaicinoids in immature fruits than in mature fruits grown both under open sky and in 70% shade; on the other hand, the PIP genotype presented a higher content of capsaicinoids in mature fruits. No differences in DHC content by season or light level were observed in immature or mature fruits. At times, the mature fruits of the two genotypes of *C. annuum* var. *glabriusculum* showed higher CAP content in open sky conditions during the dry season (Figure 1A); this was not observed in fruits harvested in the rainy season (Figure 1B).

Table 1. Climatic data during the phenological cycle of *Capsicum annuum* var. *glabriusculum* and *C. frutescens* in 2019 and 2020. T_{min}: Minimum temperature, T_m: average temperature, T_{max}: maximum temperature.

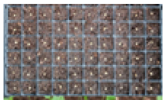
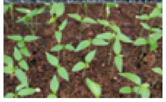
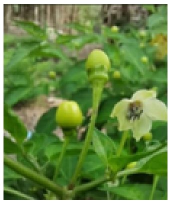
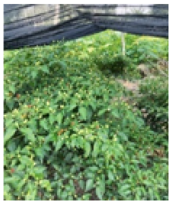
Month/Year	Rainfall	T_{min}	T_m	T_{max}	Phenological stage
	mm	°C	°C	°C	
Dec/2019	144.4	19.8	24.4	29.0	
Jan/2020	75.3	20.3	24.7	29.1	
Feb/2020	42.9	20.6	25.8	31.0	
Mar/2020	18.8	21.7	27.7	33.6	
Apr/2020	36.3	24.7	30.8	36.9	
May/2020	281.7	24.6	30.0	35.4	
Jun/2020	385.7	24.3	28.7	33.1	
Jul/2020	92.9	24.6	29.6	34.6	
Ago/2020	303.1	24.2	28.9	33.5	
Sep/2020	461.3	24.0	28.3	32.6	
Oct/2020	550.4	23.2	27.3	31.4	
Nov/2020	501.3	22.1	25.9	29.7	
Dec/2020	173.3	19.4	23.7	27.9	

Table 2. Significance levels of the ANOVA of capsaicinoid and carotenoid contents of the AMA, GAR and PIP chili genotypes grown in the shade and under open sky. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, ^{ns}Nonsignificant.

	Dry season			Rainy season		
Metabolite	Shade level (SL)	Genotype (G)	SL×G	Shade level (SL)	Genotype (G)	SL×G
Capsaicin (CAP)	ns	***	***	ns	***	*
Dihydrocapsaicin (DHC)	**	***	**	***	***	**
Lutein (LUT)	**	***	*	***	***	***
β-Carotene (BCA)	**	***	*	***	***	***

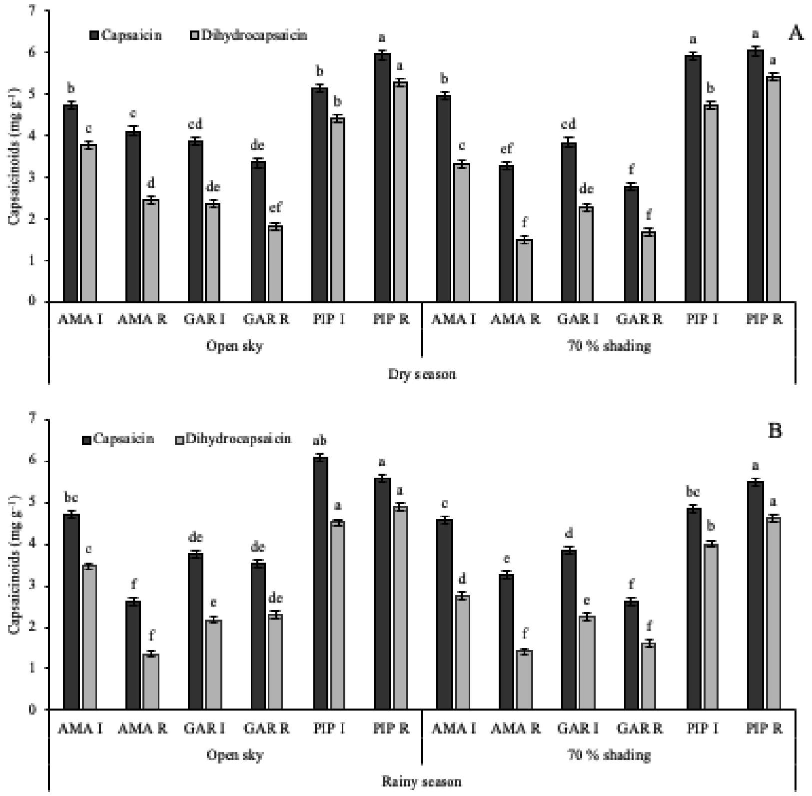


Figure. 1. Content of capsaicinoids capsaicin (CAP) and dihydrocapsaicin (DHC) in immature (I) and mature (R) fruits of the AMA, GAR and PIP chili genotypes grown in the shade and under open sky. A: Fruits collected in the dry season; B: Fruits collected in the rainy season. Different letters indicate significant differences by compound ($P \leq 0.05$). The error bars indicate the standard error.

Carotenoids composition

In immature fruits, in the three genotypes, LUT content was always higher than that of BCA in both seasons (Figure 2), and immature fruits of the PIP genotype had the highest content. The LUT content increased in the immature fruits harvested in the open sky during rainy season (Figure 2B) compared to the fruits harvested in the dry season (Figure 2A), regardless of the genotype.

The BCA increased in the ripe fruits of the three genotypes, especially in the ripe fruits of the PIP genotype (630 µg g^{-1}). This carotenoid content was affected by the time of year, and it also increased in the rainy season in mature fruits of all three genotypes (Figure 2B). Significant differences ($P < 0.05$) were observed by light level; fruits grown under open sky had the highest contents of BCA, which means that shade negatively affected the production of this metabolite in fruits of these *Capsicum* species.

Phenolic acids composition

The phytochemical profile of phenolic acids differed significantly ($P < 0.05$) by genotype, level of maturity of the fruit, light level and season (Table 3). The PIP fruits had the highest number of phenolic acids determined, both in immature and mature stages, followed by AMA and GAR. In the PIP genotype, 10 phenolic acids were detected in immature fruits and 11 in mature fruits, both under open sky and in 70% shade, in the dry season, and they decreased to 9 in the rainy season in both light conditions. In fruits of the AMA genotype, 7 phenolic acids were detected in fruit from plants grown under open sky at both maturity levels, but these were increased to 8 and 9 for immature and mature fruits grown in the shade, respectively, in the dry season. In the rainy season, the immature fruits of the AMA genotype showed increases in up to

11 phenolic acids, when grown under the open sky and in the shade. The GAR genotype exhibited the smallest number of phenolic acids: 5 and 2 for immature and ripe fruits, respectively, in both light conditions in the dry season, and 8 in immature fruits in the rainy season under both light conditions. The phenolic acids AGA and ASR had the highest content in both mature and immature fruits in the three genotypes studied (Table 3). The concentration of phenolic acids was affected significantly ($P < 0.05$) by the light level. The content of ACL and ASR was significantly increased in fruits grown in 70% shade (Table 3). Greater variations in the profile of phenolic acids between genotypes were observed in the dry season compared to the rainy season. In the dry season, in the AMA genotype of *C. annuum* var. *glabriusculum*, AFE was only found in mature fruits; in the GAR genotype, 3,5-DHB was found in immature fruit. The AMA genotype, unlike the PIP genotype, did not contain AFE and APR when grown under open sky. However, when grown in the shade, the only difference between the genotypes was the APR detected in immature fruits of AMA genotype. The AGA content increases in the dry season, due to effect of light level in the GAR and PIP genotypes in the two stages of maturity studied, while AMA was only in immature fruit. An increase in ABR acid was observed in mature fruit grown in the shade, both in the AMA and PIP genotypes. In contrast, in the rainy season, ABR acid was only present in mature fruits of the GAR and PIP genotype grown under open sky, and in the AMA genotype grown in 70% shade. The AGA content in the dry season increased in immature and mature fruit of PIP and GAR grown in the shade (Table 3). In contrast, in the AMA genotype, the increase was observed only in immature fruit grown in the shade.

In the rainy season (Table 3), AVC and APR were present in immature fruit of the AMA and GAR genotypes, when grown under open sky and in the shade. This was not observed in the dry season. The fruits of the PIP genotype did not have these phenolic acids. The APH acid was increased in immature and mature fruit of the AMA and PIP genotypes for both light levels at this time of year.

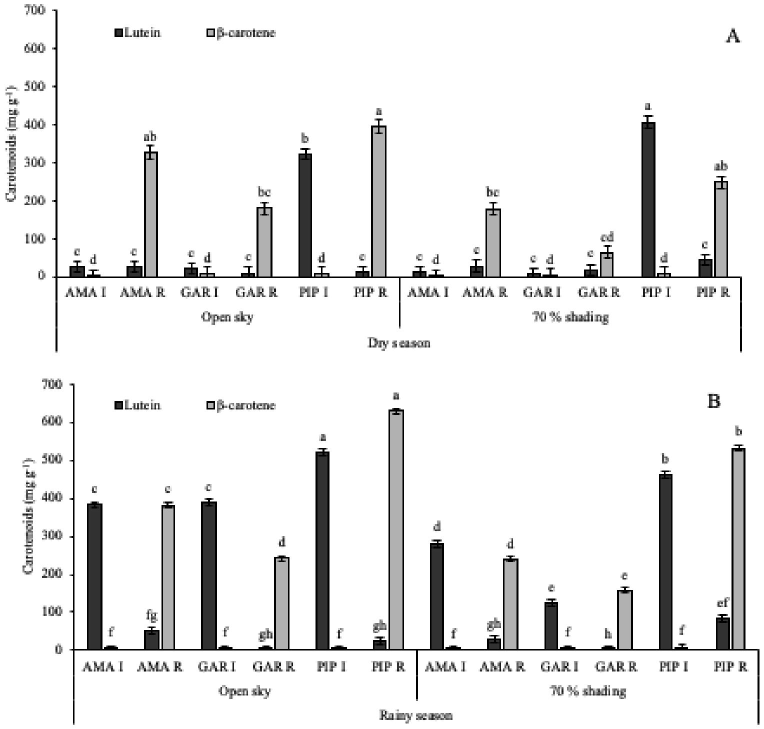


Figure. 2. Content of carotenoids lutein (LUT) and β-carotene (BCA) in immature (I) and mature (R) fruits of the AMA, GAR and PIP chili genotypes grown in the shade and under open sky. A: Fruits harvested in the dry season; B: Fruits harvested in the rainy season. Different letters indicate significant differences by compound, according to the Tukey's test ($P < 0.05$). The error bars indicate the standard error.

Table 3. Composition of phenolic acids in immature and mature fruits of three genotypes of chili peppers grown in the shade and under open sky and harvested during fruit production seasons. I: Immature fruits; R: ripe fruits; nd: Not detected. All values represent averages of four replicates ± SE.

Phenolic acids	Dry season (µg g^{-1} DW)											
	Open sky						70% shading					
	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R
Clorogenic	2.53 ± 0.06	nd	2.03 ± 0.06	nd	3.06 ± 0.17	3.42 ± 0.03	3.38 ± 0.17	3.05 ± 0.08	2.88 ± 0.17	nd	3.44 ± 0.03	3.28 ± 0.14
Ferulic	nd	10.26 ± 0.12	nd	nd	10.92 ± 0.16	10.56 ± 0.66	nd	10.92 ± 0.02	nd	nd	12.62 ± 0.26	11.37 ± 0.30
Rosmarinic	22.23 ± 1.78	22.25 ± 0.79	23.59 ± 0.78	nd	23.73 ± 1.07	44.08 ± 1.07	22.96 ± 2.08	21.15 ± 0.24	21.53 ± 0.48	nd	26.96 ± 0.73	38.94 ± 2.59
Protocatechuic	nd	nd	nd	nd	76.32 ± 0.75	71.29 ± 6.18	77.18 ± 0.69	nd	nd	nd	75.60 ± 0.23	75.33 ± 0.09
Vainillic/caffeic	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3,5-Dihydroxybenzoic	27.65 ± 1.85	26.29 ± 1.06	22.41 ± 0.12	nd	22.43 ± 0.01	22.35 ± 0.18	29.14 ± 0.09	23.91 ± 25.34	21.98 ± 0.13	nd	22.45 ± 0.09	22.42 ± 0.02
Gallic	1076.70 ± 103.25	1135.91 ± 23.38	92.11 ± 7.50	159.61 ± 18.42	605.30 ± 74.75	1133.81 ± 3.73	789.34 ± 80.92	1150.6 ± 1.79	180.95 ± 11.55	283.25 ± 37.44	799.29 ±121.72	1368.64 ± 203.04
p-Cumaric	44.4 ± 0.52	105.51 ± 4.11	nd	nd	44.95 ± 0.83	91.49 ± 8.12	55.09 ± 7.30	49.21 ± 0.48	nd	nd	47.11 ± 2.69	114.29 ± 6.24
Sinapic	25.09 ± 0.23	23.59 ± 0.14	nd	nd	25.22 ± 0.53	24.30 ± 0.10	29.27 ± 2.90	24.14 ± 0.23	nd	nd	25.43 ± 0.20	23.62 ± 0.56
p-Hydroxybenzoic	nd	nd	nd	nd	1.26 ± 0.09	0.71 ± 0.31	nd	nd	nd	nd	1.21 ± 0.10	0.79 ± 0.45
β-Resorcylic	nd	nd	nd	nd	nd	26.48 ± 5.08	nd	37.16 ± 5.19	nd	nd	nd	50.59 ± 4.91
Syringic	254.92 ± 0.72	244.89 ± 4.33	248.21 ± 0.28	259.30 ± 2.82	265.79 ± 0.47	268.71 ±1.01	265.76 ± 9.99	260.73 ± 3.58	260.71 ± 0.11	252.95 ± 0.69	266.34 ± 0.57	300.27 ± 11.55
	Rainy season (µg g^{-1} DW)											
	Open sky						70% shading					
	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R
Clorogenic	2.64 ± 0.07	2.69 ± 0.53	2.53 ± 0.45	2.19 ± 0.53	3.06 ± 0.02	3.35 ± 0.16	2.44 ± 0.07	3.42 ± 0.41	2.31 ± 0.35	2.92 ± 0.04	3.34 ± 0.03	3.63 ±0.08
Ferulic	11.08 ± 0.02	10.42 ± 0.09	nd	nd	11.91 ± 0.13	10.79 ± 0.81	13.16 ± 1.11	11.63 ± 0.50	nd	nd	13.66 ± 0.16	13.83 ± 0.53
Rosmarinic	20.73 ± 0.70	20.96 ± 1.12	22.88 ± 0.59	21.98 ± 1.36	26.40 ± 4.34	31.81 ± 2.12	25.32 ± 0.98	24.27 ± 0.42	21.73 ± 0.20	21.83 ± 0.46	26.87 ± 1.72	35.42 ± 0.29
Protocatechuic	81.31 ± 0.55	nd	75.59 ± 0.14	nd	nd	nd	82.87 ± 0.55	nd	75.67 ± 0.19	nd	nd	nd
Vanillic/caffeic	0.43 ± 0.25	nd	2.79 ± 1.05	nd	nd	nd	0.77 ± 0.39	nd	1.64 ± 0.22	nd	nd	nd
3,5-Dihydroxybenzoic	22.04 ± 0.17	26.93 ± 0.02	24.84 ± 0.57	nd	22.03 ± 0.01	22.40 ± 0.47	24.89 ± 1.17	24.56 ± 0.68	24.61 ± 0.21	nd d	22.43 ± 0.60	22.21 ± 0.58
Gallic	184.84 ± 41.68	1284.04 ± 7.31	142.72 ± 19.45	163.89 ± 5.77	644.20 ± 19.51	1157.31 ± 46.53	212.82 ± 11.64	1194.36 ± 69.25	128.98 ± 2.63	253.57 ± 19.78	787.82 ± 30.94	1190.12 ± 95.48
p-Cumaric	66.93 ± 12.24	44.12 ± 0.06	nd	nd	57.55 ± 7.07	50.82 ± 0.79	40.14 ± 1.14	48.31 ± 4.30	nd	nd	46.40 ± 0.32	52.64 ± 1.62
Sinapic	25.03 ± 0.08	24.08 ± 0.14	26.55 ± 0.19	nd	25.22 ± 0.02	24.75 ± 0.06	27.71 ± 2.27	24.25 ± 1.64	25.86 ± 0.74	nd	25.27 ± 0.36	24.60 ± 0.17
p-Hydroxybenzoic	0.72 ± 0.09	0.83 ± 0.27	nd	nd	0.60 ± 0.04	0.87 ± 0.13	1.18 ± 0.16	1.62 ± 0.15	nd	nd	0.69 ± 0.03	1.13 ± 0.16
β-Resorcylic	nd	nd	nd	42.08 ± 5.51	nd	nd	nd	31.79 ± 5.95	nd	nd	nd	nd
Syringic	253.95 ± 0.07	249.60 ± 0.46	253.15 ±7.07	251.15 ± 0.98	265.90 ± 3.09	271.76 ± 13.82	267.73 ± 7.42	259.91 ± 6.99	256.91 ± 7.31	255.76 ± 0.86	277.05 ± 3.87	293.75 ± 0.77

Flavonoids composition

The number of flavonoids detected in the fruits varied by genotype, level of maturity, light level, and season (Table 4). Immature fruit of the AMA genotype had nine flavonoids when grown under open sky in the dry season and only six flavonoids were found in mature fruit grown under the same conditions. Approximately eight flavonoids were found in the PIP genotype in both seasons and under both light conditions. The GAR phenotype had the lowest number (four and one, in immature and mature fruit, respectively) in the dry season under both light conditions; the number increased to eight (immature) and seven (mature) in the rainy season under both light conditions. The major flavonoid in the three genotypes under study in both seasons was QUE. Under open sky conditions, MIR, KAE and RUT increased in fruit of the three genotypes under study. The AMA and GAR genotypes exhibited the greatest variation in the flavonoid profile in immature and mature fruit, mainly harvested in the dry season; KAE and RUT were higher in immature fruit of the AMA genotype grown under open sky. The contents of MIR, ISO, API and MOR were reduced, and the contents of QUE, CAT and FLO were increased in fruit grown in 70% shade. In the rainy season, the contents of MIR and RUT increased in the fruit of all the genotypes under study (Table 4).

Table 4. Composition of flavonoids in ripe and immature fruits of three genotypes of chili peppers grown in the shade and under open sky and harvested in the dry and rainy seasons. I: Immature fruits; R: ripe fruits; nd: not detected. All values represent averages of four replicates ± SE.

	Dry season (μg g^{-1} DW)											
	Open sky						70% shading					
Flavonoid	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R
Myricetin	50.72 ± 1.25	nd	2.03 ± 0.06	nd	3.06 ± 0.17	3.42 ± 0.03	3.38 ± 0.17	3.05 ± 0.08	2.88 ± 0.17	nd	3.44 ± 0.03	3.28 ± 0.14
Kaempferol	85.34 ± 0.27	10.26 ± 0.12	nd	nd	10.92 ± 0.16	10.56 ± 0.66	nd	10.92 ± 0.02	nd	nd	12.62 ± 0.26	11.37 ± 0.30
Isorhamnetin	39.78 ± 0.07	22.25 ± 0.79	23.59 ± 0.78	nd	23.73 ± 1.07	44.08 ± 1.07	22.96 ± 2.08	21.15 ± 0.24	21.53 ± 0.48	nd	26.96 ± 0.73	38.94 ± 2.59
Apigenine	84.24 ± 0.02	nd	nd	nd	76.32 ± 0.75	71.29 ± 6.18	77.18 ± 0.69	nd	nd	nd	75.60 ± 0.23	75.33 ± 0.09
Rutin	114.07 ± 7.74	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Morin	48.14 ± 6.09	26.29 ± 1.06	22.41 ± 0.12	nd	22.43 ± 0.01	22.35 ± 0.18	29.14 ± 0.09	23.91 ± 25.34	21.98 ± 0.13	nd	22.45 ± 0.09	22.42 ± 0.02
Quercetin	95.18 ± 1.58	1135.91 ± 23.38	92.11 ± 7.50	159.61 ± 18.42	605.30 ± 74.75	1133.81 ± 3.73	789.34 ± 80.92	1150.6 ± 1.79	180.95 ± 11.55	283.25 ± 37.44	799.29 ±121.72	1368.64 ± 203.04
Catechin	19.46 ± 11.36	105.51 ± 4.11	nd	nd	44.95 ± 0.83	91.49 ± 8.12	55.09 ± 7.30	49.21 ± 0.48	nd	nd	47.11 ± 2.69	114.29 ± 6.24
Floridzine	8.19 ± 1.61	23.59 ± 0.14	nd	nd	25.22 ± 0.53	24.30 ± 0.10	29.27 ± 2.90	24.14 ± 0.23	nd	nd	25.43 ± 0.20	23.62 ± 0.56
	Rainy season (μg g^{-1} DW)											
	Open sky						70% shading					
	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R	AMA I	AMA R	GAR I	GAR R	PIP I	PIP R
Myricetin	49.38 ± 0.34	49.36 ± 0.13	50.47 ± 1.12	52.45 ± 2.57	49.39 ± 0.30	49.81 ± 0.68	51.65 ± 1.48	47.53 ± 0.40	49.28 ± 0.17	51.56 ± 1.08	50.97 ± 0.24	50.01 ± 2.01
Kaempferol	84.62 ± 0.57	89.09 ± 0.06	81.07 ± 0.22	85.59 ± 0.07	81.67 ± 0.46	80.73 ± 0.34	88.06 ± 2.10	87.18 ± 0.35	84.51 ± 1.75	83.73 ± 0.13	81.37 ± 0.52	80.36 ± 0.09
Isorhamnetin	38.82 ± 1.02	38.05 ± 0.03	33.42 ± 0.03	33.60 ± 0.31	38.03 ± 0.02	37.47 ± 0.83	40.48 ± 3.48	39.76 ± 0.04	36.33 ± 3.69	33.38 ± 0.58	37.97 ± 0.13	38.01 ± 0.23
Apigenine	84.37 ± 0.45	87.30 ± 0.35	88.98 ± 0.88	100.97 ± 0.82	86.96 ± 0.29	84.32 ± 0.01	88.92 ± 1.01	89.85 ± 0.04	90.91 ± 0.20	107.75 ± 2.39	89.62 ± 0.09	87.87 ± 0.67
Rutin	101.31 ± 1.04	103.30 ± 1.92	92.06 ± 4.80	107.41 ± 2.03	115.22 ± 1.01	154.70 ± 1.93	134.34 ± 21.84	119.46 ± 5.73	112.85 ± 6.27	115.60 ± 3.15	141.62 ±5.01	163.40 ± 1.26
Morin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin	133.63 ± 16.72	164.73 ± 29.06	126.13 ± 14.59	137.73 ± 3.60	117.15 ± 11.74	128.20 ± 19.93	116.91 ±2.79	160.02 ± 0.92	108.41 ± 2.08	152.52 ± 0.22	116.09 ± 4.78	130.81 ± 14.64
Catechin	23.51 ± 5.64	nd	14.67 ± 4.67	nd	10.21 ± 2.72	nd	24.12 ± 1.16	nd	5.64 ± 1.94	nd	15.70 ± 5.55	nd
Floridzine	7.29 ± 0.66	1.20 ± 0.44	20.34 ± 2.98	8.03 ± 2.94	21.33 ± 1.89	3.52 ± 0.85	34.12 ± 12.46	1.62 ± 0.58	15.62 ± 1.15	11.27 ± 1.34	4.12 ± 0.02	5.25 ± 0.68

Exploratory analysis

The distribution of secondary metabolites of *Capsicum* fruits differed depending on genotype, level of maturity, harvest season and light level, as seen in the results of the principal component analysis (PCA) (Figure 3). Genotype (Figure 3A) and the level of maturity (Figure 3C) had the greatest influence on the spatial separation of the phytochemicals analyzed. The PCA showed that the first component (PC1) and second component (PC2) represented 32.7% and 15.4% of the total variance, respectively. The percent of dispersion due to genotype (Figure 3A) shows that in PC1, phenolic acids (ACL, AFE, APR, APH, ARO, AGA, APC and ABR) contributed to the variation positively and to a greater degree, followed by the flavonoids QUE and RUT and the carotenoid BCA. These were abundantly present in the PIP genotype. The phenolic acid AVC and the flavonoid API, which characterize the GAR genotype, contributed negatively. In PC2, the flavonoids KAE, CAT, MOR and FLO positively contributed to the variation, and MIR negatively contributed. The GAR and PIP genotypes showed the greatest dispersion of their compounds.

In the case of the level of maturity of the fruits (Figure 3C), the PCA shows a spatial separation between immature and ripe fruits. The PC1 showed a positive contribution from the phenolic acids ACL, AFE, ARO, APC, ASN, APH and ASR, from the flavonoid RUT and from the capsaicinoids CAP and DHC, and a negative contribution from the flavonoid API. For PC2, the positive contribution was from phenolic acids APR and the flavonoids MOR, CAT and FLO, and the greater negative contribution was from the phenolic acid ABR, the flavonoid QUE and the carotenoid BCA. Ripe fruits were separated on the basis of a positive contribution in PC1 from the phenolic acids ABR and AGA, the flavonoid QUE and the carotenoid BCA and by a greater negative contribution from the flavonoid API.

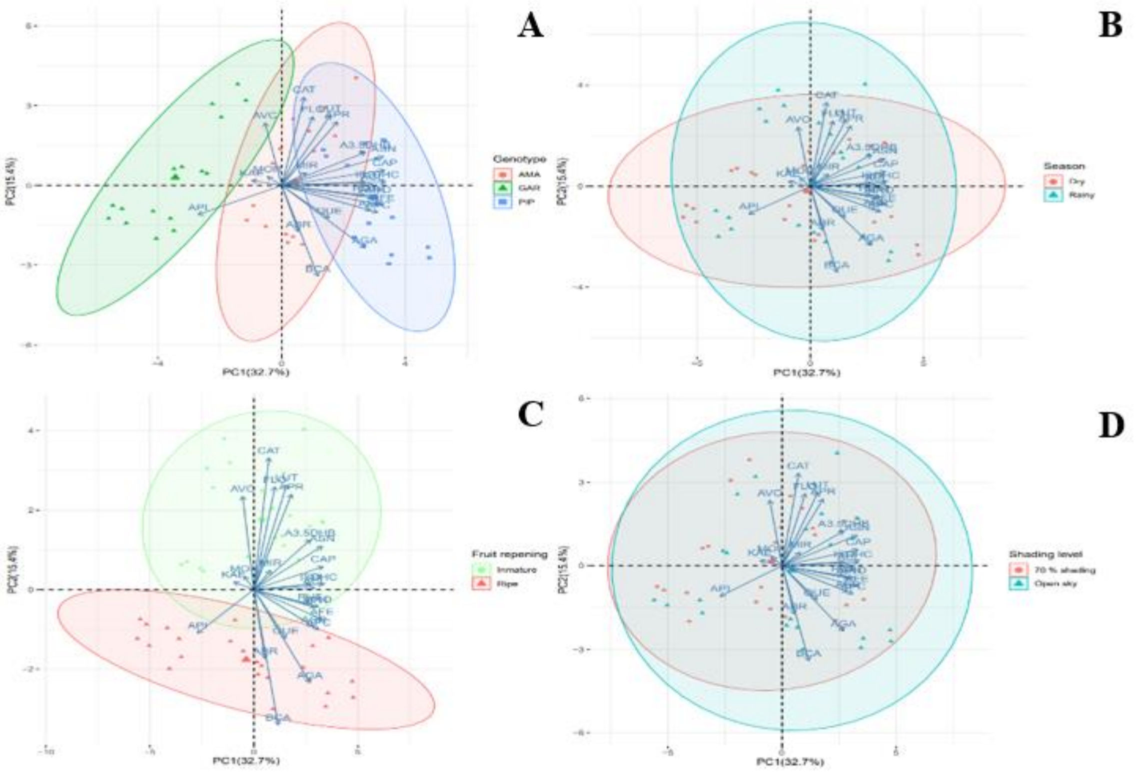


Figure 3. Principal component analysis (PCA) of carotenoids, capsaicinoids, phenolic acids and flavonoids in *Capsicum* spp. A: Effect of genotype; B: effect of season; C: effect of level of maturity; D: effect of the light level. AMA: Amashito genotype, GAR: garbanzo genotype, PIP: pico paloma genotype. Phenolic acids: ACL chlorogenic acid, AFE ferulic acid, ARO rosmarinic acid, APR protocatechuic acid, AVC vanillic/caffeic, A3.5DHB 3,5-dihydroxybenzoic acid, AGA gallic acid, APC *p*-cumaric acid, ASN synapic acid, APH *p*-hydroxybenzoic acid, APR β-resorcylic acid, ASR syringic acid. Flavonoids: MIR myricetin, KAE kaempferol, ISO isorhamnetin, API apigenin, RUT rutin, MOR morin, QUE quercetin, CAT catechin, FLO floridzine. Carotenoids: LUT lutein, BCA β-carotene. Capsaicinoids: CAP capsaicin, DHC dihydrocapsaicin.

DISCUSSION

One of the defense mechanisms that plants have in order to protect themselves against the stresses produced by environmental changes is the production of secondary metabolites (Lee et al., 2018). Environmental changes will occur more frequently and with greater intensity in the world (Bhargawa and Singh, 2019), and can modify metabolites composition in the plants and their biological activity, which would have repercussions for the use of plant extracts for agricultural or food consumption uses.

In the genotypes of chili peppers studied, phenolic acids, AGA and ASR were the compounds with the greatest presence in the fruits, and in the case of flavonoids, they were QUE and RUT, with contents close to those shown in other studies (Moreno-Ramírez et al., 2018). The AGA and RUT were affected by the light level and season in the AMA genotype; when grown under open sky and in the dry season, its concentration increased in immature fruits. The increase observed at higher incident radiation may be due, on the one hand, to the fact that both phenolic acids and flavonoids are derived from the shikimate pathway with phenylalanine or tyrosine as intermediates, where the key enzyme of the phenylpropanoid pathway is phenylalanine ammonium lyase (PAL), which is highly sensitive to the quantity and quality of incident radiation on plants (Da Costa et al., 2020). On the other hand, in immature (green) fruits, one of the main functions of phenolic and flavonoid compounds is the photoprotection of the cells involved in the photosynthetic process to avoid oxidative damage, so that the variation in their content can be affected by incident solar radiation and the prevailing climatic conditions during the development of fruits in the plants (Meckelmann et al., 2015; Lekala et al., 2019; Dobón-Suárez et al., 2021).

Differences in the number and content of phenolic acids were observed between genotypes and seasons. Kim et al. (2021) also observed variation in the content phytochemicals in 11 varieties of chili peppers grown at two sites during 2016, 2017 and 2018, where the contents of all compounds were lower in 2016 than in 2017 and 2018.

Phenolic acids such as AGA, AFE, APR, APH and hydroxycinnamic have been shown to have antimicrobial effects against the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Listeria monocytogenes*, where they produce irreversible changes in the permeability and physicochemical properties of the cell membrane of these microorganisms (Borges et al., 2013). In the fruits of the chili peppers studied, the GAR genotype of *C. annuum* var. *glabriusculum* did not contain AFE under any of the conditions studied. In the AMA genotype, AFE, APR and APH were present in fruit grown in the rainy seasons in both light levels. These genotypes of *C. annuum* var. *glabriusculum* have been shown to have an antifungal effect against cocoa pathogens (De la Cruz Ricardez et al., 2020b), so the variations observed in the phytochemical profile based on light level could affect the biological activity, depending on the time of fruit harvest. So, rainy season seems the best period to harvest immature fruits of these genotypes, this season has the highest fruit yield in the study region (De la Cruz-Ricardez et al., 2023).

In the case of flavonoids, compounds that function mainly as antioxidants, they varied by the level of maturity of the fruits, the genotype and harvest season rather than by the shading conditions of the chili peppers. The QUE was the flavonoid with the highest concentration found in both mature and immature fruit grown in the shade, and it increased in the dry season in 70% shade in the three genotypes studied. These increases in the dry season, where high temperatures are frequently accompanied by a lack of water, induce the synthesis of osmoprotective compounds such as secondary metabolites to trap free radicals in cells produced by stress (Moomin et al., 2023). The QUE has also been observed as the major flavonoid in ripe fruits of 14 varieties of *Capsicum*, including 12 of *C. annuum*, one of *C. chinense*, and one of *C. baccatum*, while the MIR, API and KAE contribute to a lesser extent in both stages of maturation (Ribes-Moya et al., 2020).

In this study, the fruits developed under conditions of greater solar radiation (open sky) had a higher content of the BCA carotenoid, one of the major carotenoids found in *Capsicum* fruits (Mohd et al., 2019). The BCA content in the AMA and GAR genotypes was within that determined for *C. annuum*, which ranged from 0.3348 to 254.7 µg g^{-1} (Kim et al., 2021), while in *C. frutescens* the content was twice as high. In immature fruits, LUT, a xanthophyll, was the most abundant carotenoid among the species under study. This carotenoid is reported as the most abundant carotenoid in the green stage (Mohd et al., 2019). The starting compound for the biosynthesis of plant carotenoids is geranylgeranyl pyrophosphate (GGPP). This compound is converted through several reactions into phytoene and lycopene. From here, two routes lead to the biosynthesis of α-carotene or BCA. In immature fruits, the formation of α-carotene leads to the formation of LUT, and as ripening progresses, this step is reduced, increasing the cyclization of lycopene to form BCA in mature fruits (Mohd et al., 2019).

In *Capsicum* fruits, carotenoids are positively correlated with capsaicinoids (Giuffrida et al., 2014), which are composed of a typical molecular structure of alkaloids and are responsible for the characteristic hotness of these fruits. The proportions of CAP and DHC are not similar in all species, it depends on the cultivar, degree of maturity, and environmental factors (Giuffrida et al., 2014, Díaz-Sánchez et al., 2021). In the species under study, the content of capsaicinoids showed significant differences based on genotype. The PIP fruits showed the highest content, followed by AMA and GAR; in addition, immature fruits showed the highest content of capsaicinoids, which is consistent with previous studies for these genotypes (De la Cruz-Ricardez et al., 2020a), although there is variation among landraces of piquín pepper in the concentration of CAP during fruit ripening (Díaz-Sánchez et al., 2021). The higher concentration of CAP in immature fruits could be mainly due to the stage in which capsaicinoid biosynthesis begins in *Capsicum* fruits, according to Vázquez-Espinosa et al. (2020), a peak of concentration of these compounds occurs at 42 d after flowering, remaining until 60 d, when the fruits are in the immature stage, which is the period in which the content is greater. After this phase, the metabolism of the plant changes, and the decrease in capsaicinoids begins.

CONCLUSIONS

The phytochemical composition of the genotypes of *Capsicum annuum* var. *glabriusculum* (AMA and GAR) and *C. frutescens* (PIP) is affected by the genotype, the light level in which the fruits developed, harvest season and level of maturity of the fruit. Capsaicin is the major capsaicinoid in these genotypes, with the highest content in the rainy season under open sky conditions. Of the carotenoids analyzed, lutein showed the highest content in immature fruit, while β-carotene was in ripe fruits. Gallic and syringic acids were the phenolic acids with the highest concentration in the fruits of the genotypes studied, while the major flavonoids were quercetin and rutin. The concentration of these compounds by genotype depended on the light level, where syringic acid and rutin increased their content in fruits grown in 70% shade during rainy season. Among the genotypes, PIP presented the highest number of phytochemicals analyzed, followed by AMA and GAR, with contents differing by harvest season and light level, which must be considered in the study of the biological activity of plant extracts from these *Capsicum* species.

Author contribution

Conceptualization: D.delaC-R., L.C.L-E. Field experimentation: D.delaC-R., L.C.L-E. Data acquisition: D.delaC-R., L.C.L-E. Writing and editing: D.delaC-R., L.C.L-E. Supervision of phytochemical analysis and critical discussion of the results: R.M.S-H, E.H-N. Critical data discussion and drafts review: C.F.O-G. Analysis and discussion of datasets: R.G.A-P. All co-authors reviewed the final version and approved the manuscript before submission.

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