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Colored Shading Nets Differentially Affect the Phytochemical Profile, Antioxidant Capacity, and Fruit Quality of Piquin Peppers (*Capsicum annuum* L. var. *glabriusculum*)

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Abstract: Piquin pepper fruits, a semi-domesticated wild pepper species highly valued in Mexico, currently face the threat of unsustainable harvesting practices that endanger the species. For this reason, it is necessary to establish sustainable agricultural practices for the cultivation of these peppers. Solar radiation, a critical determinant in crop production, plays a crucial role in plant development, influencing a spectrum of physiological and morphological processes, including the synthesis of phytochemicals. Our study evaluated the effect of light manipulation through colored shading nets on the phytochemical profile, antioxidant capacity, and fruit quality of semi-domesticated piquin peppers at two maturation stages: immature and mature (green and red fruits). Our hypothesis posits that these shading treatments may induce changes in these fruits' phytochemical composition and antioxidant properties, as well as quality. Our results indicate that the shading treatments and maturity stage have significant on capsaicinoid and carotenoid levels, with the highest levels observed in mature fruits. Notably, red fruits grown under black shading treatments resulted in the highest capsaicinoid levels. Carotenoid levels were higher in the black shading treatment during the first cycle, while in the second cycle, the blue shading treatment showed elevated carotenoid levels, suggesting that high irradiance conditions could reduce carotenoid contents. Although no significant differences were observed among the treatments in green fruits, in red fruits, both black and blue treatments exhibited the highest total phenolic compounds in both production cycles. Furthermore, the antioxidant capacity revealed that red fruits exhibited higher antioxidant levels than green fruits. Color analysis showed that red fruits had higher chroma and hue angle values, indicating their brighter and more intense red color than green fruits. The morphological changes in fruit width, length, and weight can be attributed to shading treatments and maturation stages. These results indicate the potential of piquin peppers to act as rich sources of bioactive compounds, emphasizing the benefits of shading as an effective strategy to improve the quality and quantity of phytochemical compounds in piquin peppers. Our findings provide substantial insights into the intricate relationship between maturation, shading treatments, and phytochemical composition, offering a path to improve the nutritional value and quality of piquin peppers.

Keywords: capsaicinoids; carotenoids; light quality; plant secondary metabolites; phenolic compounds; pungency; shading nets; solar radiation; wild peppers

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1. Introduction

Piquin pepper (*Capsicum annuum* L. var. *glabriusculum)*, also known as *chiltepín*, is a semi-domesticated pepper widely distributed on the American continent. In northern Mexico, piquin peppers are considered to be of high commercial value [1] and are appreciated for their flavor, aromatic profile, and high pungency [2]. Currently, most piquin peppers are collected from wild specimens, which has drastically reduced the populations of wild pepper plants. [3] The Mexican states reported to have the highest production of piquin peppers include Nuevo León, Tamaulipas, and Coahuila in northern Mexico (Figure 1) [4].

Figure 1. Main growing regions of piquin pepper (*Capsicum annuum* L. var. *glabriusculum*) in Mexico, and location of the experimental site: CAETEC experimental station of Tecnologico de Monterrey in Pedro Escobedo, Querétaro, Mexico (20.535169 N, −100.211472 W).

Piquin peppers have a phytochemical profile associated with health benefits, including analgesic, anti-inflammatory, and anti-cancer properties, among others [5]. The bioactive compounds related to these health benefits include carotenoids, flavonoids, and phenolic compounds. These phytochemicals are biosynthesized by distinct cellular and physiological mechanisms that occur during regular fruit development and maturation. The level of accumulation is regulated by internal signals (including plant hormones) and external factors [6].

The accumulation of phytochemicals in peppers involves complex cellular and physiological mechanisms. Capsaicinoids, responsible for the pungency of peppers, are biosynthesized within the placental epidermis cells, where they are secreted to the outer cell wall, and then accumulate within specialized structures known as "blisters" [7]. Capsaicinoid biosynthesis involves enzymes such as capsaicin synthase and fatty acid synthase, stemming from the phenylpropanoid pathway. The regulation of capsaicinoid production can be mediated by hormonal signals like jasmonic acid and may also be influenced by environmental factors such as temperature and light exposure [8,9].

On the other hand, carotenoids, which contribute to the color and nutritional value of peppers, play a pivotal role as pigments and antioxidants. They are synthesized in the plastids (chromoplasts) of fruit cells using isopentenyl pyrophosphate (IPP) as a precursor, which is generated from the methylerythritol-4-phosphate (MET) pathway. Enzymes such as phytoene synthase and phytoene desaturase are involved in carotenoid biosynthesis and accumulation [10]. At the cellular level, carotenoids are crucial for capturing and transferring light energy in photosynthesis. The accumulation of carotenoids can be affected by environmental factors, including light exposure and temperature fluctuations [11].

Finally, phenolic compounds, known for their antioxidant properties, play a significant role in safeguarding plants against oxidative stress and their defense mechanisms. These compounds are produced in various plant tissues through the phenylpropanoid pathway, relying on key enzymes such as phenylalanine ammonia-lyase (PAL) and chalcone synthase [12]. At the cellular level, phenolic compounds protect cells from damage caused by free radicals and have various other functions. The accumulation of phytochemicals can increase in response to environmental factors such as light exposure and temperature variations [13].

Light intensity is one of the environmental factors that affect the phytochemical profile of peppers [13,14]. In recent years, pepper cultivation has been carried out primarily under shaded conditions to enhance horticultural productivity and fruit quality [15]. Nowadays, most pepper production occurs under protected horticultural conditions, where light conditions are manipulated with photo-selective shading nets or plastic covers [16,17]. Shading nets may be needed in regions with intense solar radiation and high temperatures [18], where excessive irradiance can result in photodamage and adverse effects on plant growth and development [19]. Shading induces changes in the physiology and biochemistry of plants [20], leading to changes in secondary metabolites [21].

Previous studies on piquin peppers have indicated that the use of black shading nets (the most used in horticulture) has a direct effect on productivity, fruit quality, and vegetative growth, and that these effects are dependent on shade levels [17]. Colored shading nets selectively filter sunlight and modify the spectral composition of light, promoting specific wavelengths [22]. We hypothesize that colored shading nets may induce changes in the phytochemical profile of peppers, thus leading to variations in the antioxidant activity and potential health benefits of piquin peppers.

The objective of this study was to determine how shading nets of different colors can influence the phytochemical profile of peppers, in particular capsaicinoids, carotenoids, and phenolic compounds in piquin pepper fruits, as well as their antioxidant activity. The effects of shading on color and fruit quality were also determined.

2. Materials and Methods

2.1. Plant Material and Experimental Site

This study was carried out at the CAETEC experimental station of Tecnológico de Monterrey in Pedro Escobedo, Qro, Mexico (20.535169 N, −100.211472 W) during the 2021 and 2022 production cycles (Figure 1). This location has a tropical and subtropical steppe climate (BSk) according to the Köppen climate classification system [23]. The experimental site has a vertisol soil type according to the FAO/UNESCO soil classification system [24]. Analysis of soil particles indicates that the study was conducted in a clay loam soil containing 38.2% sand, 30.02% silt, and 31.78% clay (Fertilab, 2018. Laboratorio de Nutrición Vegetal Celaya, Guanajuato).

The piquin pepper plants (*Capsicum annuum* L. var. *glabriusculum*) used in this study were obtained from eight selection cycles of a wild ecotype originally from San Fernando, Tamaulipas, Mexico. Before the study, the experimental plants had been selected based on their productivity and phenological attributes.

The seeds of piquin peppers have naturally low germination rates, which makes it necessary to apply pre-germination treatments (Figure 2). These treatments consisted of seed imbibition in a solution with 5000 ppm of gibberellic acid (Cyto-Gibb® CbM, Tlalnepantla de Baz, Mexico) for 24 h, with constant stirring at room temperature. After the treatments, the seeds were planted in trays containing peat moss as container media and placed in moist conditions in a greenhouse. The planting dates were 15 November 2020 and 1 December 2021 for the 2021 and 2022 cycles, respectively. Seedlings were transplanted approximately 125 d after sowing to the experimental site when they reached an average size of 10–15 cm. The seedlings were planted at 90 cm intervals under the shading treatments. Plants were irrigated using a nutrient solution to provide the necessary elements for plant growth. Irrigation was carried out three times a day for five minutes every day. The nutrient solution used for irrigation consisted of 15 $mM \cdot L^{-1}$ nitrates, 1 $mM \cdot L^{-1}$ ammonium, 1.5 $mM \cdot L^{-1}$ phosphates, 8 $mM \cdot L^{-1}$ potassium, 4 $mM \cdot L^{-1}$ calcium, 2 $mM \cdot L^{-1}$ magnesium, and 3 $mM \cdot L^{-1}$ sulfur. The electrical conductivity of the solution was 1.5 $dS \cdot m^{-1}$, and the pH was set at 6.0.

Air temperatures were recorded during the production cycles in each treatment using a data logger (RC-51H waterproof USB temperature humidity data logger, Elitech, San Jose, CA, USA). The data loggers were programmed to record temperature data once every 30 min.

Light transmission at the canopy level was quantified for each color shade treatment in terms of photosynthetically active radiation (PAR, µmol m^{-2} s^{-1}) using the LI-190R quantum sensor (LI-COR, Lincoln, NE, USA). These measurements were conducted at two time points at 12:00 and 14:00 h.

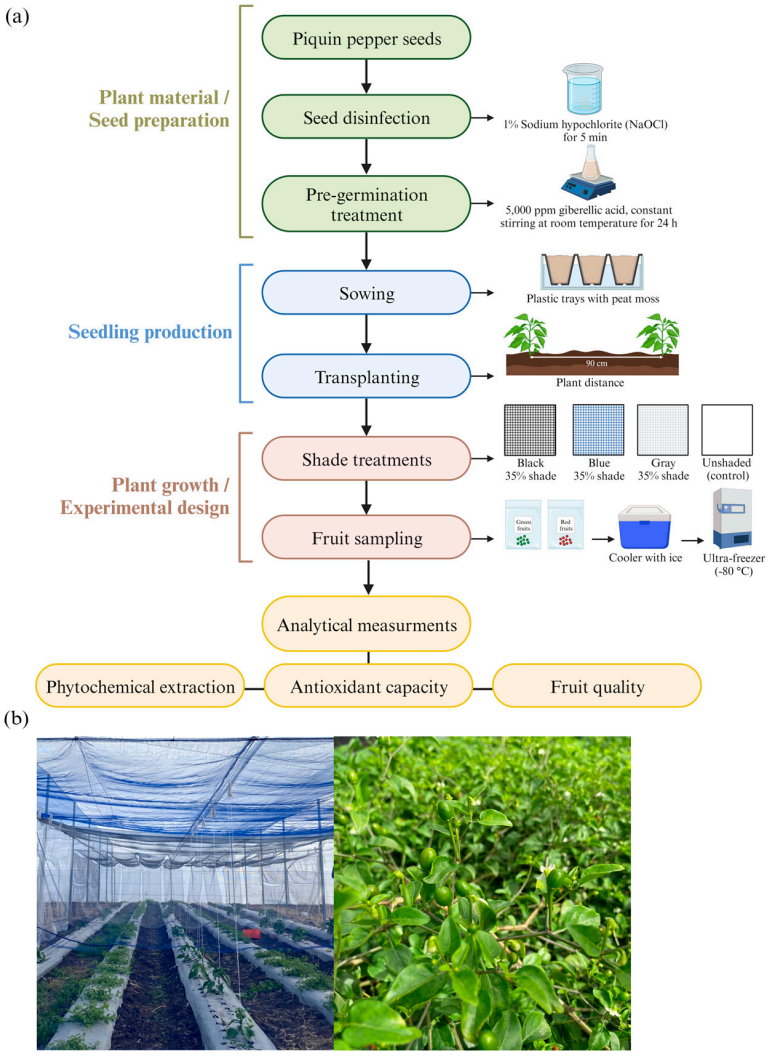

Figure 2. (**a**) Methodology flowchart: assessing the effects of shading treatment on phytochemical profile, antioxidant capacity, and fruit quality of piquin pepper fruits (*Capsicum annuum* L. var. *glabriusculum*) at two maturation stages. (**b**) Pictures of experimental setup and piquin pepper plants with fruits.

2.2. Experimental Design

The study was conducted using a factorial experimental design. The treatments were applied by growing the plants under black, blue, and gray shading nets. The nets used were high-density polyethylene (HDPE) with 35% light interception (Eurosol 54, EURAM, Santiago, Chile). An additional unshaded treatment was used as the control. The nets were placed at 3 m above the ground, covering all sides of the structure. The experimental units consisted of four randomly selected plants per treatment. A 200 g fruit sample was obtained from the plants. The sample fruits were collected at two different maturation stages: immature (green) and mature (red); homogeneity in size and color was ensured within the samples. Fruit collection was conducted during the months of June for green fruits, approximately 35–40 days after flowering (DAF), and in August for mature (red) fruits, approximately 70–75 DAF in both cycles. All analytical measurements were tested in triplicate.

2.3. Sample Preparation

After harvest, the peppers were placed in labeled polyethylene bags and stored in a cooler with ice to keep the fruits fresh. Once in the laboratory, the fruits were placed initially in a −20 °C freezer, and then in an ultra-freezer at −80 °C until further analysis.

Prior to phytochemical extractions, the moisture content of the piquin pepper fruits was determined at both maturation stages using a convection oven (Binder ED, Tuttlingen, Germany). A total of 20 fruits from each category were individually weighed on an analytical balance (Mettler Toledo, ME54E, Columbus, OH, USA) to record initial weights. All fruits were placed in the oven at 70 °C until constant weight, demonstrated by minimal mass fluctuations over time. The fruits were subsequently allowed to cool in a desiccator. After cooling, the fruits were weighed. The moisture content was calculated for each group using the following formula: Moisture Content (%) = [(Initial Weight − Final Weight) × Initial Weight^{-1}] × 100. This method ensures an accurate determination of moisture content, which was used to report all phytochemical results on a dry weight basis (DWB).

Before the extraction procedures, all fruits were washed with water and soap and rinsed with distilled water. The analysis of capsaicinoids and the total phenolic compounds were determined using fresh fruits. For carotenoid analysis, fruits were dried in a convection oven at 65 °C for 24 h. After drying, the seeds were removed and the placenta and pericarp were pulverized with an electric mill (Krups GX4100®®, Mexico City, Mexico). The extraction procedures for the different phytochemicals varied depending on the compound of interest.

2.4. HPLC Analysis for Capsaicinoid Content

The extraction procedure used to determine capsaicinoids used 2 g of fresh fruits that included pericarp, placenta, and seeds. Samples were homogenized using diatomaceous earth (1:1 w/w) and ground using a porcelain mortar. The extraction solvent was 6 mL of 100% methanol. The resulting mixture was sonicated (Ultrasonic Cleaner 8890, Cole-Parmer, Niles, IL, USA) for 15 min and then centrifuged (Centrifuge Multifuge X1R, Thermo Fisher, Walthman, MA, USA) at 22,830 times gravity (× *g*) at 4 °C for 5 min. The supernatant was filtered using Whatman N° 2 paper.

Capsaicinoid analyses were carried out via HPLC using the method described by Wahyuni et al. (2011) [25], with minor modifications. The HPLC system was an Agilent Technologies 1200 series with a UV–Vis detector. Extracts were filtered through a 0.2 µm PTFE membrane filter into a 1.5 mL amber vial. For each HPLC determination, 10 µL samples were injected in triplicate. Compounds were separated using an analytical column (ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 Micron, Agilent, Santa Clara CA, USA). The mobile phases consisted of formic acid and ultrapure water (1:10^3, v/v eluent A), and formic acid and acetonitrile (1:10^3, v/v, eluent B). The gradient applied started at 25% B for 5 min and increased linearly to 75% B for 10 min, and preequilibrated for 2 min to the initial conditions before the next injection. The column temperature was set at 40 °C, and the flow rate was 1 mL·min^{-1}. Capsaicinoids were detected at a 280 nm wavelength and quantified using external standards for capsaicin (CAP) and dihydrocapsaicin (DHC) from Sigma (St. Louis, MO, USA) at different concentrations.

2.5. HPLC Analysis for Carotenoid Content

The extraction process for carotenoid determinations used 0.5 g of peppers (dry weight basis or DW) that included the pericarp and placenta. Then, 10 mL of chloroform and methanol (1:1, v/v) was added as the extraction solvent. The resulting mixture was sonicated for 30 min, followed by centrifugation at 7000× *g* for 15 min. The entire process was carried out twice under dark conditions and at room temperature to ensure maximum carotenoid extraction. The supernatant was filtered using Whatman N° 2 paper. The solvent of the extracts was evaporated using a SpeedVac concentrator (Thermo Scientific, San Jose, CA, USA) and reconcentrated by adding 2 mL of the same solvent.

The analyses of carotenoids were performed via HPLC using the methods described by Mínguez-Mosquera and Hornero-Méndez (1993) [26] and Blanco Ríos et al. (2013) [27], with minor modifications. The HPLC system used was the Agilent Technologies 1200 series equipped with a UV–Vis detector. Extracts were filtered through a 0.2 µm PTFE membrane filter into a 1.5 mL amber vial. For each determination, 20 µL of sample was injected in triplicate for HPLC analysis. Compounds were separated using an analytical column (ZORBAX Eclipse XDB-C18, 4.6 × 150 mm, 5 Micron, Agilent, Santa Clara, CA, USA). The mobile phases consisted of acetone (eluent A) and ultrapure water (eluent B). The gradient applied started at 25% B for 5 min, increased linearly to 75% B for 10 min, and preequilibrated for 2 min to the initial conditions before the next injection. The temperature in the column was maintained at 25 °C, and the flow rate was set at 1.7 mL·min^{-1}. Carotenoid levels were detected at a wavelength of 450 nm and quantified using an external standard for β-carotene from Sigma (St. Louis, MO, USA) at different concentrations.

2.6. Total Phenolic Compounds

The total phenolic compounds were determined spectrophotometrically using the Folin–Ciocalteu method [28]. Compounds were extracted by adding 10 mL of a solution of methanol and water (70:30 v/v) to 5 g of ground fresh fruits. This mixture was sonicated for 30 min and centrifuged at 7000× *g* for 15 min. This process was performed twice to maximize the compound extraction. All extracts were stored at −20 °C until further analysis.

The Folin–Ciocalteu assay was performed in a 96-microwell plate. In each microwell, 20 µL of sample was added, followed by 50 µL of 0.5 N of the Folin–Ciocalteu reagent and 150 µL of water. After five minutes of incubation at room temperature, the reaction was neutralized with 50 µL of sodium carbonate (Na_2CO_3 20%, p·v^{-1}). Subsequently, the mixture was incubated for 2 h at room temperature under dark conditions. After this incubation time, the absorbances were read at 765 nm using a microplate absorbance spectrophotometer (xMark^{TM}, Bio-Rad, Hercules, CA, USA).

The spectrophotometric readings were compared against a Gallic acid standard. The calibration curve was obtained using dilutions of Gallic acid with concentrations ranging from 0 to 1000 µM. All samples were measured in triplicate and the final concentration was expressed as milligrams of gallic acid equivalents (GAE) on a dry weight basis.

2.7. Antioxidant Capacity

A comprehensive analysis of the antioxidant capacity of piquin pepper fruits was determined using in vitro assays comparing the antioxidant effects of the fruits at two different maturation levels using the ABTS and DPPH methods [29]. These methods yield complementary information about the antioxidant properties of the samples, and their combined results allow for a more complete determination of the overall antioxidant capacity. The same extracts obtained for the phenolic compound analysis were employed to assess antioxidant activity.

2.7.1. ABTS Method

The ABTS method measures the Trolox equivalent antioxidant capacity (TEAC), which compares the antioxidant capacity to cleave the radical cation of ABTS and Trolox [30]. The ABTS stock solution was obtained by reacting 7 mMol·L^{-1} and 2.45 mMol·L^{-1} of potassium persulfate after incubation in the dark for 16 h. The stock solution was subsequently diluted in ethanol to an absorbance of 0.8 ± 0.1 at 734 nm. Trolox standard solutions were prepared in methanol from 0 to 700 µmol·L^{-1} and assayed under the same conditions. In each well of a 96-microplate plate, 200 µL of reagent and 20 µL of the sample extracts were added and incubated for 6 min with constant agitation. Each sample was assessed in triplicate. The measurements were taken at 734 nm using xMark™ Microplate Absorbance Spectrophotometer (xMark^{TM}, Bio-Rad, Hercules, CA, USA). The calibration curve for the ABTS method was estimated using Trolox standard solutions from 0 to 700 µmol·L^{-1}. These solutions were prepared in methanol and assayed under the same conditions. Based

on these solutions, the calibration curve was estimated as The TEAC was reported as $y = 0.0015x + 0.0426$, $R^2 = 0.9941$. Trolox equivalents (mM TE·g^{-1} DW). These units quantify the antioxidant capacity of the sample based on its ability to neutralize the ABTS radicals relative to Trolox.

2.7.2. DPPH Method

The DPPH method is based on the radical unpaired electron yield of an antioxidant substance, in which DPPH is demoted from a blue–purple color to light yellow [31]. For this assay, a stock solution of 125 µM of DPPH (1,1-diphenyl-2-picrylhydrazyl) was prepared. For the assay, 20 µL of sample extracts and 200 µL of DPPH were added to a well in a 96-microwell plate and mixed; analysis was carried out in triplicate. The plaque was stored in the dark for 90 min at room temperature. After incubation, DPPH stock solution was added as a control to the plaque. Absorbance readings were taken at 520 nm using an xMark™ Microplate Absorbance Spectrophotometer (xMark^{TM}, Bio-Rad, Hercules, CA, USA). Scavenging was expressed in the mg of dry sample needed to decolorate 50% of the reagent and as Trolox equivalents (mM TE·g^{-1} DW). The calibration curve for the DPPH method was estimated in a similar way to the ABTS method. The standard curve equation obtained for the ABTS method was $y = 0.0015x + 0.0622$, $R^2 = 0.9966$).

2.8. Fruit Quality

2.8.1. Analysis of Color

The colorimetric determinations of piquin pepper fruits were carried out using a Konica Minolta spectrophotometer (CM-5, Ramsey, NJ, USA). Ten fruits per treatment were placed in a glass petri dish, and the measurements were taken using the spectrophotometer in triplicate. Color results were reported using the CIE L*a*b* (CIELAB) color space parameters, as these parameters allow an accurate description of the color of fruits [32]. In the CIELAB parameters, L* represents the lightness value, ranging from 0 (black) to 100 (white), a* represents the chromaticity value between green (−) and red (+), and b* represents the chromaticity value between blue (−) and yellow (+) [33].

2.8.2. Morphological Analysis

The morphological analyses of peppers were conducted on samples of ten individual fruits per treatment. Individual fruit sizes (width and height) were determined using a digital vernier. Additionally, the weight of ten fruits was determined using an analytical balance (Mettler Toledo, ME54E, Columbus, OH, USA).

2.9. Statistical Analysis

The results were analyzed using a one-way analysis of variance (ANOVA) to determine the significant differences between treatments. Significance levels were set at $p < 0.05$ throughout the study. When significant differences were detected, the means were separated using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p < 0.05$. The statistical analyses were performed using Minitab Statistical Software (version 21.4). All results are presented as the mean ± standard deviation (SD) of three replicates.

3. Results and Discussion

3.1. Temperature

The effect of the shading treatments on air temperature (Figure 3) indicated that the highest temperatures were registered in the unshaded treatments in comparison to the rest of the treatments. The largest differences in mean temperature occurred in the middle of the day but were less than 4 °C across treatments. Shading treatments can effectively reduce the air temperature and elevate air humidity. Shading nets represent an impactful approach to creating an optimal environment for crop cultivation, leading to improved quality and increased crop productivity in regions with high levels of solar radiation [34].

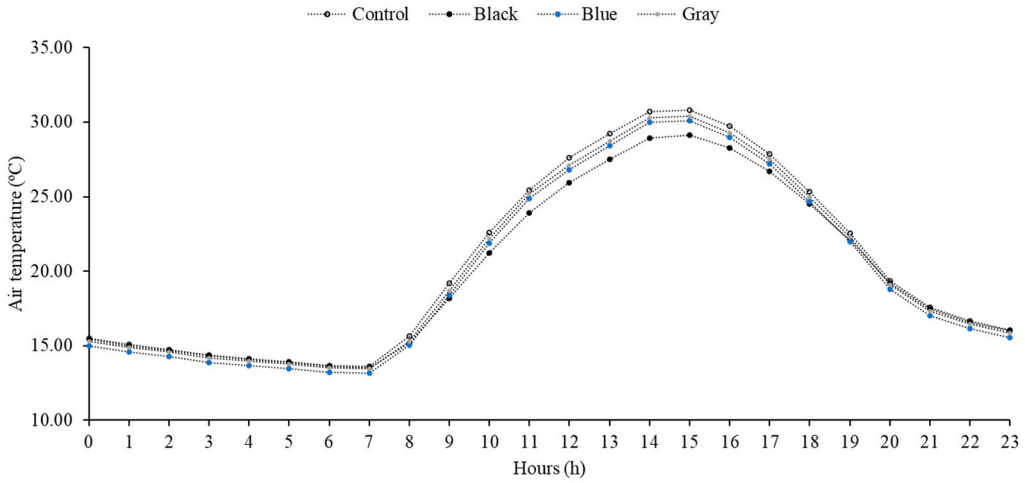

Figure 3. Mean hourly air temperature (°C) for each treatment during the production cycles of piquin peppers (*Capsicum annuum* L. var. *glabriusculum*) in Querétaro, México.

3.2. Photosynthetically Active Radiation (PAR)

All colored shading nets exhibited a reduction in light transmittance compared to the control (Figure 4). The black shading treatment reduced transmittance by an average of 31%, while the blue and gray nets showed reductions of 27% and 25%, respectively. The differences in variability in the modulation of light transmission may have implications for photosynthesis and plant development. The microclimatic impact of colored shading nets on the reduction in photosynthetically active radiation (PAR) has the potential to significantly impact the key physiological processes determining fruit yield and quality in crops, including photosynthesis and carbon allocation [35]. The variations in PAR availability observed among the different nets stem from the color of threads, which alters the proportion of diffuse light compared to the total light transmitted under nets [36].

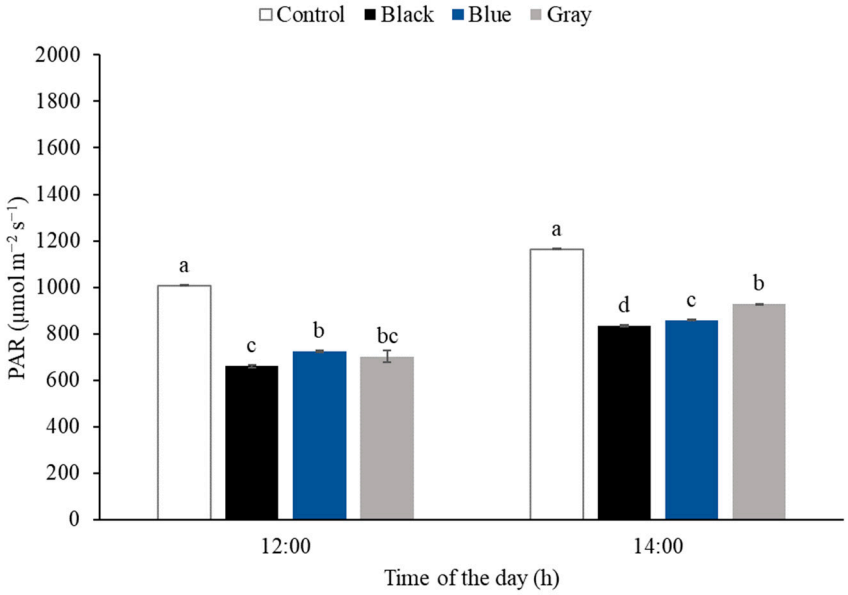

Figure 4. Mean solar radiation as photosynthetically active radiation (PAR, $\mu mol \cdot m^{-2} \cdot s^{-1}$) measured in experimental treatments at two different times of the day. Effect of the black, blue, and grey shading treatments on light transmission compared to the control (unshaded treatment). Error bars represent standard error of replicates. Values with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

3.3. Capsaicinoid Content of Piquin Peppers

The effect of the different shading nets on the contents of capsaicinoids in piquin peppers showed significant differences, and the results varied depending on the shading treatments and the maturation stage of the peppers. Capsaicinoid levels increased in red fruits of both production cycles (Figure 5). An increase in capsaicinoids during maturation has been reported in other peppers, where capsaicinoids start to accumulate in the early stages of fruit development, followed by an increase during fruit maturation [37]. The results of the second production cycle showed a similar increase in capsaicinoids, but the final contents were higher in all treatments compared to the first cycle.

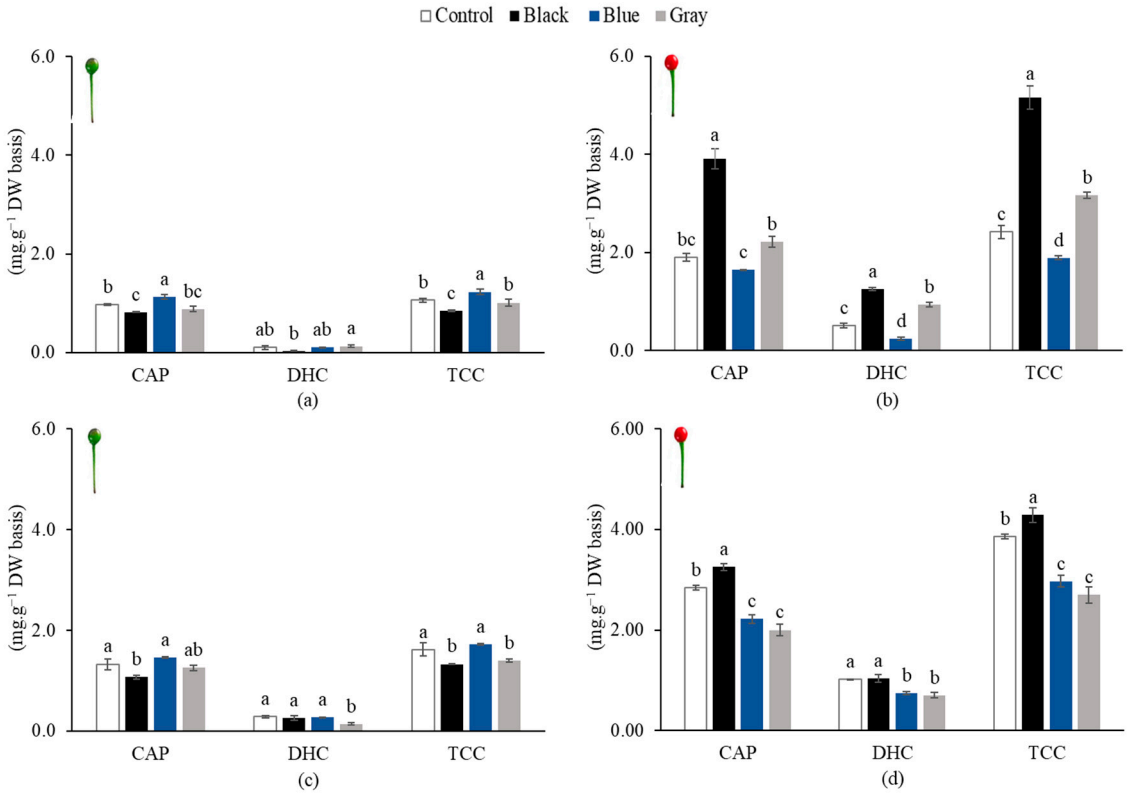

Figure 5. Mean capsaicinoid contents: capsaicinoids (CAPs), dihydrocapsaicin (DHC), and total capsaicinoid content (TCC) (mg·g^{-1} DW basis) of green and red fruits of piquin peppers grown under colored shading net treatments in Queretaro, Mexico, across two production cycles (2021–2022). (**a**) Green fruits, first production cycle (2021). (**b**) Red fruits, first production cycle (2021). (**c**) Green fruits, second production cycle (2022). (**d**) Red fruits, second production cycle (2022). Error bars represent standard error of replicates. Values with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

The immature fruits grown under the blue shade treatment had the highest capsaicinoid contents in both production cycles. However, as the fruits matured, the black shading treatment presented the highest capsaicinoid contents, measured as capsaicin (CAP), dihydrocapsaicin (DHC), and the total contents of capsaicinoids (TCC). These results were significantly higher than the control and the other shading treatments. These results are consistent in both cycles and indicate that the black shading treatment increased the capsaicinoid content of piquin peppers in the red maturation stage. Black shading nets reduce the light intensity reaching the plant canopy, creating a shaded environment, which, in turn, may influence the expression of genes involved in capsaicinoid biosynthesis [9,14].

As mentioned, the capsaicinoid levels were higher in mature fruits than in green fruits, confirming previous reports in which the capsaicinoid contents of piquin peppers increased as the fruit matured. Nonetheless, the observed differential effect of the colored

shading treatments on capsaicinoid levels [17] could be related to the modulation of gene expression associated with capsaicinoid biosynthesis, where light plays a role in the expression of the capsaicin synthase gene (*CS*). The promoter regions of the *CS* gene contain light-responsive motifs, indicating that light can influence its expression [9]. In a similar study on *C. annuum* 'Star Flame' and 'Fire flame,' the interaction of reduced light intensity using different colored shading nets at different harvest times significantly impacted capsaicinoid contents [38]. The reduced light intensity and modified light qualities caused by colored shading nets might influence the gene expression responsible for capsaicin production [39,40]. Nonetheless, the higher capsaicinoid contents associated with green nets could also be related to higher temperatures [41].

Overall, light intensity and changes in its spectrum seem to be critical environmental factors that influence capsaicinoid accumulation in pepper plants. The duration of light exposure may also impact the synthesis of capsaicinoids, leading to variations in the pungency levels of the different pepper cultivars.

3.4. Carotenoid Content of Piquin Peppers

The analysis of carotenoid content, reported as β-carotene equivalents, showed significant differences between shading treatments for green and red fruits in both production cycles (Figure 6). In the first production cycle, the black shading treatment resulted in the highest carotenoid content for green and red fruits, while the gray treatment had the lowest carotenoid content for red fruits. In the second production cycle, the blue shading treatment caused the highest carotenoid content for red fruits.

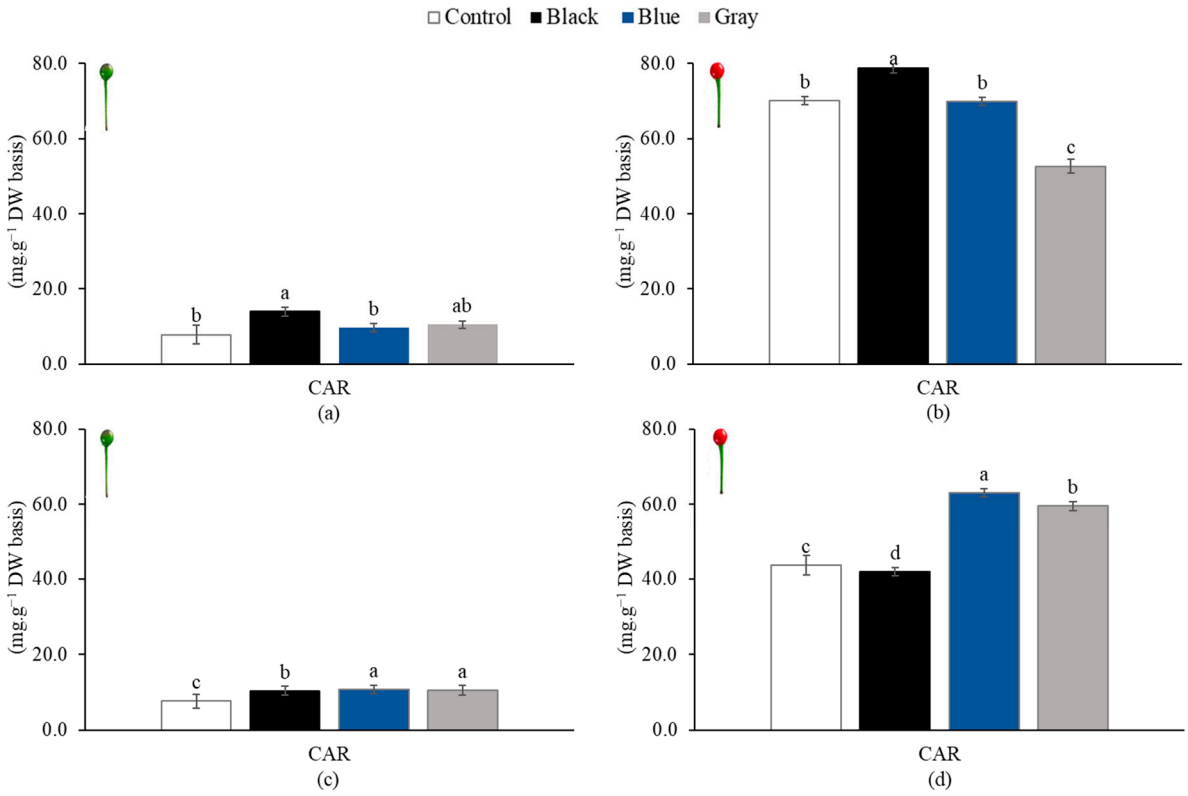

Figure 6. Mean carotenoid contents (β-carotene) (CAR, mg·g^{-1} DW basis) of green and red fruits of piquin peppers grown under colored shading net treatments in Queretaro, Mexico, across two production cycles (2021–2022). (**a**) Green fruits, first production cycle (2021). (**b**) Red fruits, first production cycle (2021). (**c**) Green fruits, second production cycle (2022). (**d**) Red fruits, second production cycle (2022). Error bars represent standard error of replicates. Values with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

The maturation process of peppers brings about increased carotenoids in *Capsicum* fruits. In this process, chloroplasts differentiate into chromoplasts in the epicarp of the fruit, which leads to an accumulation of carotenoids. Increased carotenoids contribute to a change in the coloration of peppers, in which more than thirty types of carotenoids are involved [42–44]. In piquin peppers, this process starts at the initial stages of fruit development, where fruits exhibit a green color, indicating their immature stage. As they progress to the breaker stage, their color transitions to purple or orange, and finally, at full maturity, piquin peppers acquire a vibrant red color [1,45].

In our results, the shaded treatments affected the carotenoid content of piquin peppers. The black and blue shading treatments led to higher carotenoid contents than the unshaded control. Our findings coincide with previous studies on other *Capsicum* fruits that showed a significant increase in the carotenoid content in the shaded treatments compared to the unshaded conditions [46,47].

When bell pepper plants were grown in shaded conditions, there was a reduction in the exposure of plants to light. Reduced light intensity can increase carotenoid levels in sweet peppers grown under black nets, particularly β-carotene and lycopene [48]. In another cultivar of sweet pepper, unshaded treatments produced over 50% lower carotenoid levels than those grown under white or colored nets [49]. Nonetheless, the optimal shading treatment for increasing carotenoid contents may vary based on the specific cultivar and prevailing environmental conditions during production [50].

The reduced carotenoid contents in the unshaded nets (control treatment) could be related to the rapid destruction of carotenoids by high-intensity illumination [51]. Thus, the use of shade nets reduces light-intensity stress and protects the leaves from thylakoid damage caused by high irradiance [52]. Increased carotenoids enhance the nutritional value of peppers and enrich the vibrant colors, with a positive impact on fruit quality.

3.5. Total Phenolic Compounds of Piquin Peppers

The general effects of the different shading net treatments on the total phenolic compounds (TPC) of piquin peppers indicate that the shading treatments did not significantly affect the phenolic contents of the immature (green) fruits (Figure 7). In mature fruits, there was an increase in TPC compared to immature fruits in both production cycles. For mature fruits in the first production cycle, the control and black treatments showed slightly higher phenolic contents than the other treatments. In the second production cycle, the grey treatment showed a slightly higher phenolic content than the control and black treatments. Higher TPC in mature fruits has been previously described during the maturation process of habanero peppers and other *Capsicum* cultivars [53,54].

Our results indicate that the different shading net treatments affected the production of TPC as the piquin peppers reached maturity, but did not have a significant effect on immature fruits. Nonetheless, the specific effects of the colored shading treatments on the TPC of mature fruits are not clear, as the results varied from the 2021 to the 2022 cycle. In other shading studies using cultivars of sweet peppers, the unshaded, white, and pearl shade treatments increased the phenolic compounds and antioxidant activity, whereas black shade nets caused a reduction in phenolic compounds [15,50]. The higher phenolic compounds in the unshaded environments could be attributed to higher irradiance, which triggers a stress response in the plant, leading to the production of higher levels of phenolic compounds as a protective mechanism. Phenolic compounds can act as antioxidants and protect the plant from oxidative damage caused by increased irradiance. UV radiation is a stress factor that stimulates the biosynthesis of flavonoids and phenolic compounds, resulting in higher contents as a response to UV radiation [55].

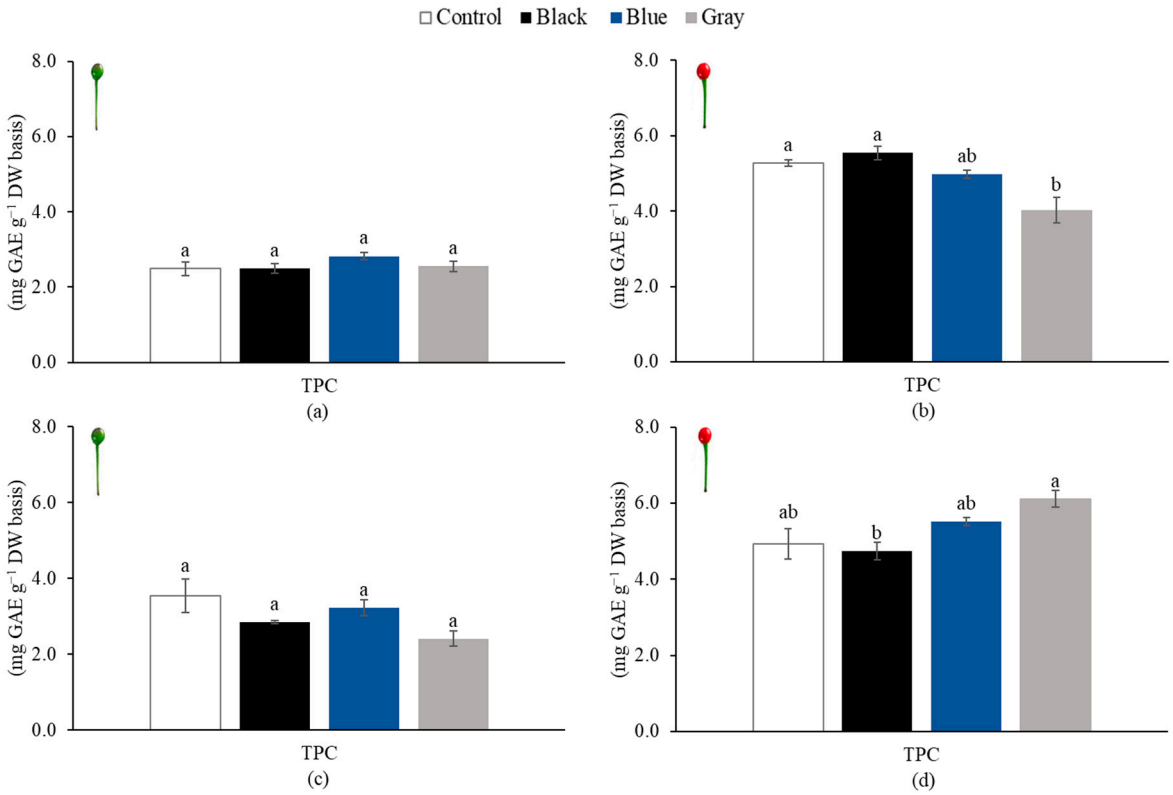

Figure 7. Mean total phenolic compounds (TPC, mg GAE·g^{-1} DW basis) of green and red fruits of piquin peppers grown under colored shading net treatments in Queretaro, Mexico, across two production cycles (2021–2022). (**a**) Green fruits, first production cycle (2021). (**b**) Red fruits, first production cycle (2021). (**c**) Green fruits, second production cycle (2022). (**d**) Red fruits, second production cycle (2022). Error bars represent standard error of replicates. Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

3.6. Antioxidant Capacity

The antioxidant capacity of immature (green) and mature (red) peppers determined using the ABTS method showed that the blue treatment caused the highest antioxidant capacity at both maturation stages, followed by the gray and black shades (Figure 8). The control treatment had the lowest antioxidant capacity in both maturation stages. Comparable results were obtained in the second production cycle, in which the blue and gray treatments also exhibited a higher antioxidant capacity than the control fruits in both maturation stages. The black shade treatment also increased the antioxidant capacity in immature fruits in both production cycles. However, the differences in antioxidant capacity were not statistically significant in the mature fruits of the second production cycle, and the black shading treatment showed a reduction in antioxidant capacity compared to the control group.

The determination of antioxidant capacity by DPPH showed a similar trend, with the blue shade net treatment having the highest antioxidant capacity in most cases; the control treatment had the lowest antioxidant capacity. However, some differences between the results from the ABTS and DPPH could be identified, with some treatments showing different antioxidant capacity levels depending on the method. These methods served as complementary assays to explore a wide range of antioxidant compounds; ABTS can estimate both hydrophilic and lipophilic radicals [56], while DPPH is particularly sensitive to lipophilic radicals [57]. Antioxidants in peppers predominantly arise from a combination of hydrophilic compounds, mainly phenolic compounds, and lipophilic compounds such as carotenoids [43,58].

During maturation, the red fruits generally had a higher antioxidant capacity than the green fruits, as expected. The antioxidant capacity and ascorbic acid content significantly increased in different pepper cultivars during the growth and maturation of the fruits; the highest levels were found in the last stage of maturity [53,54]. This difference was more pronounced in the DPPH results, where the differences between green and red fruits were higher than in the ABTS results.

Our results indicate that the shading treatments had an enhancing effect on the antioxidant capacity of the piquin peppers, particularly the blue shade treatment. The specific effects varied depending on the shade color and fruit maturation stage. The results confirmed the potential health benefits of consuming piquin peppers, particularly when they are fully ripe. Previous results regarding piquin peppers have reported that differences in antioxidant capacity are a result of more than 32 compounds identified, mainly phenolic compounds that contribute to the free radical scavenging of fruits [59]. The antioxidant capacity of *Capsicum* fruits is directly related to the total phenolic compounds [60].

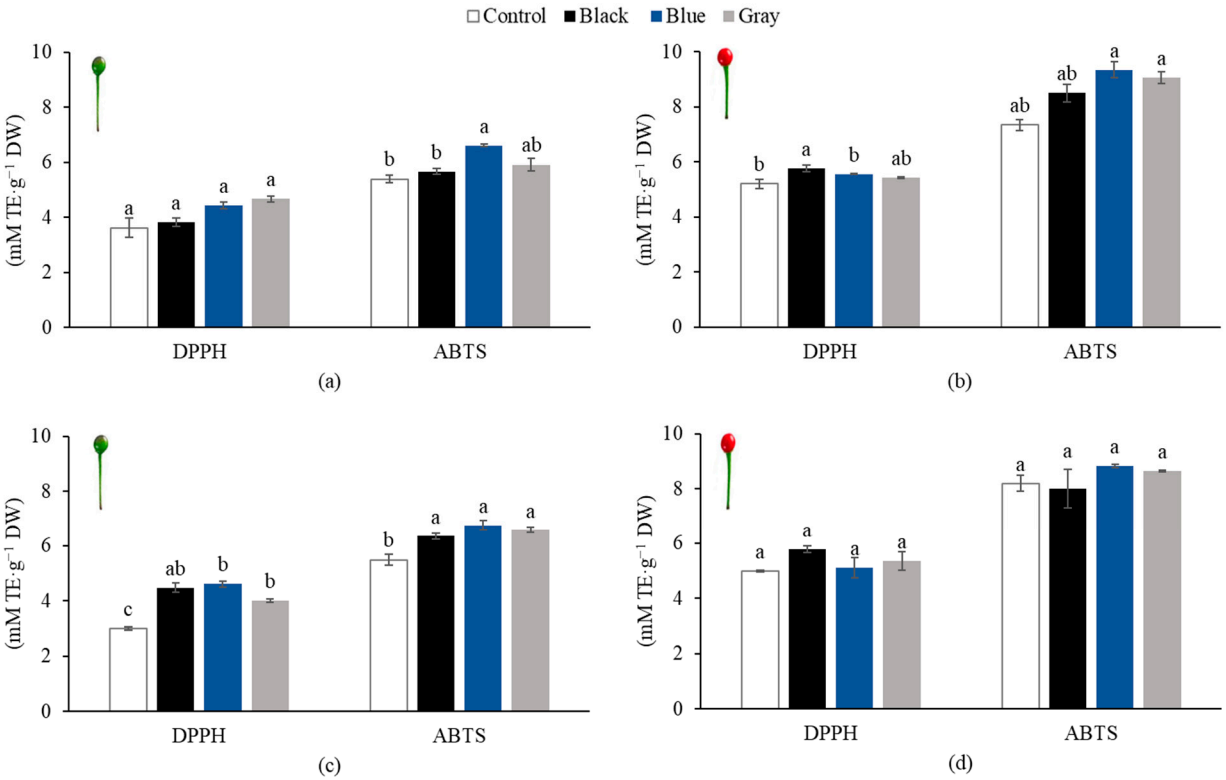

Figure 8. Mean antioxidant activity of green and red piquin peppers grown under colored shading net treatments in Queretaro, Mexico, across two production cycles (2021–2022) using DPPH and ABTS methods. (**a**) Green fruits, first production cycle (2021). (**b**) Red fruits, first production cycle (2021). (**c**) Green fruits, second production cycle (2022). (**d**) Red fruits, second production cycle (2022). Error bars represent standard error of replicates. Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

3.7. Fruit Quality

3.7.1. Effects on Pepper Color

The color of piquin peppers was significantly affected by the shading treatments in both production cycles (Tables 1 and 2). In the first cycle, the control fruits had a lighter color (the highest L* value) for both immature and red peppers in relation to the other treatments. In immature peppers, the black treatment had the highest a* and b* values, indicating a more intense green color. The blue treatment had the highest a* and b* values for mature peppers, indicating a more intense red color. Color values in fruits may be used

as predictors of pigment concentrations, and the reduced a* values observed in unshaded treatments can be related to a reduction in carotenoids [50].

Table 1. Mean colorimetric values of green and red piquin peppers grown under colored shading net treatments in Queretaro, Mexico, during the first production cycle (2021).

Treatment	Green Fruits			Red Fruits		
	L*	a*	b*	L*	a*	b*
Control	30.88 ± 2.32 a	−8.67 ± 5.09 a	31.20 ± 2.28 a	28.51 ± 0.88 a	43.69 ± 4.92 a	38.24 ± 1.72 b
Black	30.26 ± 2.69 a	−15.45 ± 21.48 a	28.04 ± 3.07 b	27.64 ± 2.42 a	43.39 ± 1.40 a	38.60 ± 3.07 b
Blue	29.33 ± 1.57 a	−9.98 ± 0.38 a	28.46 ± 1.83 b	28.83 ± 1.60 a	45.00 ± 1.07 a	40.50 ± 2.12 ab
Gray	30.16 ± 1.58 a	−10.24 ± 0.31 a	31.73 ± 1.85 a	28.88 ± 1.58 a	44.55 ± 1.07 a	42.06 ± 2.92 a

L*: indicates lightness, a*: is the red/green coordinate, and b*: is the yellow/blue coordinate. ± Standard deviation (n = 4). Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

Table 2. Mean colorimetric values of green and red piquin peppers grown under colored shading net treatments in Queretaro, Mexico., during the second production cycle (2022).

Treatment	Green Fruits			Red Fruits		
	L*	a*	b*	L*	a*	b*
Control	28.64 ± 1.78 a	−7.43 ± 1.60 a	40.47 ± 2.15 a	36.77 ± 2.29 a	35.02 ± 2.23 a	20.84 ± 1.97 a
Black	24.81 ± 1.59 b	−9.03 ± 0.94 b	35.57 ± 2.70 bc	38.06 ± 0.60 a	34.00 ± 1.40 a	19.22 ± 1.63 a
Blue	23.73 ± 2.20 b	−10.29 ± 0.78 c	36.90 ± 1.61 b	35.30 ± 8.86 a	21.49 ± 19.67 b	22.36 ± 7.29 a
Gray	24.69 ± 2.56 b	−9.82 ± 0.38 bc	34.61 ± 2.28 c	38.19 ± 1.16 a	34.17 ± 0.77 a	20.25 ± 1.10 a

L*: indicates lightness, a*: is the red/green coordinate, and b*: is the yellow/blue coordinate. ± Standard deviation (n = 4). Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

In the second production cycle, the control fruits also had the highest L* value for both maturation stages, indicating a lighter color than in the other treatments. The black treatment had a more intense red color (highest a* value) for mature peppers, while for green peppers, the blue treatment caused a more intense yellowish-green color (highest b* value). Consistent findings have been observed in bell peppers, where the L* value was highest in unshaded treatments, while the a* value was the lowest in the unshaded treatments and reached the highest values under black and red shading nets [50]. The intense red color may be related to the higher concentration of carotenoids and anthocyanins, which are responsible for the red coloration of mature fruits [38]. Our results suggest that the differential effects of the colored shade treatments on pepper color could be related to an effect on the pigment biosynthesis (particularly carotenoids), but it is not clear whether the treatments affected chlorophyll degradation, a process that occurs during fruit maturation [61].

Nonetheless, our results indicate that the different shading treatments affected the color of piquin peppers, with some treatments resulting in more intense and vibrant colors than others. These findings could be useful for understanding the factors that influence the color of piquin peppers and to develop commercial strategies to improve color and fruit quality.

3.7.2. Size and Weight

In general, the immature (green) fruits were longer and narrower than the mature fruits (Tables 3 and 4). During fruit maturation, peppers undergo a highly intense metabolism, the emission of volatile compounds associated with fruit respiration, and changes in their cellular structure, leading to water loss, thus becoming denser [53,62]. This can result in a reduction in overall size, even as the fruit reaches its full flavor and nutrient potential. Additionally, size reduction may also be influenced by genetic factors [62], environmental conditions [48], and cultural practices [63].

Table 3. Mean morphological analysis, width, length, and weight of green and red piquin peppers grown under colored shading net treatments in Queretaro, Mexico, during the first production cycle (2021).

Treatment	Green Fruits			Red Fruits		
	Fruit Width	Fruit Length	Weight	Fruit Width	Fruit Length	Weight
Control	8.73 ± 0.32 ab	10.22 ± 0.40 ab	2683.83 ± 344.82 a	8.84 ± 0.33 a	9.98 ± 0.40 a	2553.73 ± 180.66 a
Black	8.91 ± 0.24 a	10.81 ± 0.49 a	2883.33 ± 336.51 a	8.33 ± 0.42 bc	9.57 ± 0.58 a	2242.59 ± 423.48 a
Blue	8.23 ± 0.35 c	10.42 ± 0.80 ab	2711.35 ± 319.59 a	8.62 ± 0.33 ab	10.01 ± 0.57 a	2348.14 ± 428.44 a
Gray	8.39 ± 0.26 bc	10.01 ± 0.38 b	2802.46 ± 306.35 a	8.14 ± 0.41 c	9.99 ± 0.57 a	2731.55 ± 411.68 a

Width and length, average value (mm) ± Standard deviation (n = 10). Weight, average value (mg) ± Standard deviation (n = 10). Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

Table 4. Mean morphological analysis, width, length, and weight of green and red piquin peppers grown under colored shading net treatments in Queretaro, Mexico, during the second production cycle (2022).

Treatment	Green Fruits			Red Fruits		
	Fruit Width	Fruit Length	Weight	Fruit Width	Fruit Length	Weight
Control	8.56 ± 0.28 a	10.32 ± 0.43 a	2977.50 ± 283.44 a	7.93 ± 0.19 b	9.54 ± 0.20 b	2924.50 ± 162.81 b
Black	8.47 ± 0.27 a	10.02 ± 0.34 a	2852.78 ± 187.14 ab	8.54 ± 0.17 a	9.84 ± 0.41 ab	3046.45 ± 232.93 b
Blue	8.02 ± 0.33 b	8.91 ± 0.31 b	2484.24 ± 107.96 b	8.40 ± 0.15 a	10.21 ± 0.33 a	3375.51 ± 46.85 a
Gray	8.24 ± 0.38 ab	9.26 ± 0.33 b	2586.40 ± 154.60 ab	8.37 ± 0.26 a	10.27 ± 0.50 a	2555.88 ± 99.13 c

Width and length, average value (mm) ± Standard deviation (n = 10). Weight, average value (mg) ± Standard deviation (n = 10). Means with the same letters are statistically similar. Tukey's mean comparison test (a = 0.05).

The colored shade treatments had a significant effect on the morphology of piquin peppers. While the control and black shade treatments produced the widest fruits in both production cycles, the blue shade treatment caused the thinnest fruits. In terms of fruit length, the black and the blue shade treatment caused the longest fruits in the first and second cycles, respectively. Our results seem to concur with previous studies on piquin peppers that indicate that intermediate black shading (50% shade) increases yield in terms of fruit size and the number of fruits [17].

Previous studies have reported that the use of shading nets can increase the size of *Capsicum* fruits [64], although the specific conditions and types of nets used were different. In sweet peppers, the use of red and pearl shade nets favored fruit growth, which resulted in an increased fruit yield, producing fruits with a thicker pericarp [16]. As for fruit weight, the blue shade treatment had the heaviest fruits in the second production cycle after maturation.

4. Conclusions

Our results highlight the effects of the different color shading treatments on the capsaicinoids, carotenoids, and phenolic compounds of piquin pepper fruits at two maturation stages. The maturation process naturally increased the compound levels and antioxidant capacity, as mature red fruits exhibited the highest levels of phytochemicals and showed a greater antioxidant capacity than immature green fruits. Black shading was determined to boost capsaicinoid content, while black and blue shading led to an increase in carotenoid levels. The use of black and gray shading resulted in elevated phenolic compound levels. Notably, no significant variations in antioxidant capacity were observed in the different treatments. Morphological attributes such as fruit size and weight were affected by both colored shading and fruit maturity. These findings emphasize the potential health benefits of mature red piquin peppers, suggesting their utility in the development of functional foods, nutraceuticals, and pharmaceuticals. Shading can be considered a promising technique to enhance the phytochemical contents of piquin pepper fruits. Our results suggest

promising avenues for future research and applications. In the agricultural context, we recommend the further exploration of innovative light management techniques to increase the phytochemical content of piquin or other peppers. Given the limited existing agricultural practices in this area, our work opens the door to a wide range of possibilities for researchers and farmers engaged in the cultivation of piquin peppers.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to laboratory policy.

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