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Capsaicinoid Content in the Pericarp and Placenta of Bolilla Peppers (*Capsicum annuum* L.) throughout the Ripening of the Fruit at Two Different Stages of Plant Maturation

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Abstract: Peppers are a very popular ingredient in many dishes, either as the fruit itself or as derived products. They are not only consumed because of their organoleptic characteristics, but also because of their high content of bioactive compounds. The aim of this work was to determine the optimal time to harvest the Bolilla pepper, based on the capsaicinoid concentration in the pericarp and placenta at two different plant maturities (young and adult), in order to maximize their potential biological properties. In the case of the pericarp, the maximum capsaicinoid concentration was reached at 30 days post-anthesis (dpa) (with values of 1565.98 and 2158.82 µmol/kg, for the young and adult plant, respectively), while in the placenta it was registered at 41 dpa with greater values (5898.12 and 2349.67 µmol/kg). In either case, from that moment on, there was a drastic reduction in the capsaicinoid content. With regard to the capsaicinoid content levels in the pericarp, this was greater in older plants, while the placenta presented greater content in younger plants, which is of considerable importance from an economic point of view. This work allows a better selection of the final product, taking full advantage of its beneficial effects on health and taste, which would be very interesting for analytical laboratories or industries.

Keywords: capsaicinoids; *Capsicum* spp.; fruit maturity; young plant; adult plant; pericarp; placenta; Bolilla pepper

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1. Introduction

Pepper fruits are a horticultural plant used as an ingredient or condiment in certain dishes for their flavor, aroma, and color, and, regarding some varieties, to provide a characteristic spicy flavor [1]. They belong to the genus *Capsicum*, within the Solanaceae family, which comprises more than 35 recognized species, of which only *C. annuum*, *C. chinense*, *C. baccatum*, *C. frutescens*, and *C. pubescens* have been domesticated [2]. They are native to Central and South America, but they are now cultivated in mild and subtropical climate regions all over the world [3,4]. A wide diversity of peppers with different colors, sizes, and shapes can be found. In addition, consumers' demand has led to a wide diversity of their organoleptic characteristics [5]. This diversity makes peppers extremely valuable for the production of oleoresins [6] and in the agrifood industry, either as colorants or flavoring agents, or for their biological properties and beneficial health effects associated with their consumption [7]. The chemical composition of peppers is influenced by several factors, such as genotype, growth and ripening conditions, and climate [8].

The bioactive compounds responsible for the pungent sensation of peppers are capsaicinoids, which are non-volatile alkaloids. Capsaicinoids are acid amides, via branched-chain fatty acids (C_9-C_{11}) and vanillylamines [9]. The main compounds are capsaicin

(C) (*trans*-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (DHC) (8-methyl-N-vanillylnonamide), together comprising 97% of the total capsaicinoid content. The remaining 3% corresponds to minor capsaicinoids that differ in the number of carbon atoms on the side chain and because of the presence of unsaturations. These compounds can be nor-dihydrocapsaicin I and II (n-DHC), homo-capsaicin I and II (h-C), homo-dihydrocapsaicin I and II (h-DHC) [10], among others. Several studies have shown that these compounds can behave as chemopreventive agents, preventing cancer development through inhibition of malignant cell proliferation and increased apoptosis [11]. They also promote vascular health, preventing cardiovascular disorders, by increasing nitric oxide (NO) production and reducing inflammatory responses [12,13]. Finally, they have been shown to protect the liver by reducing oxidative stress [14] and have demonstrated analgesic properties [15].

The biosynthesis of capsaicinoids takes place in the placenta of the peppers; capsaicinoids accumulate in the vacuoles of the placental epidermis until the metabolization of the cells occurs and, subsequently, their extracellular secretion occurs [16]. Capsaicinoids have also been found in other parts of the peppers, such as the pericarp, seeds, or even in the leaves or stems, but in considerably smaller amounts. This is attributed to the diffusion and adhesion of the capsaicinoids from the placenta through its epidermal walls [17]. Several studies have shown that the synthesis process of the capsaicinoids takes place during the ripening of the fruit. As peppers mature, they undergo biochemical, physiological, and structural changes that have a significant impact on their quality [18]. Because the peppers are harvested and consumed at different ripening stages, from immature to over-ripening, the study of the accumulation of these bioactive compounds during fruit development is crucial.

Iwai et al. were the first to suggest a general behavior pattern according to which capsaicinoid production increased during ripening until a maximum value was reached. Then, a rapid reversal of the trend took place as these compounds were degraded by about 60% [19]. Later, Bernal et al. confirmed that the peroxidases were the enzymes responsible for this degradation by catalyzing the oxidation of C and DHC [20,21]. Although this is the general accumulation pattern, several recent studies of the accumulation of capsaicinoids during fruit development have revealed that there is not a single pattern, so that each variety of pepper studied follows a different ripening trend [22,23]. Moreover, in some cases that follow the same trend, the maximum concentration is reached on different days, causing the collection to be on different days. Therefore, in order to gain a more comprehensive understanding of the behavior of these health-promoting bioactive compounds, more in-depth studies with a larger number of varieties will need to be completed. This paper focuses specifically on Bolilla pepper (*Capsicum annuum*), a variety with a rounded shape, resembling that of tomatoes, with a diameter of around 2–3.5 cm [24]. This pepper variety is widely used in several areas in Spain, not only for the flavoring of dishes, but also for the production of paprika.

In order to enhance the added value of the product, it is necessary to determine the optimum harvest time of the peppers, i.e., the time when they show the maximum content of bioactive compounds. Therefore, the aim of this work was to determine the concentration of capsaicinoids in Bolilla peppers at different stages of plant maturation (young and adult), during 100 days of fruit development. The concentration of the compounds in the different plant tissues (placenta and pericarp) was also studied. This should allow us to identify the optimum time to harvest the peppers, taking into account the point of the maturation process, the part of the fruit, and the state of plant maturity at which the concentration of bioactive compounds of interest is at its maximum value, thus obtaining higher quality extracts and making the most of their health benefits. This would be of great value for analytical laboratories, as well as for companies and industries, since it would allow a better selection of the final products.

2. Materials and Methods

2.1. Reagents

The capsaicinoid reference standards, capsaicin (97%) and dihidrocapsaicin (90%) and the internal standard 2,5-dihydroxybenzaldehyde were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The methanol and glacial acetic acid were purchased from Merck (Darmstadt, Germany), both being of HPLC grade. The water was obtained through a Milli-Q water deionization system (Millipore, Bedford, MA, USA).

2.2. Growing and Fertilizing of the Peppers

The seeds were supplied by a paprika producer of the Protected Designation of Origin 'Pimentón de La Vera'. Subsequently, the peppers were grown in a greenhouse at the *Centro de Investigación y Formación Agraria* (CIFA/Center for Agricultural Research and Training) in Chipiona (Spain), a section of the *Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica* (IFAPA/Andalusian Institute for Agricultural, Fisheries, Food and Ecological Production Research and Training) (located about 8 m above sea level and having geographic coordinates of 36.7728, −6.3978 latitude and longitude, respectively), which belongs to the Andalusian Regional Government. The Bolilla pepper variety was sown in a 15 × 70 m seed bed, previously fertilized using 20 kg of magnesium sulphate, 15 kg of lime superphosphate, 20 kg of potassium sulphate, and 20 kg of ammonium sulphate. The sowing took place during the month of September, and peppers were transferred to the greenhouse in November. Each pot contained a substrate mixture of Humin Substrat (Klasman-Deilmann, Geeste, Germany) (1:1:1:1, *v*/*v*) peat, sand, clay-loam, and soil, enriched with 2 g of a slow-release fertilizer (Oscomote 16N-4P-9K, Scotts, Tarragona, Spain), and the pepper plants were irrigated using a drip system using nutrient-enriched water. In addition, fertigation was used according to the maturity stage of the plant (Table S1). The greenhouse temperature was monitored. Thus, over the winter months, when temperatures dropped below 10 °C, a heating system was activated, so that the temperature did not fall any further. During the hot periods, the greenhouse walls were raised to allow ventilation and prevent any excessive heating of the greenhouse (35–40 °C). It should be noted that over the entire period of the plants' growth and maturation, the greenhouse was affected by a whitefly plague (*Trialeurodes vaporariorum*). The direct damage caused by this infestation (yellowing and weakening of the plants) is due to larvae and adults feeding on the sap absorbed from the leaves. Indirect damage is also caused due to the proliferation of the black sooty mold growing on the honeydew produced during feeding, which stains and spoils the fruits and hinders the normal development of the plants. As a result, spraying to control this pest was quite frequent.

2.3. Ripening and Harvesting of the Peppers

The ripening of the hot peppers was tracked by attaching wool ribbons of different colors to the peppers when they were just beginning to emerge, just after the flowering period. In this way, when the peppers were harvested, depending on the color of the ribbon on each one, their age could be determined. The plants began to blossom at the beginning of January. From this date on, different colored ribbons were attached to the new peppers as they were born, with a spacing of 10 days. The first harvest was carried out on 17 April (plant ≈7 months old), these peppers being considered as those coming from the young plant (first maturity stage). The second harvest of peppers was conducted on 26 June, these peppers being considered to be from the adult plant (≈9.5 months old) (second maturity stage). From this date on, the plants stopped producing peppers, so the sampling tracking was concluded.

At least 30 peppers at each ripening stage, after being collected from the greenhouse, separated, and classified according to their stage of development, were peeled; the pericarp was separated from the placenta and the pips and peduncle were discarded. The pericarp and placenta were crushed and homogenized separately using a conventional electric

grinder. Once a homogeneous sample was obtained, it was kept at a temperature of −32 °C until further analysis.

2.4. Harvesting Conditions and Appearance of Bolilla Peppers at the Two Stages of Plant Maturity (Young and Adult)

The Bolilla pepper plants showed quite remarkable growth, reaching heights of around 150 cm. As already mentioned in the Materials and Methods section, this variety was severely affected by a whitefly infestation [25,26] and regular fumigation of the plants was necessary. To carry out the fumigation, a specific insecticide, Juvinal 10 EC (Pyriproxyfen 18% EC), was used. It was applied by spraying in a fixed installation in the greenhouse, in a dose of 50–75 mL/hL, with a safety period of 3 days. This affected the normal development of the plants and fruits, which resulted in poor production.

The Bolilla pepper plants started to produce peppers on 7 January. Thus, the youngest peppers harvested had been ripening for 10 dpa on the plant (M-1). Some of the peppers were left on the plant until over-ripening (M-9 and M-10). The oldest peppers harvested were 100 dpa (M-10) and were, therefore, in a state of over-ripeness, with significant water loss and a very intense red coloration. The visual state of the peppers at the time of collection is shown in Table 1.

Table 1. State of Bolilla peppers at the first maturity stage of the plant.

Code	Fruit Sprouting Date	Days Post-Anthesis (dpa)	Visual State
M-10	17 April	100	Over-ripening
M-9	07 April	90	Over-ripening
M-8	28 March	80	Red color
M-7	17 March	70	Red color
M-6	08 March	59	Red color
M-5	27 February	50	Red color
M-4	17 February	41	Green-Red color
M-3	06 February	30	Green color
M-2	27 January	20	Green color
M-1	17 January	10	Green color

Similarly to the first plant maturity stage, the study of the evolution of capsaicinoid content was carried out on peppers from the adult plants. The monitoring of maturation was the same as that for the first stage of maturation (Table 2).

Table 2. State of Bolilla peppers at the second maturity stage of the plant.

Code	Fruit Sprouting Date	Days Post-Anthesis (dpa)	Visual State
M-10	26 June	100	Over-ripening
M-9	16 June	90	Over-ripening
M-8	6 June	80	Red color
M-7	26 May	70	Red color
M-6	17 May	59	Red color
M-5	08 May	50	Red color
M-4	28 April	41	Green-Red color
M-3	17 April	30	Green color
M-2	07 April	20	Green color
M-1	28 March	10	Green color

2.5. Extraction Procedure

The pericarp and placenta extracts were obtained by Ultrasound Assisted Extraction (UAE). With this methodology, better yields are obtained with less use of solvents and time. It is thus referred to as a green technique [27], and is widely used for the extraction of capsaicinoids, and has been employed in a large number of previous studies [28–30]. For this purpose, a Probe UP 200S (Ultraschallprozessor Dr. Hielscher, Gmbh, Berlin,

Germany) was used, coupled to a temperature-controlled thermostatic bath (J.P. Selecta, Barcelona, Spain).

The extraction conditions were as follows: 0.2 g was weighed in 25 mL of methanol. The extraction was carried out at a temperature of 50 °C and at 200 W, for 15 min. A concentration of 0.5 mL of the 2,5-dihydroxybenzaldehyde internal standard was added to the resulting extract at a concentration of 1300 mg L^{-1}. The extracts were filtered through a 0.45 µm nylon syringe filter (Millex-HN, Merck, Darmstadt, Germany) before chromatographic analysis.

2.6. Identifying the Capsaicinoids through HPLC-MS

The main capsaicinoids detected and analyzed by HPLC-MS were n-DHC, C, DHC, h-C, and h-DHC. The following conditions were used for the analysis: two different solutions A (water) and B (methanol), acidified at 0.1% using acetic acid, 0.2 mL min^{-1} flow rate, 25 µL injection volume, and the following chromatographic separation gradient (time, solvent B): 0 min, 0% B; 1 min, 0% B; 5 min, 30% B; 8 min, 50% B; 16 min, 70% B; 20 min, 70% B; 28 min, 90% B; 30 min, 90% B; 32 min, 100% B; 42 min, 100% B. A Finnigan LCQ-coupled LC-MS system (Termo Electron Co., San Jose, CA, USA), consisting of a Spectra SYSTEM 2000 gradient pump (Thermo Separation Products, Fremont, CA, USA), a C-18 analytical column (Luna 5 µm, 150 × 3 mm, Phenomenex, Torrance, CA, USA), an electrospray system as the ionization source, and an ion trap mass analyzer were employed. The software Xcalibur version 1.2 was used for both equipment control and the subsequent analyses. All the instruments' parameters were configured according to the method previously developed by our research group [31].

2.7. Developing the HPLC-FLR Quantification Method

Once the capsaicinoids had been identified, they were separated, quantified, and analyzed by HPLC-FLR. A dionex chromatographic system (Sunnyvale, CA, USA), consisting of an automated sample injector (ASI-100), a pump (P680), a thermostatic column compartment (TCC-100), a Chromolith TH Performance RP-18e (100 mm × 4.6 mm) monolithic column (Merck, Darmstadt, Germany), a photodiode array detector (PDA-100), a fluorescence detector (RF 2000), a universal chromatography interface (UCI-50), and the software application Chromaleon 6.60 were employed. The operating conditions, the parameters of the equipment components, and the variables used were the same as in our previously published works [32].

In order to allow the quantification of the compounds of interest, the calibration curves corresponding to the capsaicinoids for which we have available standards, capsaicin (C) and dihydrocapsaicin (DHC), were elaborated and the following results were obtained: y = 112.901x + 187, r^2 = 0.9995, LOD = 0.008 mg L^{-1}, and LOQ = 0.028 mg L^{-1} for C; y = 151.770x + 4589, r^2 = 0.9995, LOD = 0.011 mg L^{-1}, and LOQ = 0.036 mg L^{-1} for DHC. Since there are no commercially available standards for n-DHC, h-C, and h-DHC, these were quantified based on the calibration curve of DHC (for n-DHC and h-DHC) and C (for h-C), given the structural similarities between these molecules. All the analyses were performed in duplicate.

2.8. Statistical Analysis

The data from the duplicate analyses were expressed as the mean ± standard deviation (SD). Firstly, these data were subjected to a Shapiro–Wilk test to verify the normality and homogeneity of variances of the data. Subsequently, they were subjected to an Analysis of Variance (ANOVA), together with Tukey's test, to determine whether the different capsaicinoid contents throughout the ripening of the peppers presented statistically significant differences at a significance level of 95%. Accordingly, the results with a *p*-value lower than 0.05 were considered statistically different, as has been indicated by a different letter throughout the manuscript. The software application Statgraphic Centurion Version XVIII (Statgraphics Technologies, Inc., Los Llanos, VA, USA) was used for the statistical analyses.

3. Results and Discussion

3.1. Changes of Capsaicinoid Content in the Pericarp of Bolilla Pepper at Two Different Plant-Maturity Stages

Figure 1 shows a comparison between the concentration of total capsaicinoids in the peppers' pericarp at the two stages of plant maturity studied.

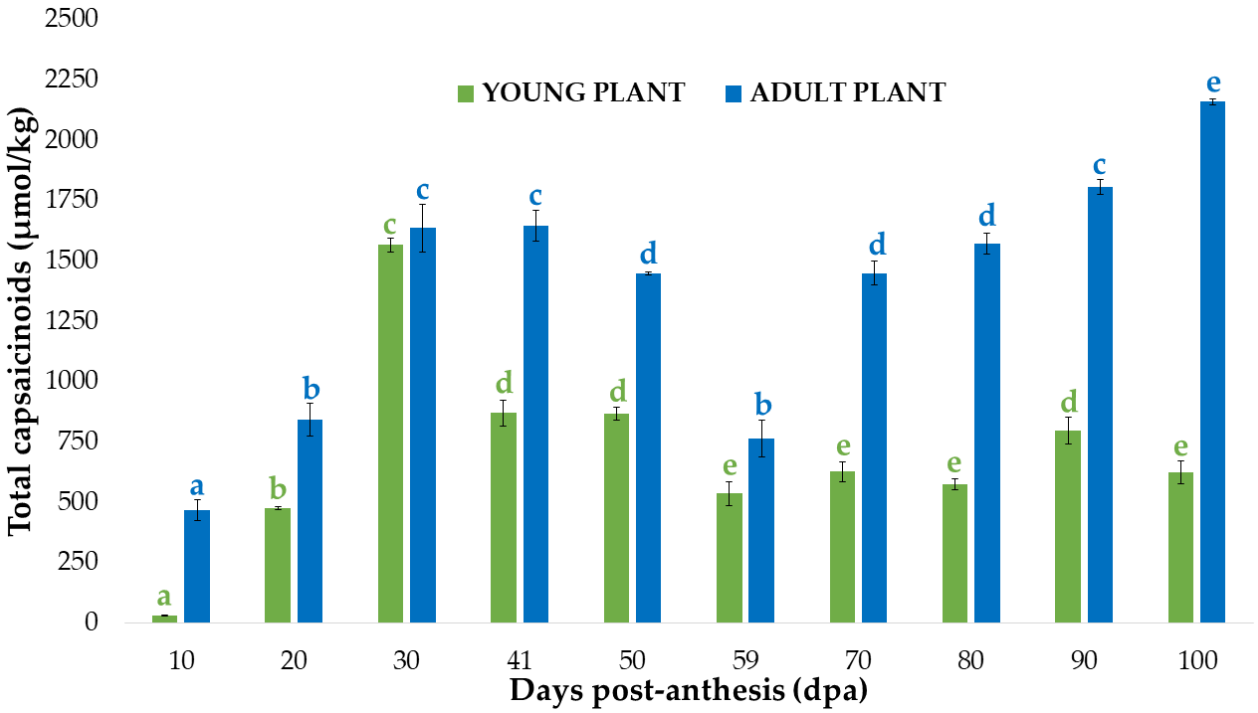


Figure 1. Changes in total capsaicinoid content (µmol/kg) during the ripening process in the pericarp of Bolilla peppers from both the young and adult plants (n = 2). (Different letters above the bars indicate a significant difference between results at a 95% confidence level according to Tukey's test).

As can be seen, for the young plant, there was a very sharp increment in the amount of total capsaicinoids up to 30 dpa (M-3). On 41 dpa (M-4) there was a rather sharp drop in the concentration of capsaicinoids in the peppers' pericarp, which continued until 59 dpa. This drop can be explained, as previously mentioned, by the oxidation caused by the peroxidases that are present in the peppers, since these enzymes are involved in the degradation of capsaicinoids [33]. This oxidation of C and DHC by the peroxidases was strictly dependent on the presence of H_2O_2 [20,21]. From this stage of maturation onwards, the concentration of total capsaicinoids remained fairly steady with no significant fluctuations.

With respect to the amount of total capsaicinoids present in the pericarp of Bolilla peppers from adult plants, it was observed that the concentration of capsaicinoids increased up to 30 dpa (M-3). From this point, a decrease in the concentration of capsaicinoids was observed, with a rather pronounced minimum concentration on 59 dpa (M-6). From 70 dpa (M-7) onwards, a moderate rise in capsaicinoid concentration was observed, which became slightly more pronounced towards the end of the fruit ripening process. This effect was not observed in the peppers from young plants, which can be explained by the higher temperature levels in the greenhouse [34] that adult plants were subjected to, and which resulted in a more pronounced dehydration process [8,35].

Subsequently, a comparison between the total capsaicinoid content in the peppers from young and adult plants was conducted to determine if there were any differences in their content related to the maturity of the plants. Up to 70 dpa, similar trends were observed in both cases, with total capsaicinoid concentrations increasing with the ripening of the fruits, and reaching maximum values, before then dropping drastically. In the case of the peppers from young plants, this maximum concentration was reached on

30 dpa with a value of 1565.98 µmol/kg; the peppers from the adult plants reached their 2158.82 µmol/kg maximum value on 41 dpa, although this maximum value was not significantly higher than the concentration previously registered on 30 dpa. Several authors previously reported similar results throughout the development of the fruit from other pepper varieties, both from plants of different maturities or from plants of the same age [36,37]. It should be highlighted that, in our study, the concentration of capsaicinoids increased with the maturity of the plant, i.e., the adult plants produced peppers with a greater quantity of bioactive compounds than the young plants. This is in agreement with the data corresponding to the Jeromín pepper variety, which had also been analyzed by our research group [32].

Finally, it is worth mentioning that, unlike other pepper varieties when ripened and harvested under the same conditions, the Bolilla peppers from the adult plants exhibited a considerable increase in the concentration of capsaicinoids. Nevertheless, this behavior has also been observed in certain varieties such as Habanero Roxo [23] or Malgueta [38].

3.2. Changes of Capsaicinoids Content in the Placenta of the Bolilla Pepper at Two Different Plant-Maturity Stages

The changes in the total capsaicinoid content in the placenta of Bolilla pepper were studied, both in peppers from young plants and those from adult plants. The results are shown in Figure 2.

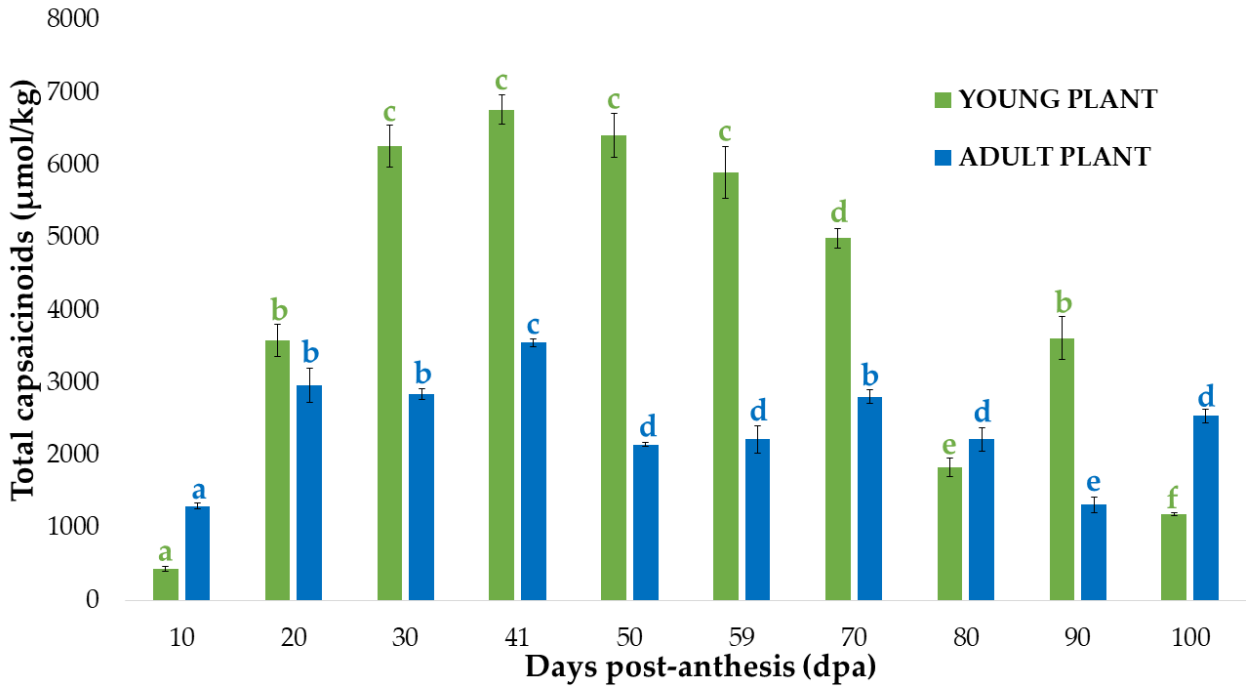


Figure 2. Changes in total capsaicinoid content (µmol/kg) during the ripening process in the placenta of Bolilla pepper from both young and adult plants (n = 2). (Different letters above the bars indicate significant differences between results at the 95% confidence level according to Tukey's test).

When the results for peppers from the young plants are observed, it can be seen that the concentration of capsaicinoids increased until 41 dpa (M-4). From this moment onward, there was a gentle decrease in the concentration of capsaicinoids until 70 dpa (M-7), which became much more pronounced on 80 dpa, where it reached its minimum value. On 90 dpa (M-9), a considerable increment can be seen again, and then the concentration decreased towards the end of the process after 100 days of development. Several studies have reported that the accumulation of capsaicinoids in peppers reached its highest concentration during the first stages of the fruit ripening, generally between 40 and 60 dpa. [36,39].

Regarding the changes in the total capsaicinoid content in the placenta of Bolilla peppers from the adult plants, a quite similar behavior was observed. Thus, the concentration of capsaicinoids increased until 41 dpa (M-4), and then decreased sharply on 50 dpa, to remain relatively constant until day 60 (M-6). During the last days of fruit development, there were slight decreases and increases in capsaicinoid concentration, although intermediate values were always exhibited. This variability during the more mature stages of the fruit can be explained by the activity of the enzyme capsaicin synthase, which allows the synthesis of new bioactive compounds in competition with the action of the peroxisome enzymes that cause their degradation [40].

Finally, a completely different behavior from that previously observed was noticed, both in Bolilla peppers' pericarp and in the Jeromin peppers previously analyzed [32]. In this case, the highest concentration of total capsaicinoids corresponded to the peppers from the young plants, and not to those from the adult ones, i.e., as the maturity of the plant increased, the quantity of these bioactive compounds decreased. This suggests that, in addition to climatic factors (highlighting water availability, growing conditions, mineral supply, plant infection, light, and temperature [34]) and plant maturity, there are certain genetic factors, and other factors related to defensive behavior against environmental aggressors or pests (whitefly), that affect the production of capsaicinoids in the fruits [26,41]. To confirm this aspect, it would be necessary to monitor healthy plants, so that any influence of pests can be left out. It would be extremely important to confirm this adverse effect of pests on the plants because of its economic relevance regarding the production of capsaicinoids.

3.3. Comparison of Total Capsaicinoid Content in the Pericarp and Placenta of Bolilla Peppers from Young and Adult Plants

The concentration of total capsaicinoids, expressed in (µmol/kg), both in the pericarp and placenta of peppers from young plants, can be seen in Figure 3.

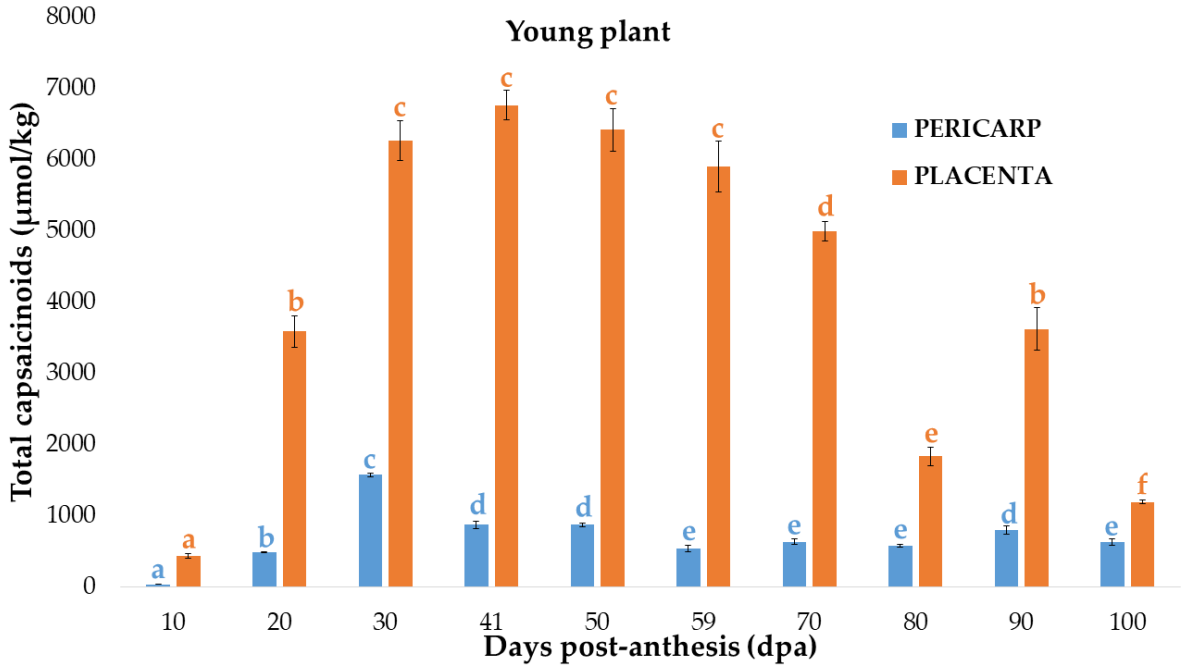


Figure 3. Changes in total capsaicinoid (µmol/kg) content in the pericarp and placenta of Bolilla peppers from the young plants (n = 2). (Different letters above the bars indicate significant differences between results at the 95% confidence level according to Tukey's test).

It can be observed that the total capsaicinoid content in the placenta was considerably greater than that in the pericarp, since the placental epidermal tissue is the only part of the plant where the synthesis and accumulation of capsaicinoids takes place [42]. This differ-

ence in capsaicinoid content between pericarp and placenta may be due to several factors such as the peroxidase enzyme activity or the competition for diffusion by the capsaicinoids in the different parts of the peppers [43,44]. However, this difference was more pronounced in certain ripening stages than in others, so that the ratio of total capsaicinoids between the placenta and the pericarp of the peppers was not constant and varied according to the ripening stage of the fruit. Other authors have also reported a higher concentration of capsaicinoids in the placenta of the peppers with respect to that in the pericarp, and this difference varies depending on the stage of development [43,44].

Similar results were observed in the peppers from adult plants, with capsaicinoid content being higher in the placenta than in the pericarp, as shown in Figure 4. In this case, the maximum concentration of capsaicinoids in the placenta was reached 10 days later than in the peppers from young plants, and, at the end of the fruit ripening, capsaicinoid content was almost twice as much as that in the peppers from young plants. This can be explained by the lower activity of the degrading agents as the plant matures. [31,39].

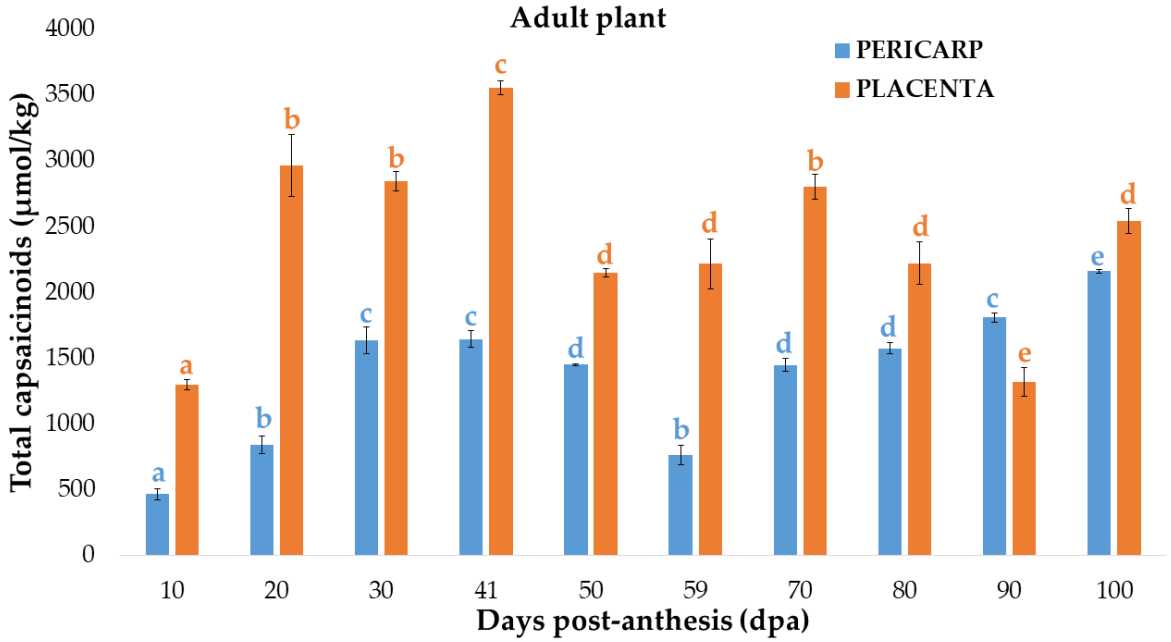


Figure 4. Changes in total capsaicinoid (µmol/kg) content in the pericarp and placenta of Bolilla peppers from adult plants (n = 2). (Different letters above the bars indicate significant differences between results at the 95% confidence level according to Tukey's test).

3.4. Individual Capsaicinoid Contents

As previously mentioned, individual capsaicinoids were determined in the pericarp and placenta of peppers from young and adult plants. These concentrations are expressed in µmol/kg pepper. The results corresponding to the five major capsaicinoids (n-DHC, C, DHC, h-C, and h-DHC) are given in Table 3. All the analyses were performed in duplicate.

C and DHC presented the highest capsaicinoid content both in the pericarp and in the placenta during the ripening stages of the peppers. Both of these capsaicinoids are responsible for the pungent sensation of the peppers [45].

Like in other studies on capsaicinoids in peppers, small amounts of h-C, h-DHC, and n-DHC were detected [46,47].

Finally, it is worth mentioning that the individual capsaicinoid concentrations followed the same trend as the concentration of total capsaicinoids, both in peppers from young and adult plants, and both the pericarp and the placenta of the peppers.

Table 3. Individual capsaicinoid concentrations in the pericarp and placenta of peppers from young and adult plants.

Concentration (µmol/kg)		n-DHC	C	DHC	h-C	h-DHC
			Pericarp			
	M-1	5.05 ± 0.46 [a]	10.26 ± 0.36 [a]	9.97 ± 0.44 [a]	2.09 ± 0.04 [a]	2.23 ± 0.16 [a]
	M-2	49.42 ± 0.41 [b]	217.52 ± 3.11 [b]	186.08 ± 2.63 [b]	8.23 ± 0.11 [b]	14.43 ± 0.34 [b]
	M-3	197.52 ± 2.86 [c]	677.98 ± 14.75 [c]	622.03 ± 10.66 [c]	19.15 ± 1.07 [c]	49.31 ± 0.47 [c]
	M-4	117.51 ± 6.35 [d]	434.80 ± 15.61 [d]	361.95 ± 23.77 [d]	10.16 ± 1.13 [d]	21.79 ± 1.12 [d]
	M-5	106.30 ± 3.68 [d]	390.72 ± 10.96 [e]	327.02 ± 10.00 [d]	11.61 ± 2.56 [d]	28.51 ± 1.29 [e]
	M-6	59.01 ± 7.44 [e]	272.88 ± 37.30 [f]	176.19 ± 22.87 [e]	8.52 ± 0.47 [e]	18.30 ± 1.96 [f]
	M-7	76.17 ± 9.53 [f]	301.92 ± 36.33 [f]	206.06 ± 28.48 [e]	9.72 ± 0.16 [f]	23.56 ± 3.29 [g]
	M-8	71.78 ± 3.17 [f]	286.55 ± 13.48 [f]	183.87 ± 6.76 [e]	9.37 ± 0.20 [f]	22.81 ± 0.47 [g]
	M-9	66.75 ± 4.59 [f]	266.79 ± 15.26 [f]	177.04 ± 4.18 [e]	8.52 ± 0.64 [g]	22.32 ± 1.93 [g]
Young plant	M-10	67.26 ± 2.56 [f]	347.82 ± 6.35 [g]	252.35 ± 24.88 [f]	11.01 ± 2.19 [g]	30.62 ± 2.44 [h]
			Placenta			
	M-1	53.13 ± 7.34 [a]	180.50 ± 22.42 [a]	164.22 ± 17.55 [a]	16.80 ± 1.48 [a]	13.89 ± 1.28 [a]
	M-2	364.47 ± 41.67 [b]	1640.18 ± 185.05 [b]	1399.41 ± 172.50 [b]	81.46 ± 9.32 [b]	96.87 ± 12.09 [b]
	M-3	908.80 ± 20.81 [c]	3164.30 ± 99.76 [c]	2597.84 ± 342.46 [c]	96.09 ± 5.68 [b]	85.17 ± 5.63 [b]
	M-4	750.74 ± 54.64 [d]	2580.06 ± 240.86 [d]	2279.49 ± 128.16 [c]	107.09 ± 1.30 [c]	180.74±13.01 [c]
	M-5	804.13 ± 35.86 [d]	2900.02 ± 135.09 [e]	2408.40 ± 118.93 [c]	102.33 ± 5.66 [c]	196.49 ± 6.56 [c]
	M-6	716.12 ± 16.51 [d]	3189.83 ± 203.06 [e]	2249.87 ± 151.32 [c]	99.22 ± 2.32 [c]	178.74±24.12 [c]
	M-7	640.04 ± 7.98 [e]	2477.59 ± 22.20 [d]	1608.74 ± 94.22 [d]	85.67 ± 4.74 [b]	177.08 ± 2.53 [c]
	M-8	240.98 ± 8.34 [f]	882.03 ± 78.04 [f]	597.29 ± 35.21 [e]	35.41 ± 1.54 [d]	71.77 ± 3.41 [d]
	M-9	259.03 ± 14,46 [f]	1179.06 ± 243.45 [f]	782.38 ± 29.57 [f]	39.02 ± 4.82 [d]	88.48 ± 5.88 [e]
	M-10	164.89 ± 12.73 [g]	461.78 ± 53.74 [g]	449.26 ± 2.12 [g]	14.61 ± 1.41 [e]	51.04 ± 1.41 [f]
			Pericarp			
	M-1	28.92 ± 3.43 [a]	270.11 ± 33.92 [a]	139.69 ± 9.29 [a]	7.00 ± 0.07 [a]	8.77 ± 0.43 [a]
	M-2	65.14 ± 6.33 [b]	462.23 ± 37.79 [b]	280.16 ± 22.09 [b]	13.87 ± 1.62 [b]	19.67 ± 1.18 [b]
	M-3	136.36 ± 17.36 [c]	836.05 ± 105.30 [c]	603.80 ± 70.93 [c]	18.30 ± 2.27 [c]	41.13 ± 3.94 [c]
	M-4	176.62 ± 7.89 [d]	750.46 ± 25.63 [d]	649.78 ± 25.92 [c]	20.49 ± 1.06 [c]	47.71 ± 2.66 [c]
	M-5	134.11 ± 0.36 [c]	768.15 ± 2.31 [d]	486.84 ± 5.71 [d]	19.95 ± 1.08 [c]	39.72 ± 0.15 [c]
	M-6	76.71 ± 9.09 [b]	371.38 ± 37.67 [b]	254.60 ± 18.04 [b]	10.67 ± 1.04 [d]	23.24 ± 3.96 [b]
	M-7	153.42 ± 4.63 [c]	700.31 ± 24.35 [d]	525.54 ± 18.18 [d]	23.93 ± 1.44 [e]	44.68 ± 1.09 [c]
	M-8	199.35 ± 4.72 [e]	629.41 ± 16.19 [e]	662.81 ± 19.64 [c]	26.69 ± 1.20 [e]	54.58 ± 1.81 [d]
	M-9	243.58 ± 6.95 [f]	722.09 ± 8.97 [d]	741.26 ± 13.55 [e]	30.35 ± 0.28 [f]	69.60 ± 2.13 [e]
Adult plant	M-10	309.40 ± 6.60 [g]	851.42 ± 17.63 [c]	874.30 ± 2.33 [f]	41.81 ± 0.21 [g]	81.89 ± 0.02 [f]
			Placenta			
	M-1	75.79 ± 7.43 [a]	410.16 ± 62.24 [a]	9.97 ± 20.77 [a]	21.50 ± 2.46 [a]	39.57 ± 1.39 [a]
	M-2	230.39 ± 16.90 [b]	1020.43 ± 129.85 [b]	186.08 ± 80.98 [b]	46.97 ± 2.62 [b]	68.29 ± 4.66 [b]
	M-3	272.81 ± 5.79 [c]	1089.28 ± 37.61 [b]	622.03 ± 26.35 [c]	35.08 ± 0.67 [c]	82.83 ± 2.07 [c]
	M-4	418.52 ± 5.69 [d]	1422.40 ± 41.36 [c]	361.95 ± 18.59 [d]	39.46 ± 0.65 [d]	107.34 ± 2.24 [d]
	M-5	212.59 ± 1.44 [b]	749.23 ± 18.89 [d]	327.02 ± 9.55 [d]	28.12 ± 1.88 [e]	60.57 ± 0.71 [e]
	M-6	233.76 ± 26.69 [b]	816.22 ± 89.56 [d]	176.19 ± 61.86 [b]	37.78 ± 4.87 [d]	70.46 ± 7.66 [b]
	M-7	340.63 ± 2.81 [e]	1046.50 ± 58.31 [b]	206.06 ± 29.57 [b]	45.31 ± 2.08 [b]	99.08 ± 1.32 [f]
	M-8	278.97 ± 32.90 [f]	954.76 ± 31.94 [b]	183.87 ± 83.62 [b]	37.67 ± 3.45 [d]	77.16 ± 9.43 [b]
	M-9	108.55 ± 1.55 [g]	326.66 ± 4.29 [e]	177.04 ± 6.59 [b]	15.89 ± 0.15 [f]	34.69 ± 0.95 [g]
	M-10	322.98 ± 12.72 [h]	971.29 ± 125.28 [b]	252.35 ± 102.96 [b]	47.60 ± 15.08 [b]	93.16 ± 2.42 [h]

Different letters in each column of the same part of the fruit and plant maturity indicate significant differences between results at the 95% confidence level according to Tukey's test.

4. Conclusions

It was demonstrated that the maturation stage is a crucial factor that determines the ideal time for harvesting, since drastic changes in the capsaicinoid content, both in the pericarp and placenta of the peppers, and in the two stages of plant maturity associated with this factor, were observed. The data presented in this study should allow harvesting

of Bolilla peppers at the time when their capsaicinoid content is at its maximum level. This would be on 30 dpa for the pericarp and 40 dpa in the case of the placenta.

The total capsaicinoid content in the placenta has always been greater than that in the pericarp, since the synthesis of these compounds takes place in the placenta of the pepper and then diffuses to other parts of the plant. It can also be observed that the capsaicinoid concentration differences between the pericarp and placenta of the adult plant were not so pronounced, probably due to a lower activity of the degrading enzymes. On the other hand, both young and adult plants followed similar capsaicinoid accumulation patterns and trends for either part of the fruits. In the case of the pericarp, as the plant matures, a higher concentration of capsaicinoids was obtained. In contrast, in the placenta the opposite behavior was revealed, with the young plant presenting a greater quantity of bioactive compounds. Finally, the individual capsaicinoids accumulated in the Bolilla peppers were quantified and it was observed that the major compound was C with a maximum value of 3189.83 µmol/kg, followed by DHC, reaching 2408.40 µmol/kg. We can conclude that the optimal time for the harvesting of Bolilla peppers in order to maximize their total capsaicinoid concentration was established.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020435/s1, Table S1: Fertirrigation applied to the pepper plants.

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