Dynamics of carotenoids and total phenolic compounds in commercial chilli pepper varieties across ripening stages

Evolución de los carotenoides y los compuestos fenólicos totales en variedades comerciales de pimiento picante a lo largo de las etapas de maduración

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Abstract

Chilli peppers (*Capsicum* spp.) are valued for their flavour, colour, and bioactive compounds. Their global production has increased due to gastronomic and functional importance. This study aimed to quantify the total carotenoids and phenolic compounds in six chilli varieties cultivated in Ecuador, including giant yellow (*Capsicum baccatum*), chile (*Capsicum chinense*), criollo (*Capsicum annuum*), gallinazo (*Capsicum frutescens*), habanero manzano (*Capsicum chinense*), and jalapeño (*Capsicum annuum*). These are at different stages of ripeness, represented by the change in colour of the peel (M10%, M50%, M80%). Bioactive compounds were extracted by ultrasound-assisted microextraction and quantified by spectrophotometry and microplate reader, with phenols determined using the Folin–Ciocalteu reagent. Results revealed species- and ripening-dependent trends. 'gallinazo' and 'habanero manzano' showed the highest phenolic content at 80%. Carotenoids increased progressively during ripening, with 'habanero manzano' and 'gallinazo' reaching maximum levels at M80%. These findings highlight the influence of genotype and maturity on the accumulation of bioactive compounds, which is relevant for food and nutraceutical applications.

Keywords: Bioactive compounds; Functional foods; Microextraction; Microplates

Introduction

The development of fruit is a complex process comprising three main stages, such as growth, development, and ripening, followed by softening and senescence. During the initial phase, cells divide and then increase in size due to the expansion of vacuoles. During ripening, the fruit reaches its maximum size and undergoes physiological and biochemical transformations, including changes in colour, texture, and chemical composition (e.g., pigments, sugars, and secondary metabolites). Genetic and environmental factors regulate these transformations, and some external interventions can delay softening (Martínez-González et al., 2017).

In this context, chillies or hot peppers (*Capsicum* spp.) are an important group of fruits, with commercially significant species including *C. annuum, C. chinense, C. frutescens, C. baccatum* and *C. pubescens* (Osorio et al., 2021). This genus is native to South America, but thanks to improved agricultural practices and growing demand for its fruits, its cultivation has expanded globally. It is currently the second most economically important vegetable in the world (Holguín-Burgos et al., 2022).

The attractive colour, aroma and spiciness of chillies make them popular natural seasonings and colourings in the food industry. They are also notable for their nutritional value and phytochemical content, including vitamins, phenolic compounds, and carotenoids. These compounds have been associated with health benefits, including the prevention of cardiovascular disease, relief from arthritis pain, and modulation of the immune system (Liu et al., 2024).

A distinctive feature of the *Capsicum* genus is its ability to synthesise capsaicinoids, which give the fruit its spicy flavour. To date, around 23 capsaicinoid compounds have been identified, with capsaicin and dihydrocapsaicin accounting for approximately 95% of these. In particular, capsaicin has been extensively studied for its analgesic, antioxidant and anti-inflammatory properties (Guevara et al., 2021). Within this framework, the focus of this research was to evaluate the dynamics of bioactive compounds, such as total phenols and carotenoids, in chillies of various varieties, sizes and colours at different stages of ripeness. The results of the study demonstrate the functional value and potential applications of chillies in the food and nutraceutical industries.

Materials and methods

For the study, six varieties of peppers were collected at three stages of ripeness, defined by changes in peel colour, thus 10% (10% of the final colour), 50% (50% of the final colour),

and 80% (80% of the final colour) (Figure 1). Additionally, plant material was collected for botanical identification. This was carried out in Nayón, Quito, Ecuador. Fruits were selected at random in accordance with the sampling protocol established by Agrocalidad-INT/CPA/01 (INEN, 2014) and the NTE-INEN-1750 standard (INEN, 2012).

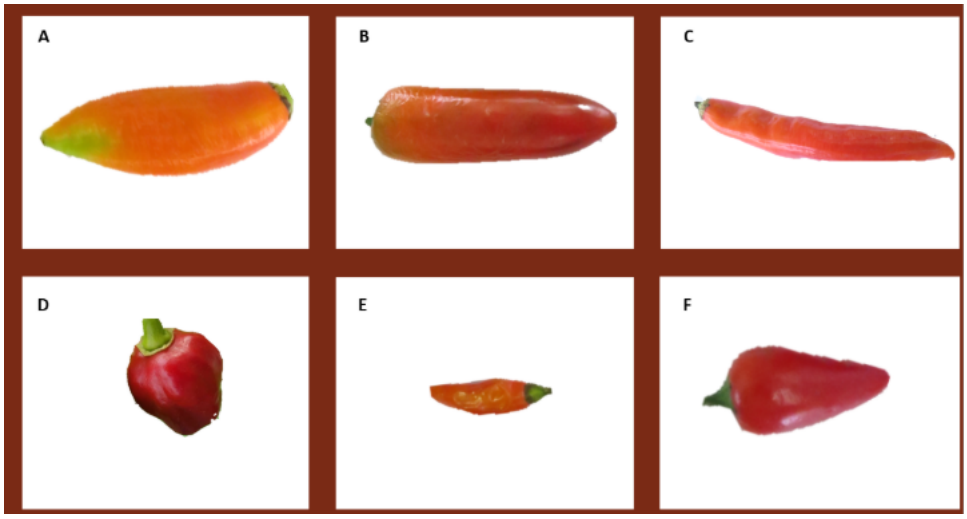

Figure 1. Photograph of different species of pipper at 80% maturity.

Note: A) Giant yellow pepper (*Capsicum baccatum*); B) Jalapeño pepper (*Capsicum annuum*); C) Criollo pepper (*Capsicum annuum*); D) Habanero manzano pepper (*Capsicum chinense*); E) Gallinazo pepper (*Capsicum frutescens*); F) Chile pepper (*Capsicum chinense*).

The collected samples were washed, and the seeds were removed before being cut into small pieces. To prevent the degradation of bioactive compounds, they were frozen at –80 °C and then freeze-dried in a Christ Alpha 1-4 LDplus freeze dryer (Martin Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

Carotenoids were determined using the methodology described by Coyago-Cruz et al. (2023), with slight modifications. Twenty milligrams of freeze-dried sample powder were weighed and then 250 µL of methanol, 500 µL of trichloromethane and 250 µL of water were added to this.

The mixture was homogenised and subjected to ultrasonic agitation using a Fisher Scientific FS60 (Fisher Scientific, Waltham, MA, USA) for two minutes. The supernatant was recovered, and the solid residue was re-extracted with 500 µL of trichloromethane. This process was repeated as many times as necessary until all the pigments had been obtained. The combined extracts were evaporated to dryness in a Buchi R-100 rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland) at a temperature below 40 °C and in the dark to prevent degradation

of the compounds. The dry residue was redissolved in 200 µL of HPLC-grade ethanol, and the absorbance was measured using a BioTek Epoch microplate reader spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA) at 450 nm. A calibration curve was prepared using β-carotene as a standard, and the results were expressed in milligrams of β-carotene per 100 g of dry weight.

Total phenolic compounds were determined following the methodology described by Coyago-Cruz et al. (2024), with slight modifications. Twenty milligrams of lyophilised powder were weighed and extracted with 1 ml of methanol acidified with 0.1% HCl. The mixture was homogenised and subjected to ultrasound for three minutes. The supernatant was recovered, and the solid residue was re-extracted twice more with 500 µL of acidified methanol. The extracts obtained were combined. To quantify the compounds, 20 µL of the extract, 100 µL of Folin–Ciocalteu reagent (diluted 1:4) and 75 µL of 100 g/L sodium carbonate were placed in each well of a 96-well plate. The reaction was kept in the dark for two hours, after which the absorbance was measured at 750 nm using a spectrophotometer with a microplate reader. A calibration curve was prepared using gallic acid as a standard, and the results were expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

Finally, the results obtained were analysed by one-way analysis of variance (ANOVA) using STATGRAPHICS Centurion XVII. Tukey´s test was used to compare means, with a significance level of $p < 0.05$. Graphs of results were generated using SigmaPlot version 14.0 software.

Results and discussion

Figure 2 shows the average carotenoid concentration for different varieties of chilli pepper at various stages of ripeness. In all cases, the highest concentration was recorded at the most advanced stage of ripeness (80%). The highest concentrations were found in Capsicum chinense (habanero) and C. chinense (chilli) varieties. These variations are related to the biosynthesis and progressive accumulation of carotenoids during ripening, a process that is regulated by physiological factors and the genetics of each variety (Ionica et al., 2017).

The total carotenoid concentrations obtained in this study far exceeded those reported in previous studies. For instance, a range of 184.4 to 675.8 mg/100 g dry weight has been reported for Capsicum annuum 'Paprika' (*Capsicum annuum*) (Yang et al., 2024). By contrast, a study of the 'Z1' variety (*C. annuum*) under different photoperiod conditions recorded much lower concentrations of between 0.1 and 2.4 mg/L (Ma et al., 2024). A literature review also compiled a narrower range of 1.0–1.3 mg/100 g dry weight for total carotenoids in various

Capsicum accessions (Mengistu & Beri, 2024).

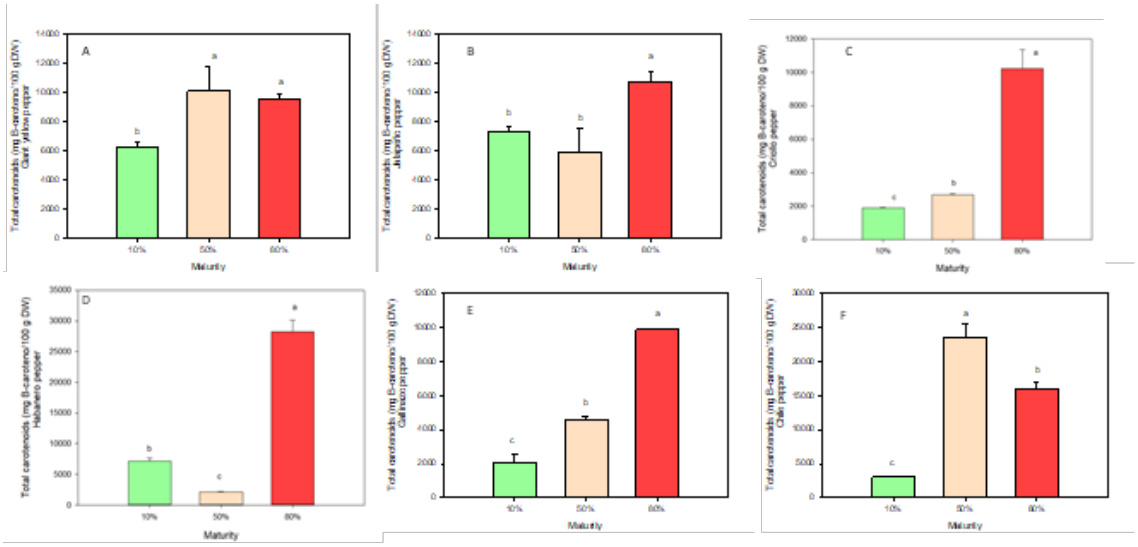

Figure 2. Average total carotenoid concentration values of pipper at different degrees of ripeness.

Note: A) Giant yellow pepper (*Capsicum baccatum*); B) Jalapeño pepper (*Capsicum annuum*); C) Criollo pepper (*Capsicum annuum*); D) Habanero manzano pepper (*Capsicum chinense*); E) Gallinazo pepper (*Capsicum frutescens*); F) Chile pepper (*Capsicum chinense*). The vertical bars indicate the standard deviation, and the capital letters above the bars indicate homogeneous groups with Tukey's *p*-value < 0.05.

Figure 3 shows the average carotenoid concentration for different varieties of chilli pepper at various stages of ripeness. In all cases, the highest concentration was recorded at the most advanced stage of ripeness (80%). In terms of total phenols, the concentrations determined in this study exceeded those reported in previous studies. For instance, a range of 3.6–4.2 mg EAG/g dry weight was reported for *Capsicum annuum* 'Paprika' (Yang et al., 2024), while values between 3.1–3.8 mg EAG/g dry weight were observed in three Korean varieties ('Dokbulwang', 'Subicho' and 'Eumseong')(Jang et al., 2024). A literature review, meanwhile, documented a much wider range: 720.5–852.0 mg EAG/100 g dry weight in various *C. annuum* varieties (Mengistu & Beri, 2024). These discrepancies can be attributed to methodological factors in phenol quantification, the type of analysed tissue, the geographical origin of the samples, and the harvest season, as suggested by other authors (Coyago-Cruz, Méndez, et al., 2023).

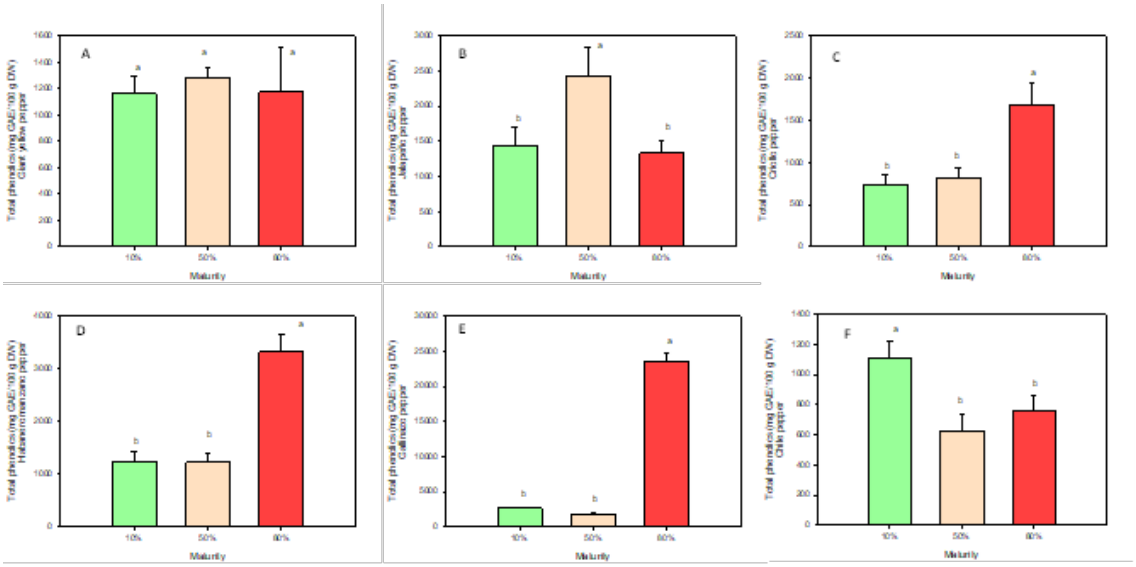

Figure 3. Average total phenolic concentration values of pipper at different degrees of ripeness.

Note: A) Giant yellow pepper (*Capsicum baccatum*); B) Jalapeño pepper (*Capsicum annuum*); C) Criollo pepper (*Capsicum annuum*); D) Habanero manzano pepper (*Capsicum chinense*); E) Gallinazo pepper (*Capsicum frutescens*); F) Chile pepper (*Capsicum chinense*). The vertical bars indicate the standard deviation, and the capital letters above the bars indicate homogeneous groups with Tukey's *p*-value < 0.05.

Conclusions

This study demonstrated that the concentrations of total carotenoids and phenols in commercially grown chilli peppers in Ecuador vary according to the ripeness stage and genetics of each species. The highest carotenoid content was consistently observed in the advanced stage of ripeness (80%), with habanero and chile (*Capsicum chinense*) varieties emerging as the highest levels. Similarly, total phenol content varied, with higher levels observed in *C. frutescens* (gallinazo) at advanced stages, while *C. chinense* (chile) exhibited higher concentrations at the early stage (10%). These findings underscore the importance of considering variety and the degree of maturity in the accumulation of bioactive compounds. This information is valuable for selecting raw materials in the food and nutraceutical industries.

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