Influence of ripening stages on carotenoid and total phenolic accumulation in multicoloured cherry tomato varieties

Influencia de los estadios de maduración sobre la acumulación de carotenoides y compuestos fenólicos totales en variedades multicolores de tomate cherry

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Abstract

Tomatoes (*Solanum lycopersicum*) are widely consumed worldwide, with cherry tomato varieties gaining interest for their nutritional and sensory attributes. Nevertheless, little is known in Ecuador about their bioactive properties. This study aimed to quantify carotenoids and total phenols in coloured cherry tomato varieties (yellow, green, black, oval red, and elongated red) at three ripening stages (M0%, M50%, M100%) defined by skin colour. Bioactive compounds were extracted using microextraction, and their quantification was performed using a spectrophotometer with a microplate reader. Total phenols were determined using the Folin-Ciocalteu method. Results showed that black, oval red, and red spotted tomatoes reached the highest carotenoid levels at full maturity (1172.06, 959.31, and 845.01 mg/100 g DW). In contrast, total phenols decreased during ripening in most varieties, with yellow tomatoes showing the highest initial value (5.69 mg GAE/100 g DW). These findings highlight the influence of colour and ripeness on bioactive compound profiles, supporting their nutritional and functional potential.

Keywords: *Solanum lycopersicum*; Microextraction; Bioactive compounds; Folin.

Resumen

Los tomates (*Solanum lycopersicum*) son ampliamente consumidos a nivel mundial, y las variedades de tomate cherry han despertado interés debido a sus atributos nutricionales y sensoriales. Sin embargo, en Ecuador existe escasa información sobre sus propiedades bioactivas. El objetivo de este estudio fue cuantificar carotenoides y fenoles totales en variedades coloreadas de tomate cherry (amarillo, verde, negro, rojo ovalado y rojo alargado) en tres estadios de maduración (M0 %, M50 % y M100 %), definidos según el color de la epidermis. Los compuestos bioactivos fueron extraídos mediante microextracción y cuantificados utilizando un espectrofotómetro con lector de microplacas. Los fenoles totales se determinaron mediante el método de Folin-Ciocalteu. Los resultados mostraron que los tomates negros, rojos ovalados y rojos moteados alcanzaron los niveles más altos de carotenoides en madurez completa (1172,06; 959,31 y 845,01 mg/100 g de peso seco, respectivamente). En contraste, los fenoles totales disminuyeron durante la maduración en la mayoría de las variedades, siendo los tomates amarillos los que presentaron el valor inicial más alto (5,69 mg EAG/100 g de peso seco). Estos hallazgos evidencian la influencia del color y del grado de madurez sobre los perfiles de compuestos bioactivos, respaldando su potencial nutricional y funcional.

Palabras clave: *Solanum lycopersicum*; microextracción; compuestos bioactivos; Folin.

Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide, valued not only for its economic relevance and high consumer demand but also for its nutritional properties and versatility in the human diet (Allende, 2018). Among the available varieties, cherry tomatoes stand out for their diversity of colours, shapes, and flavours. Particularly, dark-coloured genotypes have recently attracted attention due to their potential nutraceutical properties, as they are rich in bioactive compounds such as flavonoids, anthocyanins, phenolic acids, and carotenoids, in addition to essential nutrients including sugars, vitamins, and minerals (Guzmán et al., 2021; Raza et al., 2022). The wild form *S. lycopersicum*, considered the ancestor of modern cultivated tomatoes, is distributed naturally in South America (Peru, Ecuador, Chile, and the Galápagos Islands) and represents a valuable genetic reservoir contributing to the current diversity (Coyago-Cruz et al., 2017; Délices et al., 2019).

Carotenoids are lipophilic pigments widely distributed in fruits and vegetables, responsible for colours ranging from yellow to deep red. In tomato, they accumulate in plastids (chloroplasts and chromoplasts) and include carotenes, such as β-carotene and lycopene, and xanthophylls, such as lutein and zeaxanthin (Osamede et al., 2024). Their biosynthesis starts from geranylgeranyl pyrophosphate (GGPP), leading to the sequential formation of phytoene, ζ-carotene, and tetra-cis-lycopene, which is isomerised to trans-lycopene, the main pigment responsible for the red colour of ripe tomatoes (Meléndez, 2017). Lycopene, β-carotene, lutein, and zeaxanthin not only determine fruit colour but also exert multiple health benefits, including antioxidant, anticancer, cardioprotective, and ocular protective effects (Daphnee et al., 2022).

Phenolic compounds represent another major group of secondary metabolites in tomatoes, including phenolic acids, flavonoids, and tannins. They are synthesised mainly through the shikimate pathway, with phenylalanine acting as a precursor for multiple phenolic derivatives (Herrera et al., 2016). Tomato fruits contain relevant phenolics such as chlorogenic acid, caffeic acid, ferulic acid, and p-coumaric acid, all associated with antioxidant and protective effects against oxidative stress (Cizmarova et al., 2020; Li et al., 2021; Santana et al., 2017). Overall, these compounds are of nutritional and functional importance, as their regular intake has been linked to a lower risk of chronic diseases such as cardiovascular disorders, diabetes, and cancer (Cereceres et al., 2019).

Recent studies have demonstrated that the colour diversity of cherry tomatoes reflects differences in their phytochemical composition, particularly in carotenoids and phenolics, which directly influence both sensory and functional properties. However, there is still limited information on how these compounds vary among different coloured cherry tomato varieties and across ripening stages. Thus, the objective of this study was to investigate the concentration of carotenoids and total phenols in cherry tomato varieties of different colours at different degrees of ripeness grown in Ecuador.

Materials and methods

For the study, five varieties of cherry tomatoes were collected at three stages of ripeness, defined by changes in peel colour, thus 0% (green), 50% (halfway to the final colour), and 100% (fully ripe) (Figure 1). Additionally, plant material was collected for botanical identification. This was carried out in Checa, 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).

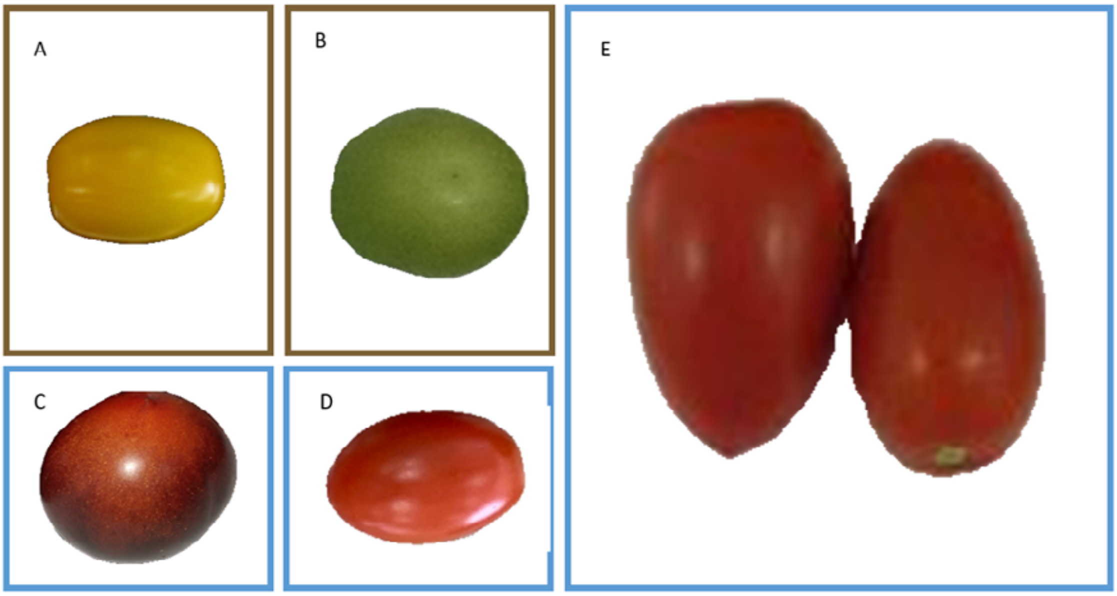

Figure 1. Photograph of different species of cherry tomatoes at 100% maturity.

Note: A) Yellow cherry tomatoes; B) Green cherry tomatoes; C) Black cherry tomatoes; D) Oval red cherry tomatoes; E) Elongated red cherry tomatoes.

The collected samples were washed and cut into small pieces. 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 concentrations in the different cherry tomato varieties evaluated throughout the different stages of ripeness. The results showed that the red varieties exhibited a progressive increase in total carotenoid concentration as ripeness progressed. In contrast, the yellow and green varieties exhibited a decrease in yield that was correlated with the degree of ripeness. This behaviour can be explained by the dynamics of carotenoid biosynthesis, in which the accumulation of these pigments predominates during the ripening process, particularly in red-coloured fruits (Coyago-Cruz et al., 2018; Meléndez, 2017).

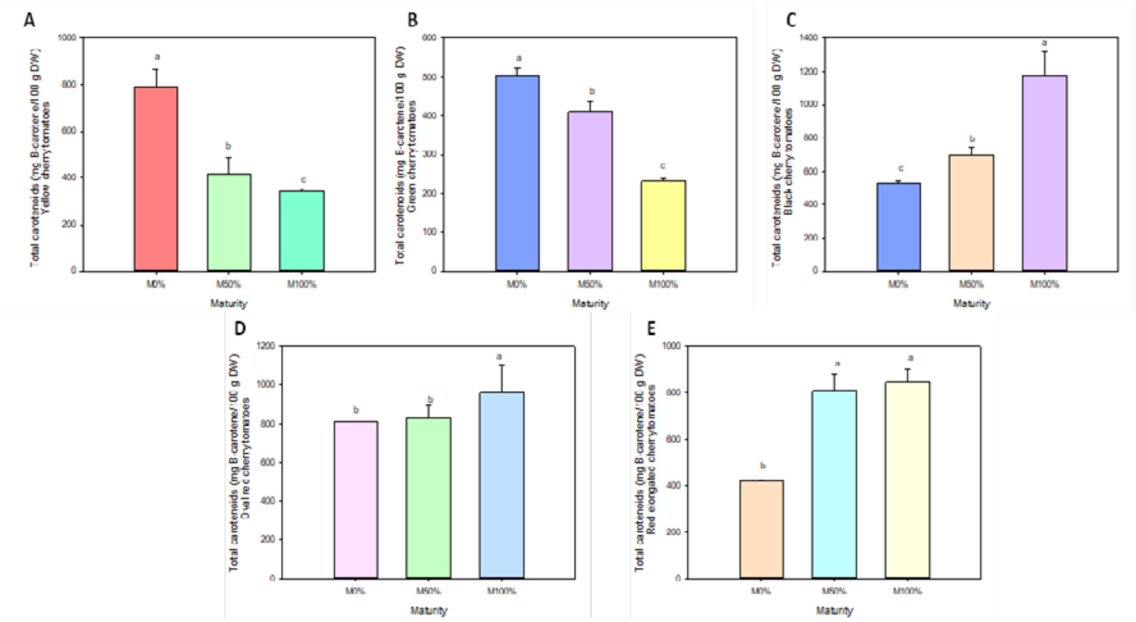

Figure 2. Average total carotenoid concentration of cherry varieties at different degrees of ripeness.

Note: A) Yellow cherry tomatoes; B) Green cherry tomatoes; C) Black cherry tomatoes; D) Oval red cherry tomatoes; E) Red elongated cherry tomatoes. 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 2 shows the average concentrations of total phenols in five cherry tomato varieties evaluated at three stages of ripeness. In the yellow cherry tomato variety (Figure 2-A), a progressive decrease in total phenols was observed from 0% to 100% ripeness, indicating a significant loss of phenolic compounds during advanced ripening. In the green cherry tomato variety (Figure 2-B), the highest values were found in unripe fruits (M0%), with a reduction at M100%. In contrast, the black cherry tomato variety (Figure 2-C) showed the opposite behaviour, with a significant increase from M0% to M50% and M100%.

In the oval red cherry tomato variety (Figure 2-D), values remained stable between M0% and M50%, but with a marked decrease at M100%. Finally, the red, elongated cherry tomato variety (Figure 2-E) exhibited a consistent reduction in concentration, depending on the degree of ripeness, with significantly higher concentrations at M0% and lower concentrations at M100%. These results show that the dynamics of total phenols depend not only on the state of ripeness, but also on the colour and genetics of each cherry tomato variety. In general, unripe fruits tend to have higher concentrations of phenols, which is consistent with what has been reported in the literature, where the synthesis of these compounds is associated with defence mechanisms against environmental and oxidative stress in the early stages of development (Coyago-Cruz et al., 2018; Guzmán et al., 2021; Raza et al., 2022) As ripening progresses, some of the phenols may be transformed into other secondary metabolites, such as anthocyanins and flavonoids, which would explain the reduction in some cases and the increase in dark-coloured varieties.

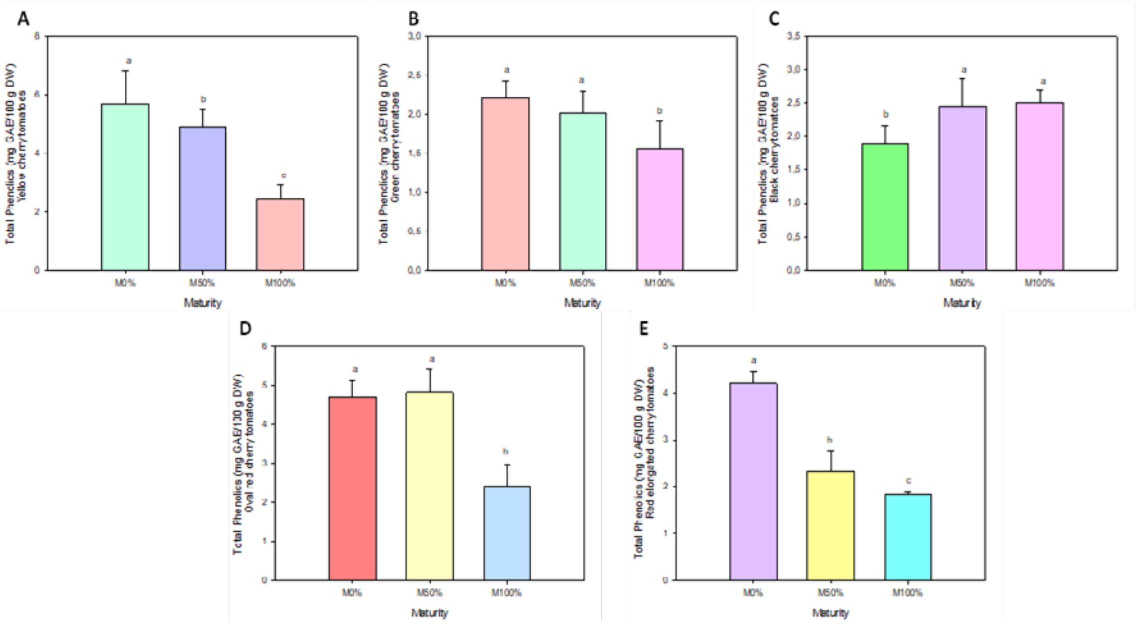

Figure 3. Average total phenolic concentration of cherry varieties at different degrees of ripeness.

Note: A) Yellow cherry tomatoes; B) Green cherry tomatoes; C) Black cherry tomatoes; D) Oval red cherry tomatoes; E) Red elongated cherry tomatoes. 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

The study revealed that phenolic compounds were predominant in immature cherry tomatoes, decreasing with ripening in most varieties, while carotenoids tended to accumulate at later stages, especially in red and dark-coloured types. These contrasting dynamics highlight genotype-dependent regulation of bioactive compounds and suggest that both immature and ripe fruits represent valuable sources of antioxidants with potential applications in functional foods and nutraceuticals.

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