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Assessment of Tocopherols Content in Five Tunisian Pepper Landraces

Marwa Chouikhi[1*], Riadh Ilahy[1], Imen Tlili[1] and Thouraya R'him[1]

[1]Laboratory of Horticulture, National Institute of Agronomic Research of Tunisia, University of Carthage, Hèdi Karray Street, 2049 Ariana, Tunisia

Abstract

Peppers (*Capsicum annuum* L.) are an important source of bioactive compounds, including tocopherols, which are lipid-soluble antioxidants essential for human health. This study evaluated the tocopherol composition (α-, β-, γ-, and total tocopherols) in five Tunisian pepper genotypes (Baklouti, Beldi, Nabeul, Marconi, and Super Marconi) grown under open-field conditions. α-Tocopherol was the predominant isoform, with the highest levels observed in Beldi (30.93 mg kg^{-1} FW) and Nabeul (26.92 mg kg^{-1} FW), whereas β- and γ-tocopherols were present in lower amounts. Significant variation in total tocopherol content among genotypes reflects the combined influence of genetic and environmental factors. The results highlight the nutritional and antioxidant potential of local Tunisian pepper landraces and provide valuable information for breeding programs aimed at enhancing vitamin E content. These findings support the role of pepper fruits as functional foods capable of contributing to human health and oxidative stress prevention.

Key Words: *Capsicum annuum, pepper landraces, tocopherols, α-tocopherol and genotypic variation.*

Introduction

Peppers (*Capsicum annuum* L.) are among the most widely cultivated and consumed vegetable crops worldwide, valued for their sensory attributes, nutritional quality and health-promoting properties. In addition to their rich content of vitamins, carotenoids, and phenolic compounds, pepper fruits are an important source of vitamin E, a group of lipid-soluble antioxidants collectively known as tocopherols (Qin et al., 2024; Meena et al., 2025).

Tocopherols, including α-, β-, γ-, and δ-isomers, play a crucial role in protecting cellular membranes against oxidative damage by scavenging reactive oxygen species (ROS) and preventing lipid peroxidation. Among them, α-tocopherol is recognized as the most biologically active form and the predominant isoform in plant tissues, particularly in photosynthetic organs and fruits (Mesa & Munné-Bosch, 2023). Beyond their antioxidant function, tocopherols are also involved in plant stress tolerance, chloroplast stability and regulation of physiological processes (Meena et al., 2025).

The accumulation and composition of tocopherols in plant tissues are influenced by multiple factors, including genotype, environmental conditions, and agronomic practices. Recent studies have highlighted the importance of genotype × environment interactions in determining tocopherol content, as well as the role of metabolic pathways linked to chlorophyll biosynthesis and plastid development (Hussain et al., 2024; Herr et al., 2025). This variability offers opportunities for the selection and improvement of crop varieties with enhanced nutritional quality.

In Tunisia, pepper landraces represent a valuable genetic resource characterized by high diversity and adaptation to local agro-climatic conditions. However, limited information is available regarding their tocopherol composition and variability. Therefore, the present study aimed to assess the tocopherol content (α-, β-, γ-, and total tocopherols) in five Tunisian pepper genotypes cultivated under open-field conditions, in order to better understand their nutritional potential and contribute to the valorization of local germplasm.

Materials and Methods

The field-experiment was conducted during 2022 growing season at the Research and Experimental Station of Teboulba, Monastir, Tunisia. Five pepper genotypes were used in this experiment consisting of Baklouti, Beldi, Nabeul, Marconi and Super Marconi. Sowing was carried out in plug-seedling trays during May. Pepper seedlings were transplanted in open-field in a sandy soil at the beginning of June with a spacing of approximately 0.4 m within the row and 0.7 m between rows, matching a density of about 4 plants.m^{-2} and grown until maturity. Drip irrigation was applied during 1-3 h at different days intervals in agreement with the local evapotranspiration potential, climatic conditions, crop coefficient and growth stage. The growing calendar was also in accordance with the techniques used by the Research station and high-yielding neighbouring farmers. The method included the use of synthetic chemical fertilizers (145 kg N ha^{-1}, 140 kg P_2O_5 ha^{-1}, 210 kg K_2O ha^{-1}). Chemical fertilizer solution was added to water irrigation by pump injection twice a week. The production method also consists of hand weeding and plant-pathogen control with synthetic chemical pesticides. Pesticides were applied once a cycle. The experimental design was a randomized complete block design with three blocks (replicates). During 2022 growing season, the average temperature ranged between 24.88-33°C, relative humidity ranged between 30-88% and rainfall ranged between 0-5 mm.

Fruit sampling

Peppers were collected from each plant at the commercial red mature ripening stage. Fresh pepper fruits were harvested from each bloc and delivered to the laboratory. The selected pepper fruits were washed with deionized water, cut into small pieces and homogenized in a laboratory blender (Waring Laboratory Science, Torrington, CT, US). The obtained homogenates were used to assess tocopherols.

Determination of Tocopherol Content

The procedure of Abushita et al. (1997) was adopted for the extraction of tocopherol using n−hexane. The analysis was conducted by the mean of an HPLC (Hitachi Chromaster, Tokyo, Japan). The system was composed of a model 5110 gradient pump, a model 5210 autosampler, and a 5440-fluorescence detector. The Nucleosil 5 mm (250 × 4.6 mm i.d.) column was also used for separation with a mobile phase (99.5:0.5 n-hexane: ethanol). The excitation and emission wavelengths were detected at 295 nm and 320 nm, respectively, as outlined in Duah et al. (2021). The isomers of α-, β-, and γ-tocopherols were determined by means of external standards (Sigma-Aldrich, Budapest, Hungary) and cochromatographed with the samples. The retention times were 16.75, 14.41, and 13.34 min for α-, β-, and γ−tocopherols, respectively and the content was expressed as mg kg^{-1} fw.

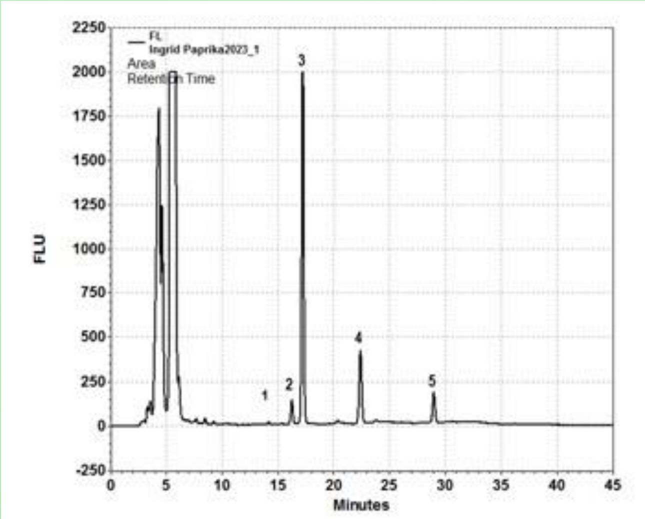

Figure 1. HPLC profile of tocopherols from pungent red pepper fruits Peak identification.
1: mixture Figure 5. HPLC profile of tocopherols from pungent red pepper fruits Peak identification. 1: mixture of β- and γ-tocopherol, 2: α-tocopherol hydroquinone, 3: α-tocopherol, 4: α-tocopherol acetate, 5: α tocopherol ester. of β- and γ-tocopherol, 2: α-tocopherol hydroquinone, 3: α-tocopherol, 4: α-tocopherol acetate, 5: α-tocopherol ester.

Statistical Analysis

The variability influencing tocopherols content of different pepper landraces were determined by variance (ANOVA) analysis. The means comparison was performed by the Duncan test ($p < 0.05$) if significant differences were spotted. The statistical evaluations were conducted using the IBM SPSS Statistics software for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

Results and Discussion

The α-tocopherol was the predominant tocopherol isomer in all studied pepper genotypes. Its content showed significant variation among cultivars, with the highest levels recorded in Beldi (30.93 mg kg^{-1} FW) and Nabeul (26.92 mg kg^{-1} FW), whereas the lowest concentration was observed in Super Marconi (14.91 mg kg^{-1} FW). This predominance is consistent with previous studies reporting that α-tocopherol is the most abundant and biologically active form of vitamin E in plant tissues, particularly in photosynthetic organs and fruits (Mesa et al., 2023). α-tocopherol acts as a major lipid-soluble antioxidant, protecting cellular membranes from lipid peroxidation caused by reactive oxygen species (ROS). Therefore, pepper genotypes with higher α-tocopherol content may exhibit enhanced oxidative stress tolerance and improved nutritional quality (Mesa et al., 2023). The observed variability among genotypes highlights the strong influence of genetic factors on α-tocopherol accumulation, as reported in recent studies on horticultural crops (Hussain et al., 2024 ; Kazemzadeh et al., 2024 ; Meena et al., 2025) . In addition, environmental conditions such as temperature, light intensity, and agronomic practices may also affect its biosynthesis.

β-Tocopherol was detected in lower amounts compared to α-tocopherol across the studied genotypes, confirming its minor contribution to total tocopherol content. This observation is consistent with previous studies indicating that β-tocopherol is generally present in smaller quantities in plant tissues due to differences in methylation patterns and biosynthetic pathway regulation (Qin et al., 2024). Despite its lower abundance, β-tocopherol still contributes to antioxidant defense by scavenging reactive oxygen species and protecting membrane lipids from oxidative damage, although its biological activity is considered lower than that of α-tocopherol (Meena et al., 2025). The variation observed among genotypes suggests that β-tocopherol accumulation is influenced by both genetic factors and enzymatic conversion processes within the tocopherol biosynthetic pathway, particularly the methylation steps that preferentially lead to α-tocopherol formation. Additionally, environmental conditions may further modulate the relative proportions of tocopherol isoforms, resulting in differential accumulation patterns in plant tissues (Hussain et al., 2024).

γ-Tocopherol was detected in relatively low amounts compared to α-tocopherol in the studied genotypes, confirming its minor contribution to total tocopherol content. This distribution is consistent with previous findings indicating that α-tocopherol is the predominant isoform in plant tissues, while γ-tocopherol generally occurs in lower concentrations due to its partial conversion during the tocopherol biosynthetic pathway. Despite its lower abundance, γ-tocopherol plays a significant physiological role as an effective lipid-soluble antioxidant, contributing to the protection of cellular membranes against oxidative damage. Notably, it exhibits a unique capacity to neutralize not only reactive oxygen species (ROS) but also reactive nitrogen species (RNS), such as peroxynitrite, which distinguishes it from other tocopherol isoforms (Es-Sai et al., 2025). Furthermore, γ-tocopherol has been reported to participate in the regulation of oxidative stress and inflammatory responses, enhancing the overall antioxidant defense system in plant and biological tissues. Its presence, even at low levels, may therefore contribute to the stability of lipid membranes and improve the nutritional and functional quality of plant-derived foods (Es-Sai et al., 2025).

Total tocopherol content represents the cumulative concentration of all tocopherol isoforms (α, β, γ, and δ), and is therefore considered a key indicator of the overall antioxidant capacity and nutritional value of plant-derived foods. Higher total tocopherol levels reflect a stronger ability to scavenge reactive oxygen species (ROS) and protect cellular structures, particularly membrane lipids, from oxidative damage (Meena et al., 2025; Qin et al., 2024). The variation observed in total tocopherol content among genotypes highlights the combined effects of genetic regulation and metabolic pathways controlling tocopherol biosynthesis. Recent studies have demonstrated that total tocopherol accumulation is closely linked to chlorophyll metabolism and plastid development, indicating a strong connection between photosynthetic activity and vitamin E biosynthesis (Herr et al., 2025). Furthermore, total tocopherol levels are highly responsive to environmental conditions such as light intensity, temperature, drought, and salinity, which can significantly alter both the concentration and composition of tocopherols in plant tissues (Meena et al., 2025). Genotypes with higher total tocopherol content may provide enhanced oxidative stability and greater health-promoting potential, reinforcing the importance of selecting and breeding varieties with elevated vitamin E levels for both nutritional and functional quality.

Table 1. Tocopherol composition (α-, β-, γ-, and total tocopherols) in five Tunisian pepper (*Capsicum annuum* L.) genotypes (mg kg^{-1} FW)

	α-Tocopherol	*β-Tocopherol*	*γ-Tocopherol*	*Total tocopherols*
Baklouti	19.5±2.04 c	0.46±0.1 b	n.d	19.96±2.11 c
Beldi	30.93±1.23 a	0.25±0.02 c	0.18±0.02 a	31.36±1.26 a
Nabeul	26.92±1.03 b	0.59±0.03 a	0.14±0.09 a	27.65±1.07 b
Marconi	23.53±1.11 bc	0.25±0.004 c	0.04±0.02 b	23.82±4.13 bc
Super marconi	14.91±1.76 d	n.d	n.d	14.91±1.76 d

n.d.: not detected.

Conclusion

This study provides a comprehensive assessment of tocopherol content in five Tunisian pepper genotypes, highlighting significant variation among cultivars. α-Tocopherol was the predominant isoform, confirming its major contribution to the nutritional and antioxidant value of pepper fruits, while β- and γ-tocopherols were present in lower amounts but still contributed to the overall antioxidant potential. The observed differences among genotypes indicate a strong influence of genetic factors, with additional modulation by environmental conditions and agronomic practices. Total tocopherol content varied significantly, emphasizing the importance of genotype selection for enhancing the functional and nutritional quality of pepper fruits. These findings underscore the potential of local Tunisian landraces as valuable sources of vitamin E and provide a foundation for future breeding programs aimed at improving antioxidant content. Overall, the study reinforces the role of peppers as a functional food with the capacity to contribute to human health and oxidative stress prevention.

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