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Effect of Pepper Genotype and Processing Conditions on Phytochemical Composition and Antioxidant Activity of Tunisian Harissa

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Abstract

Harissa, a traditional Tunisian red pepper paste, is widely appreciated for its sensory attributes and potential health benefits linked to its rich phytochemical composition. This study investigated the combined effects of pepper genotype (local vs. hybrid) and processing conditions on physicochemical properties, phytochemical content, and antioxidant activity of harissa. Two types of harissa were produced: LTH (local traditional harissa) and HTH (hybrid traditional harissa). Physicochemical analysis revealed no significant differences in pH (4.96) and titratable acidity (0.14%) between samples, while LTH exhibited higher total soluble solids and slightly greater ascorbic acid content. Phytochemical analysis showed that LTH had significantly higher total phenolic (325.74 ± 20.55 mg GAE/kg FW) and carotenoid contents (573.95 ± 10.11 mg β-CarE/kg FW) compared to HTH (270.42 ± 20.66 mg GAE/kg FW and 541.88 ± 8.61 mg β-CarE/kg FW, respectively), indicating better preservation of heat-sensitive bioactive compounds under milder processing conditions. In contrast, HTH demonstrated significantly higher hydrophilic and lipophilic antioxidant activities, suggesting that more intense processing may enhance antioxidant capacity through the formation of new antioxidant compounds or improved extractability. These findings highlight the complex interplay between raw material and processing conditions in determining the functional quality of harissa. Optimizing processing parameters may therefore allow for improved retention of bioactive compounds while maintaining or enhancing antioxidant functionality.

Key Words: *Harissa, pepper genotype, phytochemical composition; hydrophilic and lipophilic antioxidants and food processing*

Introduction

Harissa, a bright red pepper paste native to Tunisia and considered the country's national condiment, plays a central role in Tunisian cuisine and cultural identity, recently being inscribed on UNESCO's Representative List of the Intangible Cultural Heritage of Humanity. Its production, from sun-dried *Capsicum* peppers blended with spices, vinegar, and olive oil, supports rural livelihoods, contributes significantly to Tunisia's agro-food economy, and underpins its export market.

Peppers are rich in phenolic compounds and carotenoids, which contribute to antioxidant capacity and human health benefits; however, these phytochemicals are sensitive to genotype and processing conditions, often undergoing degradation, isomerization, or transformation during thermal treatments. Contemporary studies on Tunisian harissa and related pepper pastes reveal substantial variability in phenolic and carotenoid contents and antioxidant activities across traditional and commercial products, underscoring the influence of raw material and processing on functional quality (Ferrando et al., 2024 ; Chouikhi et al., 2025).

Despite its cultural and economic importance, there is limited detailed comparison of how pepper genotype (local vs. hybrid) and traditional processing interact to shape the phytochemical profile and antioxidant functionality of harissa. This study aims to address this gap by examining the effects of pepper type on total phenolic and carotenoid content, along with hydrophilic and lipophilic antioxidant activities, providing insights for optimizing processing strategies to enhance the nutritional and functional quality of this emblematic Tunisian product.

Materials and Methods

Plant culture

The field experiment was carried out during the 2023 growing season at the Teboulba Research Station (Monastir, Tunisia). Eight pepper cultivars, including four hybrids (Starter, Essifi, Sahraoui, and Necessario) and four hot pepper landraces (Beldi, Baklouti, Nabeul, and Kairouan), were grown under open-field conditions. Seedlings were transplanted in early June with a density of 4 plants/m² and managed using drip irrigation according to climatic conditions and crop requirements. Standard agronomic practices were applied, including fertigation,

weeding, and pest control. The trial followed a randomized complete block design with three replicates. During the season, temperatures ranged from 20 to 34 °C, relative humidity from 56 to 90%, and rainfall remained low.

Traditional red-hot pepper paste harissa processing

Pepper fruits were harvested from each plant at the commercial red-ripe stage. Only healthy fruits were collected from each block and immediately transported to the laboratory. The selected fruits were washed with deionised water, cut into small pieces, and homogenised using a laboratory blender (Waring Laboratory Science, Torrington, CT, USA). The resulting pepper puree was thermally treated at approximately 70 °C, after which seeds and skins were removed to improve texture. The puree was subsequently refined and vacuum-concentrated to a dry matter content of 14% at 65-70 °C. Finally, fresh red garlic paste (≥4%), coriander (≥4%), caraway powder (≥2%), and salt (≥2%) were added to the concentrated paste, followed by thorough mixing and homogenisation to obtain the final product, referred to as traditional Harissa (TH). Two types of harissa were produced, namely harissa prepared from local pepper varieties (LTH) and harissa prepared from hybrid pepper varieties (HTH).

Determination of pH, Total Soluble Solids, Titratable Acidity and Ascorbic Acid Contents

pH was measured using a digital pH meter (PCE-228) by directly immersing the electrode into the harissa homogenate. Total soluble solids (TSS) content was determined using a hand-held digital refractometer (ATAGO™ PAL-22S) by placing a small aliquot of the homogenised harissa on the prism surface, and results were expressed as °Brix. Titratable acidity was assessed by titration with 0.1 N NaOH to an endpoint of pH 8.1 and expressed as grams of citric acid per 100 g of fresh weight. Ascorbic acid content was determined by titration according to Miller (1998). 5 g of harissa sample were homogenised with 20 mL of distilled water; an aliquot (5 mL) of the extract was mixed with 5 mL of 2% acetic acid solution and titrated with 2,6-dichlorophenol-indophenol.

Quantification of total phenolic content

Total phenolics were extracted from a sample of 0.1 g of each replicate sample using a mixture of 80% aqueous methanol and 37% HCl according to the method described by Martínez-Valverde et al. (2002). The mixture was continuously stirred at 4 8C for 2h, and then centrifuged for 15min at 10,000g to obtain the supernatant. The supernatant was mixed with the Folin Ciocalteu reagent, and absorbance at 750nm was measured using a BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). To quantify the total phenol content, a linear calibration curve was generated using freshly prepared gallic acid solution, and the data were reported in milligrams of gallic acid equivalents per kilogram of fresh weight (mg GAE/kg FW).

Quantification of carotenoid content

The carotenoid content of the harissa was analysed following the method described by Sadler et al. (1990) modified by Perkins-Veazie et al. (2001). The extraction process utilised a mixture of hexane, ethanol, and acetone in a 2:1:1 ratio, with the addition of 0.05% butylated hydroxy toluene. Carotenoid levels were quantified by measuring the absorbance at 450 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). The carotenoid content was expressed in milligrams of β-carotene-equivalent per kilogram of fresh weight (mg β-CarE/kg FW).

Quantification of hydrophilic and lipophilic antioxidant activities

The ABTS radical scavenging capacity of the samples was determined following the method of Miller and Rice-Evans (1997). The hydrophilic and lipophilic antioxidant activities (HAA and LAA, respectively) were measured using the Trolox Equivalent Antioxidant Activity assay. After centrifugation of the sample at 10,000 g for 7 min, the supernatant was collected for antioxidant activity measurements. The samples were analysed for their absorbance values at 734 nm using a Cecil spectrophotometer. Fresh Trolox solutions were used to create two different calibration curves for the measurements of HAA and LAA. Trolox solution with concentrations ranging from 0 to 16M Trolox was used to create a linear calibration curve. The results were reported as µM of Trolox per 100g of fresh weight (µM Trolox/100g FW).

Data analysis

The statistical analysis of the data was conducted using the IBM SPSS Statistics software for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA). To compare the mean values, the least significant difference (LSD) procedure was utilised, with statistical significance set at $P < 0.05$.

Results and Discussion

The pH values of both LTH and HTH harissa were identical (4.96), indicating comparable acidity levels. This pH range is characteristic of processed pepper-based products and plays a key role in ensuring product stability

and microbial safety. The absence of significant differences suggests that processing conditions and acid-base equilibria were similar for both formulations. In contrast, soluble solids content (°Brix) showed a significant variation between the two samples. LTH exhibited a higher °Brix value (16.13) than HTH (15.77), reflecting a greater concentration of soluble sugars and dissolved solids. This difference may contribute to enhanced sweetness perception and a more pronounced flavor profile in LTH harissa. Titratable acidity was comparable in both products (0.14%), indicating similar levels of organic acids. This consistency is in agreement with the identical pH values and suggests that the acid composition and buffering capacity remained stable across both harissa types. With respect to ascorbic acid content, LTH showed a slightly higher concentration (206 mg/100 g FW) compared to HTH (202.8 mg/100 g FW). Although the difference is modest, it may indicate a better preservation of vitamin C in LTH, potentially linked to variations in raw material quality or processing intensity. Overall, while both harissa types displayed similar acid-related characteristics, LTH was distinguished by higher soluble solids and marginally greater vitamin C content, which could positively influence both sensory attributes and nutritional value.

Table 1. Physicochemical parameters of LTH and HTH harissa.

Harissa	*pH*	*Soluble Solids (°Brix)*	*Titratable Acidity (%)*	*Ascorbic acid (mg/100 g FW)*
LTH	4.96±0.02a	16.13±0.22 a	0.14±0.005 a	206±0.36 a
HTH	4.96±0.05a	15.77±0.24 b	0.14±0.005 a	202.8±0.53 b

Total phenolic content varied significantly between the two harissa types, with LTH exhibiting a higher concentration (325.74 ± 20.55 mg GAE/kg FW) compared to HTH (270.42 ± 20.66 mg GAE/kg FW). This result suggests a better preservation of phenolic compounds under LTH processing conditions. Phenolics are important secondary metabolites known for their strong antioxidant activity, contributing substantially to the nutritional and functional quality of food products through their ability to scavenge free radicals and reduce oxidative stress. Their stability is highly dependent on processing factors, particularly temperature and duration, which may induce degradation or structural modifications (Jia et al., 2025 ; Collins et al., 2024). Consequently, the higher phenolic content observed in LTH may reflect milder or more favorable processing conditions, ultimately enhancing the antioxidant potential and associated health benefits of the final product. (Arya et al., 2024 ; Kahkashan et al., 2024 ; Kalita et al., 2025).

Total carotenoid content was significantly higher in LTH (573.95 ± 10.11 mg β-CarE/kg FW) than in HTH (541.88 ± 8.61 mg β-CarE/kg FW), suggesting that LTH processing conditions are more effective in preserving these lipophilic pigments. Carotenoids, which are responsible for the characteristic red-orange coloration of pepper-based products, also act as potent fat-soluble antioxidants and contribute to various health benefits through their role in mitigating oxidative stress. However, these compounds are highly sensitive to processing conditions, particularly temperature and treatment duration, which can induce degradation or isomerization and consequently reduce their functional properties. The higher carotenoid retention observed in LTH therefore indicates reduced thermal degradation compared to HTH, potentially enhancing both the nutritional value and lipophilic antioxidant capacity of the final product (Ferrando et al., 2024).

LTH harissa exhibited significantly higher total phenolic (325.74 ± 20.55 mg GAE/kg FW) and carotenoid contents (573.95 ± 10.11 mg β-CarE/kg FW) compared to HTH (270.42 ± 20.66 mg GAE/kg FW and 541.88 ± 8.61 mg β-CarE/kg FW, respectively), indicating that milder processing conditions favor the preservation of these heat-sensitive bioactive compounds (González-Peña et al., 2021). Phenolics and carotenoids are major contributors to antioxidant capacity; however, both are susceptible to thermal degradation. Despite their lower phytochemical contents, HTH showed significantly higher hydrophilic and lipophilic antioxidant activities than LTH, suggesting that more intense processing may enhance antioxidant activity through the formation of Maillard reaction products or by increasing the extractability and reactivity of certain compounds. These findings highlight the complex relationship between processing conditions, phytochemical retention, and antioxidant functionality, where increased thermal intensity may reduce native compounds but simultaneously enhance overall measured antioxidant activity.

Table 2. Comparison of phytochemical composition (total phenolics and carotenoids) and hydrophilic/lipophilic antioxidant activities in LTH and HTH harissa.

Harissa	*Total Phenolic (mg GAE/kg FW)*	*Total Carotenoids (mg β-CarE/kg FW)*	*Hydrophilic Antioxidant Activity (μM Trolox/100g FW)*	*Lipophilic Antioxidant Activity (μM Trolox/100g FW)*
LTH	325.74±20.55 a	573.95±10.11 a	1072.86±16.94 b	1170.63±12.03 b
HTH	270.42±20.66 b	541.88±8.61 b	1943.3±22.49 a	1732.53±10.41 a

Conclusion

Harissa produced from local pepper varieties showed higher phenolic and carotenoid contents, indicating better preservation of native bioactive compounds, whereas harissa from hybrid varieties exhibited substantially higher hydrophilic and lipophilic antioxidant activities. These contrasting trends highlight that antioxidant capacity is not solely dependent on phytochemical concentration but is strongly influenced by processing-induced transformations and compound interactions. The results emphasize the combined roles of pepper genotype and processing conditions in shaping the nutritional and functional quality of harissa and provide a basis for optimizing processing strategies to enhance antioxidant functionality.

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