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Research Article

POLYPHENOLS AND PHENOLIC COMPOUNDS IN MOUNTAIN VERSUS LOWLAND FOOD

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ABSTRACT

The objective of the research demonstrates that important differences consists between mountainous and lowland products, particularly concerning polyphenols and phenolic compounds. The research aims to establish the importance of certain agronomic practices in ensuring the agricultural management applicable to the development of mountain and lowland value chains. The reasons for addressing the importance of polyphenols and phenolic compounds in vegetables for the development of agriculture include multiple factors, with one of the most relevant involving the complexity of the health benefits of polyphenols. This class of pigments appears widely in nature, contributing vibrant tastes to various fruits and vegetables. The results provide insight into the polyphenols content of different vegetables, as well as phenolic compounds, highlighting their nutritional significance. The synthesis of these findings serves as a foundation for understanding the far-reaching implications on the economic viability, environmental sustainability and strategic positioning of individual agricultural producers from lowland and mountain areas.

1.Introduction

The current research is highly relevant in the context of food security and safety. As the global population seeks more effective solutions to these challenges, mountain regions offer a promising opportunity. Mountain products presents highly valued compared to lowland counterparts due to their distinct physicochemical properties, including the presence of bioactive compounds such as phenolic compounds and polyphenols found in mountain-sourced foods. Beyond their role as natural colorants, carotenoids and polyphenols play a crucial role in human nutrition, providing a number of health benefits. Two prominent examples of carotenoid-rich crops are tomatoes and corn, where these compounds contribute not only to the visual appeal but also to the nutritional profile of these staple foods. In photosynthetic systems, "carotenoids are essential for photoprotection against excess light and contribute to light harvesting, but are perhaps best known for their properties as natural pigments in the yellow to red range"

(Rodriguez-Concepcion *et al.,* 2018; Covaci *et al.,* 2024).

Clinical studies suggest that the use of carotenoids and polyphenols is associated with a "lower risk of cardiovascular disease, cancer and eye disease. Another issue discussed is the role of carotenoids in animals and their feed, with emphasis on birds, fish and crustaceans, livestock and poultry". (Langi *et al.,* 2018; Covaci *et al.,* 2024)

Appropriate conditions and methods of growing, storing, and processing fruits and vegetables to help delay "carotenoid degradation and enhance carotenoid biosynthesis are also reviewed and identified". (Ngamwonglumlert *et al.,* 2020; Covaci *et al.,* 2024)

Some of the health benefits are antioxidant protection, anti-inflammatory effects, etc. Carotenoses are of critical importance to "humans as precursors of vitamin A synthesis and as dietary antioxidants. The vital roles of carotenoids in plants and humans have led to significant advances in our understanding of carotenoid metabolism and regulation". (Sun *et al.,* 2022; Covaci *et al.,* 2024)

From an agronomic point of view, it was observed that the results improve qualitatively and quantitatively the products by applying appropriate irrigation techniques. During the three years of application of agronomic treatments, carotenoids and phenolic compounds in tomatoes, corn, beans and wheat increased through water stress, high water quality, appropriate soil selection for each crop and appropriate irrigation system. (Ciotea *et al.,* 2023; Covaci *et al.,* 2024)

Water supply practice changes the quality of the crops, especially in the mountain area (Covaci B *et al.,* 2024). In tomatoes the content of soluble solids together with a slight reduction in the amount of production could be undeniable affected. Different studies from the specific literature show the effect of the mountain altitude on the composition of carotenoids, polyphenols, and lycopene. (Takács *et al.,* 2020; Covaci *et al.,* 2024)

Tomatoes and corn are important mountain crops that contain abundant bioactive physico-chemical compounds. However, they are sometimes affected by drought stress in mountainous areas. (Klunklin and Savage, 2017; Beta and Hwang 2018; Covaci *et al.,* 2024)

A study on carotenoids and phenolic compounds in maize shows that under water deficit conditions, growth traits, relative water content, total chlorophyll and carotenoids, and grain yield were significantly decreased in both lines compared to control conditions. (Moharramnejad *et al.,* 2019; Covaci *et al.,* 2024)

Maintaining the correct and accurate amount of water through a drip system can promote efficient harvesting and crop quality. An investigation carried out in Hungary on four pepper varieties under different water supply treatments demonstrates this. (Agyemang Duah *et al.,* 2021; Covaci *et al.,* 2024)

Durum wheat represent a staple crop for the temperate-continental and Mediterranean diet due to its adaptation to "environmental pressure and its wide use in cereal-based foods such as pasta and bread as a source of calories and protein. Whole durum wheat grains are also highly valued for their particular amount of dietary fiber and minerals, as well as bioactive compounds of particular interest for their purported health-beneficial properties, including polyphenols, carotenoids, tocopherols, tocotrienols and phytosterols". (De Santis *et al.,* 2021; Covaci *et al.,* 2024)

2. Materials and methods

The study employed various physico-chemical methods in conjunction with agronomic investigations as part of the Research and Consultancy Agreement entitled "Development and Optimization of Probiotic Bacteria Production", issued by the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania, and it is correlated with the methodology from the article Ciotea et al. (2023) (both articles being a result of this project).

Figures and tables presented in the work were created and processed by the author in alignment with the terms of the research and consultancy agreement.

The research was carried out in compliance with the standard rules for physical-chemical analysis of vegetable products (Abedi et al. 2022; Lalithamba et al. 2023; Loghmanifar et al. 2022)

For mountain vegetable products (*Solanum lycopersicum* - tomatoes, *Zea mays* – maize/corn, *Capsicum annuum* - peppers), phenolic compounds and polyphenols were assessed using the Folin-Ciocalteu method, similar to related research (Adebo et al. 2022; Fibiani et al. 2022; Majkowska-Gadomska et al. 2021).

Polyphenols for these products were determined through the HPLC-DAD-ESI+ Method.

This study focuses on the quantification of polyphenols and phenolic compounds in tomatoes, corn and peppers collected from Cheșa and Dumbrăvița, two distinct regions of Bihor - one lowland and one mountainous - using the HPLC-DAD method. The extraction procedure was systematically conducted as detailed below. A 2 g sample underwent extraction with a solvent mixture formed from methanol/ethyl acetate/petroleum ether. The process of separation includ vortex agitation for one min using a Heidolph Reax top vortex mixer, afterwards 15 min of sonication in an Elmasonic E 15 H ultrasonic water bath and centrifugation at 8000 rpm for 10 min using an Eppendorf AG 5804 centrifuge. This sequence was repeated three to four times, discoloration being the final stage. The collected extract was transferred into a separatory funnel and washed three times with a 15% NaCl solution. The organic phase was initially filtered using anhydrous Na2SO4 and further purified with petroleum ether until complete decolorization was obtained. The final extract present stages from evaporation to dryness under reduced pressure using a Heidolph Hei-VAP Expert rotary evaporator. The residue performed in re-dissolving. Phenolic compounds and polyphenols resolution was resolved through an Agilent 1200 HPLC system equipped with a solvent degasser (first), quaternary pumps (second), a diode-array detector (DAD) (third), and an automatic injector (Agilent Technologies, USA) (fourth). The mobile phases consisted of acetonitrile/water/ triethylamine as solvent B, employing the following gradient program: at 0 min, 90% A; at 10 min, 50% A; decreasing further to 10% A at 20 min (**R_t**). The parameters consist in flow rate of 1 mL/min, and chromatograms for wavelength of λ = 450 nm (mean), then interpreted through Agilent ChemStation software. Reagents presents acetonitrile and ethyl acetate procured from Merck (Germany), and ultrapure water obtained using a Direct-Q UV from Millipore (USA). Standard references for polyphenols and phenolic compounds were purchased from Sigma-Aldrich (Germany) for identification and quantification purposes. To quantify polyphenols and phenolic compounds in the analyzed samples, validation curves establish the injection of miscellaneous concentrations of polyphenols and phenolic compounds. The meticulous use of high-purity reagents and standards, combined with precise chromatographic conditions, ensures the accuracy and reliability of polyphenols and phenolic compounds quantification. The constructed calibration curves provide a robust basis for the precise determination of polyphenols and phenolic compounds content in the analyzed samples. (Ciotea et al. 2023)

Laboratory analyses pertaining to phenolic compounds in *Solanum lycopersicum* (tomatoes) were conducted using the aforementioned methodology, comparing tomatoes from mountainous and lowland areas.

Calibration curves were established for quantifying phenolic compounds using standard substances dissolved in methanol, including Gallic acid, Chlorogenic acid, and Rutin.

The interpretation present statistical methods. The polyphenols and phenolic compounds represent, including retention time (R_t), wavelength (λ) and frequency ($[M+H]^+$), the means of four repeated measurements (as described previously), analyzed using ANOVA together with Tukey's complex comparison tests (tables 1-3). Thus, *p < 0.001 indicates an important incongruity from Dumbravita, and **p < 0.001 show a significant unconformity from Chesa.

The quantitative calculation for each phenolic compound was performed using the equations derived from these calibration curves, specifying hydroxybenzoic acid as gallic acid equivalent, hydroxycinnamic acids as chlorogenic acid equivalent, and flavonols as rutin equivalent (Figure 1 – USAMV-CJ 2023; Covaci et al. 2024).

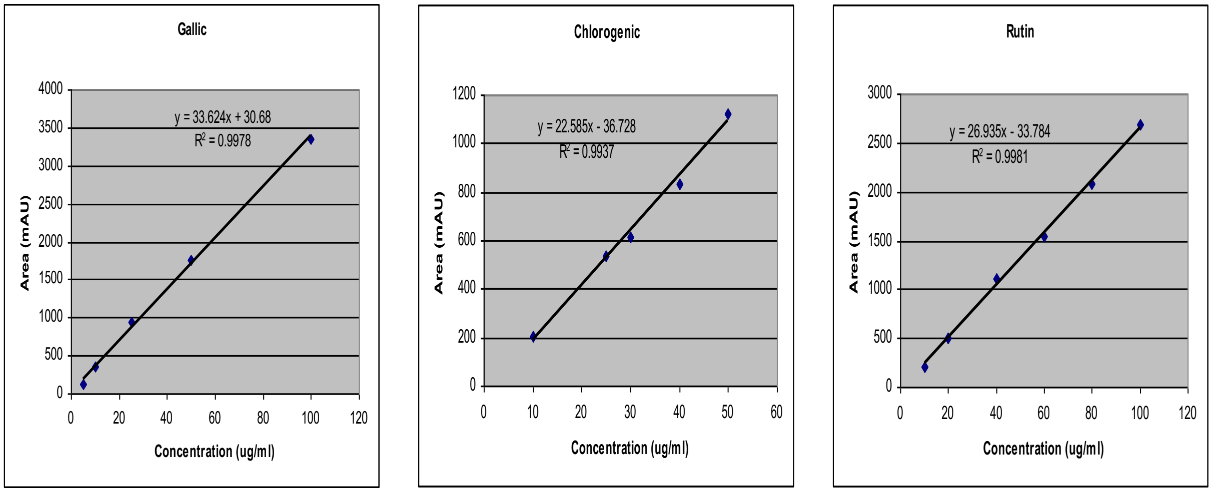
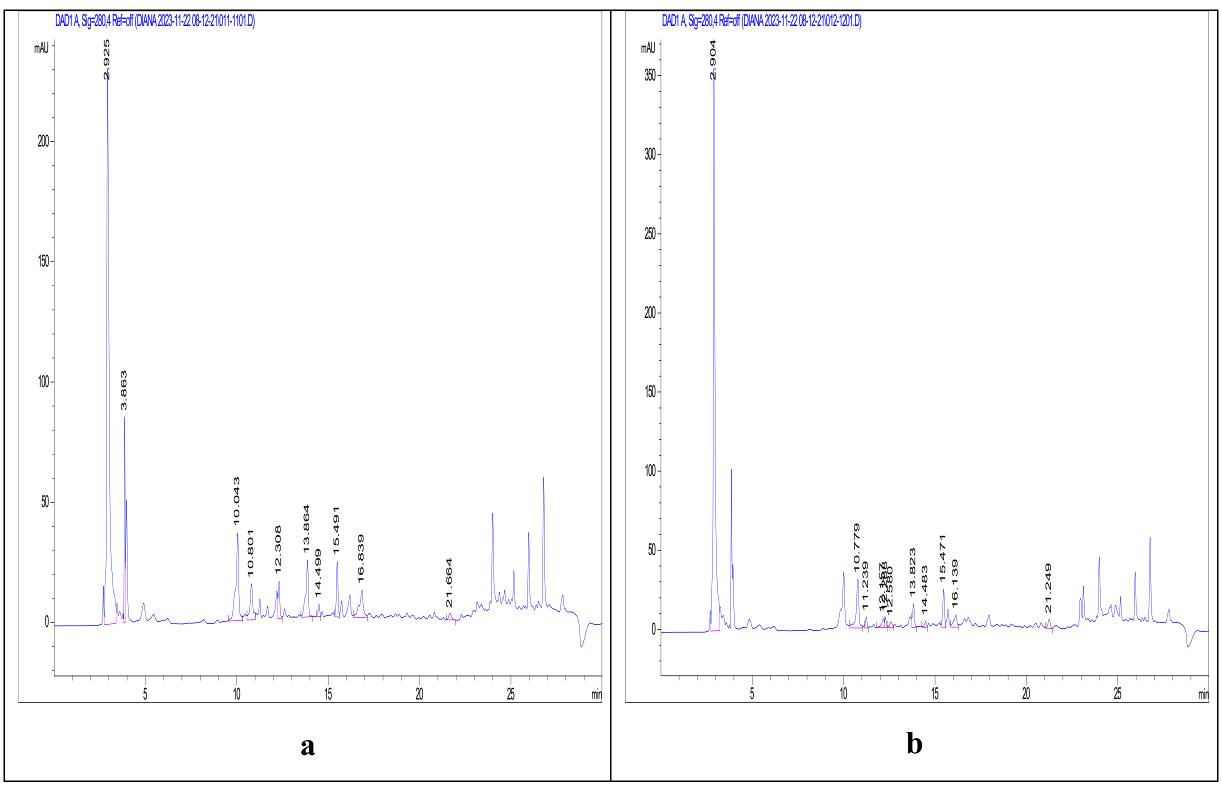


Figure 1. Calibration curve for tomato phenolic compounds – gallic / chlorogenic acid, rutine

3. Results and discussions



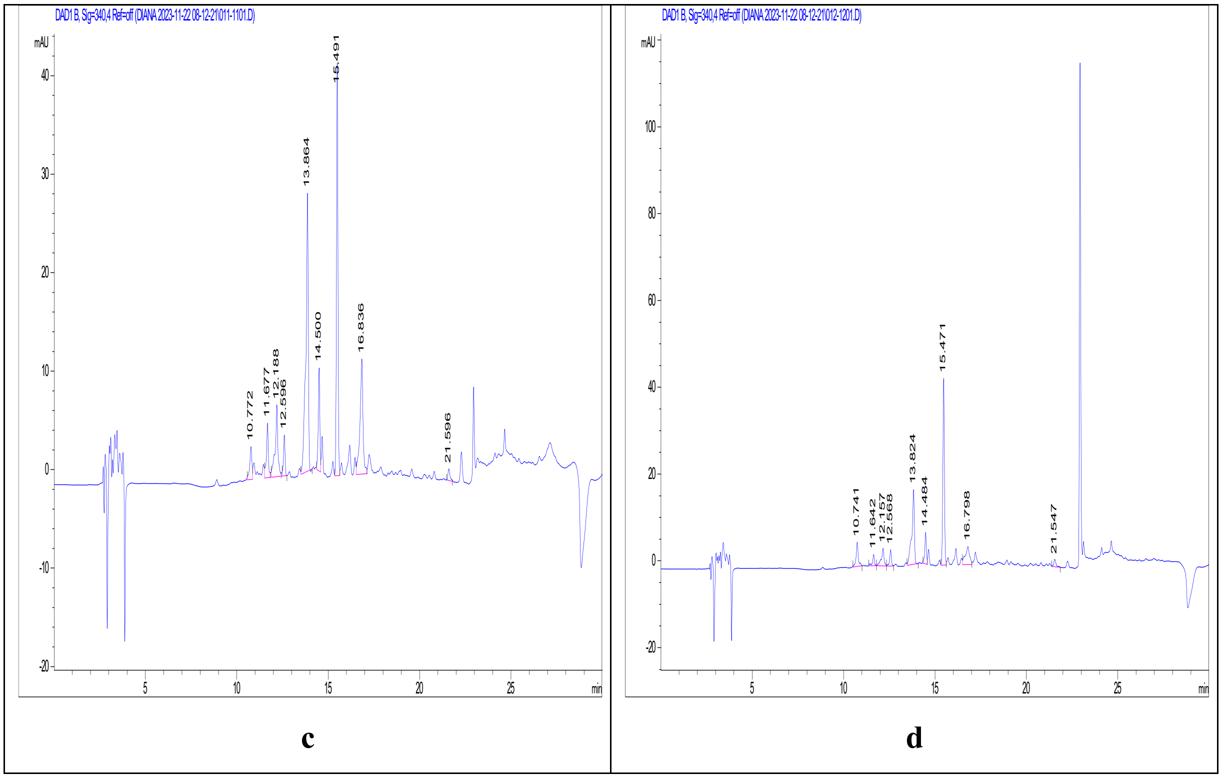


Figure 2. Chromatogram of phenolic compounds for Dumbravita tomatoes – 280 nm (a), Chesa tomatoes – 280 nm (b), Dumbravita tomatoes – 340 nm (c), Chesa tomatoes – 340 nm (d)

Data, performed under the wavelength λ=280 nm for phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acids and flavonols) and λ=340 nm specific to hydroxycinnamic acids and flavonols, presents important results for mountain products.

The chromatograms, analyzed at the frequency λ=280 nm, related to the Dumbravita mountain tomatoes (2925 mAU) show values of phenolic compounds superior to those of the Chesa lowland (2904 mAU) (Figure 2 - a and b). (USAMV-CJ, 2023)

The comparative analysis extends to the chromatograms scrutinized at the wavelength λ=340 nm, concerning Dumbravita mountain tomatoes (15491 mAU - Figure 2c). These chromatograms distinctly manifest elevated levels of phenolic compounds when juxtaposed with the counterparts from the Chesa lowland (15471 mAU - Figure 2d).

The discernible divergence in phenolic content underscores the potential influence of geographic and edaphic factors on the chemical composition of tomatoes, accentuating the distinctiveness in phytochemical profiles between mountainous and lowland cultivation environments. (USAMV-CJ, 2023)

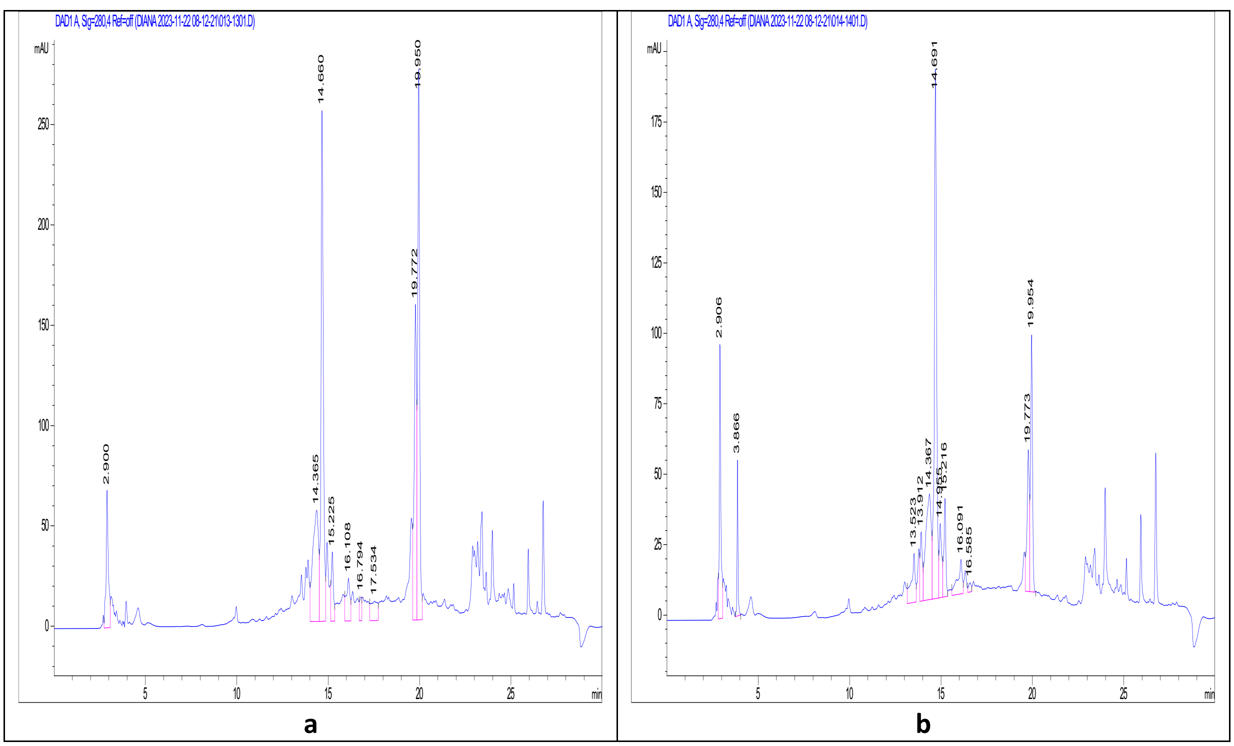
The spectrum of analyzes for phenolic compounds in tomato samples showed superior values for many tomatoes originating from the mountain area. (Table 1 - USAMV-CJ, 2023

Evidently, it is apparent that mountain tomatoes exhibit an augmented presence of phenolic compounds in comparison to their lowland counterparts. Noteworthy constituents among these compounds include Caffeic acid-glucosides, 3-Caffeoylquinic acid (Neochlorogenic acid), 5-Caffeoylquinic acid (Chlorogenic acid), Caffeic acid, Quercetin-triglucosides, and Ferulic acid, as elucidated in detail in Table 1. This divergence in phenolic profiles between mountain and lowland tomatoes underscores the substantial impact of environmental factors on the phytochemical composition of tomatoes. (USAMV-CJ, 2023)

In the case of *Zea mays* (maize), the chromatograms scrutinized at the wavelength λ=280 nm, specifically focusing on the phenolic compounds in mountain maize (19960 mAU, Figure 3a), distinctly reveal elevated values in comparison to their lowland maize counterparts (14691 mAU, Figure 3b).

Table 1. Identification and quantification of phenolic compounds, retention time (R_t), wavelength (λ) and frequency $[M+H]^+$ in tomato samples, quantity expressed in mg/100g sample

Peak	R_t (minute)	λ_{max} (nm)	$[M+H]^+$ (m/z)	Phenolic compound	Subclass	Tomato Dumbravita *	Tomato Chesa **
1	2.93	265	139	2-Hydroxybenzoic acid	Hydroxybenzoic	34,675	37,731
2	10.07	270	139	4-Hydroxybenzoic acid	Hydroxybenzoic	1,433	1,876
3	11.68	312, 248	343	Caffeic acid- glucosides	Hydroxycinnamic acid	1,721	1,256
4	12.11	326, 248	355	3-Caffeoylquinic acid (Neochlorogenic acid)	Hydroxycinnamic	2,496	1,765
5	12.59	326, 248	355	5-Caffeoylquinic acid (chlorogenic acid)	Hydroxycinnamic	1,344	1,367
6	13.86	324, 248	181	Caffeic acid	Hydroxycinnamic	6,613	4,532
7	14.50	355, 255	743	Quercetin-triglucosides	Flavonols	1,815	1,444
8	15.49	354, 256	611	Quercetin-rutinosides (Routine)	Flavonols	5,045	5,379
9	16.83	326, 248	195	Ferulic acid	Hydroxycinnamic	3,780	2,319
10	21.58	355, 255	303	Quercetin	Flavonols	0.813	0.924
				Total phenolics		*59,735*	*58,592*



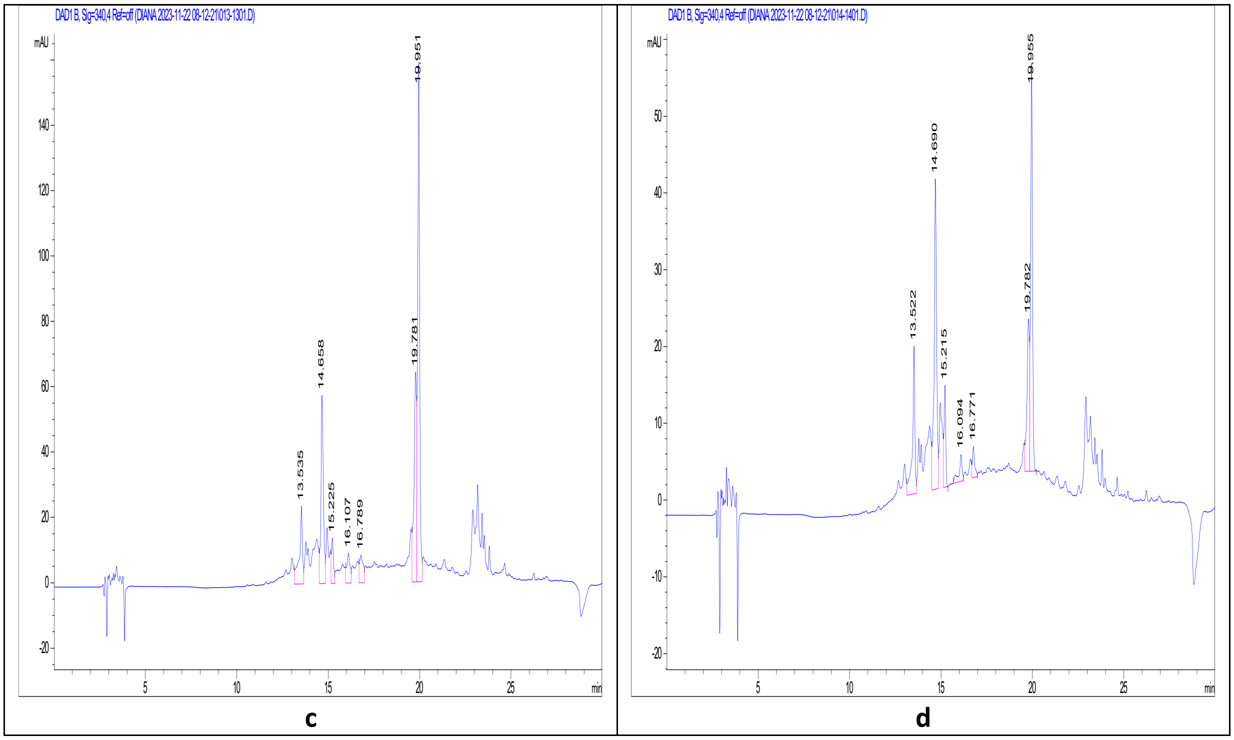


Figure 3. Phenolic compounds chromatogram for Dumbravita corn - 280 nm (a), Chesa corn - 280 nm (b), Dumbravita corn -340 nm (c), Chesa corn – 340 nm (d)

This discernible disparity accentuates the potential influence of cultivation environment on the phenolic content of maize, emphasizing the significance of geographic and edaphic conditions in shaping the phytochemical profiles of agricultural produce. (USAMV-CJ, 2023)

The species preserve in the analysis to a frequency λ=340 nm and the chromatograms for the phenolic compounds of the mountain corn show higher values compared to the lowland one (Figure 3c-d). (USAMV-CJ, 2023)

Within the realm of phenolic compounds, it is discernible that mountain maize manifests augmented quantities of specific entities. Notably, the phenolic compounds exhibiting higher values in mountain corn include 2-Hydroxybenzoic acid, Caffeic acid, Syringic acid, p-Coumaric acid, Ferulic acid, and Di-Caffeoylquinic acid, as meticulously detailed in Table 2 (USAMV-CJ, 2023). This distinctiveness in the phenolic composition highlights the potential superiority of mountain corn in augmenting various physiological functions, such as antioxidant and anti-inflammatory effects.

Table 2. Identification and quantification of phenolic compounds, retention time (R_t), wavelength (λ) and frequency $[M+H]^+$ in corn samples, amount expressed in mg/100g sample

Peak	R_t (minute)	λ_{max} (nm)	$[M+H]^+$ (m/z)	Phenolic compound	Subclass	Maize Dumbravita *	Maize Chesa **
1	2.93	265	139	2-Hydroxybenzoic acid	Hydroxybenzoic	8,347	7,886
2	3.86	265	139	3-Hydroxybenzoic acid	Hydroxybenzoic	0.719	2,622
3	13.54	322	181	Caffeic acid	Hydroxycinnamic	33,359	24,050
4	14.69	280	199	Syringic acid	Hydoxybenzoic	6,990	4,754
5	16.12	300	165	p-Coumaric acid	Hydroxycinnamic	3,359	1,743
6	16.69	323	195	Ferulic acid	Hydroxycinnamic	3,381	1,610
7	19.95	322	517	Di-Caffeoylquinic acid	Hydroxycinnamic	41,327	13,786
				Total phenolics		*97,481*	*56,451*

The observation that a solitary phenolic compound exhibits lower values in mountain corn accentuates the comprehensive and nuanced impact of the cultivation environment on the phytochemical makeup of agricultural produce.

The implication of these findings extends beyond mere compositional disparities, suggesting that the consumption of mountain products present a preference not only for those seeking a wholesome lifestyle but also a pragmatic necessity for bolstering the body's immunity.

The interplay of diverse phenolic compounds in mountain maize underscores the multifaceted benefits of incorporating such produce into dietary regimens, emphasizing the intricate relationship between agricultural cultivation practices and the potential health-enhancing properties of the resultant food products.

The ascendancy of mountain agricultural products shows further substantiated through the examination of the aggregate phenolic compound content. In this context, the corn, tomatoes, and mountain peppers cultivated in the Dumbravita region exhibit conspicuously elevated levels of phenolic compounds in comparison to their counterparts from the Chesa lowland, as meticulously detailed in Table 3 (USAMV-CJ, 2023). This discernible disparity underscores the pivotal role that geographical and environmental factors play in influencing the phytochemical composition of agricultural yields.

Table 3. Quantification of the total content of phenolic compounds in corn, tomato and pepper samples, amount expressed in mg/100g sample

Sample	Total content of phenolic compounds mg/100g
Chesa corns	77,395
Dumbravita corns	99,663
Chesa tomatoes	65,446
Dumbravita tomatoes	69,248
Chesa peppers	134,966
Dumbravita peppers	176,787

The heightened concentration of phenolic compounds in mountain produce stands as a testament to the intricate interplay between altitude, soil characteristics, and climate, which collectively contribute to the distinctive chemical profile observed in these crops. Such empirical findings not only accentuate the intrinsic value of mountainous cultivation, together with phenolic-rich agricultural products. This elucidation aligns with broader scholarly discourse on the impact of geographical origin on the nutritional and bioactive attributes of food commodities.

4. Conclusions

The results underscore the significance of developing tomato, corn, and pepper crops, given consumer behavior in both lowland and mountainous areas, where consumers prefer these vegetables over others. The paper highlights the importance of noting that the health benefits of polyphenols and phenolic compounds reach their maximum potential when people consume these foods as part of a balanced and varied diet. Incorporating a wide range of colorful fruits and vegetables, such as tomatoes and corn, ensures a diverse intake of polyphenols and phenolic compounds, contributing to overall health and well-being.

Mountain regions play a pivotal role in the global agricultural landscape, characterized by well-distributed terrain and considerable potential for mountain-based production. However, some mountainous areas face challenges related to biodiversity pollution together with careful balance between agricultural practices and renewable development and sustainability. Mountain regions emerge as rich in biodiversity, making a substantial contribution to European mountain productivity. The complex relationship between diverse landscapes, flora, and fauna positions these regions as vital ecological reservoirs.

As we examine individual agricultural enterprises in mountain areas, the challenges faced by farmers highlight the intricate navigation required between market dynamics and environmental sustainability. These insights provide a foundation for strategic interventions to ensure the sustainable development of these regions and the prosperity of individual agricultural producers. Moreover, the analysis of polyphenols and phenolic compounds in tomatoes, corn, and pepper emphasizes the nutritional importance of these crops, underscoring the value of diverse and balanced dietary choices for overall health and well-being.

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Abbreviations

ETc - crop evapotranspiration under standard conditions

GAE - gallic acid equivalents

HCl - Hydrochloric acid

HPLC - high-performance liquid chromatography

HPLC-DAD-ESI - high-performance liquid chromatography with diode-array detection, electrospray ionisation process

UV - ultraviolet