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Studies of bioactive compounds of wild tomatoes in the context of functional food and genetic improvement of Ararat Valley plants

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ABSTRACT

Wild tomato species (*Lycopersicon pennellii* and *L. cheesmanii*) are rich sources of bioactive compounds with potential functional food applications. Six accessions (three L. *cheesmanii* and three L. *pennellii*) cultivated in the Ararat Valley, Armenia, were analyzed for dry matter, vitamin C, β-carotene, lycopene, lutein, and pectin. Yellow and orange fruits (L. *cheesmanii*) exhibited higher β-carotene, lutein, and pectin, while red fruits (L. *pennellii*) were richer in lycopene. Significant variation in vitamin C and dry matter was also observed, reflecting genotype and pigmentation effects. These findings highlight the high functional and nutritional potential of wild tomato accessions, forming a scientific basis for the selection of valuable germplasm aimed at developing health-promoting tomato cultivars. The novelty of this study lies in the first cultivation and comprehensive evaluation of these wild tomato species under the agro-climatic conditions of the Ararat Valley (Republic of Armenia), with an emphasis on their potential use in functional food production and breeding programs.

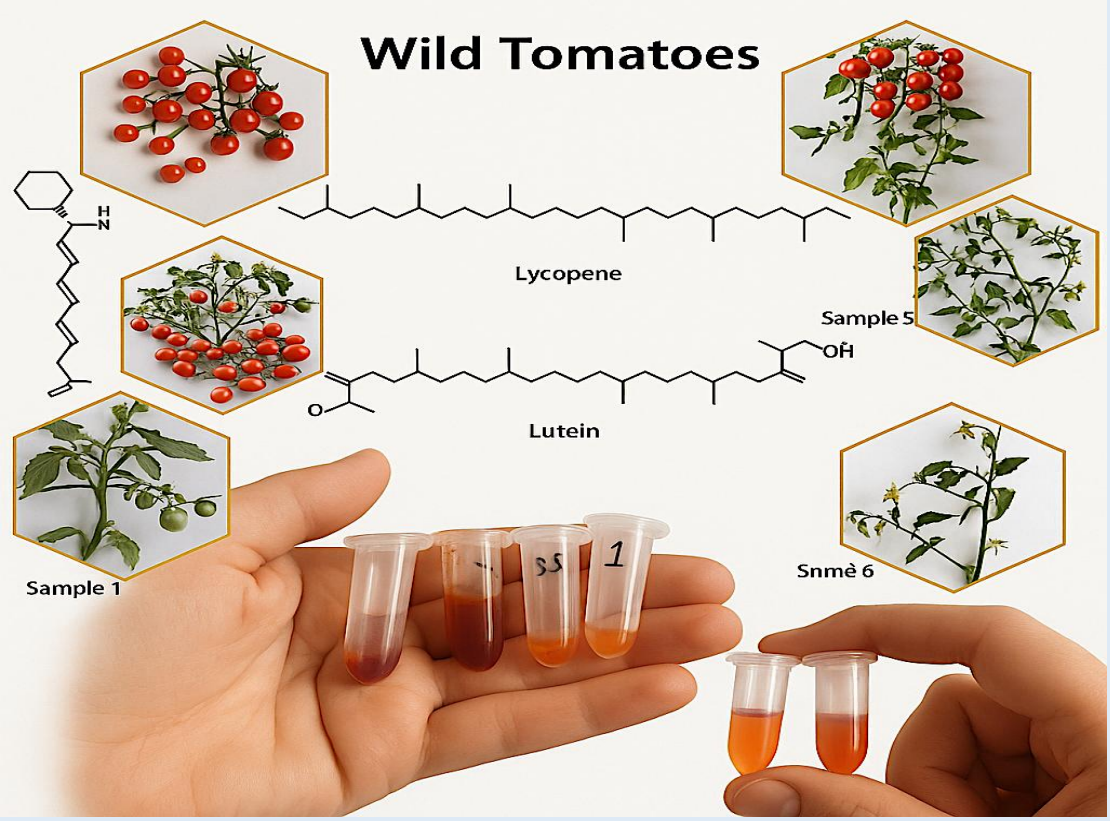
Objective: To evaluate key bioactive and nutritional compounds in wild tomato accessions for their potential use in functional food production.

Methods: Fruits from six wild tomato accessions were analyzed for dry matter, vitamin C, β-carotene, lycopene, lutein, and pectin using standard biochemical assays. Quantitative data were statistically processed to assess variation among accessions.

Results: Yellow and orange fruits (L. *cheesmanii*) contained higher levels of β-carotene, lutein, and pectin, whereas red fruits (*pennellii*) were richer in lycopene. Vitamin C and dry matter also varied significantly among accessions, reflecting the influence of genotype and fruit pigmentation.

Conclusion: Wild tomato accessions exhibit substantial diversity in bioactive compounds, emphasizing their functional and nutritional potential. These results can guide the selection of promising germplasm for breeding nutritionally enhanced tomato varieties.

Keywords: wild tomato, *Lycopersicon pennellii*, *Lycopersicon cheesmanii*, bioactive compounds, functional food, β-carotene, lycopene, lutein, vitamin C, pectin.



Graphical Abstract: Studies of bioactive compounds of wild tomatoes in the context of functional food and genetic improvement of Ararat Valley plants.

INTRODUCTION

Tomato is considered one of the most widespread and economically valuable crops in the world, for whichconsumer demand is constantly growing [1-3]. Meeting this demand is possible by creating new varieties and hybrids with high nutritional value and introducing them into production. Tomatoes are used both fresh and processed in the form of paste, juice, and other products [4-5]. The widespread consumption of this crop is due not only to its palatability, but also to the high content of important bioactive components: lycopene, β-carotene, vitamin C, lutein, dry matter, and soluble sugars, which enhances its role as a functional food [6-12].

Wild tomato varieties are distinguished by high levels of the above substances, which make them promising not only for genetic improvement but also for therapeutic and nutritional applications. These forms are a valuable gene pool due to their adaptability to extreme environmental conditions, such as drought or saline soils, as well as their high content of important biochemical compounds. These features can be used not only for the creation of new varieties but also for the search for sources of functional foods [13-14].

Wild tomato fruits naturally contain a wide range of carotenoids—biologically active conjugated isoprenoid compounds that have high nutritional value. They are divided into two main groups: hydrocarbon carotenes (e.g., lycopene, β-carotene) and oxygen-rich xanthophylls (e.g., lutein) [15-17]. These compounds have powerful antioxidant properties and play an important role in strengthening the body's defense system and preventing chronic degenerative diseases, including cancer [18]. In addition, β-carotene is a biologically active precursor of vitamin A, the deficiency of which is considered a common global nutritional problem among children [19].

Quantitative assessment and comparison of the content of carotenoids in wild tomato genotypes is important not only for nutritional studies but also for assessing the stability of the accumulation of these substances during the stages of cultivation, storage, and processing [20-24]. The ripening process can substantially increase lycopene content, which is an important indicator for selection [25-27]. Laboratory analysis of these compounds is mainly carried out by high-performance liquid chromatography (HPLC) with C18 or C30 columns, which provides the necessary sensitivity and analytical precision for comparing the biochemical composition of wild and cultivated tomatoes [28]. These methods provide a basis for fully revealing the nutritional potential of wild tomato species and evaluating new prospects for their use in functional foods and selection.

Additionally, the biomorphological and biochemical properties of six wild tomato samples, including *Lycopersicon pennellii* (115-22, 1, 17-24) and *Lycopersicon cheesmanii* (K-8473, K-3970, K-5027), cultivated under the conditions of the Ararat Valley, were studied. The growth and developmental characteristics of the plants were analyzed, as were the carotenoids present in the fruits, particularly lycopene. The results obtained indicate significant differences between the samples, highlighting their genetic and nutritional potential. The scientific novelty of the article lies in the fact that for the first time these wild tomato species were cultivated in the Ararat Valley of the Republic of Armenia and evaluated in terms of food functionality.

MATERIALS AND METHODS

Scientific research was conducted at the Darakert Experimental Farm of the Scientific Center for Vegetables and Technical Crops of the Ministry of Economy of the Republic of Armenia (SCVIC, MEofRA) during 2023-2024.

Research material: A total of 10 wild forms were studied, which are quite diverse in geographical origin, from which the best six were selected (*Lycopersicon pennellii* (115-22, 1, 17-24) and *Lycopersicon cheesmanii* (K-8473, K-3970, K-5027)). The samples were collected from botanical scientific collections.

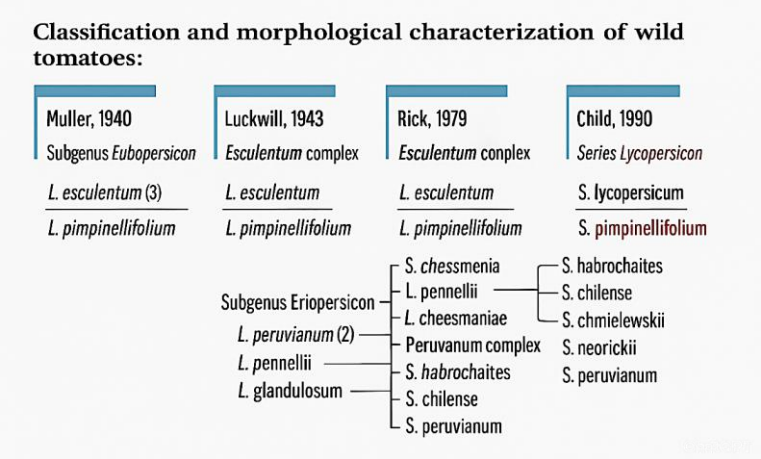


Figure 1. Classification and morphological characterization of wild tomatoes.

Experimental planting scheme: The experiments were set up with 4 replications, the size of the experimental plots was 50 sq.m, the planting scheme was 90+60x30 cm.

The variety testing was conducted in accordance with the state and AVRDC methodologies for agricultural crop variety testing.

Soil cultivation, fertilization, and crop care were carried out in accordance with agrotechnical measures typical for the given zone and wild tomatoes. The agrochemical indicators of the experimental plot served as the basis for the application of the correct doses of the main mineral fertilizers during cultivation.

Sowing was carried out within the agrotechnical requirements: March 25-30, and transplanting was carried out on May 1-5. Winter wheat was used as a predecessor.

Basic studies of experiments: For the research, six samples of wild tomato were studied, including the species

1. Phenological stages recording (flowering, fruiting, ripening)

2. Biomorphological measurements: stem height, leaf structure, branching

3. Morphological description of fruits: shape, size, color, mass

4. Yield calculation: g/plant, ha/indicator

5. Biochemical analyses: Dry matter, Lycopene, Lutein, Vitamin C,β-carotene, Pectin.

Dry matter: The dry matter content of ripe fruits was determined using a refractometer.

Lycopene: The fruits of the samples were collected at the fully ripe stage for biochemical analysis. Ten ripe fruits were taken from each sample. Following accurate weighing, the tomato samples were homogenized, after which a measured volume of HPLC-grade acetone was added while continuously agitating the mixture. Subsequently, a methanolic sodium hydroxide (NaOH) solution was introduced, and a reflux condenser was attached to the reaction vessel. The mixture was heated in a water bath for a specified duration and then rapidly cooled to room temperature. Distilled water was added to the hydrolyzed mixture, and the resulting solution was filtered.

To isolate the target compounds, the filtrate was subjected to three extractions with HPLC-grade acetone in the presence of potassium sulfate (K_2SO_4) to facilitate phase separation. The absorbance of the lycopene-rich extract was measured using a Spectronic 21D spectrophotometer at 340 nm [29-30].

Lutein: The fruits of the samples were collected at the fully ripe stage for biochemical analysis. Ten ripe fruits from each sample were selected, accurately weighed, and homogenized to obtain a uniform paste. A known volume of HPLC-grade ethanol was added to the homogenate while continuously stirring the mixture to facilitate pigment extraction. The homogenate was then treated with a methanolic potassium hydroxide (KOH) solution to induce saponification of lipids and release of esterified lutein.

The reaction vessel was equipped with a reflux condenser and heated in a water bath at 60 °C for 30 minutes. After the mixture was allowed to cool to room temperature, distilled water was added to stop the reaction. The hydrolyzed solution was filtered using Whatman filter paper, and the filtrate was extracted three times with HPLC-grade hexane in the presence of sodium chloride (NaCl) to enhance phase separation.

The hexane layers containing the lutein were collected and pooled. The combined extract was dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was redissolved in ethanol, and the lutein content was determined spectrophotometrically at 445 nm using a calibrated standard curve [31-32].

Vitamin C: The content of ascorbic acid was measured spectrophotometrically using a Carry 60 UV-Vis spectrophotometer (Agilent Technologies, USA), following the standard procedure with 2,4-dinitrophenylhydrazine and absorption measurement at λ = 520 nm. Calibration solutions were prepared based on L-ascorbic acid [33-34].

β-carotene: Beta-carotene, a fat-soluble carotenoid, is quantitatively determined using High-Performance Liquid Chromatography (HPLC). Ripe tomato fruits are washed, cut into small pieces, and stored frozen to preserve the chemical stability of the carotenoids. Approximately 5 g of homogenized sample is mixed with ethanol (or methanol) containing 0.1% BHT (butylated hydroxytoluene) to prevent oxidation.

Then, hexane and distilled water are added to create a biphasic system. The mixture is vigorously shaken and centrifuged at 4,000 rpm for 10 minutes to facilitate phase separation. The upper hexane layer, containing β-carotene, is carefully collected.

An aliquot (1 mL) of the extract is filtered and injected into the HPLC system equipped with a C18 reversed-phase column. The mobile phase typically consists of methanol, acetonitrile, and dichloromethane in optimized proportions to achieve efficient separation.

Detection is carried out at 450 nm using a diode-array detector (DAD). The concentration of β-carotene is calculated using external standard calibration with a pure β-carotene reference standard (e.g., Sigma-Aldrich). Results are expressed as milligrams per 100 grams of fresh weight (mg/100 g FW).

Data collected from spectrophotometric and chromatographic analyses were statistically evaluated using analysis of variance (ANOVA). When significant differences were found among treatment means, comparisons were made using Duncan's multiple-range test (DMRT) at the 5% significance level. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) [35-36].

RESULTS

Morphological description: During the studies, the analysis of morphological indicators revealed that the plants of the Cheesman wild specimens were mainly of ordinary average, ordinary dwarf and shtambovi dwarf appearance, the vegetation period was 83-85 days. The fruits were mainly round in shape, yellow-orange, red, and reddish orange in color, with an average fruit weight of 12-35 g, with a 2-4-stem structure, while the plants of

the Pennelli wild specimens were mainly of ordinary indeterminate and ordinary Potato form, the vegetation period ranged from 87-90 days. The fruits were round in shape, red, weighing 6-35 g, with 2-3 stems.

Table 1. Morphological and phenological characteristics of tomato wild forms

Samples	vegetation period	bush	leaf	fruit			
				weight	coloring	form	number of nests
L. pennellii 115-22	88	ordinary indeterminate	green	6-7	red	round	2-3
L. pennellii 86	87	ordinary potato	dark green	30-35	red	round	3-4
L. pennellii 17-24	90	ordinary indeterminate	green	15-17	red	round	2-3
L. cheesmanii K-8473	85	ordinary average	light green	20-25	yellowish orange	round	2-3
L. cheesmanii K-3970	83	ordinary dwarf	green	30-35	red	round	2-4
L. cheesmanii K-5027	84	shtambovi dwarf	green	12-15	reddish-orange	round	2

Phenological data showed that the specimens of the L. Pennellii group are late-ripening, while the L. cheesmanii group is characterized by early ripening. The stem height ranged from 45 to 90 cm, and the degree of branching was from medium to high.

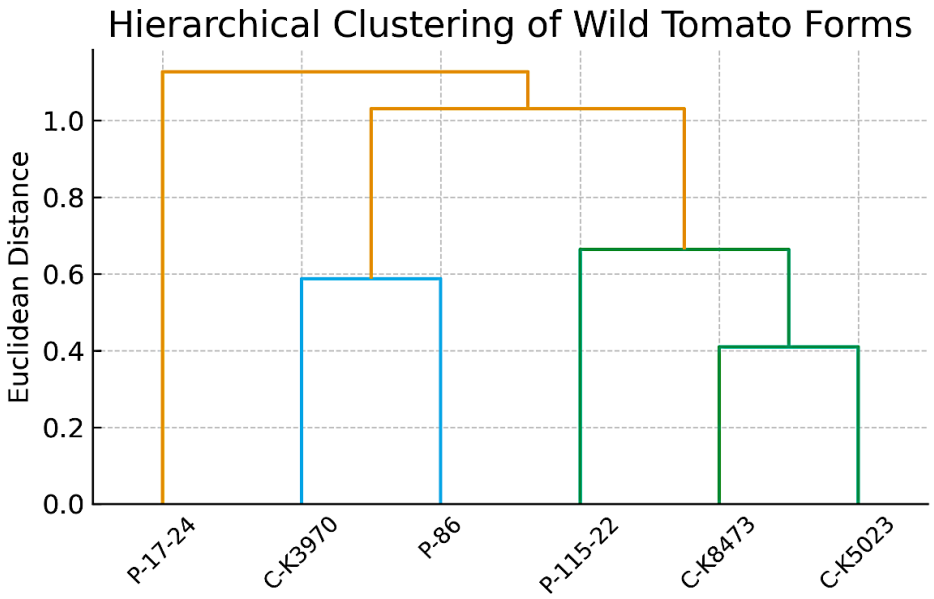


Figure 2. Hierarchical Cluster Analysis Interpretation

We performed hierarchical cluster analysis to group the six Lycopersicon pennellii samples into distinct clusters based on their morphological characteristics. Clustering was performed using an agglomerative method, where individual samples were initially considered as separate units and then gradually merged based on their level of similarity.

In the first stage of the dendrogram, the closest similarity was observed between samples LP-3 and LP-4, indicating that these two acolytes share the closest morphological features within the studied group.

This subcluster subsequently merged with LP-2, suggesting that LP-2 also exhibits features of LP-3 and LP-4, albeit with slightly lower similarity.

Another separate branch was formed by LP-5 and LP-6, which showed a high degree of similarity to each other, but were clearly distinguished from the LP-2–LP-4 cluster.

The most divergent sample, LP-1, joined the main group only at the highest level of divergence. This suggests that LP-1 has unique characteristics compared to the other accessions.

Overall, the dendrogram reveals two main groups:

1. A larger cluster consisting of LP-2, LP-3, and LP-4, with LP-1 being a distant member.

2. A smaller, more homogeneous cluster consisting of LP-5 and LP-6.

These results may be valuable for selecting parental lines in breeding programs, as adaptations

from different clusters may provide higher genetic variability in hybrid combinations.

Analysis of the bioactive substances of ripe, fresh fruits of wild tomato shows that the dry matter content of all forms belonging to the *L. cheesmanii* species was, on average, 0.63% higher than that of *L. pennellii* forms. This is a very important indicator both for evaluating this form as a functional food and for its use as breeding material in subsequent breeding work. Since the heritability coefficient of dry matter varies from 0.25 to 0.95 when pollinated with different combinations.

Dry matter analysis shows that all forms belonging to the *L. cheesmanii* species are on average 0.63 percent higher than *L. pennellii* forms, respectively. This is a very important indicator both for evaluating a given form as a functional food and for using it as a breeding material in further breeding work. Since the dry matter inheritance coefficient when pollinated with different combinations varies from 0.25 to 0.95.

The analysis showed that the vitamin C content in all studied samples was relatively high, ranging between 44.1 and 56.7 mg/100g. The highest level was recorded in *L. pennellii* 115-22 (56.7 mg/100g, while the lowest was found in *L. cheesmanii* K-5027 (44.1 mg/100g). On average, *L. pennellii* accessions exhibited higher values (51.8–56.7 mg/100g) compared to *L. cheesmanii* (44.1–48.9 mg/100g).

Table 2. Bioactive compound content of wild tomato fruits as a functional food.

Samples	Dry matter, %	Lycopene mg/100g	Lutein content (mg/100g),	Vitamin C mg/100g	β-carotene, mg/100g	Pectin, %
L. pennellii 115-22	7.7 ± 0.05	14.045 ± 0.05	0.148 ± 0.005	44.5 ± 0.1	1.5 ± 0.02	0.35 ± 0.01
L. pennellii 86	7.6 ± 0.05	14.079 ± 0.05	0.139 ± 0.005	45.2 ± 0.1	1.4 ± 0.02	0.33 ± 0.01
L. pennellii 17-24	7.4 ± 0.05	15.105 ± 0.05	0.102 ± 0.005	44.1 ± 0.1	1.4 ± 0.02	0.31 ± 0.01
L. cheesmanii K-8473	8.7 ± 0.051	8.601 ± 0.05	0.874 ± 0.005	56.7 ± 0.1	2.5 ± 0.02	0.51 ± 0.01
L. cheesmanii K-3970	7.6 ± 0.05	13.903 ± 0.05	0.115 ± 0.005	49.2 ± 0.1	1.5 ± 0.02	0.34 ± 0.01
L. cheesmanii K-5027	8.1 ± 0.051	9.021 ± 0.05	0.526 ± 0.005	55.7 ± 0.1	1.8 ± 0.02	0.49 ± 0.01

Values represent mean ± SD (n = 4). Differences among samples at $p < 0.05$ according to Tukey's HSD test.

These differences are associated with genotypic characteristics as well as fruit pigmentation, with red-fruited accessions generally containing higher amounts of vitamin C. The results indicate that *L. pennellii* samples can be considered a rich source of ascorbic acid, which is of great importance not only for improving the nutritional value but also for enhancing the functional properties of tomato fruits.

Tomato fruits exhibit a wide range of color variations, primarily determined by the accumulation of carotenoid pigments. Fruit color is closely associated with both the quantity and composition of bioactive

compounds. Yellow-colored fruits generally contain high levels of β-carotene, a provitamin A source with notable antioxidant properties. In contrast, red-colored fruits are predominantly rich in lycopene, a potent antioxidant that lacks provitamin activity but plays a significant role in reducing the harmful effects of free radicals in the human body.

Thus, β-carotene and lycopene, while belonging to the same biochemical group, confer distinct biological effects and characteristic coloration to tomato fruits. If you were to schematically present the biogenesis of β-carotenoids in tomato fruits, it would have the following form [37].

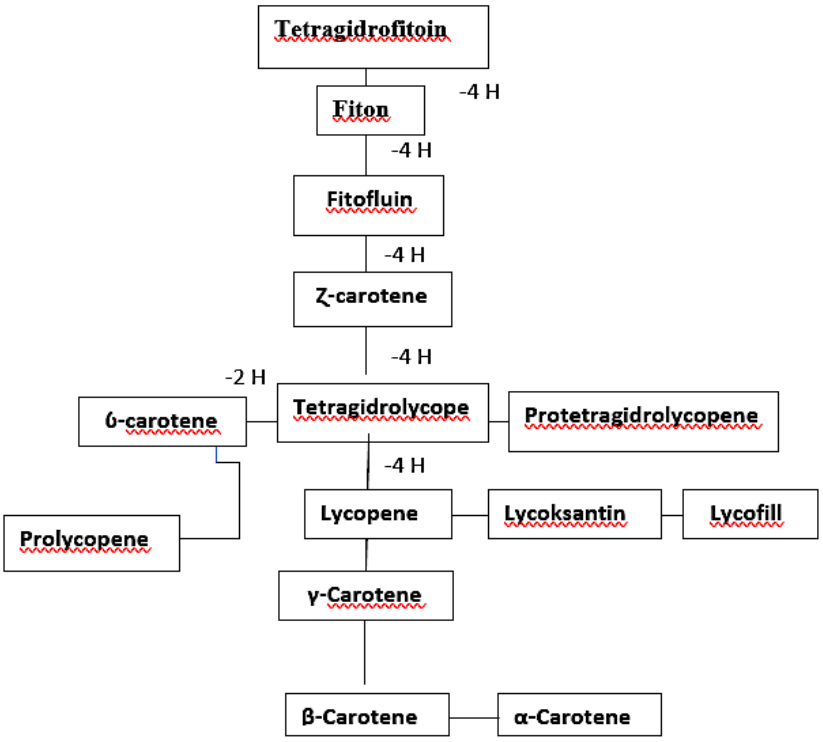


Figure 3. Biogenesis of β-carotenoids in tomato fruits.

Over the years, different points of view have been expressed on this biosynthesis, and there have been schematic changes, but the general basis has remained almost unchanged.

Our study demonstrated that the pigmentation of wild tomato fruits has a significant influence on their carotenoid composition. Analysis of the table and diagram data revealed that yellow, yellow-orange, and orange fruits (mainly characteristic of *L. cheesmanii* accessions) contained higher levels of β-carotene, ranging from 1.5 to 2.5 mg/100g per 100 g, whereas in red fruits (L. *pennellii* accessions) β-carotene levels were lower, ranging from 1.4 to 1.5 mg/100g. This finding is particularly important, as β-carotene is the primary provitamin A carotenoid, essential for vision, immune system function, and skin health.

In contrast, the analysis of lycopene content showed an opposite trend. Red fruits (L. *pennellii* accessions) exhibited significantly higher lycopene levels, ranging from 14.045 to 15.105 mg/100g, while yellow and orange fruits (L. *cheesmanii* accessions) contained lower levels, varying between 8.601 and 13.903 mg/100g. Lycopene is among the most potent natural antioxidants, known for its protective role in

cardiovascular health and its potential to reduce the risk of certain cancers.

Lutein content, however, demonstrated a reverse pattern compared to lycopene. In red fruits (L. *pennellii* accessions), lutein levels were relatively low (0.102–0.148 mg/100g), whereas in yellow and orange fruits (L. *cheesmanii* accessions) they were considerably higher, ranging from 0.526 to 0.874 mg/100g. Lutein is another key carotenoid, particularly important for eye health, as it protects the macula lutea and reduces the risk of age-related macular degeneration. Moreover, lutein contributes to cardiovascular protection and helps maintain the overall antioxidant balance in the human body.

Taken together, these results indicate that carotenoid accumulation in wild tomato fruits is closely associated with fruit pigmentation. The study of β-carotene, lycopene, and lutein content is therefore highly relevant not only for the development of functional foods but also for breeding programs, where understanding the inheritance patterns of carotenoid traits could create new opportunities to develop nutritionally enhanced cultivars and hybrids.

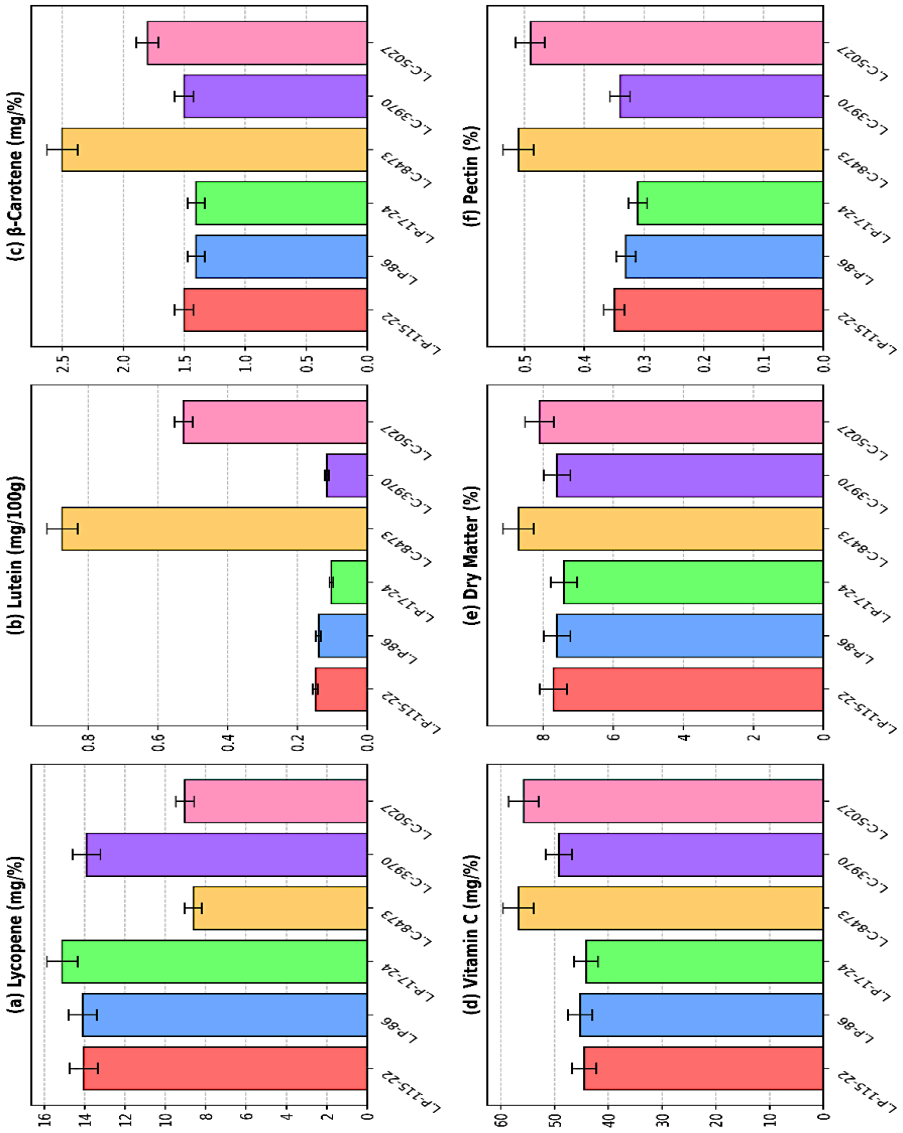


Figure 4. The content of bioactive substances in the fruits of different wild tomato samples.

Our study demonstrated that pectin content also varies among wild tomato fruits with different pigmentation. Overall, higher levels of pectin were recorded in yellow and orange fruits (0.34–0.51 mg/100g), whereas red fruits contained comparatively lower amounts (0.31–0.35 mg/100g). This observation is significant, as pectin is a type of dietary fiber widely recognized for its health-promoting and technological properties. It contributes to improving the gut microbiota, reducing cholesterol levels, and stabilizing blood glucose. Additionally, pectin is extensively used in the food industry as a natural gelling and stabilizing agent, particularly in the production of jams, jellies, and functional foods.

DISCUSSION

The comprehensive study of six wild tomato accessions — Lycopersicon pennellii (115-22, 86, 17-24) and *L. cheesmanii* (K-8473, K-3970, K-5027) — grown under the conditions of the Ararat Valley demonstrated pronounced variability in both morphological and biochemical parameters. The analysis revealed that fruit pigmentation was strongly associated with the accumulation of bioactive compounds. The red-fruited *L. pennellii* accessions were distinguished by their high lycopene content (14.0–15.1 mg/100g), which considerably exceeded that of the yellow- and orange-fruited *L. cheesmanii* accessions (1.8–2.9 mg/100g). At the same time, *L. cheesmanii* samples showed higher levels of β-carotene (1.5–2.5 mg/100g) and lutein (0.526–0.874 mg/100 g), whereas *L. pennellii* contained only 0.102–0.148 mg/100 g lutein. Pectin content also varied significantly, ranging from 0.31–0.35 % in *L. pennellii* to 0.34–0.51 % in L. cheesmanii, confirming the superior gelling potential of the latter.

Dry matter content fluctuated between 7.4 and 8.7 %, with *L. pennellii* 115-22 demonstrating the highest value (8.7 %). Vitamin C content was relatively high across all accessions, varying between 44.1 and 56.7 mg/100g, which confirms the important dietary value of these genotypes. These differences emphasize the role of genetic background and fruit pigmentation in shaping the biochemical profile of wild tomato fruits.

Vitamin C content was also relatively high in all studied accessions, ranging between 44.1–56.7 mg/100g. The highest level was observed in *L. pennellii* 115-22 (56.7 mg/100g), while the lowest was in *L. cheesmanii* K-5027 (44.1 mg/100g). Overall*, L. pennellii* samples contained higher levels of ascorbic acid, highlighting their value as a rich source of vitamin C.

Overall, the results suggest that *L. pennellii* can serve as a valuable source of lycopene for breeding programs aimed at enhancing antioxidant capacity, while *L. cheesmanii* represents a promising donor for β-carotene, lutein, and pectin enrichment. The considerable genetic and nutritional diversity of these wild tomatoes underscores their potential for functional food development, biofortification, and the creation of nutritionally superior tomato cultivars and hybrids adapted to diverse agroecological conditions.

CONCLUSION

The main objective of this study was to comprehensively evaluate the content of bioactive and nutritional compounds, including carotenoids (lycopene, β-carotene, lutein), vitamin C, dry matter, and pectin, in selected samples of wild tomatoes (*Lycopersicon pennellii* and *Lycopersicon cheesmanii*) cultivated in the Ararat plain. The aim was to identify the genetic and nutritional potential of these species and to assess their prospects for application in functional food development and future plant breeding programs. The implementation of these studies and the evaluation of functionality are

being carried out for the first time and are a scientific novelty for our republic.

List of Abbreviations: ABA — Abscisic Acid, CAR — Carotenoids, Chl — Chlorophyll, DM — Dry Matter, DW — Dry Weight, FW — Fresh Weight, HPLC — High-Performance Liquid Chromatography, LC-MS/MS — Liquid Chromatography–Mass Spectrometry, ROS — Reactive Oxygen Species, SNP — Single Nucleotide Polymorphism, TSS — Total Soluble Solids, Vit C — Vitamin C.

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Author Contributions: G. K. and K. S. designed the study and provided wild tomato seeds for the study. A. Zh. performed the biochemical analyses, G. K., M. G., G. Sh., and V. V. performed the biochemical analyses. G. K. and V. V. performed the statistical analyses. G. K. wrote the manuscript. G. K. and A. Z. edited the article. All authors read and approved the final version of the manuscript.

Competing Interests: All authors declared noncompeting interests.

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