



## Article

# Effect of Seed Spaceflight Storage on Tomato Fruit Quality and Peel/Pulp Mineral and Antioxidant Distribution

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**Abstract:** The spaceflight storage of seeds is known to cause mutations affecting both their quality and the mature plants originating from them. To study the effects of space stress, tomato seeds of two cultivars (Lotus and Autumn rhapsody) were subjected to half a year of storage at the International Space Station (ISS), and then, sown in a greenhouse to produce tomato fruits. The space-treated plants gave smaller fruits with a stable total yield not significantly different from that of the control plants. Space-treated tomatoes showed significantly higher levels of dry matter, dietary fiber, monosaccharides and citric and malic acids and lower values of oxalic acid compared to the control plants. The pulp of space-treated fruits had 1.44–1.70 times lower levels of carotenoids, while their peel contained a 1.27–1.90 times higher pigment amount compared to the control plants. No significant changes in the total antioxidant activity (AOA), photosynthetic pigments and phenolic (TP) and proline content were recorded in the fruits due to seed spaceflight storage. Contrarily, space-treated tomatoes showed decreased levels of Ca, Sr and Mo and increased Se both in the fruit pulp and peel. The concentration of Fe and especially Pb was lower in space-treated fruit pulp. Positive correlations between Se and dry matter, Ca and Sr, Ca and Co, Ca and Fe, and Cr and carotenoids, and negative correlations between Se and Mo, Se and K, and Mo and dry matter were recorded. The results indicate that seed stress caused by long-term spaceflight affects both the biochemical characteristics and mineral composition of tomato fruits and causes the peel/pulp redistribution of carotenoids as well as macro- and micro-elements, improving Se accumulation levels in the fruit peel.

**Keywords:** seed space storage; *Solanum lycopersicum* L.; adaptation; antioxidants; selenium; minerals; peel/pulp distribution



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## 1. Introduction

The effect of spaceflight on seed vigor and genetic changes has been the object of many investigations aiming to increase the rate of breeding [1–8]. The exposure of seeds to radiation and microgravity during storage at the ISS greatly affects gene expression [9], causing mutations [10], chromosome aberration [11] and morphological changes [12]. Microgravity increases the sensitivity of plants to radiation [13], and the duration of these stress factors is critical for seed viability and stress resistance. The radiation at the ISS includes the impact of galactic cosmic rays (GCRs), of the South Atlantic anomaly of the Earth's radiation belt (SAA) and of solar flares. According to literature reports, the mean

total radiation dose at the ISS per half year is about 7.5 cGy for GCRs, 2.3 cGy for SAA and up to 5 cGy during intensive large solar particle events [14]. Chronic exposure of plants to the mentioned stress factors may affect oxidative stress-related genes and genes belonging to carbohydrate and nucleic acid metabolism and photosynthesis [15].

Indeed, spaceflight is known to induce mutations in seeds [16], and may affect genes essential for root hair growth [17], improve the production of stress response proteins, and inhibit auxin metabolism [18]. Numerous investigations in this field demonstrated high varietal and species differences in plant and seed responses to microgravity and cosmic radiation, suggesting high potential of seed space treatment to improve seed germination and mature plant yield and quality [19–22]. Previous investigations by Kahn and Stoffella [23] demonstrated tomato seed tolerance to long-term spaceflight storage. Contrarily, many studies indicating genetic mutation showed the development of oxidative stress both in seeds and mature plants [24–30].

Plant tolerance to abiotic stresses depends on many factors, including genetic peculiarities, morphological modifications, the production of secondary metabolites, activation of the antioxidant defense system, hormonal changes and modulations in the mineral composition of tissues [31]. The complexity of plant responses to abiotic stresses and the significance of this phenomenon for the efficiency of crop production and quality promote intensive investigations of the mechanism of plant protection and adaptability [32]. In this respect, most of the investigations have been devoted to the widespread abiotic stresses of drought, salinity, nutrient deficiency, extreme temperatures, etc. Contrary, information about the mechanism of plant defense against stresses caused by seed exposure to spaceflight [6] is much scantier.

The aim of the present study was the evaluation of the antioxidant status and mineral profiles of two tomato cultivars grown from seeds after half a year of storage at the International Space Station (ISS).

## 2. Material and Methods

Research was carried out on tomato (*Solanum lycopersicum* L.) grown in a greenhouse at the Federal Scientific Vegetable Center in Odintsovo (Moscow region, Russia). The experimental protocol was based on the factorial combination of a seed space treatment plus an untreated control and two cultivars (Lotus and Autumn Rhapsody). A randomized complete block design was used for the treatment distribution in the greenhouse, with three replicates.

Tomato seeds of cultivars Lotus and Autumn Rhapsody (selection of Federal Scientific Vegetable Center) were exposed at the space station ISS-21-Korolev from 18 March 2022 to 29 September 2022. Seeds were stored in sealed foil bags inside the ISS, at an average temperature of 22–23 °C and 25% humidity. The untreated control seeds of the two mentioned cultivars were also stored in sealed foil bags under the same temperature and humidity conditions during the whole experiment at the Federal Scientific Vegetable Center.

Seeds were sown on 31 March 2023, and the seedlings were transplanted in 120 cm wide soil beds, spaced 30 cm along the rows (2.8 plants per m<sup>2</sup>), in an unheated polycarbonate greenhouse on 3 May. The plants were supplied with the organo-mineral fertilizer ‘Universal’, protected against the late blight by a single spray with the ‘Prophit Gold’ preparation (‘Gardener’s Green Pharmacy’ Co. Ltd., Moscow, Russia). Drip irrigation was activated when the soil humidity dropped to 80% of the available water capacity at a 20 cm depth. Regular soil loosening and weed removal were carried out during the vegetation phase. Tomato fruits were collected twice a week beginning from 17 June.

The ‘Germination—flowering’ periods of both control and space-treated plants lasted 52 days for cv. Lotus and 55 days for Autumn rhapsody; the ‘Germination—biological ripeness’ period was 94 and 101 days long for cvs. Lotus and Autumn rhapsody, respectively.

The mean day and night temperatures during plant cycles were 20–27 °C and 15–18 °C, respectively. Drip irrigation was carried out to maintain the optimal soil humidity (70%). No artificial illumination of plants was used.

### 2.1. Sample Preparation

Tomato fruits were harvested from 17 June to 16 August. The number, weight and diameter of mature fruits per plant, and plant height were measured for 15 randomly selected plants per plot. Biochemical characteristics of fruits were evaluated on random samples of three units per cluster both in control and space-treated plants.

After harvesting and calculating the fruit number and weight, a fraction of each sample was homogenized, and fresh homogenates were used for the determination of the ascorbic acid, nitrates, dietary fiber and organic acid content. The remainder of the samples were used for peel and pulp separation. To obtain fruit peel, ripe tomato fruits were immersed into hot water (85 °C) for 1–2 min, cooled down quickly under tap water, dried with filter paper and hand peeled.

Fresh homogenates of the peel and pulp of 10 fruits were used for the carotenoid profile assessment. After carotenoid analysis, peel and pulp samples were dried at 70 °C to a constant weight and used for the determination of proline, monosaccharides, total polyphenol content (TP), total antioxidant activity (AOA) and mineral composition.

Photosynthetic pigments were determined in fresh leaves.

### 2.2. Dry Matter

The dry matter content was determined gravimetrically after drying peel and pulp samples up to constant weight at 70 °C in an oven.

### 2.3. Mineral Composition

Tomato fruit elemental profiling, including Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Sr, V and Zn, was performed on dried homogenized fruit peel and pulp samples using ICP-MS on a quadruple mass-spectrometer Nexion 300D (Perkin Elmer Inc., Shelton, CT, USA) and a Berghof SW-4 DAP-40 microwave digestion system (Berghof Products + Instruments Gmb H, 72, 800 Eningen, Germany), as reported by Kharchenko et al. [33].

### 2.4. Ascorbic Acid

The ascorbic acid content was assessed using the manual titration method based on the interaction of the ascorbic acid with sodium 2,6-dichlorophenol indophenolate (Tillman's reagent) [34].

### 2.5. Total Polyphenols (TP)

To implement a unified approach to the analysis of the total antioxidant activity (AOA), polyphenol, sugar, proline and mineral content, tomato fruits were dried to a constant weight at ambient temperature before the analysis. This provided high representativeness of tomato sampling, particularly important when using low sample weight (less than 500 mg), and ensured the high storability of probes.

The Folin–Ciocalteu colorimetric method was used for total polyphenol determination utilizing 70% ethanol extracts of dry peel/pulp homogenates [35]. The concentration of polyphenols was assessed using the absorption value of the Folin reagent–sample ethanol extract reaction mixture at 730 nm (Unico 2804 UV spectrophotometer, Suite E Dayton, NJ, USA). The solution of 0.02% gallic acid was used as an external standard, and all the results were expressed in mg of Gallic Acid Equivalent per g of dry weight (mg GAE g<sup>-1</sup> d.w.).

### 2.6. Carotenoids

The determination of carotenoid content in fruit peel and pulp was carried out spectrophotometrically after the chromatographic separation of carotenoids [35]. About 0.5 g of fresh homogenized sample was ground in a mortar with ceramic powder and extracted with small portions of acetone until color disappearance. The combined extract was diluted with 9 mL of hexane and washed 4–5 times with distilled water to remove traces of acetone. The residual extract was quantitatively transferred to a volumetric flask, and the volume was adjusted to 10 mL with hexane. The resulting extract was mixed and filtered through a small portion of anhydrous  $\text{Na}_2\text{SO}_4$ . The separation of carotenoids was achieved using quantitative thin-layer chromatography on Whatman 3A chromatographic paper in two chromatographic systems: (1) hexane to separate  $\beta$ -carotene and (2) hexane–acetone, 10:0.5 for separation of lycopene and lutein. Appropriate zones of carotenoid compounds were cut out and filled with 3 mL of hexane. The determination of carotenoid content in tomato fruit was performed using appropriate specific absorption  $E^{1\%}_{1\text{cm}}$  for  $\beta$ -carotene (2580 at  $\lambda = 450$  nm), lycopene (3470 at  $\lambda = 474$  nm) and lutein (2560;  $\lambda = 447$  nm) on a Unico 2804 UV spectrophotometer. The internal standards were  $\beta$ -carotene, lutein and lycopene from Sigma Inc. (Kure, Japan).

### 2.7. Antioxidant Activity (AOA)

To evaluate the total antioxidant activity of fruit peel and pulp, 0.01 N  $\text{KMnO}_4$  solution was titrated with ethanol extracts of peel/pulp homogenates until the solution became completely discolored [35]. Gallic acid (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany) was used as an external standard, and the values obtained were expressed in  $\text{mg GAE g}^{-1}$  d.w.

### 2.8. Proline

Proline determination was carried out using 3% sulfur salicylic extract of dry homogenized tomato pulp via a reaction with ninhydrin reagent in acetic acid, as described by Ouertani et al. [36]. After heating at  $95^\circ\text{C}$  for 1 h, the probes were cooled and extracted with toluene. Proline content was calculated using the absorption value of the extract at 505 nm and a calibration curve with 5 different proline (Merck, Rahway, NJ, USA) concentrations.

### 2.9. Photosynthetic Pigments

Chlorophyll a, chlorophyll b and carotene content in tomato leaves was assessed spectrophotometrically by a Unico 2804 UV spectrophotometer using 96% ethanol extract of fresh leaf samples and the following equations developed by Lichtenthaler [37]:

$$\text{Ch-a} = 13.36A_{664} - 5.19A_{649};$$

$$\text{Ch-b} = 27.43A_{649} - 8.12A_{664};$$

$$C\ c = (1000A_{470} - 2.13\ \text{Ch-a} - 87.63\ \text{Ch-b})/209;$$

where A = absorbance, Ch-a = chlorophyll a, Ch-b = chlorophyll b and C c = carotene.

### 2.10. Organic Acids

Organic acid content was determined via HPLC (Agilent 1100) (column Zorbax Bonus-RP C18;  $4.6 \times 250$  mm,  $5\ \mu\text{M}$ ; flow rate of  $1.0\ \text{mL}\cdot\text{min}^{-1}$ ; wavelength of 210 nm) using an isocratic elution with phosphate buffer at pH 2.5 [38]. Appropriate standards of organic acids (citric, oxalic, malic) were obtained from Sigma-Aldrich (Burlington, MA, USA). The results are expressed as the means of three replications.

### 2.11. Dietary Fiber

The dietary fiber content in tomato fruits was assessed gravimetrically after the enzymatic sequential hydrolysis of starch and non-starch compounds with protease,  $\alpha$ -amylase

and amyloglucosidase (Sigma Chemical Co., St. Louis, MI, USA) to mono-, di-, oligosaccharides and peptides and the appropriate precipitation of dietary fibers with ethanol. The fiber content was determined after drying the precipitate at 70 °C to a constant weight and is expressed in % per d.w. [39].

### 2.12. Statistical Analysis

The data were statistically processed by conducting an analysis of variance (ANOVA), and the mean separation was determined through Duncan's test at  $p < 0.05$ , using the SPSS software version 28 (IBM, Armonk, NY, USA).

## 3. Results and Discussion

### 3.1. Biometrical Parameters, Dry Matter and Dietary Fiber

The chosen tomato cultivars differed both by fruit size and colour: Lotus is a spread cultivar with medium-sized red fruits smaller than those of Autumn rhapsody (Table 1), and a predominant content of lycopene while the orange fruits of Autumn rhapsody contain  $\beta$ -carotene as the main carotenoid pigment (Table 5). The UV spectrum of individual carotenoids in the latter case confirmed the structure of  $\beta$ -carotene and the absence of pro-lycopene, typical for tangerine tomatoes.

**Table 1.** Biometric, yield and growth parameters of tomato.

Parameter	Lotus		Autumn Rhapsody	
	Control	Space	Control	Space
Plant height (cm)	99 ± 10 a	97 ± 10 a	105 ± 12 a	100 ± 11 a
Fruit weight (g)	84 ± 8 b	79 ± 8 b	143 ± 14 a	138 ± 14 a
Fruit number per plant	36 ± 3 a	39 ± 4 a	14 ± 2 b	16 ± 3 b
Early fruit yield (kg m <sup>-2</sup> )	2.53 ± 0.24 a	2.76 ± 0.30 a	1.81 ± 0.20 b	2.02 ± 0.20 b
Total fruit yield (kg m <sup>-2</sup> )	9.18 ± 0.95 a	9.04 ± 0.90 a	9.25 ± 0.91 a	9.13 ± 0.90 a
Fruit diameter (cm)	58.4 ± 6 b	54.8 ± 5 b	73.9 ± 7 a	61.7 ± 6 a
Marketability (%)	80 ± 5 a	79 ± 5 a	84 ± 5 a	85 ± 5 a
Leaf dry matter (%)	10.5 ± 1.0 b	12.9 ± 1.1 a	11.5 ± 1.0 ab	13.5 ± 1.1 a
Fruit pulp dry matter (%)	5.3 ± 0.5 c	8.0 ± 0.8 a	6.0 ± 0.6 bc	6.8 ± 0.7 ab
Fruit peel dry matter (%)	9.9 ± 0.9 b	11.9 ± 1.1 ab	9.8 ± 0.9 b	13.2 ± 1.2 a
Dietary fiber (% f.w.)	5.4 ± 0.3 c	6.2 ± 0.4 b	6.1 ± 0.5 b	8.6 ± 0.7 a

f.w.: fresh weight. Along each line, values with the same letters do not differ statistically according to Duncan's test at  $p < 0.05$ .

The fruit weight and diameter of space-treated plants were tendentially lower than those of the control fruits, but with no significant differences (Table 1). The lack of yield differences between the control and space-treated plants suggests that space stress induced the formation of a higher number of lighter fruits.

A significant increase in fruit dry matter due to the seed space storage was recorded for both cultivars (Table 1). The differences in dry weight between the control and space-treated plants reached 1.5 times in Lotus pulp and 1.2 times in peel and, accordingly, 1.13 and 1.35 times in the pulp/peel of Autumn rhapsody tomatoes (Table 1). Notably, similar trends were recorded for fruit dietary fiber content and dry matter, also suggesting a presumably close relationship between the latter and total fiber. Most tomato fibers (87%) are represented by hemicellulose, cellulose and lignin. The increased dietary fiber content in space-treated tomato fruits is directly connected with plant lignification under stress conditions [40,41]. The lignin carbohydrate complex is known to increase plant resistance to biotic and abiotic stresses via the enhancement of plant tissue hardness and mechanical strength [42], and the stimulation of nutrient assimilation and water transport [43].

Significant differences in the dry matter content between the control and space-treated plants caused the need to calculate all the parameters, including photosynthetic pigments and the ascorbic acid content per dry weight, to make the appropriate comparisons.

### 3.2. Photosynthetic Pigments

Photosynthetic pigment accumulation is closely connected with the intensity of plant development, indicating the efficiency of carbon assimilation. Indeed, the evaluation of the intensity of photo-pigment accumulation in lettuce, Brassicaceae and Apiaceae species grown from the one-year storage of seeds at the ISS demonstrated significant species and varietal differences directly related to plant yield [33]. In the present study, the analysis of photosynthetic pigments showed an increase in the chlorophyll a level only in space-treated cv. Autumn rhapsody, while no significant changes were recorded for total chlorophyll content and carotene levels (Table 2). Nevertheless, a significant increase in the chl a/chl b ratio was detected in the leaves of space-treated plants.

**Table 2.** Leaf photosynthetic pigments.

Photosynthetic Pigments (mg g <sup>-1</sup> d.w.)	Lotus		Autumn Rhapsody	
	Control	Space	Control	Space
Chlorophyll a	12.08 ± 1.0 ab	12.40 ± 1.20 ab	10.52 ± 1.00 b	13.70 ± 1.21 a
Chlorophyll b	6.86 ± 0.61 ab	6.05 ± 0.60 b	8.96 ± 0.90 a	7.41 ± 0.71 a
Total chlorophyll	18.94 ± 1.71 a	18.45 ± 1.70 a	19.48 ± 1.88 a	21.11 ± 2.01 a
Carotene	2.38 ± 0.20 a	2.71 ± 0.22 a	2.35 ± 0.20 a	2.52 ± 0.21 a
Chl a/Chl b ratio	1.80	2.05	1.17	1.84

d.w.: dry weight. Along each line, values with the same letters do not differ statistically according to Duncan's test at  $p < 0.05$ .

Chl a and Chl b interconversion is known to regulate the PSI/PSII ratio. Moreover, Chl a is recorded in both core and light harvesting complexes, whereas Chl b is found only in the latter case. The increase in Chl a content and conversion of Chl b to Chl a during chlorophyll degradation is a critical point in plant survival under stress conditions [44]. Accordingly, the significant increase in the Chl a/Chl b ratio in the space-treated leaves of cv. Autumn rhapsody is consistent with the mentioned phenomenon.

### 3.3. Organic Acids

Photosynthetic pigment accumulation is closely connected with sugar and organic acid content. These compounds affect tomato fruit flavor and taste [45]. Tomato organic acids (predominantly citric and malic acids) influence fruit acidity and play an important role in regulating osmotic pressure, pH homeostasis and stress resistance. Their levels in tomato fruit are affected both by genetic peculiarities and environmental stress factors, improving photosynthesis, antioxidant defense and osmoregulation [46]. Abiotic stresses are known to affect endogenous citric and malic acid levels in plants. Indeed, drought stress was shown to significantly increase the citric acid content in tomato, *Gossypium hirsutum*, *Clusia* sp. and *Aptenia cordifolia* [46]. In the present investigation, space-treated tomato plants showed 1.26 times higher levels of citric acid and 1.16–1.19 times higher content of malic acid compared to the control fruits (Table 3).

The organic acid pool of citric and malic acids may play a significant role in decreasing oxalic acid content [46], which is in accordance with the present data indicating a 4–5 times oxalic acid decrease in space-treated tomatoes (Table 3).

Monosaccharides are also known to participate actively in plant antioxidant defense, acting as signaling molecules and ROS scavengers [47]. Varietal differences in the accumulation of these compounds in the fruit of the control and experimental tomatoes indicate a higher intensity of monosaccharide level increases in the fruit of space-treated cv. Autumn rhapsody compared to cv. Lotus (1.24 times compared to 1.12 times), while changes in the organic acid content were more pronounced in Lotus fruit (1.25 times compared to 1.18 times).

Space stress caused a decrease in nitrate content by 1.33–1.41 times.

**Table 3.** Organic acid accumulation in control and space-treated tomato fruits.

Parameter	Lotus		Autumn Rhapsody	
	Control	Space	Control	Space
Citric acid (mg 100 g <sup>-1</sup> d.w.)	7079 ± 395 b	8921 ± 480 a	7030 ± 480 b	8333 ± 430 a
Malic acid (mg 100 g <sup>-1</sup> d.w.)	1015 ± 57 c	1261 ± 61 b	1248 ± 63 b	1447 ± 67 a
Oxalic acid (mg 100 g <sup>-1</sup> d.w.)	43.4 ± 2.5 a	10.0 ± 0.6 c	33.3 ± 1.7 b	5.9 ± 0.4 d
Total (mg 100 g <sup>-1</sup> d.w.)	8137.4 ± 422 b	10,192 ± 499 a	8311 ± 405 b	9785.9 ± 512 a
Citric/malic ratio	6.97	7.07	5.63	5.76
Monosaccharides (g 100 g <sup>-1</sup> d.w.)	50.8 ± 4.8 a	56.8 ± 5.2 a	36.7 ± 3.2 b	45.6 ± 4.2 a
Nitrates (mg kg <sup>-1</sup> d.w.)	10,774 ± 780 a	7662.5 ± 540 b	9283 ± 650 a	6956 ± 430 b

Along each line, values with the same letters do not differ statistically according to Duncan's test at  $p < 0.05$ .

### 3.4. AOA, TP, Ascorbic Acid and Proline

The comparison between the polyphenol analysis results of fresh and dried tomato pulp samples revealed that the polyphenol losses did not exceed 5% in the latter case. Contrarily, dried samples retained their antioxidant characteristics for half a year with an insignificant decrease in TP (less than 1–2%), while the TP losses reached about 30–40% in the fresh samples kept at  $-10\text{ }^{\circ}\text{C}$ , in the mentioned time. These results are consistent with the literature reports, reflecting the lowest polyphenol losses during short sample drying time at ambient temperature and the highest in cases of long storage or drying time at low temperature [48].

In general, the antioxidant status of tomato fruits is determined by many parameters, such as organic acid, monosaccharide, proline and polyphenol content; total antioxidant activity; and the carotenoid profile.

The present results indicate a lack of significant changes in fruit AOA, polyphenol (TP), ascorbic acid and proline content due to seed space storage (Table 4). Only a tendency toward increasing proline and ascorbic acid levels was recorded (Table 4). Contrary, the content of polyphenols in the peel of the control and space-treated tomato fruits was 1.1–1.4 times higher compared to the fruit pulp.

**Table 4.** Antioxidant parameters of tomato fruits.

Parameter	Plant Part	Lotus		Autumn Rhapsody	
		Control	Space	Control	Space
AOA (mg GAE g <sup>-1</sup> d.w.)	Pulp	43.9 ± 3.9 b	41.9 ± 4.0 b	56.2 ± 5.2 a	56.7 ± 5.3 a
	Peel	48.9 ± 4.2 ab	50.2 ± 4.9 a	57.9 ± 5.2 a	54.6 ± 5.2 a
	Peel/pulp	1.114	1.198	1.030	0.963
Polyphenols (TP) (mg GAE g <sup>-1</sup> d.w.)	Pulp	18.0 ± 1.7 c	17.3 ± 1.6 c	18.7 ± 1.7 c	18.7 ± 1.7 c
	Peel	19.8 ± 1.8 bc	24.2 ± 2.1 a	22.6 ± 1.8 ab	22.4 ± 1.8 ab
	Peel/pulp	1.100	1.399	1.209	1.198
Ascorbic acid (mg 100 g <sup>-1</sup> d.w.)	Pulp	232 ± 23 b	272 ± 27 ab	278 ± 27 ab	328 ± 32 a
Proline (mg g <sup>-1</sup> d.w.)		8.0 ± 0.7 a	9.3 ± 0.9 a	9.1 ± 0.9 a	9.6 ± 0.9 a

Within each parameter, values with the same letters do not differ statistically according to Duncan's test at  $p < 0.05$ .

### 3.5. Carotenoids

The most significant changes in antioxidant defense were detected with regard to the carotenoid profile of fruit peel and pulp (Table 5). Among the identified carotenoids, only low lutein levels demonstrated a lack of significant differences between the control and space-treated plant fruit. Contrary, lycopene accumulation in the pulp of Lotus fruit and  $\beta$ -carotene in the pulp of cv. Autumn rhapsody showed 2.0 and 1.8 times decreases, respectively. The opposite phenomenon was recorded in fruit peel, where a significant major carotenoid content increase due to seed space storage was demonstrated (lycopene in

Lotus fruit and  $\beta$ -carotene in Autumn rhapsody fruit) (Figure 1). In a previous experiment, we recorded a moderate carotenoid content increase in experimental tomato fruit, cv. Podmoskovny grown from seeds after half a year storage at the ISS [49]. Unfortunately, no separation of fruit peel and pulp was achieved, which makes it difficult to perform an adequate comparison.



**Figure 1.** Appearance of control and space-treated tomato plants (a,b) and of pulp homogenates (c,d) of space-treated and control fruits.

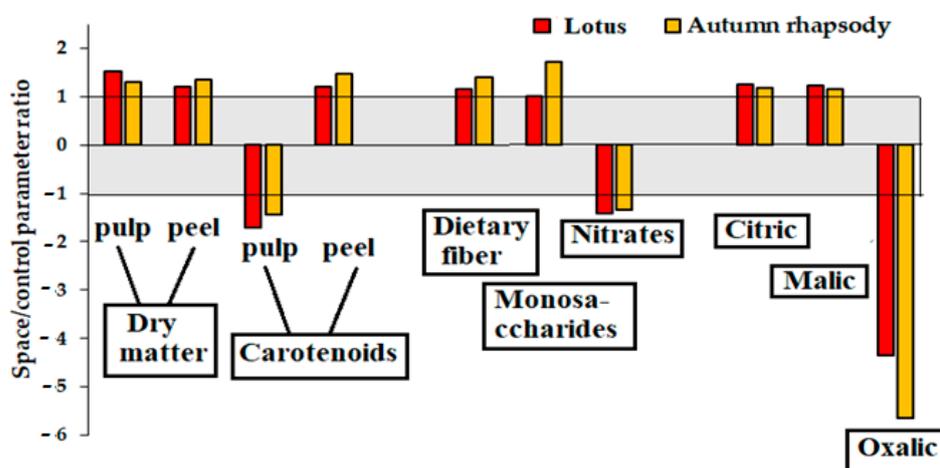
The present results indicate the significance of tomato peel/pulp carotenoid distribution due to long-term seed space stress. The existence of the 'edge effect' phenomenon in plants entails that the high antioxidant status of the outer tissues reflects a powerful mechanism of plant protection against different forms of oxidative stresses [50]. Genetic changes in tomato seeds due to long-term space stress led to enhanced carotenoid content to the fruit's outer peel and decreased carotenoid accumulation in the pulp. The total carotenoid contents in the peel of space-treated tomato fruit were 2.4–4.4 times higher than those recorded in the pulp samples (Table 5).

The phenomenon of seed spaceflight storage effect on mature tomato plant quality not only includes significant changes in the biochemical characteristics of the fruit but is also characterized by valuable peel/pulp redistribution of the dry matter, and especially carotenoids, improving plant protection (Figure 2). In this respect, the changes induced by space stress in seeds [51] and in peel/pulp may become especially important in the breeding of plants with high tolerance to abiotic stresses.

**Table 5.** Carotenoid profile of control and space-treated tomato fruits.

Parameter	Plant Part	Lotus		Autumn Rhapsody	
		Control	Space	Control	Space
Total Carotenoids (mg 100 g <sup>-1</sup> d.w.)	Pulp	117.1 ± 10.1 a	68.8 ± 6.2 b	81.5 ± 8.0 b	56.7 ± 5.1 c
β-carotene (mg 100 g <sup>-1</sup> d.w.)		Trace	Trace	62.7 ± 6.0 a	35.1 ± 3.1 b
Lutein (mg 100 g <sup>-1</sup> d.w.)		31.1 ± 2.9 a	25.9 ± 2.2 ab	18.8 ± 1.7 b	21.6 ± 2.0 b
Lycopene (mg 100 g <sup>-1</sup> d.w.)		86 ± 8 a	42.9 ± 4.0 b	traces	traces
Total Carotenoids (mg 100 g <sup>-1</sup> d.w.)	Peel	245.3 ± 23.5 b	299.1 ± 28.2 a	92.9 ± 9.1 d	136.4 ± 12.9 c
β-carotene (mg 100 g <sup>-1</sup> d.w.)		Trace	Trace	54.1 ± 5.0 b	103.8 ± 9.3 a
Lutein (mg 100 g <sup>-1</sup> d.w.)		49.5 ± 4.3 a	49.6 ± 4.4 a	38.8 ± 3.5 b	32.6 ± 2.9 b
Lycopene (mg 100 g <sup>-1</sup> d.w.)		195.8 ± 19.0 b	249.5 ± 24.2 a	Trace	Trace
Total carotenoids	Peel/ /pulp ratio	2.09	4.30	1.14	2.41
Lutein		1.592	1.915	2.064	1.509
Lycopene		2.28	5.82	-	-
β-carotene		-	-	0.86	2.96

Along each line, values with the same letters do not differ statistically according to Duncan’s test at *p* < 0.05.



**Figure 2.** Changes in tomato fruit dry matter, pigments, dietary fiber, monosaccharides, nitrates and organic acids caused by half a year of spaceflight in seeds.

**3.6. Elemental Composition**

The evaluation of ash content in tomato fruit revealed a tendency to total mineral accumulation increase in the pulp of space-treated fruit and an opposite phenomenon in peel, reaching values of 1.13 for cv. Lotus and 1.50 for Autumn rhapsody (Figure 3).

The significant decreases in peel ash content in space-treated tomato fruits were in accordance with the corresponding decreases in peel Ca levels in fruits grown from spaceflight seeds (Table 6). Interestingly, decreased Ca levels were recorded both in the peel and pulp of space-treated fruits. Considering that Ca accumulation in plant tissues directly depends on the expression of transport proteins [52], it may be supposed that the Ca decrease may reflect important space-induced genetic changes, possibly resulting in the inhibition of cell wall strength due to the important role of Ca in cell wall stability [53]. Ca is known to improve plant responses to different stresses via stimulation of the stress response genes, acting as a signaling molecule in different physiological and biochemical pathways, improving nutrient uptake and stabilizing cellular membranes [54]. In this respect, a significant decrease in Ca levels in tomato pulp, and especially peel, may reflect a negative effect of seed spaceflight storage on tomato adaptability. Conversely, the predominance of Ca accumulation in tomato peel, both in the control and space-treated plants, indicates the significance of Ca protection in bordering tissues.

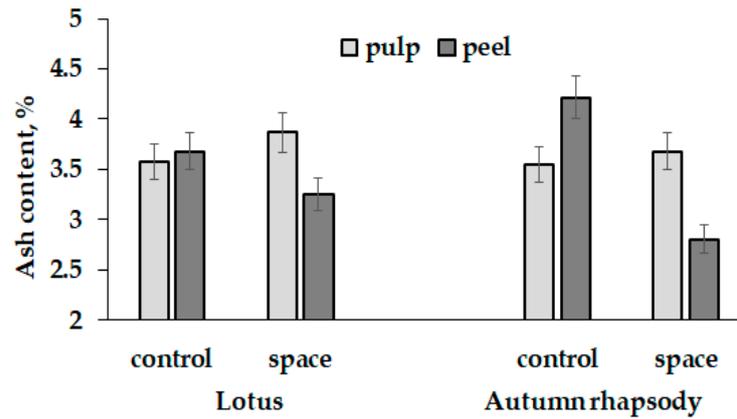


Figure 3. Ash content in peel and pulp of control and space-treated tomato fruits. Bars indicate standard deviation.

The high accumulation of Ca in tomato peel has been previously described by Elbadrawy and Sello [55]. In the present study, the chemical similarity of Ca and Sr [56] was reflected in the synchronous decrease in both Ca and Sr content in space-treated tomato fruits due to seed spaceflight storage (Figure 4). In this respect, seed spaceflight storage changed the accumulation levels of Ca and Sr but also affected the relationship between these elements (Figure 5).

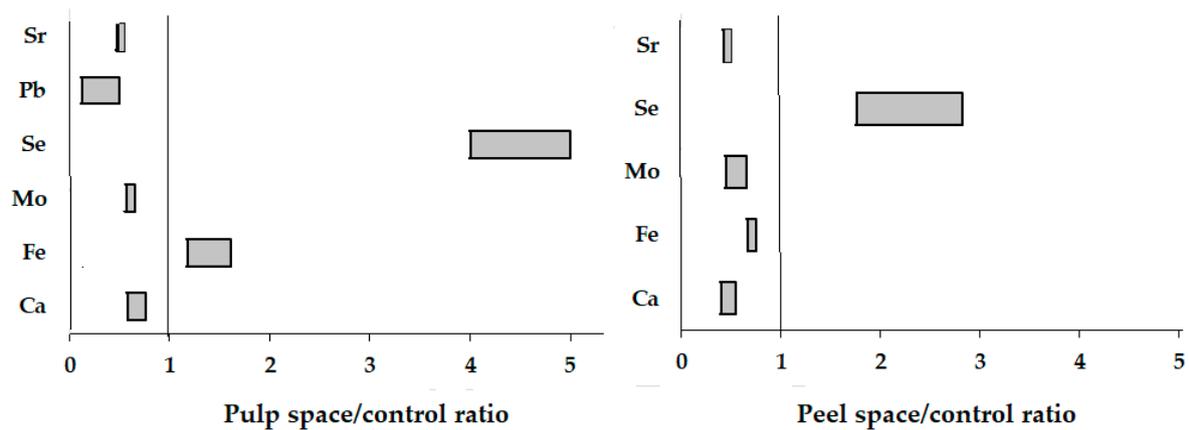


Figure 4. Differences in element accumulation between space-treated and control tomato fruits.

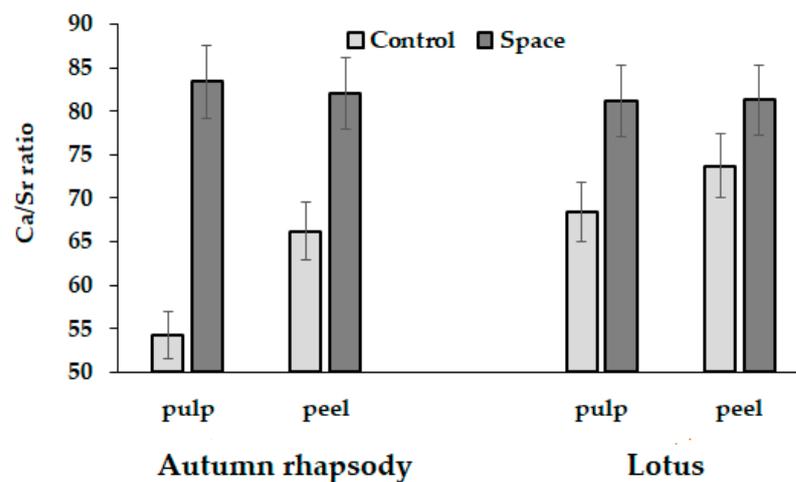


Figure 5. Differences in Ca/Sr ratio between control and space-treated plants. The bars indicate the standard deviation.

**Table 6.** Mineral composition of tomato fruits grown from seeds subjected to one-year spaceflight (mg kg<sup>-1</sup> d.w.).

	Autumn Rhapsody				Lotus			
	Control		Space Treatment		Control		Space Treatment	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Ca	1127 ± 113 c	3084 ± 302 a	851 ± 85 d	1658 ± 164 b	1313 ± 130 bc	3569 ± 360 a	766 ± 76 d	1432 ± 143 b
K	27,254 ± 2700 ab	27,805 ± 2700 an	27,722 ± 2700 ab	19,515 ± 1900 c	27,595 ± 2700 ab	27,187 ± 2700 ab	30,939 ± 3000 a	23,839 ± 2300 b
Mg	1061 ± 103 bcd	1531 ± 150 a	1254 ± 124 ab	845 ± 84 d	1071 ± 105 abc	860 ± 86 d	1204 ± 120 bc	990 ± 98 cd
Na	275 ± 27 b	363 ± 36 a	328 ± 33 a	298 ± 30 ab	248 ± 24 bc	219 ± 22 c	268 ± 27 bc	268 ± 26 bc
P	5594 ± 560 bc	9125 ± 910 a	6321 ± 630 b	5522 ± 550 bc	5226 ± 520 bc	4735 ± 470 c	5309 ± 530 bc	5780 ± 570 b
B	8.41 ± 0.80 bc	11.4 ± 1.0 ac	7.19 ± 0.70 c	8.14 ± 0.80 bc	10.7 ± 1.0 a	9.71 ± 0.95 ab	10.4 ± 1.0 a	9.92 ± 1.00 ab
Co	0.023 ± 0.002 cd	0.043 ± 0.04 a	0.026 ± 0.002 bcd	0.031 ± 0.003 b	0.021 ± 0.002 d	0.033 ± 0.003 b	0.022 ± 0.002 cd	0.028 ± 0.002 b
Cu	5.9 ± 0.6 b	4.78 ± 0.4 c	8.06 ± 0.80 a	3.54 ± 0.33 d	7.48 ± 0.75 a	2.94 ± 0.30 d	5.08 ± 0.50 bc	3.35 ± 0.33 d
Fe	30.7 ± 3.0 e	55.4 ± 5.2 a	49.3 ± 4.9 ab	37.2 ± 3.6 cde	32.2 ± 3.2 de	54.7 ± 5.4 a	38.0 ± 3.7 cd	41.0 ± 4.0 bc
Li	0.033 ± 0.003 d	0.051 ± 0.004 ab	0.019 ± 0.002 e	0.054 ± 0.005 a	0.033 ± 0.003 d	0.047 ± 0.004 abc	0.043 ± 0.004 bc	0.042 ± 0.004 c
Mn	5.22 ± 0.50 c	9.28 ± 0.91 a	8.76 ± 0.85 a	6.03 ± 0.60 b	5.25 ± 0.51 c	6.22 ± 0.61 b c	6.01 ± 0.60 bc	6.92 ± 0.70 b
Mo	1.62 ± 0.13 a	1.59 ± 0.15 a	1.07 ± 0.10 b	0.72 ± 0.07 cd	1.56 ± 0.15 a	0.95 ± 0.09 b	0.89 ± 0.08 bc	0.62 ± 0.06 d
Se	0.04 ± 0.01 e	0.23 ± 0.02 c	0.20 ± 0.02 c	0.65 ± 0.06 a	0.03 ± 0.01 e	0.21 ± 0.02 c	0.12 ± 0.01 d	0.37 ± 0.03 b
Zn	13.8 ± 1.3 b	19.2 ± 1.9 a	17.7 ± 1.8 a	17.7 ± 1.7 a	13.8 ± 1.3 b	13.9 ± 1.3 b	16.0 ± 1.6 ab	17.3 ± 1.7 a
Al	143 ± 14 b	81.5 ± 8.0 d	178 ± 17.1 a	133 ± 13 b	212 ± 21 a	51.2 ± 5.0 e	105 ± 10 c	57.7 ± 5.6 e
As	0.046 ± 0.005 bc	0.066 ± 0.006 a	0.048 ± 0.05 bc	0.043 ± 0.04 c	0.051 ± 0.005 b	0.025 ± 0.002 b	0.057 ± 0.006 ab	0.037 ± 0.003 c
Cd	0.057 ± 0.005 d	0.095 ± 0.01 ab	0.063 ± 0.006 d	0.11 ± 0.01 a	0.078 ± 0.007 c	0.092 ± 0.009 ab	0.11 ± 0.01 a	0.086 ± 0.008 bc
Cr	0.10 ± 0.01 d	0.15 ± 0.01 b	0.12 ± 0.01 cd	0.15 ± 0.01 b	0.15 ± 0.01 b	0.19 ± 0.02 a	0.12 ± 0.01 cd	0.14 ± 0.01 bc
Ni	0.13 ± 0.01 ef	0.23 ± 0.02 a	0.14 ± 0.01 de	0.18 ± 0.01 b	0.17 ± 0.01 bc	0.12 ± 0.01 f	0.14 ± 0.01 de	0.15 ± 0.01 cd
Pb	1.12 ± 0.11 b	0.75 ± 0.07 c	0.15 ± 0.01 f	0.73 ± 0.07 c	0.20 ± 0.02 e	1.82 ± 0.2 a	0.10 ± 0.01 h	0.30 ± 0.03 d
Sr	20.8 ± 0.2 b	46.6 ± 4.4 a	10.2 ± 1.0 c	20.2 ± 2.0 b	19.2 ± 1.9 b	48.4 ± 4.8 a	9.43 ± 0.92 c	20.9 ± 2.0 b
V	0.073 ± 0.007 d	0.11 ± 0.01 ac	0.086 ± 0.008 cd	0.07 ± 0.01 b	0.13 ± 0.01 a	0.071 ± 0.007 d	0.099 ± 0.01 bc	0.080 ± 0.008 d

Along each line, values with the same letters do not differ significantly according to Duncan's test at  $p < 0.05$ .

Despite the significant varietal differences in the Ca/Sr ratio, the results show a more substantial Sr decrease due to seed space storage compared to Ca (Table 6, Figure 5).

The received data also indicated significantly decreased values of Ca/Na ratios both in the experimental fruit pulp (1.6 times in cv. Autumn rhapsody and 1.8 times in cv. Lotus) and fruit peel (1.7 and 2.9 times, respectively), which suggests the possibility of predicting plant protection decreases in conditions of salt stress, where Ca is known to mitigate the negative effect of Na<sup>+</sup> [57]. Further investigations are necessary to confirm this hypothesis in space tomatoes.

Information about the effect of seed spaceflight storage on the mineral composition of mature plants is rather scarce. Indeed, a significant decrease in most minerals was recorded in leafy vegetables grown from spaceflight storage seeds, except Se, whose concentration in space-treated plants was significantly higher than in the control ones [33]. The decrease of iron and copper levels in space-treated tomato fruits were also indicated in another work [49].

The present results confirm the phenomenon of Se level increases due to seed long-term spaceflight storage (Table 6, Figure 3). An improvement in Se accumulation was recorded both in fruit pulp and peel, reaching values of 4–5 and 1.8–2.8, respectively. Varietal differences were reflected in the intensity of Se peel/pulp accumulation. While Autumn rhapsody exhibited a predominant increase in peel Se levels, cv. Lotus demonstrated the opposite trend, with a significantly more intense pulp Se increase compared to the peel data (Figure 4). The beneficial effect of Se in improving plant tolerance to abiotic stresses is largely connected with its antioxidant properties [58] and its ability to regulate the biosynthesis of phytohormones [59,60], thus affecting all biochemical reactions of the plant organism.

Among the elements tested, Fe demonstrated significant peel/pulp redistribution in tomato fruits, with a significant concentration increase in the pulp tissues and a corresponding decrease in the peel of space-treated plants (Figure 4, Table 6). Iron participates in a variety of metabolic processes, affecting biomass production and photosynthesis, improving mitochondrial respiration and protein biosynthesis, and providing high activity of antioxidant enzymes and membrane protection [54,56,61]. The significant decrease in Fe accumulation in space-treated tomato fruit described previously in the whole tomato fruit [49] was not confirmed in the present study for the cvs. Lotus and Autumn rhapsody. In this respect, cv. Autumn rhapsody demonstrated more substantial changes compared to cv. Lotus. To date, no data exist on the significance of Fe peel/pulp distribution in tomato adaptability, which indicates the necessity for further investigation.

Molybdenum is another essential element significantly affected by tomato seed space storage (Figure 4). Due to the presence of Mo in many enzymes participating in the redox reactions of plants and affecting nitrogen metabolism [62], the significant decrease in its concentration in tomato pulp and peel due to seed space storage indicates the existence of another stress factor influencing tomato plants grown from space-treated seeds.

Among the elements tested, Pb is one of the most interesting. Indeed, both cultivars showed a 2–7.5 times decrease in pulp Pb levels, while the concentration of this element in peel either increased (1.6 times in cv. Lotus) or did not change (cv. Autumn rhapsody). Interestingly, among other elements, Pb demonstrated in space-treated tomato fruits the largest increase in peel/pulp ratio: 7.27 times in cv. Autumn rhapsody (from 0.67 to 4.87) and 3.33 (from 9.1 to 30.3) in Lotus (Figure 4). Previous investigations demonstrated the possibility of high Pb accumulation in tomato fruits in conditions of high anthropogenic uptake [63] and the high Pb absorption capacity of peel, emphasizing the prospects of peel utilization in wastewater purification [64]. However, further investigations are needed to check the effectiveness of the latter phenomenon to decrease the risk of tomato fruit Pb accumulation in plants grown in contaminated soils or in conditions of wastewater utilization for irrigation.

Previously, we indicated the significance of bordering tissues and their chemical and mineral composition in plant protection against oxidative stresses. Indeed, the elemental

peel/pulp ratio is a good indicator of plant adaptability to stress factors [50]. In this respect, the predominance of Ca and Se accumulation in the peel of the control and space-treated tomato fruits may reflect the importance of these elements for plant development (Figure 6). This statement may also be applied to the predominant accumulation of Pb in the fruit peel of space-treated plants.

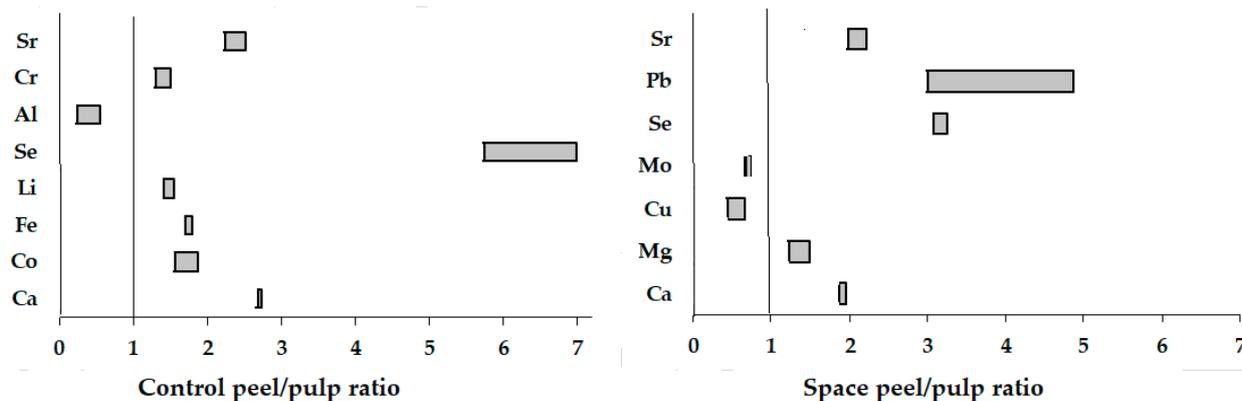


Figure 6. Differences in peel/pulp ratio between control and space-treated tomato fruits.

The analysis of the relationship between the elements and biochemical parameters demonstrated significant positive correlations between Ca-Sr, Ca-Co, Sr-Co, Sr-Cr, Se-Dry matter and Cr-carotenoids (Table 7). Significant negative correlations were recorded between Mo-dry matter, Se-K and Se-Mo. A positive correlation between Ca and Co was also recorded previously for other plant species grown in Co-contaminated soils [65].

Table 7. Correlations between the parameters measured in control and space tomato fruits.

	Ca	Fe	Mo	Se	Sr	Co	Cr	Carotenoids	Dry Matter
K	-0.080	0.149	0.454	-0.875 a	-0.022	-0.124	-0.239	0.417	-0.696 d
	Ca	0.717 d	0.096	0.133	0.983 a	0.803 b	0.420	0.647	0.376
		Fe	-0.104	0.076	0.664	0.756 c	0.189	0.337	0.403
			Mo	-0.680 d	0.231	0.180	-0.544	-0.437	-0.693 d
				Se	0.050	0.315	0.359	0.271	0.843 a
					Sr	0.794 c	0.381 d	0.584	0.299
						Co	-0.023	0.146	0.366
							Cr	0.591	0.482
							Carotenoids		0.550

p < (a) 0.001; (b) 0.005; (c) 0.01; (d) 0.05.

Contrary to the peculiarities in Ca accumulation, with a typical decrease of this element both in the peel and pulp of space-treated fruit, the potassium content in tomato pulp was rather stable compared to the peel, with a significant K level decrease in plants grown from space-treated seeds. According to literature data, Se may either stimulate or inhibit the accumulation of K in plants [66], though in most cases, no correlations between these elements have been revealed [67]. The present results indicate a significant negative correlation between the mentioned parameters. The detected phenomenon does not significantly affect the nutritional value of tomatoes considering the predominant utilization of pulp in nutrition, but it should be taken into account in the breeding process.

We also recorded a negative correlation between the peel/pulp levels of Se and Mo (Table 7). Selenium is known to affect Mo metabolism via participation as a cofactor in certain molybdoenzymes and the stimulation of Mo utilization by plants [68]. Molybdenum (Mo) is regarded as an essential trace element for plants that promotes plant growth and relieves abiotic stresses [69]. In 2012, Zhang et al. [70] revealed an antagonistic relationship between Mo and Se in the Chinese cabbage. The present results indicate that space seed stress causes a Se-Mo imbalance via a significant Mo decrease and a Se increase in fruit

tissues. This phenomenon is connected with the antioxidant properties of Se as well as its ability to improve photosynthesis and cell permeability in plants under different forms of biotic and abiotic stresses [58]. These results suggest that Se and Mo are closely related to dry matter content (Table 1) and possibly with dietary fiber accumulation. Indeed, space-treated plants demonstrated not only high levels of Se in tomato fruit but also increased dietary fiber (Table 1).

#### 4. Conclusions

The results of the present research revealed a complex response of mature tomato plants to seed anomalous space stress, including the production of secondary metabolites and dietary fiber as well as the intensive peel/pulp redistribution of carotenoids, macro- and micro-elements. Anomalous changes in Ca, Se, Pb and Fe peel/pulp concentration indicate the active participation of minerals in plant defense. Further investigations with a higher number of tomato cultivars are desirable to clarify the revealed phenomena.

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