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Variability in Chemical Profile and Bioactivities of the Flesh of Greek Pumpkin Landraces

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Abstract: The aim of this study was to evaluate the chemical profile of the flesh and yield parameters of Greek pumpkin genotypes, including nine local landraces and two commercially available cultivars, focusing on valorizing the genetic pool of *Cucurbita* sp. with high added value products. Yield parameters (mean fruit weight and total fruit yield) recorded high variability with genotypes V8 and V2 showing the highest fruit yield. Moreover, genotype V11 was the most abundant in glucose and total sugars and scored the highest sweetness index suggesting good taste and promising marketing attributes. The highest antioxidant activity (OxHLIA assay) was assessed in the V8 genotype, while the V2 genotype showed the highest α-, β- and total tocopherols content. Oxalic acid was the main organic acid, followed by malic and citric acids, while organic acid composition varied among the tested genotypes. Moreover, the flesh extracts showed varied antimicrobial activity against several bacteria and fungi, while no toxicity against non-tumor cells was recorded. In conclusion, our results make evident the presence of high innate variability in terms of crop performance, chemical composition and bioactive properties not only between the different genotypes but also at the intra-populational level. This finding is of high importance for the valorization of the local genetic pool of *Cucurbita* species through the selection of elite genotypes with high yield and quality of fruit, contributing to the conservation of valuable genetic material and limitation of the risk of genetic erosion due to neglect of local landraces.

Keywords: *Cucurbita* sp.; antimicrobial activity; local landraces; bioactive compounds; antioxidant activity; cytotoxicity; fruit quality

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1. Introduction

Among cucurbits, *Cucurbita* is considered a genus with considerable diversity in terms of the plant growth habits, fruit morphology and disease resistance, as well as the nutritional and phytochemical profile of fruit, which is mainly attributed to its complex domestication trajectories [1]. The genus presents a pre-Holocene distribution, originally in the form of bitter fruits [2], while its domestication took place in northern South and Central America ca. 10,000 B.P. [3], being included among the earliest crop domestication records. Although the relationships between wild and domesticated species are not well elucidated [4], it is

known that domestication involved traits related to fruit quality, primarily focusing on the loss of bitterness and fruit size [1]. Indeed, among all plant species cultivated for their fruit, squashes and pumpkins produce the most profoundly sizable fruit in comparison to their non-domesticated wild forms [5].

Pumpkin is the fruit of *Cucurbita* species that belongs to the Cucurbitaceae family and it is traditionally consumed all over the world. Pumpkins belong to the genus *Cucurbita* L., which encompasses 9 species—*C. maxima*, *C. pepo*, *C. moschata*, *C. argyrosperma*, *C. digitata*, *C. ficifolia*, *C. foetidissima*, *C. okeechobeensis* and *C. palmata*—the first three being the most common around the world [6]. Although these three species have common requirements for optimum growth and development, due to their wide adaptation to soil and climatic conditions, they vary remarkably in fruit morphology (e.g., fruit size, shape, skin and flesh color and flesh structure), as well as in nutritional value [7]. Moreover, interspecies hybridization results in considerable heterogeneity at the intrapopulational level with a large number of subspecies and cultivars that vary in size, shapes, colours and taste [8]. The vernacular term of pumpkin is commonly used to describe the fruit of *Cucurbita pepo* L., *C. moschata* Duchesne ex Poir, *C. maxima* Duchesne, *C. ficifolia* Bouché and *C. argyrosperma* K.Koch species, while it is interchangeably used with squash or winter squash terms [9]. In addition to the pleasant flavour and versatility, the known nutritional value and functional properties of pumpkin have gathered significant interest in scientific studies and health-conscious consumers [10]. As global consumer trends increasingly lean towards healthier and sustainable food choices, the diverse nutraceutical properties of pumpkin fruit have earned it a place of importance in modern diets [11–13].

The flesh (pulp) is the most appreciated part of the fruit, which represents more than 70% of the whole fruit [14]; it is a good source of carbohydrates, dietary fibres (especially pectins) and proteins, while it contains significant amounts of fat and minerals, varying among species and genotypes [15–17]. In nineteen accessions of *Cucurbita* ssp evaluated by Nwofia et al. [17], the crude fibres varied from 0.55% to 1.04% in fresh weight (moisture from 78.46 to 91.97%). Considering an adequate intake of 25 g/day of fibre by ESFA [2], this fruit contributes to a balanced diet. The high mineral content of iron, potassium, phosphorus, magnesium, selenium, calcium and copper has been well documented so far in different pumpkin varieties [18,19]. In the pulp of *Cucurbita maxima*, 184.34 ± 1.24 mg/100 g dw of potassium and 53.67 ± 0.19 mg/100 g dw of iron was found [17], while the "Waïgoré dollugo" dish, which consists of boiled pumpkin (78.2% of pumpkin fruit and 21.8% of water), presented 2753.0 ± 3.2 mg/100 g dw of potassium and 4.3 ± 0.2 mg/100 g dw of iron [20], which adequate intake and average requirement is, according to EFSA (European Food Safety Authority), about 3500 and 6 mg/day, respectively [21]. In addition to macronutrients, pumpkin is also a rich source of essential micronutrients such as vitamins, antioxidant compounds and other phytochemicals. Several studies have shown that pumpkins are a notable source of vitamins A, C and E, which play crucial roles in supporting immune function and acting as potent antioxidants to combat oxidative stress, in addition to the presence of alkaloids, flavonoids, polyphenols, tannins, phytosterols and cucurbitacins [18,19,22,23]. Kaur et al. [19] also highlighted the great amounts of pro-vitamin A carotenoids in pumpkin and their importance in developing important antioxidant effects, reducing the risk of certain diseases including cancer. These compounds are known as antioxidant and antimicrobial agents for their capacity to neutralize harmful free radicals in the body, preventing oxidative damage in cells and tissues, and inhibiting the growth or killing of microorganisms, such as bacteria or fungi, combating infections or preventing their spread, respectively. Contents of 171.9 µg/g of carotenoids, 2–10 mg/100 g of vitamin C, and 9–10 mg/100 g of vitamin E are reported in pumpkin flesh, which also contributes to a healthy diet (average requirement of 9 g/day of carotenoids with provitamin A activity, and 110 mg/day of vitamin C, of which adequate intake is 13 mg/day) [21].

Beyond its nutrients, pumpkins possess functional properties that are of particular interest to the food industry. Sharma et al. [24] recently reviewed the health-promoting potential of pumpkin in terms of antioxidant, anticancer, anti-inflammatory, anti-obesity,

anti-diabetes, antimicrobial and other bioactivities, as well as its application in the food industry, as a cooked, powdered or pureed ingredient in different food products. Their attractive taste and vibrant color make them suitable for the formulation of health-promoting food products, including soups and snacks [25–27].

Pumpkin cultivation is widely practiced across the globe, being generally a warm-season crop that thrives in well-drained, nutrient-rich soils with good sunlight exposure. Its cultivation and appreciation may vary according to the wide variety of pumpkin species and cross-pollinations between cultivated species and/or wide relatives, which results in high genetic diversity and inherent polymorphism [28–30]. According to data from FAO (Food and Agriculture Organization of the United Nations), in 2021 the pumpkin production (the aggregate of pumpkins, squash and gourds) was approximately 24 million tonnes harvested from 1.5 million hectares, while the world's leading producers were China (approximately 7.4 million tonnes), followed by Ukraine (approximately 1.43 million tonnes), Russian Federation (approximately 1.2 million tonnes) and USA (approximately 1.1 million tonnes) [31]. Within the European Union, pumpkin is cultivated on 84,270 hectares with a total production of 2.7 million tonnes, while the main producers are Spain (789,780 tonnes), Italy (601,660 tonnes), France (410,360 tonnes) and Poland (316,300 tonnes) [31]. In Greece, although pumpkin does not occupy many hectares of agricultural land (2170 hectares with annual production of 61,120 tonnes [31]), there is wide genetic diversity with several local landraces being cultivated all through the country. The annual world production over the last ten years fluctuated between 21.9 and 26.6 million tonnes, whereas the harvested area showed decreasing trends dropping from almost 2.0 million hectares in 2015 to 1.5 million hectares in 2021 [31]. On the other hand, in Greece, there has been a great increase in harvested area and annual production over the last ten years, since harvests have increased from 536 ha to 2170 ha and annual production from 673 tonnes to 61120 tonnes [31]. Fruit yield may also vary with values that range between 37 to 98 tonnes/ha [32], 25.2 to 55.7 tonnes/ha [33] or 7.3 to 41.8 tonnes/ha [34], depending on fertilization rate and irrigation level.

However, despite the rising pumpkin production during the last few years, the cultivation of this valuable crop faces mounting challenges due to the intensifying impacts of abiotic stress factors. The climate changes observed in the world have changed patterns of temperature and rainfall, thus increasing the occurrence of extreme weather events, affecting the yield of several crop species, including pumpkin [35,36]. Moreover, the intense exploitation of the soil without proper management also has negative effects through land degradation, drought and salinization of irrigation water and agricultural land [37]. These effects have challenged the academic community in search of alternatives that aim to provide high-yield and quality crops, through the understanding of the effects of these adversities on traditional crops as well in the search and promotion of new, more promising crops [37–39].

Considering the high fruit polymorphism, more and more studies of the characterization in terms of composition and functional properties of different pumpkin genotypes are being disseminated [40–42]. However, the genetic pool of Greek pumpkin germplasm is not adequately characterized so far, especially when considering the high genetic variability of the species which could be a valuable tool for the adaptation of modern horticulture in the changing climate conditions. Therefore, the present study aimed to provide useful information regarding the biochemical and nutritional diversity in pumpkin genotypes cultivated in Greece. For this purpose, eleven local landraces and commercially available genotypes were cultivated under the same conditions and nutritional and chemical profiles, and the functional properties of fruit flesh were investigated.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The experiment was conducted at the experimental farm of the University of Thessaly, Greece during the growing period of 2021 (e.g., May–November, 2021). Seeds from eleven

genotypes (V1–V11) were directly sown in the soil on 19 May 2021. The description of genotypes is presented in Table 1. The genotypes included 9 local landraces collected from the respective regions, while two commercial genotypes were also included (e.g., F1 Fytro FS-243, Fytro seeds S.A., Athens, Greece and Big Max, Geniki Fytotechniki S.A., Athens, Greece). Seeds were sown in rows at distances of 1.6 m within each row and 2.0 m between the rows. Each row consisted of 32 plants, with 3 rows for each genotype being used (96 plants for each genotype in total), while rows were laid out according to the completely randomized design (n = 3). Before sowing, a complex fertilizer (18-9-18, N-P-K; Complex Haifa Turbo K + Mg + S + Fe and Zn; Haifa Group, Israel) was applied as a base dressing at a rate of 250 kg/ha and was evenly distributed with a rototiller. Irrigation was applied via a drip irrigation system (one emitter per plant with a water supply of 6 L/h) at regular intervals based on the environmental conditions during the experimental period. Irrigation was scheduled based on the recordings of soil moisture content taken at regular intervals aiming to retain 100% of field capacity. Soil moisture content was recorded with PR2 Profile Probe (Delta T PR2/4 +HH2; Delta-T devices Ltd., Burwell, UK) using access tubes 40 cm long, while measurements were taken at soil depths of 10, 20, 30 and 40 cm. Access tubes were established with Del-ta-T augering and extraction kit (PR-ASK1-S, Delta-T Devices Ltd., Cambridge, UK), using one access tube per genotype (15 tubes in total).

Table 1. Genotype name of pumpkin cultivated in Greece according to the fruit type.

Pumpkin Genotypes	Sample Code	Harvest Date
Fytro FS-243	V1	26 October–19 November 2021
Landrace from the region of Trikala (Turbinate)	V2 T	25 August–19 October 2021
Landrace from the region of Trikala (Cylindrical)	V2 C	25 August–19 October 2021
Big Max	V3	25 August–19 October 2021
Local landrace "Nychaki" (Cylindrical)	V4 C	23 September 2021
Local landrace "Nychaki" (Round)	V4 R	23 September 2021
Local landrace "Leuka Melitis" (Flattened)	V5 F	5 October–19 November 2021
Local landrace "Leuka Melitis" (Round)	V5 R	5 October–19 November 2021
Local landrace from the region of Lakonia	V6	25 August 2021
Local landrace from the region of Lakonia (Pyriform)	V7 P	24 September–19 November 2021
Local landrace from the region of Lakonia (Flattened)	V7 F	24 September–19 November 2021
Local landrace from the region of Lakonia	V8	22 November 2021
Local landrace "Makedonika prasina" (Cylindrical)	V9 C	22 November 2021
Local landrace "Makedonika prasina" (Round)	V9 R	22 November 2021
Local landrace from the region of Laconia	V10	22 November 2021
Local landrace ("Voutirato")	V11	22 November 2021

After crop establishment, fertilization was applied via the drip irrigation system (fertigation). In particular, on 16 July 2023 plants received ammonium nitrate (34.5% nitrogen; 40 kg/ha), Solusop (0-0-52, N-P-K; Agri.fe.m Ltd., Athens, Greece; 20 kg/ha) and Disper bloom (Disper Bloom; Agrofarm S.A., Athens, Greece; 1.5 kg/ha). On 26 July 2023, plants were foliar sprayed with Disper Bloom (100 g/100 L of water) and Root and Leaf (20-202-20, N-P-K +TE; 200 g/100 L of water). On 28 July 2023, plants were fertigated with 40 kg of ammonium nitrate and 20 kg of Solusop. On 12 August 2023, plants were treated with 25 kg of ammonium nitrate, 25 kg/ha of Solusop and 75 kg/ha of Mannitol 3 GR (Agrofarm S.A., Greece). Weeds were controlled chemically with pre-emergence herbicides, as well as manually with a hand hoe during the growing period and until crop establishment. Pests and pathogens were chemically controlled based on the recommended practices of the crop.

Harvest took place when fruit reached maturity from 25 August 2021 to 22 November 2021, depending on the genotype. Due to polymorphism that some genotypes showed (e.g., V2, V4, V5, V7 and V9), two types of fruit that differed in their form were harvested, as described in Table 1. After harvest, fruit yield was calculated as the sum of the weight of all the fruit from each cultivar extrapolated to the harvested area of one hectare.

2.2. Samples Preparation

After collection, fruits were weighted and 15 randomly selected fruits from each genotype and fruit form were cut in half; after removing seeds and pericarp flesh samples were collected, put in an air-sealed bag and stored under freezing conditions (−19 °C). Then, the frozen samples were lyophilized, ground to powder and stored at deep-freezing conditions until further analysis. To obtain the flesh samples, the fruits were peeled and we removed the seeds and fibrous strands. The fleshes were then lyophilized (Sublimator model EKS, manufactured by Christian Zirbus Co. in Osterode am Harz, Germany), crushed (~20 mesh) and stored until subsequent analysis. The applied lyophilization program for drying the plant biomass was the following:

Step 1: −35 °C for 2 h at atmospheric pressure (1000 mbar);
Step 2: from −35 °C to −20 °C in 6 h under vacuum 0.150 mbar;
Step 3: from −20 °C to 0 °C in 12 h under vacuum 0.150 mbar;
Step 4: from 0 °C to 10 °C in 12 h under vacuum 0150 mbar;
Step 5: from 10 °C to 25 °C in 12 h under vacuum 0.150 mbar.

For the bioactivities, the hydroethanolic (80 ethanol: 20 water) flesh extracts were obtained by maceration for 1 h, twice, with a solid/liquid ratio of 1 g/30 mL, following the standard methodology of the group, previously described in Leichtweis et al. [42]. The extracts were then lyophilized as described above and stored until subsequent analysis.

2.3. Nutritional Characterization

The AOAC procedures [43] were followed to obtain the nutritional composition of flesh samples, including the total fat, crude protein, carbohydrates, dietary fibre content, ash and total energy. The fat content was extracted with petroleum ether in a Soxhlet apparatus; for the crude protein, we used the macro-Kjeldahl method (equipment Pro-Nitro A, Selecta, Barcelona, Spain) and a nitrogen-to-protein conversion factor of 6.25; the total dietary fibre content was determined using a combination of enzymatic and gravimetric method; and, the ash content was assessed by incinerating the samples at 550 ± 10 °C.

Total carbohydrates were calculated by difference, and the total energy was calculated using the following equation:

$$\text{energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrates}) + 9 \times (\text{g fat}) + 2 \times (\text{g total dietary fibre})$$

The results were expressed in g/100 dry weight (dw).

2.4. HPLC Assays

Tocopherol content was determined by the HPLC system described above coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA), following the methodology described by Pereira et al. [44]. The detector was configured to excite at 290 nm and measure emission at 330 nm and the chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 °C. Tocol was used as an internal standard (IS) and the compound isoforms (α-, β-, γ-, and δ) were identified by comparisons with authentic standards. The quantities were obtained using the following calibrations curves: α-tocopherol, $y = 1.295x$, $R^2 = 0.991$, LOD = 18.06 ng/mL, LOQ = 60.20 ng/mL); β-tocopherol, $y = 0.396x$, $R^2 = 0.992$, LOD = 25.82 ng/mL, LOQ = 86.07 ng/mL); and γ-tocopherol, $y = 0.567x$, $R^2 = 0.991$, LOD = 14.79 ng/mL, LOQ = 49.32 ng/mL. The results were expressed in mg/100 g dw.

The organic acid characterization was performed by ultra-fast liquid chromatography (UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a photodiode array detector (PDA), using 215 and 245 nm (for ascorbic acid) as preferred wavelengths [45]. Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C18 column (5 µm, 250 mm × 4.6 mm i.d.) thermostatted at 35 °C. Identification and quantification were achieved by comparison with commercial standards. The cali-

bration curves were as follows: oxalic acid, $y = 8 \times 10^6x + 331{,}789$; $R^2 = 0.9912$; malic acid, $y = 942{,}562x + 38{,}506$, $R^2 = 0.9987$; ascorbic acid, $y = 5 \times 10^7x + 449{,}262$; $R^2 = 0.9813$; shikimic acid, $y = 8 \times 10^7x + 567{,}119$; $R^2 = 0.9903$; citric acid, $y = 968{,}367x - 12{,}295$; $R^2 = 0.9974$; and fumaric acid, $y = 9 \times 10^7x - 100{,}894$, $R^2 = 0.9986$. The results were expressed in g/100 g dw, except for fumaric acid (mg/100g dw).

The analysis of free sugars was carried out using HPLC coupled to a refractive index detector, more specifically an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA) and an RI detector (Knauer Smartline 2300), following a previously described method [46]. A Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 °C (7971 R Grace oven) was used. For this analysis, melezitose was used as IS for the quantification method and the compounds were identified by chromatographic comparisons with authentic standards D(-)-fructose, D(+)-sucrose, D(+)-glucose, D(+)-trehalose and D(+)-raffinose pentahydrate. The results were expressed in g/100 g dw. The content of individual free sugars was used to calculate the sweetness index (SI) of fruit through the formula [45]:

$$SI = \textit{fructose content} \times 157.5 + \textit{glucose content} \times 67.5 + \textit{sucrose content} \times 100$$

Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID), a DANI model GC 1000 instrument and a Macherey–Nagel column (30 m × 0.32 mm ID × 0.25 µm df), following the methodology published by Pereira et al. [44]. The identification and quantification were performed by comparing the retention times of peaks detected to the ones of FAME peaks of commercial standards (FAME reference standard mixture, standard 47885-U, Sigma-Aldrich, St. Louis, MO, USA). The contents were presented in relative percentages.

2.5. Bioactivities

The antioxidant capacity of the flesh extracts was evaluated using two in vitro cell-based assays. The first screening was obtained using porcine (*Sus scrofa*) brain homogenates, to observe the ability of the extract to inhibit lipid peroxidation by the thiobarbituric acid reactive substances inhibition (TBARS) assay, as described by Pereira et al. [47], and the second one using a red cell suspension of healthy sheep blood, investigating the extracts capacity to inhibit the oxidative hemolysis (OxHLIA), as described by Backes et al. [48]. The results were expressed in IC_{50} values (µg/mL), meaning the extract concentration that inhibits 50% of oxidation and that delays 50% of erythrocyte hemolysis for 60 min, respectively. Trolox was used as a positive control in both assays.

For the antimicrobial activity, the extracts were tested against eight bacteria and two fungi related to food contamination, more specifically, *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19111), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 49741), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica subsp* (ATCC 13076), *Yersinia enterocolitica* (ATCC 8610), *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404). The microdilution and *p*-iodonitrotetrazolium chloride (INT) methods were used [49]. Streptomycin and ampicillin were used as positive controls for bacteria and ketoconazole and bifonazole for fungi. The results were expressed in mg/mL.

Additionally, the cytotoxic effects were assessed in a primary culture of non-tumor porcine liver cells (PLP2) derived from porcine liver obtained from a local slaughterhouse. The evaluation was conducted using the Sulforhodamine B (SRB) colorimetric assay [50]. The IC_{50} values (inhibiting 50% of the net cell growth) for extract concentration, presented in µg/mL, were compared to the positive control, Ellipticine.

2.6. Statistical Analysis

For chemical analysis, fruit samples were analysed in triplicate, and the results were expressed as mean ± standard deviation. For comparing two groups of data, a Student's *t*-test was used, while for three or more groups, the one-way analysis of variance (ANOVA) was applied. Prior to analysis, normal distribution and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. For homoscedastic data with $p > 0.05$, Tukey's honestly significant difference (HSD) test was employed and, for heteroscedastic data, Tamhane's T2 multiple comparison test was used. All analyses were conducted at a 5% significance level using IBM SPSS Statistics software (Version 22.0, IBM Corp, Armonk, NY, USA).

In addition, principal component analysis (PCA) was performed to identify the contribution of each variable to the total genetic diversity and classify the studied pumpkin landraces based on their chemical composition and antioxidant activities of fruit flesh. All statistical analyses were carried out using the StatGraphics Centurion-XVII statistical package (StatPoint Technologies Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Crop Performance

The results of crop performance (e.g., total fresh yield of fruit, number of fruit per plant and mean fruit weight) are presented in Table 2, where significant differences among the studied genotypes were recorded. In particular, the highest fruit yield was recorded for the V8 genotype (34,069 kg/ha), followed by V2 and V4 genotypes (30,695 kg/ha and 27,997 kg/ha, respectively), whereas the lowest overall yield was recorded for V5 genotype with a production of only 7618 kg/ha. Moreover, significant differences were recorded in the number of fruit per plant where V1 recorded the highest mean value (6.1 fruit per plant) with an average fruit weight of 1.0 kg per fruit. On the other hand, the lowest number of fruits per plant was recorded in V5 with an average weight of 3.6 kg. Significant differences were also recorded in mean fruit weight where V3 recorded the highest mean value (8.1 kg) followed by V2, V8 and V11 (6.3, 6.1 and 5.6 kg, respectively). These results indicate that the high number of fruit is accompanied by smaller fruit weight, as in the case of V2, V3, V8 and V11. The high variability recorded in the tested genotypes is mainly attributed to the differences in their genetic background, especially regarding the local landraces which are acclimatized in specific edaphoclimatic conditions through the cultivation in specific regions with particular microclimates. According to the literature, genotypic diversity in *Cucurbita* species is expected to result in differences in crop performance [51] and quality features [52,53], while differences observed in multi-site experiments can justify the acclimatization of pumpkin genotypes to specific environmental conditions and agronomic practices [54,55]. Moreover, the differences in plant morphology and phenology (e.g., vine length, fruit maturation, number of branches, etc.) may influence crop performance [56], especially when plants are grown under the same conditions and agronomic practices as those in our study.

Table 2. Crop performance (total fruit weight in kg/ha) of the tested genotypes.

Genotype	Total Fruit Weight (Tonnes/ha)	Numbers of Fruit/Plant	Mean Fruit Weight (kg)
V1	15.5 ± 6.6^{g}	6.1 ± 0.5^{a}	1.0 ± 0.1^{g}
V2	30.7 ± 5.6^{b}	0.9 ± 0.1^{e}	6.3 ± 0.6^{b}
V3	19.6 ± 7.8^{e}	$1.0 \pm 0.1^{d,e}$	8.1 ± 0.7^{a}
V4	28.0 ± 3.6^{c}	3.9 ± 0.4^{b}	2.9 ± 0.3^{f}

Table 2. *Cont.*

Genotype	Total Fruit Weight (Tonnes/ha)	Numbers of Fruit/Plant	Mean Fruit Weight (kg)
V5	7.6 ± 2.4^{j}	0.4 ± 0.1^{g}	3.6 ± 0.4^{e}
V6	10.9 ± 4.5^{i}	1.2 ± 0.2^{d}	3.8 ± 0.4^{e}
V7	24.0 ± 9.6^{d}	2.9 ± 0.3^{c}	1.7 ± 0.2^{g}
V8	34.1 ± 5.4^{a}	1.1 ± 0.1^{d}	6.1 ± 0.5^{b}
V9	16.7 ± 2.2^{f}	0.7 ± 0.1^{f}	4.8 ± 0.4^{d}
V10	27.7 ± 6.3^{c}	4.1 ± 0.3^{b}	2.7 ± 0.3^{f}
V11	11.8 ± 3.9^{h}	$0.8 \pm 0.1^{e,f}$	5.6 ± 0.5^{c}

Means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$.

3.2. Nutritional Profile

The proximate composition of the studied genotypes is presented in Table 3, including two samples of the same genotype that varied in fruit morphology as indicated in Table 1 (e.g., V2, V4, V5, V7 and V9). A variable content was recorded with significant differences not only among the studied genotypes but also between the different fruit types of the same genotype. In particular, fat content ranged between 0.38 and 1.17 g/100 g dw with the highest content being recorded in V2 T, V6 and V11, whereas the lowest content was found in the flesh of V4 R genotype. Protein and ash content ranged between 8.0–21.4 g/100 g dw and 3.5–10.95 g/100 g dw, respectively, with V9 C genotype having the highest content (21.4 g/100 g and 10.95 g/100 g dw, respectively), whereas the lowest overall content of protein and ash was recorded in V4 C and V5 R genotypes (8.6 g/100 g dw and 3.5 g/100 g dw), respectively. V1 showed the highest energy content (360.1 Kcal/100 g dw), V2 had the highest content in carbohydrates (72.2 g/100 g dw), while V9 C was the richest in fibres (27.4 g/100 g dw). The detected amounts of macronutrients were within the same range of literature reports regarding the flesh of *Cucurbita* species [40,57–59], although protein and fat content was higher and lower than other studies, respectively [60]. The high variability in macronutrients presented in our study indicates the genetic inter- and intra-population diversity of the studied germplasm, which could be due to breeding through the farmer's seed system which facilitates heterogeneity, especially in the case of local landraces as in our study and can justify the recorded differences with other reports in the literature [61,62].

The main free sugars detected in the flesh of the studied genotypes were glucose, sucrose and fructose, followed by trehalose and raffinose (Table 4), while typical chromatographs of samples rich in fructose and sucrose are presented in Figure 1. The highest amounts of the main sugars were recorded in V11 (26.9 g/100 g dw) and in both fruit types of V2 (9.7 and 10.0 g/100 g dw for T and C fruit types, respectively) and V7 (14.0 g/100 g dw for both fruit types), while trehalose was the highest in V2 C (0.67 g/100 g dw) and raffinose in V2 T, V6 and V8 genotypes (and 0.29, 0.27 and 0.25 g/100 g dw, respectively). The highest total free sugar content was recorded for the local landrace V11 due to the increased content of glucose. Moreover, the same genotype (V11) presented the highest sweetness index (3367) due to the overall composition of individual and total free sugars which indicates a sweeter taste and is highly associated with better acceptability from consumers [63]. Similarly to our study, Seroczyńska et al. [64] evaluated twelve forms of *C. maxima* fruit and identified sucrose, fructose and glucose in variable amounts depending on the genotype while they detected mannose in specific genotypes. The same sugars were detected by Dhenge et al. [65] in *C. moschata* fruit although a high fraction of total free sugars was not tentatively identified. Moreover, Kostecka-Gugała et al. [66] highlighted the intra-species variability between different cultivars of *C. pepo*, *C. maxima*, C. *moschata* and *C. ficifolia* in terms of soluble sugar content which explains the differences recorded in our study, while similar results were reported for indigenous and hybrid varieties of (*Cucurbita maxima* Linn) by Amin et al. [59].

Table 3. Proximate composition of the flesh of the tested pumpkin genotypes on a dry weight (dw) basis.

Genotype	Fat (g/100 g dw)	Protein (g/100 g dw dw)	Ash (g/100 g dw)	Energy (Kcal/100 g dw)	Carbohydrates (g/100 g dw)	Fibres (g/100 g dw)
V1	0.83 ± 0.04^{c}	$12.6 \pm 0.6^{g,h}$	4.511 ± 0.009^{l}	360.1 ± 0.4^{a}	69.1 ± 0.7^{b}	13.0 ± 0.1^{j}
V2 T	1.12 ± 0.06^{a}	$13.7 \pm 0.4^{d,e}$	6.07 ± 0.04^{j}	332.8 ± 0.3^{h}	54.9 ± 0.7^{h}	24.27 ± 0.02^{c}
V2 C	0.645 ± 0.002^{e}	$12.9 \pm 0.1^{f,g}$	7.1 ± 0.1^{h}	334.6 ± 1.2^{g}	$59.2 \pm 0.8^{f,g}$	20.2 ± 0.8^{f}
V3	0.45 ± 0.02^{h}	18.07 ± 0.03^{c}	7.7 ± 0.2^{e}	321.85 ± 0.02^{j}	49.0 ± 0.2^{j}	24.7 ± 0.5^{c}
V4 C	$0.64 \pm 0.03^{e,f}$	8.6 ± 0.1^{k}	$7.1 \pm 0.1^{g,h}$	332.5 ± 0.1^{h}	62.6 ± 0.2^{e}	$21.1 \pm 0.2^{e,f}$
V4 R	0.38 ± 0.02^{h}	17.46 ± 0.04^{c}	9.08 ± 0.07^{d}	321.7 ± 0.6^{j}	51.1 ± 0.3^{i}	$21.9 \pm 0.4^{d,e}$
V5 F	$0.58 \pm 0.03^{f,g}$	12.61 ± 0.05^{g}	5.126 ± 0.006^{k}	353.70 ± 0.07^{b}	67.3 ± 0.1^{c}	14.34 ± 0.04^{i}
V5 R	0.55 ± 0.03^{g}	9.9 ± 0.3^{j}	3.5 ± 0.1^{m}	361.0 ± 0.1^{a}	72.2 ± 0.6^{a}	$13.8 \pm 0.4^{i,j}$
V6	1.17 ± 0.02^{a}	$13.4 \pm 0.3^{e,f}$	6.98 ± 0.05^{h}	341.5 ± 0.8^{e}	60.27 ± 0.03^{f}	18.2 ± 0.3^{g}
V7 P	0.92 ± 0.04^{b}	14.1 ± 0.1^{d}	7.49 ± 0.03^{f}	343.6 ± 0.6^{d}	61.9 ± 0.6^{e}	15.5 ± 0.5^{h}
V7 F	$0.70 \pm 0.03^{d,e}$	$11.9 \pm 0.1^{h,i}$	7.27 ± 0.06^{g}	348.76 ± 0.05^{c}	67.26 ± 0.6^{c}	12.8 ± 0.2^{j}
V8	$0.76 \pm 0.03^{c,d}$	11.3 ± 0.4^{i}	7.29 ± 0.02^{g}	329.4 ± 1.6^{i}	58.0 ± 1.3^{g}	22.7 ± 0.9^{d}
V9 C	0.73 ± 0.03^{d}	21.4 ± 0.6^{a}	10.95 ± 0.05^{a}	315.05 ± 0.96^{k}	44.5 ± 0.2^{k}	22.4 ± 0.5^{d}
V9 R	0.65 ± 0.03^{e}	20.3 ± 0.3^{b}	10.19 ± 0.07^{b}	307.7 ± 1.5^{l}	41.4 ± 0.4^{l}	27.4 ± 0.8^{a}
V10	0.73 ± 0.03^{d}	9.9 ± 0.2^{j}	9.3 ± 0.1^{c}	313.8 ± 0.1^{k}	53.7 ± 0.2^{h}	26.3 ± 0.1^{b}
V11	1.12 ± 0.02^{a}	8.0 ± 0.3^{k}	6.71 ± 0.04^{i}	338.52 ± 0.34^{f}	64.0 ± 0.4^{d}	20.1 ± 0.2^{f}

Means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$; T: turbinate; C: cylindrical; R: round; F: flattened.

Table 4. Free sugar content of the flesh of the tested pumpkin genotypes (g/100 g dry weight (dw)).

Genotype	Fructose	Glucose	Sucrose	Trehalose	Raffinose	Total
V1	2.42 ± 0.03^{i}	2.50 ± 0.08^{j}	12.2 ± 0.4^{b}	$0.46 \pm 0.03^{c,d,e}$	$0.0579 \pm 0.0003^{h,i}$	17.7 ± 0.5^{g}
V2 T	9.7 ± 0.9^{a}	$21.5 \pm 0.3^{b,c}$	1.4 ± 0.2^{i}	$0.45 \pm 0.02^{c,d,e}$	0.29 ± 0.03^{a}	33 ± 1^{b}
V2 C	10.0 ± 0.3^{a}	22 ± 1^{b}	0.9 ± 0.2^{i}	0.67 ± 0.02^{a}	$0.09 \pm 0.02^{g,h,i}$	34 ± 1^{b}
V3	6.57 ± 0.02^{e}	9.6 ± 0.4^{g}	2.6 ± 0.3^{g}	0.23 ± 0.02^{i}	$0.23 \pm 0.03^{b,c,d}$	$19.2 \pm 0.8^{f,g}$
V4 C	$7.854 \pm 0.001^{c,d}$	18.6 ± 0.8^{e}	6.4 ± 0.7^{d}	$0.51 \pm 0.06^{b,c}$	$0.13 \pm 0.04^{f,g}$	33 ± 2^{b}
V4 R	6.6 ± 0.3^{e}	10.2 ± 0.6^{g}	3.47 ± 0.01^{f}	$0.47 \pm 0.03^{c,d}$	$0.24 \pm 0.03^{b,c}$	$20.9 \pm 0.9^{e,f}$
V5 F	3.790 ± 0.002^{g}	4.8 ± 0.1^{h}	3.6 ± 0.2^{f}	$0.408 \pm 0.005^{d,e,f}$	$0.18 \pm 0.01^{d,e,f}$	12.8 ± 0.4^{h}
V5 R	2.55 ± 0.07^{i}	4.66 ± 0.05^{h}	4.91 ± 0.05^{e}	$0.46 \pm 0.01^{c,d,e}$	$0.06 \pm 0.05^{h,i}$	12.6 ± 0.1^{h}
V6	8.18 ± 0.04^{c}	$20.33 \pm 0.06^{c,d}$	1.12 ± 0.06^{i}	$0.50 \pm 0.03^{b,c}$	$0.27 \pm 0.02^{a,b}$	30.41 ± 0.09^{c}
V7 P	$2.8 \pm 0.2^{h,i}$	$3.1 \pm 0.2^{i,j}$	14.0 ± 0.5^{a}	0.56 ± 0.02^{b}	0.05 ± 0.01^{i}	20.4 ± 0.8^{f}
V7 F	$3.3 \pm 0.2^{g,h}$	4.7 ± 0.2^{h}	14.0 ± 0.7^{a}	$0.50 \pm 0.02^{b,c}$	$0.057 \pm 0.008^{h,i}$	$23 \pm 1^{d,e}$
V8	7.4 ± 0.4^{d}	$19 \pm 2^{d,e}$	4.0 ± 0.2^{f}	$0.37 \pm 0.02^{f,g}$	$0.25 \pm 0.02^{a,b}$	31 ± 1^{c}
V9 C	8.23 ± 0.04^{c}	12.2 ± 0.5^{f}	$2.2 \pm 0.1^{g,h}$	$0.26 \pm 0.04^{h,i}$	$0.16 \pm 0.02^{e,f}$	23.1 ± 0.3^{d}
V9 R	4.9 ± 0.1^{f}	$4.3 \pm 0.4^{h,i}$	10.4 ± 0.3^{c}	$0.40 \pm 0.06^{e,f}$	$0.16 \pm 0.03^{e,f}$	20.2 ± 0.9^{f}
V10	6.7 ± 0.4^{e}	$19.3 \pm 0.8^{d,e}$	3.48 ± 0.08^{f}	$0.3273 \pm 0.0007^{g,h}$	$0.108 \pm 0.004^{g,h}$	30 ± 1^{c}
V11	8.9 ± 0.4^{b}	26.9 ± 0.9^{a}	$1.50 \pm 0.06^{h,i}$	0.48 ± 0.04^{c}	$0.20 \pm 0.03^{c,d,e}$	38.0 ± 0.5^{a}

Means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$. T: turbinate; C: cylindrical; R: round; F: flattened.

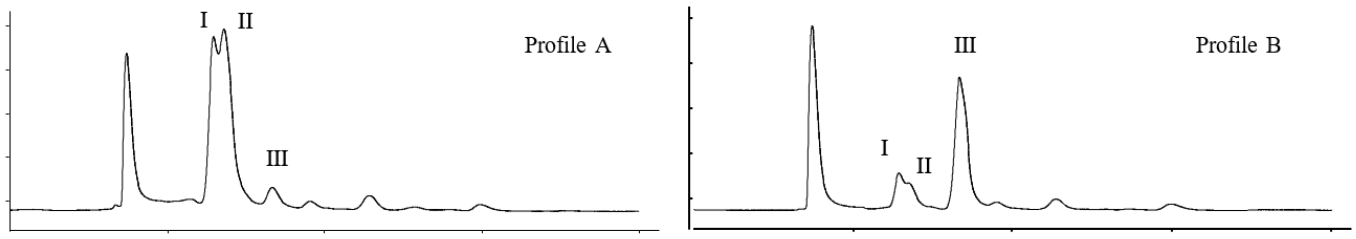

Figure 1. Profile **A** (from V2 T sample), rich in fructose (I) and glucose (II); Profile **B** (from V1 sample), rich in sucrose (III).

The fatty acids composition of the flesh of the studied genotypes is presented in Table 5. The main fatty acids were palmitic (19.6–63.4%), oleic (1.98–14.1%), linoleic (5.1–35.8%) and γ-linolenic acids (0.5–38.8%). So far, most of the reports in the literature focused on the fatty acids composition of seeds and seed oils where palmitic, oleic and linoleic acids are highlighted as the main compounds [59,60], while the edible flowers contain mostly myristic, oleic, stearic and heneicosanoic acid [67] and the leaves palmitic, linoleic and linolenic acids [68]. To the best of our knowledge, there is a lack of scientific literature regarding the composition of fatty acids in the flesh of pumpkin fruit. The majority of fatty acids were classified as saturated and polyunsaturated fatty acids which accounted for approximately 85 to 95% of total fatty acids, while the rest of the identified compounds were monounsaturated fatty acids SFA and PUFA, respectively. The only exceptions were genotypes V2 C and V4 C where SFA was the predominant class of fatty acids accounting for approximately 85% due to the high content of palmitic acid, whereas V6 and V11 genotypes had the highest content of PUFA (approximately 65%). Moreover, the PUFA/SFA and n-6/n-3 values were higher than 0.45 and lower than 4.0, respectively, for all the tested genotypes indicating high nutritional value of pumpkin pulp [69], except for genotypes V2 C and V4 C where high amounts of PUFA and very low amounts of n-3 fatty acids were recorded.

3.3. Bioactivities

3.3.1. Antioxidant Capacity

Varied lipid peroxidation and oxidative haemolysis inhibition capacity were recorded among the tested genotypes (Table 6). In particular, V4 C and V7 P genotypes recorded the highest antioxidant activity for the TBARS assay (lowest IC_{50} values), while the V8 genotype was the most effective in the case of the OxHLIA assay. In any case, none of the tested extracts was more effective than Trolox which was used as the positive control for both assays. Recently, Leichtweis et al. [42,70] determined the antioxidant activity in different parts of *Cucurbita* fruit cultivated in Portugal, Algeria and Greece suggested a variable response to TBARS and OxHLIA assays depending on the fruit type and the growing location, while seeds recorded the highest antioxidant activity compared to peels and endocarp. On the other hand, Jahan et al. [71] suggested that flowers showed the highest antioxidant activity, followed by flesh, leaves, seeds and peels, which indicates the effect of the implemented assay on the obtained results. Moreover, the addition of powder from pumpkin fruit and flowers improved the capacity of lipid peroxidation inhibition in cooked sausages and chicken [72,73]. In the studies of Rolnik et al. [71,74], where the inhibition of lipid peroxidation was determined via the TBARS assay, it was reported a significant variability among cucurbit vegetables, including pumpkin, zucchini and squash which highlights the importance of the genotypic impact on the antioxidant capacity of *Cucurbita* fruit. In contrast, Tarwadi and Angte [75] who compared fruit and root vegetables classified pumpkin fruit among the species with poor performance in terms of the TBARS assay.

Table 5. Fatty acids composition of the flesh of the tested pumpkin genotypes (relative %).

Compound	V1	V2 T	V2 C	V3	V4 C	V4 R	V5 F	V5 R	V6	V7 P	V7 F	V8	V9 C	V9 R	V10	V11
C8:0	n.d.	n.d.	0.264 ± 0.006 ^{a}	n.d.	0.24 ± 0.01 ^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.141 ± 0.001 ^{c}	n.d.	n.d.	n.d.
C10:0	0.137 ± 0.001 ^{c}	n.d.	0.1775 ± 0.0007 ^{a}	0.0830 ± 0.0000 ^{g}	0.143 ± 0.002 ^{b}	n.d.	n.d.	0.0720 ± 0.0000 ^{h}	n.d.	0.120 ± 0.001 ^{d}	0.070 ± 0.002 ^{i}	0.0680 ± 0.0000 ^{i}	0.100 ± 0.001 ^{f}	0.1125 ± 0.0007 ^{e}	n.d.	n.d.
C12:0	1.893 ± 0.004 ^{a}	0.219 ± 0.03 ^{j,k}	0.49 ± 0.02 ^{e}	0.500 ± 0.004^{e}	0.61 ± 0.01 ^{d}	0.3050 ± 0.0000 ^{g,h}	0.25 ± 0.01 ^{i,j,k}	0.330 ± 0.001 ^{f,g}	0.2135 ± 0.0007 ^{k}	1.49 ± 0.05 ^{b}	1.21 ± 0.04 ^{c}	0.259 ± 0.003 ^{i,j}	0.342 ± 0.004 ^{f,g}	0.36 ± 0.01 ^{f}	02755 ± 00007 ^{h,i}	0.125 ± 0.004 ^{l}
C13:0	n.d.	n.d.	n.d.	0.1020 ± 0.0000 ^{b}	n.d.	n.d.	0.058 ± 0.001 ^{d}	0.0935 ± 0.0007 ^{c}	0.0940 ± 0.0000 ^{c}	n.d.	n.d.	n.d.	n.d.	0.120 ± 0.006 ^{a}	n.d.	n.d.
C14:0	1.898 ± 0.007 ^{a}	1.22 ± 0.03 ^{e}	1.84 ± 0.03 ^{b}	1.0505 ± 0.0007 ^{g}	1.5980 ± 0.0000 ^{c}	0.7960 ± 0.0000 ^{j,k}	0.90 ± 0.01 ^{h}	1.053 ± 0.006 ^{g}	0.866 ± 0.001 ^{h,i}	1.55 ± 0.04 ^{c}	1.13 ± 0.05 ^{f}	0.83 ± 0.01 ^{i,j}	1.29 ± 0.02 ^{d}	1.21 ± 0.01 ^{e}	0.748 ± 0.008 ^{k}	0.83 ± 0.04 ^{i,j}
C15:0	0.079 ± 0.004 ^{k}	0.117 ± 0.004 ^{i}	0.53 ± 0.01 ^{b}	0.1690 ± 0.0000 ^{g}	0.648 ± 0.007 ^{a}	0.190 ± 0.001 ^{f}	n.d.	0.0895 ± 0.0007 ^{j}	0.1350 ± 0.0000 ^{h}	0.1095 ± 0.0007 ^{i}	0.0775 ± 0.0007 ^{k}	0.290 ± 0.003 ^{e}	0.445 ± 0.005 ^{c}	0.1945 ± 0.0007 ^{f}	0.3455 ± 0.0007 ^{d}	0.133 ± 0.006 ^{h}
C16:0	28.9 ± 0.2 ^{e}	20.9 ± 0.6 ^{k}	61.4 ± 0.5 ^{b}	36.46 ± 0.04 ^{c}	63.4 ± 0.2 ^{a}	29.8 ± 0.2 ^{d}	26.97 ± 0.03 ^{f,g}	26.3 ± 0.1 ^{h}	20.88 ± 0.03 ^{k}	25.67 ± 0.01 ^{i}	22.3 ± 0.2 ^{j}	30.2 ± 0.1 ^{d}	26.49 ± 0.05 ^{g,h}	27.3 ± 0.1 ^{f}	25.982 ± 0.007 ^{h,i}	19.6 ± 0.5 ^{l}
C16:1	0.42 ± 0.02 ^{g}	0.269 ± 0.006 ^{k}	0.586 ± 0.006 ^{b}	0.295 ± 0.004 ^{j}	0.53 ± 0.01 ^{c,d}	0.50 ± 0.02 ^{e}	0.45 ± 0.01 ^{f}	0.388 ± 0.002 ^{h}	0.61 ± 0.02 ^{a}	0.283 ± 0.009 ^{j,k}	0.36 ± 0.02 ^{i}	0.50 ± 0.01 ^{e}	0.520 ± 0.004 ^{d,e}	0.231 ± 0.008 ^{l}	0.37 ± 0.01 ^{h,i}	0.55 ± 0.02 ^{c}
C17:0	0.390 ± 0.002 ^{f,g}	0.224 ± 0.002 ^{i,j}	6.39 ± 0.05 ^{b}	1.23 ± 0.04 ^{c}	7.01 ± 0.04 ^{a}	0.969 ± 0.007 ^{d}	0.222 ± 0.001 ^{i,j}	0.237 ± 0.001 ^{i,j}	0.1520 ± 0.0000 ^{k}	0.602 ± 0.004 ^{e}	0.349 ± 0.001 ^{h}	0.93 ± 0.01 ^{d}	0.350 ± 0.004 ^{g,h}	0.26 ± 0.01 ^{i}	0.423 ± 0.001 ^{f}	0.199 ± 0.009 ^{j}
C17:1	0.182 ± 0.006 ^{d}	0.107 ± 0.002 ^{h}	0.84 ± 0.01 ^{a}	0.3770 ± 0.0000 ^{c}	0.65 ± 0.01 ^{b}	n.d.	0.132 ± 0.001 ^{g}	0.1465 ± 0.0007 ^{e,f}	0.1250 ± 0.0000 ^{g}	0.377 ± 0.009 ^{c}	n.d.	0.1355 ± 0.0007 ^{f,g}	0.0945 ± 0.0007 ^{i}	n.d.	0.1805 ± 0.0007 ^{d}	0.151 ± 0.006 ^{e}
C18:0	2.5 ± 0.1 ^{e,f,g}	1.85 ± 0.05 ^{j}	4.89 ± 0.07 ^{b}	2.45 ± 0.08 ^{f,g}	5.30 ± 0.02 ^{a}	2.614 ± 0.008 ^{e}	2.25 ± 0.06 ^{h}	2.02 ± 0.01 ^{i}	1.568 ± 0.005 ^{k}	4.2 ± 0.1 ^{c}	3.0 ± 0.1 ^{d}	2.53 ± 0.05 ^{e,f}	2.3 ± 0.1 ^{h}	1.64 ± 0.04 ^{k}	2.37 ± 0.04 ^{g,h}	1.54 ± 0.06 ^{k}
C18:1n9c	12.1 ± 0.3 ^{c}	14.1 ± 0.1 ^{a}	8.4 ± 0.2 ^{f}	6.0 ± 0.2 ^{i}	4.95 ± 0.05 ^{j}	7.6 ± 0.2 ^{g}	8.6 ± 0.2 ^{e,f}	6.53 ± 0.03 ^{h}	9.57 ± 0.04 ^{d}	13.30 ± 0.02 ^{b}	8.9 ± 0.4 ^{e}	4.720 ± 0.007 ^{j}	5.86 ± 0.01 ^{i}	1.980 ± 0.006 ^{l}	3.919 ± 0.006 ^{k}	9.46 ± 0.03 ^{d}
C18:2n6c	22.9 ± 0.2 ^{k}	34.0 ± 0.8 ^{c}	5.1 ± 0.1 ^{m}	25.8 ± 0.2 ^{i}	5.9 ± 0.2 ^{l}	28.71 ± 0.08 ^{g}	27.0 ± 0.1 ^{h}	32.2 ± 0.2 ^{e}	30.12 ± 0.03 ^{f}	24.8 ± 0.1 ^{j}	29.8 ± 0.2 ^{f}	35.78 ± 0.03 ^{a}	31.97 ± 0.04 ^{e}	33.4 ± 0.1 ^{d}	34.65 ± 0.07 ^{b}	25.09 ± 0.07 ^{j}
C18:3n6	0.162 ± 0.006 ^{c}	0.080 ± 0.002 ^{d}	n.d.	n.d.	0.54 ± 0.03 ^{a}	n.d.	0.216 ± 0.006 ^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3n3	25.9 ± 0.3 ^{f}	24.9 ± 0.4 ^{g}	0.512 ± 0.001 ^{l}	20.4 ± 0.1 ^{j}	0.60 ± 0.02 ^{l}	24.48 ± 0.02 ^{h}	29.81 ± 0.02 ^{c}	27.3 ± 0.4 ^{e}	32.87 ± 0.04 ^{b}	23.42 ± 0.07 ^{i}	29.53 ± 0.08 ^{c}	19.003 ± 0.0007 ^{k}	25.73 ± 0.07 ^{f}	29.59 ± 0.09 ^{c}	28.5 ± 0.1 ^{d}	38.8 ± 0.3 ^{a}
C20:0	n.d.	n.d.	1.03 ± 0.03 ^{a}	0.3790 ± 0.0000 ^{c}	0.75 ± 0.03 ^{b}	n.d.	n.d.	0.213 ± 0.001 ^{g}	0.1840 ± 0.0000 ^{h}	0.311 ± 0.003 ^{d,e}	0.25 ± 0.01 ^{f}	0.289 ± 0.001 ^{e}	0.335 ± 0.001 ^{d}	0.37 ± 0.01 ^{c}	0.2315 ± 0.0007 ^{f,g}	0.235 ± 0.008 ^{f,g}
C20:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2015 ± 0.0007 ^{b}	n.d.	n.d.	n.d.	0.542 ± 0.002 ^{a}	n.d.	n.d.	n.d.
C21:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1345 ± 0.0007 ^{f}	0.1455 ± 0.0007 ^{e}	0.184 ± 0.002 ^{c}	n.d.	0.241 ± 0.001 ^{a}	0.1555 ± 0.0007 ^{d}	0.2135 ± 0.0007 ^{b}	n.d.	n.d.
C20:3n6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1355 ± 0.0007	n.d.	n.d.	n.d.
C22:0	0.68 ± 0.02 ^{j}	0.811 ± 0.004 ^{i}	3.23 ± 0.01 ^{a}	1.11 ± 0.03 ^{e,f}	2.02 ± 0.02 ^{b}	1.26 ± 0.04 ^{c}	1.16 ± 0.04 ^{d,e}	1.089 ± 0.006 ^{f}	0.876 ± 0.009 ^{h}	1.16 ± 0.01 ^{d,e}	1.13 ± 0.05 ^{d,e,f}	1.18 ± 0.02 ^{d}	0.838 ± 0.003 ^{h,i}	0.973 ± 0.005 ^{g}	0.653 ± 0.002 ^{j}	1.26 ± 0.05 ^{c}
C20:5n3	0.099 ± 0.003	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C22:1	n.d.	n.d.	n.d.	0.166 ± 0.006 ^{c}	n.d.	0.602 ± 0.009 ^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.435 ± 0.002 ^{b}	n.d.	n.d.	n.d.
C22:2	0.264 ± 0.004 ^{d}	0.163 ± 0.004 ^{h}	n.d.	0.223 ± 0.002 ^{f}	0.246 ± 0.008 ^{e}	n.d.	0.152 ± 0.006 ^{i}	0.28 ± 0.01 ^{c}	0.141 ± 0.005 ^{j}	0.536 ± 0.006 ^{a}	0.240 ± 0.007 ^{e}	0.334 ± 0.002 ^{b}	0.225 ± 0.001 ^{f}	0.1840 ± 0.0000 ^{g}	0.1775 ± 0.0007 ^{g}	0.150 ± 0.004 ^{i,j}
C22:6n3	n.d	n.d	n.d	n.d	0291 ± 0006	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
C23:0	0.368 ± 0.001 ^{g,h}	0.33 ± 0.01 ^{i}	0.98 ± 0.04 ^{a}	0.519 ± 0.009 ^{d}	0.87 ± 0.03 ^{b}	0.427 ± 0.006 ^{f}	0.385 ± 0.008 ^{g,h}	0.37 ± 0.01 ^{g,h}	0.39 ± 0.01 ^{g}	0.539 ± 0.006 ^{d}	0.47 ± 0.02 ^{e}	0.438 ± 0.002 ^{e,f}	0.537 ± 0.002 ^{d}	0.615 ± 0.001 ^{c}	0.360 ± 0.001 ^{h,i}	0.598 ± 0.006 ^{c}
C24:0	1.14 ± 0.01 ^{h}	0.78 ± 0.03 ^{i}	3.4 ± 0.2 ^{b}	2.63 ± 0.06 ^{c}	3.696 ± 0.008 ^{a}	1.67 ± 0.03 ^{e}	1.38 ± 0.01 ^{f}	1.141 ± 0.006 ^{h}	0.854 ± 0.008 ^{i}	1.36 ± 0.01 ^{f}	1.25 ± 0.05 ^{g,h}	2.3 ± 0.1 ^{d}	1.136 ± 0.004 ^{h}	1.1905 ± 0.0007 ^{g,h}	0.811 ± 0.003 ^{i}	1.28 ± 0.03 ^{f,g}
C24:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0875 ± 0.0007	n.d.	n.d.	n.d.

Table 5. *Cont.*

Compound	V1	V2 T	V2 C	V3	V4 C	V4 R	V5 F	V5 R	V6	V7 P	V7 F	V8	V9 C	V9 R	V10	V11
SFA	38.0 ± 0.3 ^{e}	26.4 ± 0.5 ^{k}	84.6 ± 0.4 ^{b}	46.7 ± 0.1 ^{c}	86.3 ± 0.2 ^{a}	38.8 ± 0.09 ^{e}	33.58 ± 0.02 ^{h}	33.1 ± 0.2 ^{h}	26.36 ± 0.03 ^{k}	37.3 ± 0.2 ^{d}	31.2 ± 0.3 ^{j}	39.53 ± 0.02 ^{d}	34.41 ± 0.03 ^{g}	34.6 ± 0.2 ^{g}	32.20 ± 0.02 ^{i}	25.8 ± 0.4 ^{l}
MUFA	12.7 ± 0.2 ^{c}	14.5 ± 0.1 ^{a}	9.8 ± 0.3 ^{f}	6.9 ± 0.2 ^{j}	6.13 ± 0.07 ^{k}	8.7 ± 0.2 ^{h}	9.2 ± 0.2 ^{g}	7.07 ± 0.04 ^{j}	10.51 ± 0.05 ^{d}	13.96 ± 0.00 ^{b}	9.2 ± 0.4 ^{g}	5.36 ± 0.02 ^{l}	7.533 ± 0.006 ^{i}	2.211 ± 0.003 ^{n}	4.46 ± 0.01 ^{m}	10.157 ± 0.008 ^{e}
PUFA	49.3 ± 0.5 ^{i}	59.1 ± 0.4 ^{d}	5.6 ± 0.1 ^{m}	46.4 ± 0.3 ^{k}	7.6 ± 0.3 ^{l}	53.2 ± 0.1 ^{h}	57.2 ± 0.2 ^{f}	59.8 ± 0.2 ^{c}	63.13 ± 0.02 ^{b}	48.8 ± 0.2 ^{j}	59.59 ± 0.07 ^{c,d}	55.12 ± 0.03 ^{g}	58.05 ± 0.04 ^{e}	63.2 ± 0.2 ^{b}	63.34 ± 0.03 ^{b}	64.0 ± 0.4 ^{a}

Means of the same row followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$; n.d.: not detected. T: turbinate; C: cylindrical; R: round; F: flattened; Caprylic acid (C8:0); Capric acid (C10:0); lauric acid (C12:0); tridecylic acid (C13:0); myristic acid (C14:0); pentadecylic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); heptadecenoic acid (C17:1); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6c); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); gondoic acid (C20:1); heneicosylic acid (C21:0); arachidonic acid (C20:3n6); behenic acid (C22:0); eicosatrienoic acid (C20:5n3); erucic acid (C22:1); docosadienoic acid (C22:2); (C20:6n3); tricosylic acid (C23:0); lignoceric acid (C24:0); nervonic acid (C24:1); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 6. Lipid peroxidation inhibition capacity (TBARS) and oxidative haemolysis inhibition capacity (OxHLIA) of the flesh of the tested pumpkin genotypes (inhibition capacity (IC_{50}), μg/mL).

Genotype	TBARS (IC_{50} [1], μg/mL)	OxHLIA $_{60\ min}$ (IC_{50} [1], μg/mL)
V1	2877 ± 79 ^{i}	674 ± 21 ^{c}
V2 T	3000 ± 120 ^{g,h}	302 ± 9 ^{h,i}
V2 C	5339 ± 151 ^{b}	980 ± 44 ^{a}
V3	3328 ± 164 ^{f}	236 ± 11 ^{j,k}
V4 C	1864 ± 76 ^{m}	114 ± 7 ^{l}
V4 R	2310 ± 47 ^{k}	187 ± 7 ^{k}
V5 F	2556 ± 121 ^{j}	350 ± 13 ^{g,h}
V5 R	2218 ± 111 ^{l}	599 ± 10 ^{d}
V6	3022 ± 103 ^{g}	284 ± 8 ^{i,j}
V7 P	1816 ± 91 ^{m}	385 ± 30 ^{g}
V7 F	4035 ± 157 ^{d}	452 ± 12 ^{f}
V8	2915 ± 133 ^{h,i}	35 ± 3 ^{m}
V9 C	4149 ± 192 ^{c}	254 ± 13 ^{i,j}
V9 R	2981 ± 84 ^{g,h}	520 ± 17 ^{e}
V10	5694 ± 129 ^{a}	255 ± 8 ^{i,j}
V11	3561 ± 161 ^{e}	792 ± 31 ^{b}
Trolox	139 ± 5	21.8 ± 0.2

[1] IC_{50}: extract concentration that inhibits oxidation by 50%; means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$. T: turbinate; C: cylindrical; R: round; F: flattened.

3.3.2. Antimicrobial Activity

The antimicrobial properties of the extracts obtained from the fruit of the tested genotypes are presented in Table 7. In general, despite some slight differences among the tested extracts in terms of MIC. MBC and MFC values, none of them showed higher activity against various Gram− and Gram+ bacterial strains and two species of *Aspergillus* compared to streptomycin, ampicillin and ketoconazole. However, almost all the tested extracts showed the ability to inhibit the growth of at least one bacterial strain (MIC values up to 10 mg/mL), except for the case of V9 R where no effects were recorded. Moreover, the lowest overall MIC values were recorded for V2 T against *Salmonella enterocolitica* (5 mg/mL); V6 against and *Salmonella enterocolitica* (5 mg/mL for both bacteria); V7 F against *Yersinia enterocolitica* (2.5 mg/mL); V8 against *Escherichia coli* (5 mg/mL); V9 C against *Enterobacter cloacae* (5 mg/mL); and V10 against *Enterobacter cloacae* and *Yersinia enterocolitica* (5 mg/mL). Similarly, most of the tested extracts showed efficacy against at least one of the studied *Aspergillus* species, except for V1, V2, V4 C and V5 genotypes. Similar to our study, Leichtweis et al. [42,70] suggested significant antimicrobial properties from fruit parts (peels, endocarp and seeds) of different pumpkin genotypes. Moreover, Mokhtar et al. [76] reported varied antimicrobial properties of *C. moschata* fruit depending on their ripeness with extracts from mature fruit being more effective against various bacterial strains compared to young and ripe fruit. According to the same authors, this finding was mainly attributed to the highest content of polyphenols detected in this particular stage [76]. Badr et al. [57] also recorded moderate efficacy of rind and flesh extracts against *Bacillus subtilis* and *B cereus.* Hussain et al. [77] suggested seed extracts showed higher activity against *Candida albicans*, *Fusarium oxysporum*, *Mucor miehei* and *Trichoderma* spp. compared to peels and flesh, whereas flesh extracts recorded greater activity against *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Streptococcus aureus* compared to the other two extracts. The beneficial effects of different pumpkin powders, extracts, isolates, purified bioactives and pumpkin-based functional food products are well reported in the review performed by Hussain et al. [78], which corroborates its health promotion properties. In contrast, Saavedra et al. [7] did not record any antimicrobial effects for peel and seed extracts obtained from *C. pepo* byproducts (shells and seeds).

Table 7. Antimicrobial and antifungal activity of the flesh of different pumpkin varieties from Greece (mg/mL).

	V1		V2 T		V2 C		V3		V4 C		V4 R		V5 F		V5 R		V6		V7 P	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																				
Enterobacter cloacae	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10	>10	>10
Escherichia coli	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	>10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterocolitica	>10	>10	5	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	>10	>10
Yersinia enterocolitica	10	>10	>10	>10	10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10
Gram-positive bacteria																				
Bacillus cereus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Staphylococcus aureus	10	>10	10	>10	10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10

	V7 F		V8		V9 C		V9 R		V10	V11			Streptomycin		Methicilin		Ampicillin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MIC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																		
Enterobacter cloacae	10	>10	>10	>10	5	>10	>10	>10	5	>10	10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	5	>10	10	>10	>10	>10	>10	>10	10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterocolitica	10	>10	10	>10	>10	>10	>10	>10	>10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	2.5	>10	>10	>10	>10	>10	>10	>10	5	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																		
Bacillus cereus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	V1		V2 T		V2 C		V3		V4 C		V4 R		V5 F		V5 R		V 6	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10
Aspergillus fumigatus	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10

	V7 P		V7 F		V8		V9 C		V9 R		V10		V11		Ketoconazole	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	>10	>10	0.5	1

MIC: minimum inhibitory concentration; MBC: minimal bactericidal concentration; MFC: minimal fungicidal concentration; n.t: not tested; T: turbinate; C: cylindrical; R: round; F: flattened.3.3.3. Cytotoxicity.

The reported antimicrobial activity of the pumpkin pulp extracts assessed in the present study, together with the antioxidant activity described above, supports the importance of including this nutritious and bioactive fruit in healthy diets.

None of the tested samples presented cytotoxic activity against the non-tumor porcine liver cells (up to 400 μg/mL), which indicates the high potential of using pumpkin flesh as a natural antioxidant agent in the food industry. This finding is in agreement with the previous reports of Leichtweis et al. [42,70], who did not record any cytotoxic effect on non-tumor cells for the extracts from different parts of pumpkin fruit. Moreover, according to Gaweł-Bęben et al. [79], peel extracts did not show any toxic effects against human keratinocytes up to the concentration of 1000 μg/mL suggesting its safe use in the cosmeceutical industry. Similarly, the extracts of zucchini fruit showed a high potential for use in cosmetic emulsions since they did not show toxic effects against HaCaT cell lines or reconstructed human epidermis [80]. This satisfactory result was expected since pumpkin pulp has been used for thousands of years for animal and human consumption. However, considering mainly local varieties, which are not yet widely cultivated and consumed, these results add new safety insights, since varieties of the same species can considerably vary in terms of chemical composition. In fact, the presence of bioactive compounds with cytotoxic activity against tumour cell lines is widely reported in pumpkins, such as, for example, cucurbitin [13,81], and in the present study we aimed to confirm their safety, testing the obtained extracts against non-tumour cells.

3.4. Chemical Characterization

3.4.1. Organic Acids

The main detected organic acids were oxalic and malic acid, followed by citric and fumaric acid, whereas ascorbic and shikimic acid were either detected in traces or not detected (Table 8). The V9 C genotype recorded the highest content of oxalic and citric acids (6.4 and 3.2 g/100 g dw, respectively), while malic acid was the highest in V4 R and V10 genotypes (5.38 and 5.2 g/100 g dw, respectively) and fumaric acid in V6 genotype (0.0943 mg/100 g dw). According to Priecina and Karklina [82] oxalic acid was the major detected compound in fresh pumpkin fruit followed by malic and citric acid, while the amounts of oxalic acid were considerably higher than those of our study (approximately 15 g/100 g dw). Moreover, Iswaldi et al. [83] tentatively identified eight organic acids and derivatives in three zucchini cultivars, although the authors did not quantify the detected compounds. Abbas et al. [84] suggested that organic acid composition and content in pumpkins may be affected by the growing conditions, the genotype and the developmental stage of fruits. In particular, they indicated a significant decrease in organic acids content throughout the maturation process, while they suggested oxalic and fumaric acids as the prevalent ones at all the developmental stages [85]. In contrast, Nawirska-Olszańska et al. [85] and Zhou et al. [86] reported the presence of only malic, citric and fumaric acid with varied content among the different genotypes of pumpkin species (*C. pepo*, *C. maxima* and *C. moschata*). Therefore, any contradictions among reports from the literature could be associated with differences in the genotype, the growing conditions and the harvesting stage of fruit.

3.4.2. Tocopherols

The main detected vitamin E isoform was α-tocopherol which recorded in amounts that ranged between 0.218 (V7 F) and 4.90 mg/100 g dw (V2 T), followed by β- and γ-tocopherols (Table 9). So far, there is scarce literature regarding the tocopherol composition in the flesh of pumpkin fruit since most of the studies refer to seeds and seed oils. Similarly to our study, Kim et al. [40] suggested α-tocopherol as the main compound in the flesh of three pumpkin species (*C. pepo*, *C. moschata* and *C. maxima*), while γ-tocopherol was detected only in low amounts in *C. moschata* fruit. Kulczynski and Gramza-Michałowska [9] detected only α- and γ-tocopherols in the pulp of *C. pepo* and *C. moschata* fruit in amounts that were towards the highest range of our study, whereas the other two vitamin E isoforms

(β- and δ-tocopherols) were not detected. Therefore, the results of our study along with the literature reports suggest that pumpkin fruit flesh can be a rich source of tocopherols (especially α-tocopherol), although a high genotypic variability should be expected at the intra- or inter-species level.

Table 8. Quantification of the organic acids in the flesh of the studied pumpkin genotypes.

Genotype	Oxalic g/100 g dw	Malic g/100 g dw	Ascorbic g/100 g dw	Shikimic g/100 g dw	Citric g/100 g dw	Fumaric mg/100 g dw
V1	3.61 ± 0.04^{f}	2.7 ± 0.1^{j}	tr	tr	1.08 ± 0.02^{d}	0.0091 ± 0.0001^{m}
V2 T	4.45 ± 0.04^{e}	$3.96 \pm 0.06^{f,g}$	n.d.	tr	n.d.	0.02825 ± 0.00007^{g}
V2 C	4.8 ± 0.2^{d}	$4.1 \pm 0.2^{d,e,f}$	n.d.	tr	n.d.	0.034 ± 0.001^{e}
V3	5.50 ± 0.01^{b}	$4.0 \pm 0.2^{e,f,g}$	n.d.	n.d.	n.d.	$0.0169 \pm 0.0007^{k,l}$
V4 C	4.5 ± 0.1^{e}	4.9 ± 0.2^{b}	n.d.	n.d.	n.d.	0.0255 ± 0.0008^{h}
V4 R	5.17 ± 0.03^{c}	5.38 ± 0.07^{a}	n.d.	n.d.	n.d.	$0.0193 \pm 0.0009^{i,j}$
V5 F	5.301 ± 0.006^{c}	$4.28 \pm 0.03^{c,d,e}$	n.d.	tr	3.16 ± 0.09^{a}	0.0155 ± 0.0002^{l}
V5 R	1.80 ± 0.03^{h}	3.14 ± 0.07^{i}	tr	tr	n.d.	0.031 ± 0.002^{f}
V6	3.641 ± 0.006^{f}	$5.16 \pm 0.06^{a,b}$	n.d.	n.d.	n.d.	0.0943 ± 0.0002^{a}
V7 P	3.75 ± 0.07^{f}	$4.2 \pm 0.1^{c,d,e,f}$	tr	tr	1.38 ± 0.06^{c}	0.021 ± 0.001^{i}
V7 F	3.64 ± 0.02^{f}	$4.3 \pm 0.2^{c,d,e,f}$	tr	tr	1.62 ± 0.01^{b}	0.0161 ± 0.0003^{l}
V8	4.77 ± 0.01^{d}	3.48 ± 0.05^{h}	n.d.	tr	n.d.	0.069 ± 0.003^{b}
V9 C	6.4 ± 0.1^{a}	$3.7 \pm 0.2^{g,h}$	n.d.	n.d.	3.2 ± 0.1^{a}	n.d.
V9 R	3.40 ± 0.04^{g}	$4.3 \pm 0.2^{c,d}$	tr	n.d.	n.d.	$0.0188 \pm 0.0009^{j,k}$
V10	4.79 ± 0.05^{d}	$5.2 \pm 0.2^{a,b}$	n.d.	n.d.	n.d.	0.0393 ± 0.0004^{d}
V11	4.36 ± 0.09^{e}	4.50 ± 0.06^{c}	n.d.	n.d.	n.d.	0.061 ± 0.001^{c}

n.d.: not detected. tr: traces. Means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$. T: turbinate; C: cylindrical; R: round; F: flattened.

Table 9. Tocopherol content of the extracts of the studied pumpkin genotypes of pumpkin flesh (mg/100 g dw).

Genotype	α-Tocopherol	β-Tocopherol	γ-Tocopherol	Total Tocopherols
V1	1.8 ± 0.1^{g}	0.1496 ± 0.0005^{d}	n.d.	1.9 ± 0.1^{h}
V2 T	4.90 ± 0.08^{a}	6.59 ± 0.08^{a}	n.d.	11.5 ± 0.2^{a}
V2 C	0.81 ± 0.03^{k}	0.238 ± 0.003^{c}	n.d.	1.05 ± 0.03^{k}
V3	1.57 ± 0.02^{h}	n.d.	1.05 ± 0.02^{d}	2.62 ± 0.04^{f}
V4 C	$1.15 \pm 0.03^{i,j}$	n.d.	$0.1998 \pm 0.0002^{g,h}$	1.35 ± 0.03^{j}
V4 R	2.68 ± 0.06^{c}	n.d.	1.619 ± 0.005^{b}	4.30 ± 0.06^{d}
VP F	2.3 ± 0.1^{e}	0.85 ± 0.01^{b}	n.d.	3.19 ± 0.08^{e}
V5 R	1.03 ± 0.02^{j}	n.d.	1.02 ± 0.03^{d}	2.05 ± 0.04^{h}
V6	2.47 ± 0.01^{d}	n.d.	4.5 ± 0.1^{a}	7.0 ± 0.1^{b}
V7 P	0.70 ± 0.04^{k}	0.043 ± 0.001^{e}	0.185 ± 0.004^{h}	0.93 ± 0.04^{k}
V7 F	0.218 ± 0.008^{m}	n.d.	$0.2685 \pm 0.0007^{f,g}$	0.486 ± 0.009^{l}
V8	1.16 ± 0.04^{j}	n.d.	0.58 ± 0.02^{e}	1.73 ± 0.02^{i}
V9 C	1.70 ± 0.09^{g}	n.d.	0.572 ± 0.003^{e}	2.3 ± 0.1^{g}
V9 R	2.91 ± 0.06^{b}	n.d.	1.24 ± 0.03^{c}	4.15 ± 0.04^{d}
V10	1.97 ± 0.09^{f}	n.d.	0.28 ± 0.01^{f}	2.3 ± 0.1^{g}
V11	0.55 ± 0.01^{l}	n.d.	4.52 ± 0.07^{a}	5.07 ± 0.08^{c}

n.d.: not detected. Means of the same column followed by different Latin letters are statistically different according to Tukey's honestly significant difference (HSD) test at $p < 0.05$. T: turbinate; C: cylindrical; R: round; F: flattened.

3.5. Principal Component Analysis

The principal component analysis (PCA) was implemented to reveal groups and highlight similarities and differences based on multivariate data. The processing of our data showed that the first eleven principal components (PCs) were associated with Eigen values higher than 1, explaining 95.9% of the cumulative variance, with PC1 accounting for

24.6%, PC2 for 18.4% and PC3 for 13.3%; and PC4 for 8.8%, PC% for 7.9%, PC6 for 6.3%, PC7 for 4.7%, PC8 for 3.9%, PC9 for 3.6%, PC10 for 2.4% and PC11 for 2.1%. In particular, PC1 showed a positive correlation with C18:2n6c and PUFA and a negative correlation with C8:0, C10:0, C15:0, C16:0, C17:0, C20:0, C22:0, C23:0, C24:0 and SFA. On the other hand, PC2 showed a positive correlation with ash, fibres, fructose, oxalic acid and raffinose content, as well as with TBARS, C22:1 and C24:1, whereas a negative correlation with carbohydrates and energy content was recorded. Finally, PC3 showed a positive correlation with fat, fumaric acid, glucose, total sugars and total tocopherols content, whereas a negative correlation was observed for C20:3n6, C22:1, C24:1 and citric acid. Therefore, PCA may help to discriminate the studied landraces as depicted in the following scatterplots and loading plots. The scatterplot in Figure 2 shows four distinct groups of the studied landraces based on their chemical composition and bioactive properties of fruit flesh.

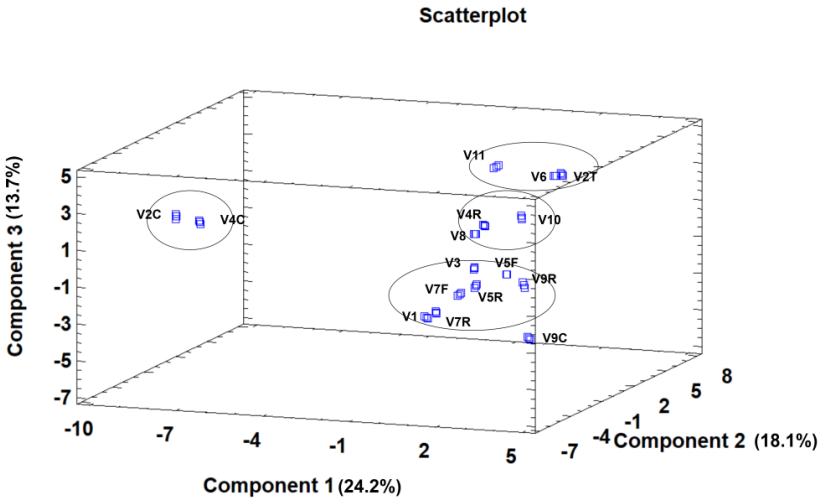

Figure 2. Three-dimensional scatterplot of principal components 1, 2 and 3 for pumpkin fruit flesh.

The loading plot of PC1 and PC2 presents the following correlations: the upper left quadrant included fibres, ash, oxalic acid, fructose, glucose, total sugars, total organic acids, malic acid, TBARS, C8:0, C15:0, C16:1, C17:1, C20:0 and C23:0; the lower left quadrant included SFA, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C22:0, C22:6n3, C24:0, trehalose, OxHLIA and carbohydrates; the upper right quadrant included C13:0, C18:2n6, C18:3n6, C20:1, C20:3n6, C21:0, C22:1, C24:1, α-tocopherol, β-tocopherol, γ-tocopherol, total tocopherols, raffinose, fumaric acid, protein, fat and PUFA; the lower right quadrant included C18:1n9c, C18:3n3, C20:5n3, C22:2, citric acid, MUFA, sucrose and energy content (Figure 3).

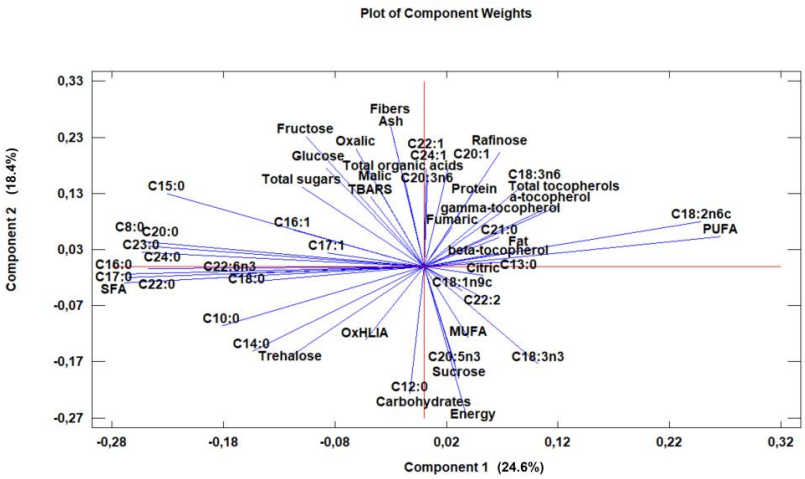

Figure 3. The loading plot of principal components 1 and 2 for pumpkin fruit flesh.

Moreover, the loading plot of PC1 and PC3 also revealed groups of positively correlated variables (Figure 4). The upper left quadrant included glucose, fructose, trehalose, total sugars, malic acid, carbohydrates, fibres, TBARS, OxHLIA, C16:1, C17:0, C17:1, C22:0 and C22:6n3; the lower left quadrant included C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C20:0, C23:0, C24:0, SFA, oxalic acid, total organic acids and ash; the upper right quadrant included fumaric acid, fat, α-tocopherol, β-tocopherol, γ-tocopherol, total tocopherols, fat, raffinose, MUFA, energy content, C13:0, C18:1n9c and C18:3n3; the lower right quadrant included C18:3n6, C18:2n6c, C20:3n6, C20:1, C20:5n3, C21:0, C22:1, C22:2, C24:1, PUFA, sucrose, protein and citric acid.

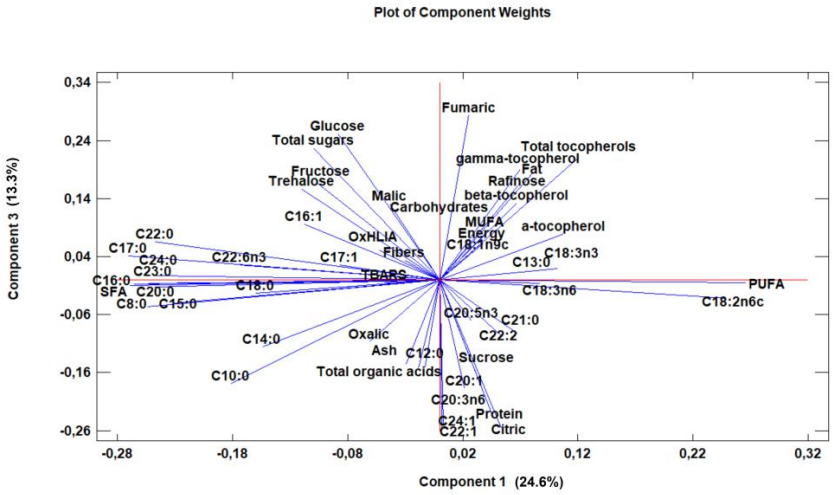

Figure 4. The loading plot of principal components 1 and 3 for pumpkin fruit flesh.

4. Conclusions

The genetic pool of Greek pumpkin germplasm is not adequately characterized so far, especially when considering the high genetic variability of the species which could be a valuable tool for the adaptation of modern horticulture in the changing climate conditions. The heterogeneity in terms of agronomic performance and chemical profile of fruit in pumpkin germplasm makes it necessary to evaluate local landraces aiming to identify promising genotypes with high yield and quality of fruit. Our results indicate that the studied local landraces and commercial genotypes had a high variability not only in fruit yield but also in all the tested parameters related to the chemical composition and bioactive properties of fruit flesh. The local landraces V2 and V8 (from "Trikala" and "Laconia", respectively) recorded the highest fruit yield, while the V2 landrace also had the highest tocopherols (α-, β- and total tocopherols) and fructose, trehalose and raffinose content. On the other hand, V8 landrace was the richest in linoleic acid and also presented the highest antioxidant activity for the OxHLIA assay. However, the rest of the tested landraces presented interesting features such as V11 ("Voutirato") which had the highest sweetness index. Finally, the flesh extracts of most of the tested genotypes showed promising antimicrobial properties, while none of them was toxic against non-tumor cells. Nevertheless, our results support the importance of the conservation and valorization of local genetic material for the selection of elite genotypes with improved nutritional value and quality. However, further research is needed in order to reveal the genetic heterogeneity of the studied material that would help to introduce local landraces in breeding programs.

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