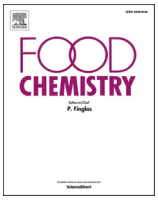
Food Chemistry 475 (2025) 143306

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Valorisation of pumpkin by-products: Chemical composition and bioactive properties of pumpkin seeds, peels, and fibrous strands from different local landraces of Greece

Maria G. Leichtweis [a,b], Adriana K. Molina [a], Maria Inês Dias [a], Ricardo C. Calhelha [a], Tânia C.S.P. Pires [a], Ourania Pavli [c], M. Beatriz P.P. Oliveira [b], Isabel C.F.R. Ferreira [a], Spyridon A. Petropoulos [d], Lillian Barros [a,*], Carla Pereira [a]

[a] CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[b] REQUIMTE—Science Chemical Department, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira no. 228, 4050-313 Porto, Portugal
[c] Laboratory of Genetics and Plant Breeding, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Fytokou Street, 38446 Volos, Greece
[d] Laboratory of Vegetable Production, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Fytokou Street, 38446 Volos, Greece

ARTICLE INFO

Keywords:
Antioxidant activity
Antimicrobial activity
Phenolic compounds
Organic acids
Tocopherols
Cytotoxicity
Pumpkin by-products

ABSTRACT

This study evaluated the fruit by-products (peels, seeds, and fibrous strands) from 11 pumpkin genotypes cultivated in Greece aiming to valorize them as natural sources of bioactive compounds. Five compounds, including (−)-epicatechin and chicoric acid isomers, were identified in peels, while seeds and fibrous strands mainly contained (−)-epicatechin. Organic acids and tocopherols varied significantly among genotypes, with oxalic, quinic, and malic acids being predominant. Total tocopherols content (mg/100 g) ranged up to 7.38 ± 0.03 in fibrous strands, 30.7 ± 0.2 in peels, and 14.58 ± 0.09 in seeds. Extracts exhibited potent antioxidant activity, particularly the seeds of genotypes "V5" and "V6", and strong antimicrobial effects, notably the peels of "V2 T" and "V11", which showed significant inhibition of microbial strains. These findings contribute to the advancement of sustainable practices in the agro-industrial waste management, as well as to the production of functional natural ingredients for various industrial applications.

1. Introduction

Pumpkin (*Cucurbita* sp. L.) is a widely cultivated vegetable crop known for the versatility and nutritional value of its fruit. Beyond its culinary applications, the most commonly used part of the pumpkin fruit is the pulp, yielding valuable by-products such as peels, seeds, and fibrous materials. These by-products have drawn increasing attention due to their rich content of bioactive compounds, including phenolic compounds, tocopherols, and carotenoids, which possess antioxidant, antimicrobial, and other beneficial properties (Chonoko & Rufai, 2011; Medjakovic, Hobiger, Ardjomand-Woelkart, Bucar, & Jungbauer, 2016; Nawirska-Olszańska, Biesiada, Sokół-Łętowska, & Kucharska, 2014; Yang et al., 2022). Utilizing these by-products for the extraction of bioactive compounds holds significant promise for various industrial applications, particularly for the development of natural additives and preservatives for the food and pharmaceutical industries (Dotto & Chacha, 2020; Leichtweis, Oliveira, Ferreira, Pereira, & Barros, 2021; Oliveira, Caleja, Oliveira, Pereira, & Barros, 2023; Salehi et al., 2019).

So far, the pumpkin's most explored part in the literature is the seeds, especially pumpkin seed oil (Dotto & Chacha, 2020; Dowidar, Ahmed, & Mohamed, 2020). Whole seeds are widely consumed in many countries as snacks, while they are used as toppings for salads and other dishes, in addition to consumption in seed oil and seed flour forms. They are a rich source of bioactive components that can benefit human health, such as antioxidant phenolic compounds, tocopherols, triterpenes, saponins, phytosterols, lignans and carotenoids (Dowidar et al., 2020). The seed oils from different pumpkin species were evaluated in terms of their chemical composition and antioxidant activity, where the richness in polyunsaturated acids (>50 %), phenolic compounds (27.52 mg gallic acid equivalents/g of methanolic extract), and tocopherols (633.51 mg/kg of total tocopherols) were verified, with consequent high antioxidant potency being demonstrated (Boujemaa, El Bernoussi, Harhar, &

* Corresponding author.
E-mail addresses: ouraniapavli@uth.gr (O. Pavli), beatoliv@ff.up.pt (M.B.P.P. Oliveira), spetropoulos@uth.gr (S.A. Petropoulos), lillian@ipb.pt (L. Barros).

https://doi.org/10.1016/j.foodchem.2025.143306
Received 8 December 2024; Received in revised form 19 January 2025; Accepted 7 February 2025
Available online 8 February 2025

Tabyaoui, 2020). Moreover, in another study the flour from pumpkin seed kernels was tested as a fat substitute and functional ingredient in beef meatballs due to its high protein (31.96 %), oil (49.87 %), oleic acid (44.78 %), and linoleic acid (39.40 %) contents (Öztürk & Turhan, 2020). However, the peels and fibrous strands, have also been shown to be a rich source of bioactive compounds. In our previous study (Leichtweis et al., 2022), the peel, fibrous strands, and seeds of different varieties of commercial pumpkins grown in Portugal and Algeria revealed important antioxidant and antimicrobial capacity, supported by the profile of phenolic compounds, with 8 individual compounds being tentatively identified (up to 3.93 ± 0.05 mg/g of extract). In fact, pumpkin peel extract has been proven to be a natural alternative to effectively prevent oxidation of lipids in edible oil (Salami, Asefi, Kenari, & Gharekhani, 2020).

However, it is important to recognize that the composition and bioactivity of these pumpkin by-products can vary significantly depending on pre- and post-harvest factors such as the genotype, growing conditions, and processing methods (Kulczyński, Gramza-Michałowska, & Królczyk, 2020; Leichtweis et al., 2023; Polyzos et al., 2022). Different pumpkin genotypes may exhibit distinct profiles of bioactive compounds and associated activities (Abbas et al., 2020; Boujemaa et al., 2020; Kostecka-Gugała, Kruczek, Ledwożyw-Smoleń, & Kaszycki, 2020). For example, Kostecka-Gugała et al. (2020) found high intra-species variability when compared eighteen cultivars of the *Cucurbita* species, where some cultivars stood out as extraordinary sources of phenolic compounds, including syringic and protocatechuic acids, catechin and kaempferol, or minerals such as zinc and copper, while other cultivars exhibited more significant antioxidant and anti-radical capacities than the others. In addition, when comparing *C. pepo* from different cultivation systems, a higher sugar content was found in the fruits from organic system compared to the conventional system, while a higher polyphenol contents, including phenolic acids and flavonoids was recorded when plants were fertilized with manure. Therefore, comprehensive studies evaluating the bioactive potential of different pumpkin parts and cultivars are crucial for maximizing the valorisation of pumpkin by-products.

In this sense, this work aims to explore the potential of utilizing pumpkin by-products, specifically peels, seeds and fibrous strands, as sources of bioactive compounds for industrial applications. Through an investigation of the bioactive profiles of different fruit parts of 11 pumpkin genotypes grown in Greece, this research seeks to reveal the intra-species variability in terms of chemical composition and bioactive properties, as well as to discover new opportunities to harness pumpkin by-products to the development of natural additives and preservatives. Furthermore, it aims to valorise local landraces, thereby fostering sustainability and promoting circular economy through the exploitation of valuable genetic material.

2. Materials and methods

2.1. Samples

Different genotypes of pumpkin, including two commercially available cultivars and 9 local landraces, were cultivated in the experimental farm of the University of Thessaly (Greece), during the growing period of 2021 (for detailed data on specific pumpkin genotypes and harvest dates, refer to Table A in the supplementary material). In the case of local landraces, some genotypes (e.g. V2, V4, V5, V7 and V9) showed high variability in terms of fruit morphology due to interspecific hybridization among the various *Cucurbita* species. Therefore, for each genotype any different types of fruit were sampled and analyzed separately, resulting in 16 distinct samples. Cultivation conditions are described in our previous study (Leichtweis et al., 2023). Fruit from each genotype and form were cut in half and the seeds, peels and fibrous strands samples were collected, after removing the pulp. Samples were then lyophilized (Sublimator model EKS, manufactured by Christian Zirbus Co. in Osterode am Harz, Germany), grounded to powder (~20 mesh), and stored until further analysis.

To evaluate bioactivities and phenolic composition, hydroethanolic extracts from the samples were obtained using standard methodology as described in the following sections. In the case of the antioxidant activity assays, the fat content of the seeds was removed before the extraction process using a Soxhlet apparatus, following AOAC procedures (AOAC, 2016). These extracts were obtained by macerating 2 g of sample in ethanol solution (80 % *v*/v, 60 mL) at room temperature. The mixture was magnetically stirred for 60 min, the extracts were then filtered, and the procedure was iterated with the residue. The ethanol from the combined extracts was rotary evaporated. Then, the extracts were freeze-dried (FreeZone 4.5, Labconco) and stored for later analysis. The extraction yield was calculated by the difference in mass before and after freeze-drying and results are presented in Table A in the supplementary material.

2.2. Individual phytochemical characterization

For the phenolic composition, the extracts were redissolved (10 mg/mL) with ethanol solution (20 % *v*/v) and assessed by high performance liquid chromatography coupled to a diode array detector, electrospray ionization and mass spectrometry (HPLC-DAD-ESI/MS), as described by Barros et al. (2013). The identification of the compounds was carried out based on multiple criteria, including retention time, UV–Vis and mass spectra, and comparison with known commercial standards and existing literature. Quantification was performed by measuring the peak areas and comparing them to the calibration curves of the most closely matching commercially available standards. The results were expressed in mg/g of extract.

The characterization of organic acids was conducted using ultra-fast liquid chromatography (UFLC, Shimadzu Corporation, Kyoto, Japan) coupled with a photodiode array detector (PDA), with wavelengths of 215 nm and 245 nm for ascorbic acid (Barros et al., 2013). Separation was performed on a SphereClone reverse phase C18 column (5 µm, 250 mm × 4.6 mm i.d.) maintained at 35 °C. Identification and quantification were accomplished by comparing the samples with commercial standards. Calibration curves were established as follows: oxalic acid, $y = 8 \times 10^6x + 331{,}789$, $R^2 = 0.9912$; quinic acid, $y = 6.9 \times 10^5x + 11{,}551$, $R^2 = 0.9983$; malic acid, $y = 942{,}562\times + 38{,}506$, $R^2 = 0.9987$; 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, $y = 968{,}367\times - 12{,}295$, $R^2 = 0.9974$; 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/100 g dw).

For tocopherol content the samples were mixed with BHT and tocol solutions and the mixture was homogenized with methanol and hexane. A saturated NaCl solution was added and the mixture was centrifuged. The supernatant was collected, and the extraction process was repeated with hexane. The extracts were dried and redissolved in *n*-hexane before being filtered for the analysis. The HPLC system was coupled with a fluorescence detector (FP-2020; Jasco, Easton, MD, USA), configured to excite at 290 nm and measure emission at 330 nm, and using a Polyamide II normal-phase column (250 × 4.6 mm; YMCWaters) at a temperature of 30 °C, as described by Pereira, Barros, Carvalho, and Ferreira (2011). Acting as an internal standard (SI), tocol facilitated the identification of isoforms (*alpha*, *beta*, *gamma* and *delta*) through comparisons with authentic standards. The results were expressed in mg/100 g dw.

2.3. Bioactivities

To evaluate the antioxidant potential of the extracts, two cell-based assays were employed. The first assay used sheep erythrocytes to assess inhibition of oxidative hemolysis (OxHLIA) (Palmeira et al., 2019), while the second assay used porcine brain homogenates to measure inhibition of lipid peroxidation (TBARS) (Pereira et al., 2011). In the

OxHLIA assay, the extract concentration required to delay 50 % of erythrocyte hemolysis for 60 min was determined and expressed as IC_{50} values (μg/mL). Likewise, in the TBARS assay, the concentration of the extract responsible for inhibiting 50 % of lipid peroxidation was determined and expressed as IC_{50} values (μg/mL). Trolox was used as a positive control in both assays to compare the antioxidant activity of the extracts.

Regarding the antimicrobial activity, the extracts were tested against eight bacteria and two fungi commonly associated with food contamination. The bacteria included *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Yersinia enterocolitica*, while the fungi were *Aspergillus fumigatus* and *Aspergillus brasiliensis*. The microdilution methods and p-iodonitrotetrazolium chloride (INT) were employed for testing, with streptomycin and ampicillin used as positive controls for bacteria and ketoconazole for fungi (Heleno et al., 2013). The results were expressed in mg/mL.

The cytotoxic effect of the extracts was tested on three human tumour cell lines, more specifically the gastric (AGS), colorectal (CaCo2), and breast (MCF-7) adenocarcinoma cells, and on two non-tumour cells, being a primary culture from porcine liver cells (PLP2) and a kidney epithelial cell line of an African green monkey (VERO). The analysis was performed by the sulforhodamine B (SRB) assay (Corrêa et al., 2015), with ellipticine as positive control. The results were expressed as the concentration of extract that inhibited 50 % of cell proliferation (GI_{50} in μg/ml).

2.4. Statistical analysis

The chemical analysis was conducted in triplicate, with results presented as mean ± standard deviation. Statistical analysis involved one-way analysis of variance (ANOVA) using Student's *t*-test for the comparison of two means and Tukey's honestly significant difference (HSD) test for the comparison of three or more means. Before analysis, normal distribution and homogeneity of variance were assessed using Shapiro-Wilk and Levene tests, respectively. For homoscedastic data ($p > 0.05$), Tukey's honestly significant difference (HSD) test was employed, while 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).

A principal component analysis (PCA) was carried out to evaluate the contribution of each variable to the total diversity, aiming to classify the studied samples based on their chemical profile and bioactive properties. For this analysis, we did not consider the different forms of fruit for the genotypes that show polymorphism in fruit shape, while only ten genotypes were considered (we excluded genotype V11 because there were not enough seeds available to perform the analysis of chemical profile and bioactive properties). The analysis was performed with the statistical software Statgraphics 5.1.plus (Statpoint Technologies, Inc., Warrenton, VA, USA). Data was also analyzed using IBM SPSS Statistics software (Version 22.0, IBM Corp, Armonk, NY, USA) to determine whether there were any correlations among different compounds and bioactive properties using Pearson correlation.

3. Results and discussion

3.1. Phytochemical characterization

3.1.1. Phenolic compounds

In all the seeds and fibrous strands samples under investigation, the sole phenolic compound that was identified was (−)-epicatechin (peak 1). This compound was identified by HPLC-DAD-ESI/MS with a retention time of 7.69 min and a maximum absorption wavelength at 280 nm, exhibiting a mass-to-charge ratio of 289 *m/z* and major fragment ions at 245 (100 %) and 205 (45 %) m/z, by comparison with the available standard compound (Table 1). As for the peels, a more diverse profile was found, with five phenolic compounds being tentatively identified, where apart from (−)-epicatechin, two chicoric acids (*cis* and *trans*; peaks 2 and 3) and two flavonoids were also detected (peaks 4 and 5). The chicoric acids compounds were tentatively identified with a retention time of 14.93 and 15.01 min, for *cis* and *trans* respectively, and a maximum absorption wavelength at 328 nm, exhibiting a mass-to-charge ratio of 473 m/z and major fragment ion at 311 (100 %) m/z. Peak 5 (with $[M-H]^-$ at m/z 593) displayed a sole MS^2 fragment at *m/z* 285 (kaempferol aglycone), that indicate the simultaneous loss of a deoxyhexosyl and hexosyl moiety ($[M-H-146-162]^-$). For peak 4 (with $[M-H]^-$ at *m/z* 769) the fragment at *m/z* 315 (100 %) corresponds to an isorhamnetin aglycone and the jointed loss of a dideoxyhexosyl and hexosyl moiety. Chicoric acid and a diversified profile of flavonoids were previously reported by Iswaldi et al. (2013) in zucchini (*Cucurbita pepo* L.). Fig. 1 shows the chromatographic representation of the profile of phenolic compounds obtained, while Table 2 presents the quantification of the phenolic compounds and the total phenolic compounds in peels.

In the case of seeds, the (−)-epicatechin ranged from 6.3 ± 0.3 mg/g extract ("V2" genotype) to 1.00 ± 0.05 mg/g extract ("V4" genotype), whereas this particular compound was not detected in "V8" and "V10" genotypes (Table 2). In fibrous strands, the highest amounts of (−)-epicatechin were recorded for "V2 T" and "V5 R" genotypes (7.6 ± 0.3 and 7.30 ± 0.05 mg/g extract respectively), whereas the lowest content was found in "V11" (~~1.532 ± 0.008~~1.53 ± 0.01 mg/g extract) (Table 2**)**. Meanwhile, in the peels, these compounds showed a narrower range, from 1.922 ± 0.002 to 0.49 ± 0.01 mg/g extract, in "V9 R" and "V7 F", respectively. Moreover, for peel extracts the total flavonoids content was higher than the content of (−)-epicatechin (total flavan-3-ols) in genotypes "V4 C", "V7 P", and "V11", while for all the samples, phenolic acids were present in less amounts. The presence of (−)-epicatechin in almost all the studied samples highlights its importance as the predominant phenolic compound. This compound is a potent flavan-3-ol which is highly recognized for its antioxidant properties. Epicatechin stands out for its well-documented health benefits, contributing to improved cardiovascular health by enhancing blood flow and potentially mitigating certain heart-related risks. Moreover, its association with enhanced exercise performance and muscle recovery underscores its relevance in physiological well-being (Slavova-Kazakova, Janiak, Sulewska, Kancheva, & Karamać, 2021). Epicatechin was also detected in higher concentrations in samples of pumpkin seeds and peels, reported by Yang et al. (2022), as well as in peels and in seed and pulp of *Momordica caranthia* (Busuioc et al., 2020), known as bitter melon, a species that also belongs to Cucurbitaceae family.

So far, literature reports a high variability in terms of total phenolic compounds content between various pumpkin (*C. maxima*) cultivars

Table 1
Phenolic compounds characterized by HPLC-DAD-ESI/MS in the samples of pumpkin by-products (peels, seeds and fibrous strands).

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	7.69	280	289	245(100), 205 (45)	(−)-epicatechin
2	14.93	328	473	311(100), 293 (76), 179(5), 149(5)	*cis*-Chicoric acid
3	15.01	328	473	311(100), 293 (89), 179(6), 149(7)	*trans*-Chicoric acid
4	15.85	354	769	315(100)	Isorhamnetin-*O*-dideoxyhexosyl-hexoside
5	17.12	348	593	285(100)	Kaempferol-*O*-deoxyhexosyl-hexoside

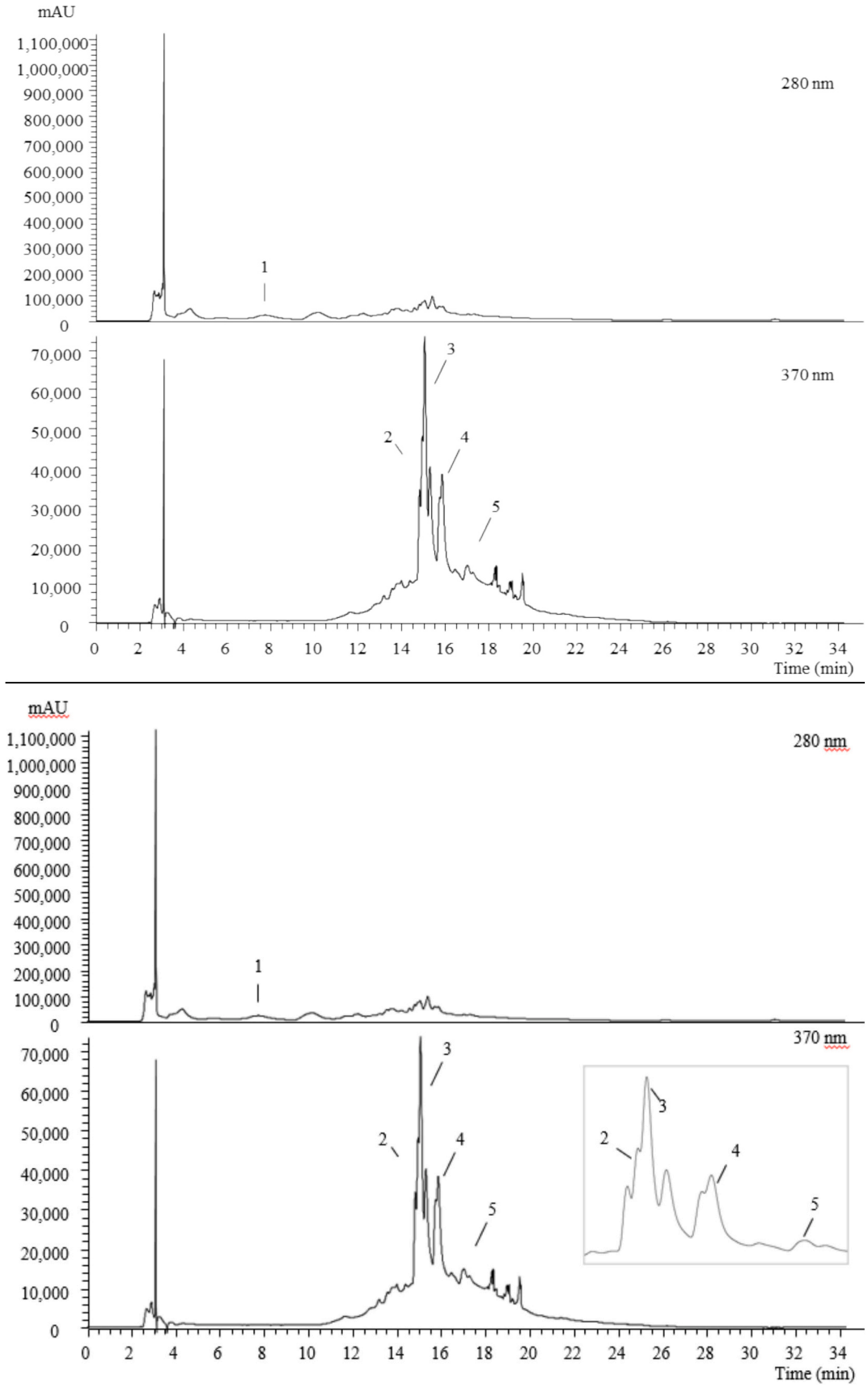


Fig. 1. Chromatographic representation of the phenolic compounds profile obtained by HPLC-DAD-ESI/MS for the peels of the "V10" genotype.

Table 2
Quantification of phenolic compounds found in the extracts of pumpkin by-products (seeds, fibrous strands and peels) expressed in mg/g of extract.

Pumpkin by-product	Genotype	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Total Flavan-3-ols	Total Phenolic Acids	Total Flavonoids	Total Phenolic Compounds
Seeds	V1	1.54 ± 0.07 ^{d}	n.d.	n.d.	n.d.	n.d.	1.54 ± 0.07 ^{d}	n.d.	n.d.	1.54 ± 0.07 ^{d}
	V2	6.3 ± 0.3 ^{a}	n.d.	n.d.	n.d.	n.d.	6.3 ± 0.3 ^{a}	n.d.	n.d.	6.3 ± 0.3 ^{a}
	V3	3.7 ± 0.2 ^{c}	n.d.	n.d.	n.d.	n.d.	3.7 ± 0.2 ^{c}	n.d.	n.d.	3.7 ± 0.2 ^{c}
	V4	1.00 ± 0.05 ^{f}	n.d.	n.d.	n.d.	n.d.	1.00 ± 0.05 ^{f}	n.d.	n.d.	1.00 ± 0.05 ^{f}
	V5	1.12 ± 0.06 ^{e,f}	n.d.	n.d.	n.d.	n.d.	1.12 ± 0.06 ^{e,f}	n.d.	n.d.	1.12 ± 0.06 ^{e,f}
	V6	4.1 ± 0.2 ^{b}	n.d.	n.d.	n.d.	n.d.	4.1 ± 0.2 ^{b}	n.d.	n.d.	4.1 ± 0.2 ^{b}
	V7	1.52 ± 0.02 ^{d}	n.d.	n.d.	n.d.	n.d.	1.52 ± 0.02 ^{d}	n.d.	n.d.	1.52 ± 0.02 ^{d}
	V8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	V9	1.4 ± 0.1 ^{d,e}	n.d.	n.d.	n.d.	n.d.	1.4 ± 0.1 ^{d,e}	n.d.	n.d.	1.4 ± 0.1 ^{d,e}
	V10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fibers	V1	3.7 ± 0.1 ^{h}	n.d.	n.d.	n.d.	n.d.	3.7 ± 0.1 ^{h}	n.d.	n.d.	3.7 ± 0.1 ^{h}
	V2 T	7.6 ± 0.3 ^{a}	n.d.	n.d.	n.d.	n.d.	7.6 ± 0.3 ^{a}	n.d.	n.d.	7.6 ± 0.3 ^{a}
	V2 C	4.39 ± 0.03 ^{e}	n.d.	n.d.	n.d.	n.d.	4.39 ± 0.03 ^{e}	n.d.	n.d.	4.39 ± 0.03 ^{e}
	V3	3.97 ± 0.01 ^{g}	n.d.	n.d.	n.d.	n.d.	3.97 ± 0.01 ^{g}	n.d.	n.d.	3.97 ± 0.01 ^{g}
	V4 C	3.14 ± 0.02 ^{i,j}	n.d.	n.d.	n.d.	n.d.	3.14 ± 0.02 ^{i,j}	n.d.	n.d.	3.14 ± 0.02 ^{i,j}
	V4 R	4.25 ± 0.07 ^{e,f}	n.d.	n.d.	n.d.	n.d.	4.25 ± 0.07 ^{e,f}	n.d.	n.d.	4.25 ± 0.07 ^{e,f}
	V5 F	5.42 ± 0.06 ^{c}	n.d.	n.d.	n.d.	n.d.	5.42 ± 0.06 ^{c}	n.d.	n.d.	5.42 ± 0.06 ^{c}
	V5 R	7.30 ± 0.05 ^{b}	n.d.	n.d.	n.d.	n.d.	7.30 ± 0.05 ^{b}	n.d.	n.d.	7.30 ± 0.05 ^{b}
	V6	4.46 ± 0.07 ^{e}	n.d.	n.d.	n.d.	n.d.	4.46 ± 0.07 ^{e}	n.d.	n.d.	4.46 ± 0.07 ^{e}
	V7 P	3.33 ± 0.09 ^{i}	n.d.	n.d.	n.d.	n.d.	3.33 ± 0.09 ^{i}	n.d.	n.d.	3.33 ± 0.09 ^{i}
	V7 F	3.0 ± 0.1 ^{j}	n.d.	n.d.	n.d.	n.d.	3.0 ± 0.1 ^{j}	n.d.	n.d.	3.0 ± 0.1 ^{j}
	V8	4.09 ± 0.07 ^{f,g}	n.d.	n.d.	n.d.	n.d.	4.09 ± 0.07 ^{f,g}	n.d.	n.d.	4.09 ± 0.07 ^{f,g}
	V9 C	3.7 ± 0.1 ^{h}	n.d.	n.d.	n.d.	n.d.	3.7 ± 0.1 ^{h}	n.d.	n.d.	3.7 ± 0.1 ^{h}
	V9 R	4.87 ± 0.01 ^{d}	n.d.	n.d.	n.d.	n.d.	4.87 ± 0.01 ^{d}	n.d.	n.d.	4.87 ± 0.01 ^{d}
	V10	4.11 ± 0.04 ^{f,g}	n.d.	n.d.	n.d.	n.d.	4.11 ± 0.04 ^{f,g}	n.d.	n.d.	4.11 ± 0.04 ^{f,g}
	V11	1.53 ± 0.01 ^{k}	n.d.	n.d.	n.d.	n.d.	1.53 ± 0.01 ^{k}	n.d.	n.d.	1.53 ± 0.01 ^{k}
Peels	V1	n.d.	tr.	0.0032 ± 0.0001 ^{f}	0.4801 ± 0.0001 ^{d}	0.4695 ± 0.0003 ^{e}	n.d.	0.0032 ± 0.0001 ^{f}	0.9496 ± 0.0002 ^{f}	0.9528 ± 0.0003 ^{j}
	V2 T	1.65 ± 0.06 ^{c}	tr.	0.0236 ± 0.0002 ^{c}	0.4712 ± 0.0003 ^{e}	0.507 ± 0.001 ^{c}	1.65 ± 0.06 ^{c}	0.0236 ± 0.0002 ^{c}	0.979 ± 0.001 ^{d}	2.65 ± 0.06 ^{b}
	V2 C	1.79 ± 0.04 ^{b}	tr.	0.0109 ± 0.0004 ^{e}	0.48 ± 0.01 ^{d}	n.d.	1.79 ± 0.04 ^{b}	0.0109 ± 0.0004 ^{e}	0.48 ± 0.01 ^{h}	2.29 ± 0.05 ^{d}
	V3	1.37 ± 0.04 ^{d}	tr.	tr.	0.577 ± 0.004 ^{a}	0.472 ± 0.001 ^{e}	1.37 ± 0.04 ^{d}	tr.	1.048 ± 0.003 ^{c}	2.41 ± 0.04 ^{c}
	V4 C	n.d.	tr.	tr.	0.4597 ± 0.0001 ^{f}	n.d.	n.d.	tr.	0.4597 ± 0.0001 ^{i}	0.4597 ± 0.0001 ^{k}
	V4 R	1.26 ± 0.01 ^{e}	tr.	tr.	n.d.	n.d.	1.260 ± 0.006 ^{e}	tr.	n.d.	1.26 ± 0.01 ^{h}
	V5 F	1.14 ± 0.01 ^{f}	n.d.	tr.	n.d.	0.456 ± 0.001 ^{f}	1.141 ± 0.007 ^{f}	tr.	0.456 ± 0.001 ^{i}	1.60 ± 0.01 ^{g}
	V5 R	1.23 ± 0.05 ^{e}	n.d.	n.d.	n.d.	n.d.	1.23 ± 0.05 ^{e}	n.d.	n.d.	1.23 ± 0.05 ^{h}
	V6	1.40 ± 0.06 ^{d}	tr.	0.015 ± 0.001 ^{d}	0.485 ± 0.001 ^{d}	0.4964 ± 0.0003 ^{d}	1.40 ± 0.06 ^{d}	0.015 ± 0.001 ^{d}	0.982 ± 0.002 ^{d}	2.40 ± 0.06 ^{c}

(*continued on next page*)

Table 2 (*continued*)

Pumpkin by-product	Genotype	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Total Flavan-3-ols	Total Phenolic Acids	Total Flavonoids	Total Phenolic Compounds
	V7 P	n.d.	0.052 ± 0.002 [a]	0.012 ± 0.001 [d,e]	0.520 ± 0.003 [b]	0.539 ± 0.003 [a]	n.d.	0.064 ± 0.002 [c]	1.0591 ± 0.0001 [b]	1.123 ± 0.003 [i]
	V7 F	0.49 ± 0.01 [h]	tr.	tr.	n.d.	0.4589 ± 0.0002 [f]	0.49 ± 0.01 [h]	tr.	0.4589 ± 0.0002 [i]	0.95 ± 0.01 [j]
	V8	1.82 ± 0.02 [b]	0.011 ± 0.001 [c]	0.086 ± 0.003 [b]	n.d.	n.d.	1.82 ± 0.02 [b]	0.097 ± 0.003 [b]	n.d.	1.92 ± 0.02 [e]
	V9 C	1.11 ± 0.04 [f]	tr.	0.0057 ± 0.0002 [f]	0.5008 ± 0.0001 [c]	n.d.	1.11 ± 0.04 [f]	0.0057 ± 0.0002 [f]	0.5008 ± 0.0001 [g]	1.62 ± 0.04 [g]
	V9 R	1.922 ± 0.002 [a]	tr.	tr.	n.d.	n.d.	1.922 ± 0.002 [a]	tr.	n.d.	1.922 ± 0.002 [e]
	V10	1.71 ± 0.08 [c]	0.017 ± 0.001 [b]	0.101 ± 0.005 [a]	0.58 ± 0.01 [a]	0.519 ± 0.002 [b]	1.71 ± 0.08 [c]	0.12 ± 0.01 [a]	1.10 ± 0.01 [a]	2.92 ± 0.07 [a]
	V11	0.86 ± 0.01 [g]	n.d.	n.d.	0.468 ± 0.002 [e,f]	0.493 ± 0.006 [d]	0.861 ± 0.009 [g]	n.d.	0.962 ± 0.007 [e]	1.823 ± 0.002 [f]

n.d. – not detected. tr. – traces. Calibration curves used for quantification: (−)-catechin (y = 84,950× - 23,200; R^2 = 0.999; LOD = 0.17 g/mL; LOQ = 0.68 g/mL; peak 1); caffeic acid (y = 388,345× + 406,369; R^2 = 0.994; LOD = 0.78 g/mL; LOQ = 1.97 g/mL; peaks 2 and 3) and quercetin 3-*O*-glucoside (y = 34,843× - 160,173; R^2 = 0.9998; LOD = 0.21 g/mL; LOQ = 0.71 g/mL; peaks 4 and 5). ANOVA analysis – In each column and for the same fruit part, different letters mean significant differences among the tested genotypes according to Tukey's HSD test ($p < 0.05$).

(Kulczyński et al., 2020), while the extraction solvent may also affect the extraction efficiency of phenolic compounds (Singh, Singh, Shukla, Rai, & A., 2016). In fact, in a previous study of our team (Leichtweis, Molina, Dias, et al., 2023) the extraction protocol was optimized for three pumpkin genotypes, resulting in significant differences in the extraction yield depending on the extraction parameters (extraction temperature, solvent concentration and extraction time) or the extraction technique (ultrasound-assisted or heat-assisted extraction). In contrast to the present study, Stryjecka, Krochmal-Marczak, Cebulak, and Kiełtyka-Dadasiewicz (2023) detected ten phenolic compounds in the pulp of five *Cucurbita* species; mostly phenolic acids such as syringic, protocatechuic and salicylic acid, while a great variability in individual compounds content was recorded among the species. Moreover, in the same study the amount of catechin was quite low compared to the most abundant phenolic acids. Similar results were reported in two cultivars of *C. pepo* fruit, where the main phenolic compounds were phenolic acids (gallic, p-coumaric, chlorogenic, ferulic and caffeic acid), whereas the main flavonoids (quercetin and kaempferol derivatives) were detected in lower amounts (Kopczyńska et al., 2021). Interestingly, these authors did not observe any differences in phenolic compounds content between the two cultivars, although they suggested that the cropping system (use of organic fertilizers, manure, or mineral fertilizers) had a significant impact on the content of individual compounds. Similarly, Dragovic-Uzelac, Delonga, Levaj, Djakovic, and Pospisil (2005) who studied raw pumpkin (*C. pepo*, *C. maxima* and *C. moschata*) fruit and their puree detected only chlorogenic and syringic acid and low amounts or caffeic and p-coumaric acids. Kulczynski and Gramza-Michałowska (2019) evaluated the chemical composition of the pulp of several pumpkin cultivars belonging to *C. pepo* and *C. moschata* and they detected several phenolic acids and flavonoids in amounts that varied greatly among the cultivars. Salehi et al. (2019) suggested the presence of various phenolic acids and flavonoids, while several studies have reported the phenolic profile of seeds (Eleiwa, Bakr, & Mohamed, 2014; Fa-Sheng et al., 2009). In the same line, Iswaldi et al. (2013) detected nine phenolic acids, nine hydroxybenzoic acids and fifteen flavonoids in the fruit of three zucchini (*C. pepo*) cultivars. Moreover, in a preliminary study by our group (Leichtweis et al., 2022) the extraction of phenolic compounds in the fruit by-products (peels, seeds and fibrous strands) of three pumpkin genotypes resulted in the identification of eight phenolic compounds in quantities that varied depending on the genotype and the part of the fruit. These contrasting results in the literature indicate the variability in phenolic compounds composition which can be influenced by pre- and post-harvest factors such as species, cultivar, agronomic practices, growing conditions and extraction methods. These findings underscore the diverse and valuable composition of compounds found in various parts of pumpkins, as well as the importance of valorizing valuable genetic material through the utilization of local landraces.

3.1.2. Organic acids

The quantification of organic acids in the studied pumpkin by-product is shown in Table 3. It is notable that the studied samples showed considerable variation in the composition of the organic acids analyzed. On average, pumpkin seeds presented malic acid concentrations around 1.00 g/100 g dw, while fibrous strands showed concentrations around 9.46 g/100 g dw of this compound. Meanwhile, oxalic acid ranged from approximately 0.07 g/100 g dw in seeds to 4.97 g/100 g dw in peels. Malic and quinic acids were the most abundant in the seeds and fibrous strands, with concentrations varying between 0.34 ± 0.01 to 2.4 ± 0.1 g/100 g dw and 0. 33 ± 0.01 to 1.76 ± 0.08 g/100 g dw, respectively, in the seeds, and between 5.8 ± 0.3 to 15.2 ± 0.6 and 4.9 ± 0.2 to 12.2 ± 0.6, respectively, in fibrous strands. On the other hand, in the peels quinic acid was detected in just 3 samples at an average amount of approximately 1.928 g/100 g dw. Among these samples, malic acid reached concentrations of up to 7.1 ± 0.2 g/100 g dw in sample "V9 R", followed by oxalic acid, which exhibited concentrations of up to 5.9 ± 0.2 g/100 g dw in sample "V3".

The high content of malic and quinic acids in pumpkin by-products is a significant aspect to consider due to their nutritional implications and potential health benefits. Malic acid is recognized for its detoxifying properties and its crucial involvement in the energy production processes of all cells in the body. Supporting this, Yousefi et al. (2023) conducted a study where they found that supplementing diets of *Oncorhynchus mykiss* with acidic malic acid led to notable improvements in the antioxidant status and immune responses of the fish, without adverse effects on its growth performance. On the other hand, quinic acid, responsible for the characteristic bitter taste of certain foods, has antioxidant, antidiabetic, and anticancer properties, along with potential positive effects on liver health and blood sugar regulation (Benali et al., 2022). Thus, the presence of these compounds in the studied pumpkin by-products in high amounts not only contributes to their organoleptic characteristics, but also adds nutritional value and health beneficially properties.

Regarding citric acid, although it was only detected in the seeds of "V8", and the fibrous strands of "V2 C", it was also found in 10 out of the 16 peel samples, ranging from 0.38 ± 0.02 to 2.2 ± 0.1 g/100 g dw (Table 3). According to the literature, evaluating the organic acid content in eight pumpkin cultivars, Nawirska-Olszańska et al. (2014) found that at post-harvest the average citric acid content was 1.62 g/kg fresh

Table 3
Quantification of the organic acids in pumpkin by-products (seeds, fibrous strands and peels) of the studied genotypes (mean ± SD).

Pumpkin by-product	Genotype	Oxalic g/100 g dw	Quinic g/100 g dw	Malic g/100 g dw	Ascorbic g/100 g dw	Shikinic g/100 g dw	Citric g/100 g dw	Fumaric mg/100 g dw
Seeds	V1	0.051 ± 0.002 ^{f}	1.76 ± 0.08 ^{a}	0.34 ± 0.01 ^{f}	n.d.	n.d.	n.d.	0.017 ± 0.001 ^{b}
	V2	0.029 ± 0.001 ^{g,h}	0.33 ± 0.01 ^{h}	n.d.	n.d.	tr.	n.d.	0.0110 ± 0.0001 ^{d,e}
	V3	0.117 ± 0.002 ^{c}	1.13 ± 0.05 ^{d}	0.97 ± 0.05 ^{d}	n.d.	tr.	n.d.	0.010 ± 0.001 ^{e}
	V4	0.017 ± 0.001 ^{i}	n.d.	n.d.	n.d.	tr.	n.d.	0.0093 ± 0.0003 ^{f}
	V5	0.025 ± 0.001 ^{h}	0.40 ± 0.02 ^{g}	0.99 ± 0.05 ^{d}	n.d.	n.d.	n.d.	0.00649 ± 0.00003 ^{g}
	V6	0.093 ± 0.003 ^{d}	0.68 ± 0.03 ^{e}	2.4 ± 0.1 ^{a}	n.d.	0.063 ± 0.003	n.d.	0.014 ± 0.001 ^{c}
	V7	0.13 ± 0.01 ^{b}	1.32 ± 0.05 ^{b}	1.08 ± 0.05 ^{c}	n.d.	tr.	n.d.	0.0132 ± 0.0001 ^{c}
	V8	0.032 ± 0.001 ^{g}	0.53 ± 0.02 ^{f}	0.64 ± 0.02 ^{e}	n.d.	tr.	0.18 ± 0.01	0.0096 ± 0.0004 ^{f}
	V9	0.14 ± 0.01 ^{a}	1.20 ± 0.06 ^{c}	1.185 ± 0.002 ^{b}	n.d.	n.d.	n.d.	0.01846 ± 0.00003 ^{a}
	V10	0.060 ± 0.002 ^{e}	0.66 ± 0.03 ^{e}	0.37 ± 0.01 ^{f}	n.d.	tr.	n.d.	0.01125 ± 0.00001 ^{d}
Fibrous strands	V1	2.9 ± 0.1 ^{g,h}	8.2 ± 0.4 ^{c}	5.8 ± 0.3 ^{f}	n.d.	n.d.	n.d.	0.220 ± 0.003 ^{k}
	V2 T	2.628 ± 0.004 ^{i,j}	n.d.	15.2 ± 0.6 ^{a}	n.d.	n.d.	n.d.	0.72 ± 0.01 ^{a}
	V2 C	4.44 ± 0.09 ^{c}	n.d.	12.2 ± 0.6 ^{b}	n.d.	n.d.	2.8 ± 0.1	0.44 ± 0.01 ^{h}
	V3	4.75 ± 0.08 ^{b}	9.3 ± 0.3 ^{b}	8.6 ± 0.4 ^{d}	n.d.	n.d.	n.d.	0.394 ± 0.004 ^{i}
	V4 C	3.95 ± 0.09 ^{f}	7.8 ± 0.4 ^{c,d}	10.4 ± 0.4 ^{c}	n.d.	n.d.	n.d.	0.612 ± 0.002 ^{c}
	V4 R	4.455 ± 0.001 ^{c}	9.6 ± 0.5 ^{b}	11.83 ± 0.07 ^{b}	n.d.	n.d.	n.d.	0.61 ± 0.01 ^{c}
	V5 F	3.12 ± 0.09 ^{g}	7.4 ± 0.1 ^{d,e}	7.9 ± 0.4 ^{e}	n.d.	n.d.	n.d.	0.47 ± 0.01 ^{f}
	V5 R	2.519 ± 0.001 ^{j}	7.5 ± 0.4 ^{d,e}	9.2 ± 0.4 ^{d}	n.d.	n.d.	n.d.	0.488 ± 0.003 ^{e}
	V6	2.73 ± 0.04 ^{i}	4.9 ± 0.2 ^{f}	7.8 ± 0.4 ^{e}	n.d.	n.d.	n.d.	0.551 ± 0.003 ^{d}
	V7 P	2.79 ± 0.04 ^{h,i}	7.0 ± 0.3 ^{e}	6.40 ± 0.07 ^{f}	n.d.	0.025 ± 0.001 ^{a}	n.d.	0.284 ± 0.001 ^{j}
	V7 F	4.16 ± 0.08 ^{d,e}	9.7 ± 0.3 ^{b}	8.99 ± 0.09 ^{d}	n.d.	n.d.	n.d.	0.48 ± 0.01 ^{e,f}
	V8	3.93 ± 0.07 ^{f}	n.d.	7.6 ± 0.1 ^{e}	n.d.	0.021 ± 0.001 ^{b}	n.d.	0.479 ± 0.002 ^{e}
	V9 C	5.0 ± 0.2 ^{a}	12.2 ± 0.6 ^{a}	11.6 ± 0.2 ^{b}	n.d.	n.d.	n.d.	0.457 ± 0.004 ^{g}
	V9 R	4.01 ± 0.01 ^{e,f}	9.1 ± 0.4 ^{b}	10.7 ± 0.2 ^{c}	n.d.	n.d.	n.d.	0.608 ± 0.002 ^{c}
	V10	4.3 ± 0.1 ^{c,d}	9.28 ± 0.03 ^{b}	11.5 ± 0.5 ^{b}	n.d.	n.d.	n.d.	0.433 ± 0.004 ^{h}
Peels	V11	1.00 ± 0.02 ^{k}	n.d.	9.00 ± 0.04 ^{d}	n.d.	n.d.	n.d.	0.631 ± 0.003 ^{b}
	V1	4.89 ± 0.07 ^{d,e}	n.d.	4.5 ± 0.2 ^{d}	tr.	n.d.	n.d.	n.d.
	V2 T	5.08 ± 0.02 ^{d}	n.d.	2.61 ± 0.03 ^{j,k}	tr.	tr.	0.92 ± 0.04 ^{e}	0.012 ± 0.001 ^{e,f}
	V2 C	4.9 ± 0.1 ^{d}	n.d.	2.4 ± 0.1 ^{k}	n.d.	n.d.	n.d.	0.012 ± 0.001 ^{e,f}
	V3	5.9 ± 0.2 ^{a}	n.d.	3.0 ± 0.1 ^{i}	tr.	n.d.	1.03 ± 0.05 ^{d}	0.013 ± 0.001 ^{d,e,f}
	V4 C	5.76 ± 0.05 ^{a}	n.d.	3.3 ± 0.2 ^{g,h}	tr.	n.d.	1.13 ± 0.05 ^{c}	n.d.
	V4 R	5.7 ± 0.3 ^{a,b}	n.d.	4.07 ± 0.04 ^{e}	tr.	tr.	n.d.	0.026 ± 0.001 ^{c}
	V5 F	4.06 ± 0.02 ^{f}	2.15 ± 0.09 ^{a}	3.6 ± 0.2 ^{f}	tr.	n.d.	0.571 ± 0.005 ^{g}	0.01211 ± 0.00001 ^{e,f}
	V5 R	2.93 ± 0.01 ^{g}	n.d.	2.8 ± 0.1 ^{i,j}	n.d.	n.d.	0.38 ± 0.02 ^{h}	0.014 ± 0.001 ^{d,e,f}
	V6	5.43 ± 0.04 ^{c}	n.d.	3.10 ± 0.02 ^{h,i}	n.d.	tr.	1.06 ± 0.05 ^{c,d}	0.0106 ± 0.0001 ^{f}
	V7 P	4.93 ± 0.09 ^{d}	n.d.	5.5 ± 0.2 ^{b}	tr.	n.d.	0.62 ± 0.03 ^{f,g}	0.017 ± 0.001 ^{d}
	V7 F	4.7 ± 0.2 ^{e}	1.65 ± 0.01 ^{c}	5.0 ± 0.2 ^{c}	tr.	tr.	1.81 ± 0.03 ^{b}	0.016 ± 0.001 ^{d,e}
	V8	5.51 ± 0.06 ^{b,c}	n.d.	3.53 ± 0.06 ^{f,g}	tr.	0.0016 ± 0.0001	n.d.	0.014 ± 0.001 ^{d,e,f}
	V9 C	5.48 ± 0.04 ^{c}	n.d.	5.1 ± 0.3 ^{c}	n.d.	n.d.	n.d.	0.38 ± 0.01 ^{a}
	V9 R	4.99 ± 0.02 ^{d}	n.d.	7.1 ± 0.2 ^{a}	n.d.	n.d.	0.66 ± 0.03 ^{f}	0.17 ± 0.01 ^{b}
	V10	5.39 ± 0.04 ^{c}	n.d.	2.40 ± 0.09 ^{k}	tr.	tr.	n.d.	0.012 ± 0.001 ^{e,f}
	V11	4.26 ± 0.03 ^{f}	1.98 ± 0.09 ^{b}	4.49 ± 0.02 ^{d}	n.d.	tr.	2.2 ± 0.1 ^{a}	0.015 ± 0.001 ^{d,e,f}

weight (fw), while malic acid had a higher concentration, averaging 3.34 g/kg fw. In contrast, fumaric acid was present in comparatively lower amounts, averaging 0.22 g/kg fw. Furthermore, in melon (*Cucumis melo* L.) cultivars, malic acid was found at an average of 1.31 mg/g without significant variation, while citric acid ranged from 5.15 to 1.19 mg/g, in the melon cultivar *Yuniang* grafted onto different pumpkin rootstocks (Kaleem et al., 2022).

To a lesser extent, the presence of fumaric acid was found in average amounts of 0.012 mg/100 g dw for seeds, 0.479 mg/100 g dw for fibrous strands and 0.05 mg/100 g dw for peels (Table 3). Shikimic acid was detected in very low or only trace amounts in some samples, while ascorbic acid was identified only in trace amounts in some samples (Table 3).

n.d. – not detected. tr. – traces. ANOVA analysis – In each column and for the same fruit part, different letters mean significant differences among the tested genotypes according to Tukey's HSD test ($p < 0.05$).

3.1.3. Tocopherols

In Table 4, the tocopherol content in various pumpkin by-products is presented. The profiles of these compounds were more similar between the seeds and peels, with *gamma*-tocopherol being the most prominent vitamin E isoform for almost all the samples, followed by *alpha*-tocopherol, while *beta*- and *delta*-tocopherols were found only in a few samples. In fibrous strands, *gamma*-tocopherol was detected in just 5 out of the 16 studied samples (e.g. "V4 C", "V6", "V7 P", "V8", and "V10") in low concentrations, whereas *alpha*-tocopherol was the most prevalent compound in these samples.

Regarding the seeds, the total tocopherol content ranged from 4.35 ± 0.03 mg/100 g dw in "V10" to 14.58 ± 0.09 mg/100 g dw in "V8", with an average amount of *gamma*-tocopherol of 6.76 mg/100 g dw and an average amount of *alpha*-tocopherol of 1.17 mg/100 g dw. Concerning the peels, the average content of *gamma*-tocopherol was 9.73 mg/100 g dw, varying from 24.1 in "V2 T" and "V10" (with no significant difference, $p > 0.05$) to 0.82 ± 0.02 mg/100 g dw in "V9" and 0.70 ± 0.03 mg/100 g dw in "V4 C" ($p > 0.05$). The *alpha*- and total tocopherol average content in these samples was, 1.43 and 11.28 mg/100 g dw, respectively. Meanwhile, in the fibrous strands, the *alpha*-tocopherol average content was 2.95 mg/100 g dw, and the average total tocopherol content was 3.20 mg/100 g dw, ranging from 0.077 ± 0.001 to 7.38 ± 0.03 mg/100 g dw in the samples "V5 F" and "V7 P", respectively.

In our previous study (Leichtweis et al., 2023), we observed that *alpha*-tocopherol was the predominant form of vitamin E in the pulp of these pumpkin genotypes, ranging from 0.218 ("V7 F") to 4.90 mg/100 g dw ("V2 T"), followed by *beta*- and *gamma*-tocopherols. In their review, Dotto and Chacha (2020) summarized several studies on pumpkin seed

Table 4
Quantification of tocopherol content in pumpkin by-products (seeds, fibrous strands and peels) of the studied genotypes expressed in mg/100 g dw (mean ± SD).

Pumpkin by-product	Genotype	*alpha*-tocopherol	*beta*-tocopherol	*gamma*-tocopherol	*delta*-tocopherol	Total tocopherols
Seeds	V1	0.1028 ± 0.0003 ^{g}	n.d.	6.5 ± 0.1 ^{f}	n.d.	6.6 ± 0.1 ^{f}
	V2	2.03 ± 0.04 ^{d}	n.d.	3.02 ± 0.06 ^{g}	n.d.	5.0 ± 0.1 ^{g}
	V3	0.371 ± 0.004 ^{e}	n.d.	12.3 ± 0.2 ^{b}	n.d.	12.7 ± 0.2 ^{b}
	V4	2.10 ± 0.06 ^{c}	n.d.	9.0 ± 0.2 ^{d}	n.d.	11.0 ± 0.3 ^{c}
	V5	2.98 ± 0.09 ^{a}	n.d.	0.86 ± 0.02 ^{j}	n.d.	3.8 ± 0.1 ^{i}
	V6	0.116 ± 0.002 ^{g}	n.d.	8.1 ± 0.2 ^{e}	n.d.	8.2 ± 0.2 ^{e}
	V7	0.37 ± 0.02 ^{e}	n.d.	9.89 ± 0.07 ^{c}	n.d.	10.26 ± 0.06 ^{d}
	V8	0.21 ± 0.01 ^{f}	n.d.	14.20 ± 0.08 ^{a}	0.169 ± 0.006	14.58 ± 0.09 ^{a}
	V9	n.d.	n.d.	1.75 ± 0.03 ^{i}	n.d.	1.75 ± 0.03 ^{j}
	V10	2.29 ± 0.03 ^{b}	n.d.	2.057 ± 0.004 ^{h}	n.d.	4.35 ± 0.03 ^{h}
Peels	V1	0.110 ± 0.001 ^{l}	n.d.	4.44 ± 0.09 ^{j}	0.343 ± 0.005 ^{c}	4.89 ± 0.09 ^{k}
	V2 T	0.55 ± 0.02 ^{h}	n.d.	24.1 ± 0.2 ^{a}	n.d.	24.6 ± 0.2 ^{b}
	V2 C	1.486 ± 0.001 ^{e}	n.d.	13.78 ± 0.04 ^{d}	n.d.	15.27 ± 0.04 ^{f}
	V3	1.35 ± 0.02 ^{f}	0.228 ± 0.008 ^{a}	5.49 ± 0.01 ^{h}	0.56 ± 0.08 ^{b}	7.62 ± 0.06 ^{h}
	V4 C	0.24 ± 0.01 ^{k}	n.d.	0.70 ± 0.03 ^{m}	n.d.	0.94 ± 0.04 ^{n}
	V4 R	1.663 ± 0.001 ^{d}	n.d.	3.40 ± 0.03 ^{l}	n.d.	5.07 ± 0.03 ^{k}
	V5 F	0.389 ± 0.003 ^{i}	n.d.	3.96 ± 0.01 ^{k}	n.d.	4.35 ± 0.01 ^{l}
	V5 R	1.002 ± 0.004 ^{g}	n.d.	5.03 ± 0.1 ^{i}	n.d.	6.3 ± 0.1 ^{i}
	V6	0.61 ± 0.02 ^{h}	n.d.	19.0 ± 0.2 ^{c}	n.d.	19.6 ± 0.2 ^{d}
	V7 P	0.19 ± 0.01 ^{k}	n.d.	5.98 ± 0.05 ^{g}	n.d.	6.17 ± 0.04 ^{i}
	V7 F	0.227 ± 0.001 ^{k}	n.d.	5.59 ± 0.02 ^{h}	n.d.	5.81 ± 0.03 ^{j}
	V8	1.02 ± 0.02 ^{g}	n.d.	0.82 ± 0.02 ^{m}	n.d.	1.83 ± 0.03 ^{m}
	V9 C	4.178 ± 0.004 ^{c}	0.116 ± 0.002 ^{b}	13.20 ± 0.01 ^{e}	0.26 ± 0.02 ^{d}	17.76 ± 0.02 ^{e}
	V9 R	4.71 ± 0.02 ^{b}	n.d.	8.97 ± 0.01 ^{f}	n.d.	13.68 ± 0.03 ^{g}
	V10	5.9 ± 0.1 ^{a}	n.d.	24.1 ± 0.1 ^{a}	0.682 ± 0.001 ^{a}	30.7 ± 0.2 ^{a}
	V11	0.362 ± 0.001 ^{i,j}	n.d.	20.55 ± 0.09 ^{b}	n.d.	20.91 ± 0.09 ^{c}
Fibrous strands	V1	4.29 ± 0.06 ^{e}	0.303 ± 0.006 ^{b}	n.d.	n.d.	4.59 ± 0.07 ^{d}
	V2 T	4.37 ± 0.01 ^{d}	n.d.	n.d.	n.d.	4.37 ± 0.01 ^{e}
	V2 C	0.529 ± 0.003 ^{m}	0.62 ± 0.03 ^{a}	n.d.	n.d.	1.15 ± 0.02 ^{k}
	V3	6.03 ± 0.01 ^{b}	n.d.	n.d.	n.d.	6.03 ± 0.01 ^{b}
	V4 C	1.61 ± 0.01 ^{k}	n.d.	0.254 ± 0.003 ^{e}	n.d.	1.86 ± 0.01 ^{j}
	V4 R	2.18 ± 0.01 ^{i}	n.d.	n.d.	n.d.	2.18 ± 0.001 ^{i}
	V5 F	0.077 ± 0.001 ^{o}	n.d.	n.d.	n.d.	0.077 ± 0.001 ^{m}
	V5 R	0.391 ± 0.001 ^{n}	n.d.	n.d.	n.d.	0.391 ± 0.001 ^{l}
	V6	1.91 ± 0.02 ^{i}	n.d.	0.76 ± 0.01 ^{b}	n.d.	2.67 ± 0.01 ^{h}
	V7 P	6.83 ± 0.03 ^{a}	n.d.	0.554 ± 0.001 ^{d}	n.d.	7.38 ± 0.03 ^{a}
	V7 F	5.56 ± 0.06 ^{c}	n.d.	n.d.	n.d.	5.56 ± 0.06 ^{c}
	V8	2.30 ± 0.03 ^{h}	n.d.	1.18 ± 0.04 ^{a}	n.d.	3.48 ± 0.01 ^{g}
	V9 C	1.87 ± 0.06 ^{j}	n.d.	n.d.	n.d.	1.87 ± 0.06 ^{j}
	V9 R	2.70 ± 0.01 ^{g}	n.d.	n.d.	n.d.	2.70 ± 0.01 ^{h}
	V10	1.25 ± 0.01 ^{l}	n.d.	0.589 ± 0.003 ^{c}	n.d.	1.83 ± 0.01 ^{j}
	V11	4.20 ± 0.06 ^{f}	n.d.	n.d.	n.d.	4.20 ± 0.06 ^{f}

n.d. – not detected. ANOVA analysis – In each column and for the same fruit part, different letters mean significant differences among the tested genotypes according to Tukey's HSD test ($p < 0.05$).

oil and reported tocopherols content in the following proportions: *alpha*-tocopherol at 0.204–35.300 mg/100 g; *beta*-tocopherol at 0.54 mg/100 g; *gamma*-tocopherol at 9.715–89.300 mg/100 g, and *delta*-tocopherol at 0.232–2.25 mg/100 g of oil. In the exocarp of summer squash fruit of four genotypes, tocopherols were found in two forms, *alpha*- and *gamma*-tocopherol, with the latter being the predominant one (Rodov et al., 2020). Additionally, in pumpkin fruit from five cultivars of *C. moschata* Duch., the seeds presented *alpha*-tocopherol content which ranged from 3.76 ± 0.48 to 5.65 ± 0.25 mg/100 g, while *beta*- and *gamma*-tocopherol content ranged from 35.83 ± 0.45 to 47.69 ± 0.43 mg/100 g. Moreover, the seed kernels exhibited *alpha*-tocopherol content ranging from 2.39 ± 0.56 to 4.19 ± 0.2 mg/100 g, and *beta*- and *gamma*-tocopherols content ranging from 28.82 ± 0.29 to 33.37 ± 0.39 mg/100 g (A. Singh & Kumar, 2022b).

This analysis of the tocopherol profile and content in different parts of pumpkin fruit by-products is important to characterize their nutritional profile and bioactive properties. Tocopherol, especially *alpha*-tocopherol, is known for its antioxidant properties, which play a crucial role in protecting against oxidative stress and combating cellular damage caused by free radicals (Rozanowska et al., 2019). The predominant presence of the *gamma*-tocopherol isoform, followed by *alpha*-tocopherol, suggests significant potential for the health benefits of these pumpkin by-products, as these forms of tocopherol have demonstrated significant antioxidant and anti-inflammatory benefits in previous studies (Polyzos et al., 2022; Rozanowska et al., 2019; Singh & Kumar, 2022a). Furthermore, variation in tocopherol contents between different parts of pumpkin fruit may influence the formulation of food products and nutritional supplements that provide these bioactive compounds aiming to improve health and prevent diseases related to oxidative stress.

3.2. Bioactivities

3.2.1. Antioxidant capacity

The antioxidant activity of the hydroethanolic extracts of the peels, seeds, and fibrous strands of the studied genotypes was determined by two in vitro assays, namely the lipid peroxidation inhibition assay (TBARS) and the oxidative hemolysis inhibition assay (OxHLIA), and the results are presented in Table 5. In the TBARS assay, all samples revealed important antioxidant activity, especially the seeds of genotypes "V5" and "V6" which presented activity (IC_{50}: 208 ± 9 and 125 ± 1 μg/mL, respectively) similar ($p > 0.05$) to the positive control Trolox (IC_{50}: 139 ± 5 μg/mL). In addition, the seeds of the rest of the genotypes also stood out compared to the peels and fibrous strands of the respective

Table 5
Lipid peroxidation inhibition capacity (TBARS) and oxidative hemolysis inhibition capacity (OxHLIA) of pumpkin by-products (seeds, fibrous strands and peels) of the studied genotypes expressed in IC_{50}; μg/mL; Δt = 60 min for OxHLIA; (mean ± SD).

Genotype	TBARS			OxHLIA		
	Deffated seeds	Fibrous strands	Peels	Deffated seeds	Fibrous strands	Peels
V1	1133 ± 54 ^{b}	1312 ± 51 ^{j}	1589 ± 63 ^{h}	52 ± 2 ^{g,h}	210 ± 9 ^{f}	475 ± 24 ^{e}
V2	640 ± 28 ^{d}	n.a.	n.a.	101 ± 3 ^{f}	n.a.	n.a.
V2 T	n.a.	1998 ± 97 ^{e}	825 ± 25 ^{l}	n.a.	168 ± 6 ^{g}	822 ± 34 ^{a}
V2 C	n.a.	3054 ± 142 ^{c}	850 ± 40 ^{k,l}	n.a.	390 ± 5 ^{d}	555 ± 8 ^{d}
V3	434 ± 7 ^{f}	3900 ± 165 ^{a}	885 ± 18 ^{j,k,l}	171 ± 5 ^{e}	638 ± 12 ^{a}	294 ± 11 ^{f}
V4	468 ± 12 ^{e}	n.a.	n.a.	431 ± 9 ^{b}	n.a.	n.a.
V4 C	n.a.	1723 ± 62 ^{h}	1285 ± 13 ^{i}	n.a.	128 ± 5 ^{h,i}	755 ± 33 ^{b}
V4 R	n.a.	1825 ± 84 ^{f,g}	4984 ± 221 ^{b}	n.a.	106 ± 6 ^{i}	197 ± 12 ^{g,h}
V5	208 ± 9 ^{h}	n.a.	n.a.	84 ± 3 ^{f,g}	n.a.	n.a.
V5 F	n.a.	3023 ± 126 ^{c}	1963 ± 94 ^{f}	n.a.	132 ± 9 ^{h}	219 ± 8 ^{g}
V5 R	n.a.	1739 ± 83 ^{g,h}	985 ± 11 ^{j}	n.a.	123 ± 2 ^{h,i}	98 ± 4 ^{j}
V6	125 ± 1 ^{i}	1911 ± 62 ^{e,f}	4973 ± 84 ^{b}	315 ± 14 ^{c}	393 ± 18 ^{d}	243 ± 17 ^{g}
V7	1007 ± 51 ^{c}	n.a.	n.a.	795 ± 31 ^{a}	n.a.	n.a.
V7 P	n.a.	630 ± 25 ^{l}	953 ± 21 ^{j,k}	n.a.	104 ± 6 ^{i}	612 ± 25 ^{c}
V7 F	n.a.	2267 ± 90 ^{d}	2749 ± 127 ^{e}	n.a.	348 ± 8 ^{e}	41 ± 2 ^{k}
V8	405 ± 19 ^{g}	3059 ± 141 ^{c}	5733 ± 260 ^{a}	289 ± 14 ^{c}	140 ± 5 ^{h}	427 ± 8 ^{e}
V9	1771 ± 59 ^{a}	n.a.	n.a.	409 ± 23 ^{b}	n.a.	n.a.
V9 C	n.a.	1645 ± 39 ^{h,i}	1796 ± 62 ^{g}	n.a.	359 ± 11 ^{e}	122 ± 2 ^{i,j}
V9 R	n.a.	1610 ± 59 ^{i}	1710 ± 84 ^{g}	n.a.	444 ± 16 ^{c}	157 ± 7 ^{h,i}
V10	640 ± 31 ^{d}	1188 ± 53 ^{k}	4216 ± 204 ^{c}	241 ± 10 ^{d}	371 ± 4 ^{d,e}	441 ± 18 ^{e}
V11	n.a.	3768 ± 190 ^{b}	3085 ± 135 ^{d}	n.a.	485 ± 7 ^{b}	139 ± 13 ^{i,j}
Trolox	139 ± 5 ^{h,i}	139 ± 5 ^{m}	139 ± 5 ^{m}	21.8 ± 0.2 ^{h}	21.8 ± 0.2 ^{j}	21.8 ± 0.2 ^{k}

n.a. – not applicable. ANOVA analysis – In each column and for the same fruit part, different letters mean significant differences among the tested genotypes according to Tukey's HSD test ($p < 0.05$).

genotypes, with IC_{50} values varying from approximately 1 to 10 times higher than the positive control. On the other hand, the samples the peels of genotypes "V2 T" and V2 C" recorded higher antioxidant activity compared to the other fruit parts (e.g IC_{50}: 825 ± 25 μg/mL and 850 ± 40 μg/mL, respectively). Finally, fibrous strands and peels showed IC_{50} values ranging from 5 to 28 times higher than Trolox, thus demonstrating that the studied by-products, especially the seeds, possess a significant capacity to inhibit lipid peroxidation.

In the OxHLIA assay, a varied response was recorded depending on the fruit part and the genotype tested. The highest and lowest antihemolytic activity was recorded for the peels of genotypes "V7 F" (IC_{50}: 41 ± 2 μg/mL) and "V2 T" (IC_{50}: 822 ± 34 μg/mL), respectively. Moreover, fibrous strands recorded higher antioxidant activity than the other two by-products in 7 out of 16 samples, followed by seeds (4 out of 10 samples) and peels (6 out of 16 samples).

The bioactive properties, particularly the antioxidant activity, of pumpkins have been well documented in the literature (Abbas et al., 2020; Diop et al., 2020; Martínez, Valenzuela, & Jamilena, 2021). Salehi et al. (2021) conducted a comprehensive review focusing on the antioxidant potential of the Cucurbitaceae family, with special attention to the genus *Cucurbita*. In addition to discussing the anti-inflammatory, antidiabetic, anticarcinogenic and hepatoprotective activities, the authors compiled several studies demonstrating antioxidant activity of *Cucurbita* spp. through in vitro and in vivo assays. It has to be noted that most in vitro analyses used chemical methodologies, notably DPPH (2,2-diphenyl-1-picrylhydrazyl), which makes the direct comparison with the results presented here cumbersome. Furthermore, the utilization of cell-based methods in the current study proves to be highly valuable for pumpkin research, as these assays offer a significant complementary approach for assessing the antioxidant activity of vegetable extracts in a biologically relevant context. Moreover, they are still relatively underexplored for this particular matrix which highlights the innovative aspect of the present study.

Apart from the variable response to the antioxidant activity observed through different methodologies, the variation in antioxidant capacity between different pumpkin genotypes has also been reported by other authors. When evaluating 18 pumpkin cultivars belonging to the species *C. maxima* Duch., *C. moschata* Duch., *C. pepo* L., and *C. ficifolia* Bouché, using DPPH, FRAP (ferric reducing antioxidant power) and CUPRAC (cupric ion reducing antioxidant capacity) assays, Kostecka-Gugała et al. (2020) reported a high intra-species variability. In particular, the results (expressed in μmol Trolox per 100 g of fresh weight) ranged from 1.01 ± 0.03 to 32.46 ± 1.06 for DPPH, from 11.7 ± 1.7 to 139.9 ± 13 for FRAP, and from 64.3 ± 3.6 to 256.6 ± 2.6 for CUPRAC assay. Moreover, the differences observed among the different parts of the pumpkin in this study are consistent with findings reported by Hussain et al. (2021). The authors compared the DPPH radical scavenging activity of peel, flesh, and seed powders extracted from a commercially available variety from Sargodha, Pakistan. The results indicated antioxidant capacities of 13.00 ± 0.08, 10.58 ± 0.06, and 16.53 ± 0.09 mg of ascorbic acid equivalent (AAE) per 100 g for peel, flesh, and seeds, respectively, thus indicating that fruit part may also affect the antioxidant capacity due to the variability in the content and the profile of bioactive compounds.

3.2.2. Antimicrobial capacity

Extracts obtained from vegetable by-products such as seeds, leaves, peels, etc., have been of great interest as a defence against microorganisms in different industries (Añibarro-Ortega et al., 2019; Oliveira et al., 2023). In this study, antibacterial and antifungal analysis ~~was~~were developed using 8 bacterial and 2 fungal strains of importance in the food industry, and the detailed results are provided in the supplementary material (Tables B, C and D). None of the samples showed bactericidal nor fungicidal capacity, meaning that none of the samples could kill the fungi nor the bacteria tested; therefore, all the activities discussed below refer to growth inhibition.

Among all the 42 samples (16 samples of peels and fibrous strands and 10 samples of seeds), the most affected bacteria were *Escherichia coli* and *Salmonella enterica*, with 31 samples showing activity against each of these strains, followed by *Staphylococcus aureus*, that was inhibited by 30 samples, *Enterobacter cloacae* (25 samples), and *Yersinia enterocolitica* (24 samples), whereas *Pseudomonas aeruginosa* was the most resistant bacteria against pumpkin extracts, where only fibrous strands of

genotype "V10" and peels of genotypes "V2 T", "V2 C", and "V3" were able to inhibit them.

Regarding the different types of pumpkin by-products, all the seed samples were effective against the *E. cloacae*, *S. enterica*, *Y. enterocolitica*, and *Listeria monocytogenes* growth, showing microbial inhibition concentrations (MIC) of 5 mg/mL, 10 mg/mL and 5–10 mg/mL, respectively. It is also worth to highlight the seeds of "V1", "V2", "V3" and "V4" genotypes, which presented the lowest MIC value of 2.5 mg/mL, against *Y. enterocolitica*. Concerning the peels, all samples, except those of "V7 P" genotype, were capable of inhibiting the *E. coli* growth, 12 samples inhibited *S. aureus*, and 9 were effective against *E. cloacae* growth. Regarding the peel samples, the "V2 T" and "V11" genotypes stood out, presenting an MIC of 2.5 mg/mL against 4 and 5 of the 8 strains evaluated, respectively. Meanwhile, none of the fibrous strands samples exhibited significant antibacterial activity, with the lowest MIC value (5 mg/Ml) being observed in genotype "V11" against the growth of *E. coli*. In fact, fibrous strands were, in general, less effective against the microorganisms tested, where none of the samples was able to inhibit the growth of *Bacillus cereus* and L. *monocytogenes*. On the other hand, these by-products showed greater effectiveness against *S. enterica* compared to the peels, and against *E. coli* compared to the seeds.

Regarding fungal growth inhibition, the peels have also exhibited the greatest overall effectiveness compared to the other by-products. While all peels and all fibrous strands samples inhibited the growth of at least one fungus, 14 peel samples were effective against both fungi tested, compared to only 5 fibrous strands samples. With the exception of the peels of "V9 C", "V7 P" and "V5 R" genotypes, all the rest of the peel samples were effective against *Aspergillus brasiliensis* with MIC values of 5 mg/mL, especially the peels of genotype "V11", which inhibited both *A. brasiliensis* and *A. fumigatus* at this concentration. On the other hand, only the seeds from "V7", "V8", "V9" and "V10" genotypes showed antifungal activity, against *A. brasiliensis* at MIC of 10 mg/mL.

These results corroborate other studies available in the literature where different pumpkin extracts also showed antimicrobial activity. The antimicrobial potential of pumpkin (*C. pepo*) was explored through the extraction of its fruit body and leaves using aqueous and methanol solutions. In the fruit body extract, antimicrobial activity was observed against 6 bacteria and 4 fungi using the agar well diffusion method. Notably, all extracts exhibited antimicrobial effects, with significant inhibition zones of 23 mm observed against *Escherichia coli*, 22 mm against *S. aureus*, and 20 mm against *Klebsiella pneumoniae* (Dubey, Mishra, & Singh, 2010). Similarly, the methanolic extract of *C. pepo* leaves was tested against 29 bacterial strains, revealing inhibitory effects against 28 bacteria with MIC values ranging from 128 to 1024 μg/ml. Additionally, bactericidal activity was observed against 17 bacterial strains, with MBC values ranging from 256 to 1024 μg/ml (Noumedem et al., 2013). Moreover, the peel and seeds of *C. pepo* also exhibited antimicrobial activity. In particular, ethanolic extracts from both seeds and peels showed activity against *S. aureus* and *Salmonella typhi*, whereas methanolic extracts were effective against *S. aureus* at a concentration of 500 μg/disc (Chonoko & Rufai, 2011). Furthermore, pumpkin seeds oil demonstrated inhibitory effects against the growth of 5 bacteria and 4 fungi, with MIC values ranging from 0.5 to 3.0 mg/mL (EI-Aziz & EI-Kalek, 2011). Although these results are not directly comparable, it is possible to conclude the remarkable activity presented by extracts obtained from pumpkin fruit parts.

3.2.3. Cytotoxic activities

None of the tested samples of peel and fibrous strands showed cytotoxic activities up to 400 μg/mL. Among the seed samples, those of the genotypes "V1", "V9", and "V10" exhibited activity against all the cell lines tested, while "V2", "V3", and "V8" genotypes affected all cell lines except VERO (Table 6). Therefore, it could be suggested that these samples were capable to inhibit the proliferation of tumour cells without affecting normal liver cells. The best activities were presented by seeds of "V2" genotype against AGS, and "V9" and "V10" genotypes against MCF-7, at concentrations of 59.2 ± 0.8, 59 ± 5, and 58 ± 2 μg/mL respectively, thus highlighting that hepatotoxicity was detected at higher concentrations. Also "V4" seeds recorded GI_{50} values of 140 ± 1 μg/mL against MCF-7, without presenting hepatotoxicity up to 400 μg/mL against both VERO and PLP2 cell lines.

Table 6
Toxicity of different pumpkin seeds expressed in μg/mL.

Genotype	AGS	CaCo2	MCF-7	PLP2	VERO
V1	229 ± 23 ^{a}	173 ± 9 ^{b}	203 ± 18 ^{b}	215 ± 1 ^{b}	233 ± 23 ^{a}
V2	59.2 ± 0.8 ^{e}	148 ± 7 ^{c}	232 ± 3 ^{a}	209 ± 15 ^{b}	n.d.
V3	168 ± 16 ^{d}	161 ± 13 ^{b,c}	240 ± 7 ^{a}	285 ± 8 ^{a}	n.d.
V4	n.d.	n.d.	140 ± 1 ^{c}	n.d.	n.d.
V5	n.d.	n.d.	n.d.	n.d.	n.d.
V6	n.d.	n.d.	n.d.	n.d.	n.d.
V7	n.d.	n.d.	n.d.	n.d.	n.d.
V8	204 ± 18 ^{b,c}	208 ± 9 ^{a}	129 ± 11 ^{c}	191 ± 16 ^{c}	n.d.
V9	223 ± 4 ^{a,b}	90 ± 6 ^{d}	59 ± 5 ^{d}	125 ± 6 ^{d}	206 ± 11 ^{b}
V10	187 ± 16 ^{c,d}	63 ± 1 ^{e}	58 ± 2 ^{d}	72 ± 7 ^{e}	235 ± 6 ^{a}

n.d. – not detected. ANOVA analysis – In each column and for the same fruit part, different letters mean significant differences among the tested genotypes according to Tukey's HSD test ($p < 0.05$).

The lack of toxicity against normal cells and the toxic effects against tumour cells for different parts of pumpkin fruit such as peels, fibrous strands, seeds, and pulp, has been described in the literature (Gaweł-Bęben et al., 2022; Leichtweis et al., 2022; Leichtweis et al., 2023; Piccolella et al., 2019). For example, Shokrzadeh, Azadbakht, Ahangar, Hashemi, and Saeedi Saravi (2010) detected activity of *C. pepo* leaves against two cancer cell lines, e.g. human hepatocarcinoma (HepG2) and human colon carcinoma (CT26), and two normal cell lines, e.g. Chinese hamster ovary (CHO) and hamster fibroblast, in concentrations between 132.6 ± 4.3 to 241.4 ± 9.6 μg/ml. The methanolic extracts of pulp of *Cucurbita maxima* fruit showed a moderate cytotoxic effect against human cervical carcinoma (HeLa) cell line (Krstić et al., 2023). In another study, pumpkin seeds extracts were assessed for the treatment of benign prostatic hyperplasia, being verified the cell growth inhibition of prostate-, breast- and colon cancer cells (Medjakovic et al., 2016). These results highlight the importance of conducting thorough studies on the toxicity of different pumpkin varieties and their fruit parts. They also emphasize the significance of integrating the findings from this research aiming to understand how pumpkin by-product extracts can be valorised through industrial applications.

3.3. Correlation analysis

Correlation analyses indicated statistically significant contributions ($p < 0.05$). In the peel, the compounds *cis*-Chicoric acid (peak 2) and *beta*-tocopherol demonstrated a strong negative correlation with TBARS ($r = -0.9703$ and -0.9969, respectively), indicating that their higher concentration is associated with greater antioxidant activity, reducing lipid peroxidation, while they presented a strong positive correlation with OxHLIA ($r = 0.9805$ and 0.9931, respectively), which suggests specific characteristics in the antioxidant mechanisms of action of these compounds. Quinic acid showed a strong and significant positive correlation with OxHLIA ($r = 0.977$), while, despite being negative, it presented a low correlation with TBARS ($r = -0.5182$). *Beta*-tocopherol was also a highlight in fiber samples, showing a strong positive correlation, in this case with both TBARS ($r = 0.9987$) and OxHLIA ($r = 0.9946$). On the other hand, shikimic acid showed a very strong negative correlation with TBARS ($r = -0.9715$) and OxHLIA ($r = -0.9102$), suggesting an important antioxidant role in this extract. Finally, in

seeds, (−)-epicatechin (peak 1) correlated strongly and negatively with cytotoxicity in AGS cells ($r = -0.9809$), indicating an important influence on the cytotoxic effect of the extract. While the other compounds had low correlation values for the bioactivities of this matrix. Complete correlation values are presented in Table E of the supplemental material.

3.4. Principal components analysis

Principal component analysis (PCA) is widely used to reduce the complexity of multivariate data, as well as to identify patterns and express data in ways that highlight similarities and differences among the evaluated treatments. In our study, the aim was to identify groups of samples with similarities in terms of chemical profile and bioactive properties. The first seven principal components (PCs) were associated with Eigen values higher than 1 and explained 82.9 % of the cumulative variance, with PC1 accounting for 32.7 %, PC2 for 16.0 %, PC3 for 9.1 %, PC4 for 8.1 %, PC5 for 6.8 %, PC6 for 5.6 % and PC7 for 4.7 %.

PC1 was positively correlated with *gamma*-tocopherol, total tocopherols, isorhamnetin-*O*-dideoxyhexosyl-hexoside, kaempferol-*O*-deoxyhexosyl-hexoside, total flaval-3-ols, total flavonoids, and total phenolic compounds content, and negatively correlated with malic acid and (−)-epicatechin content. PC2 was positively correlated to fumaric acid, malic acid, oxalic acid, quinic acid, total organic acids, TBARS, and (−)-epicatechin content. Finally, PC3 was positively correlated to α-tocopherol and *trans*-chicoric acid and negatively correlated to citric acid, isorhamnetin-O-dideoxyhexosyl-hexoside, kaempferol-*O*-deoxyhexosyl-hexoside, and total flavonoids content. These results indicate the correct implementation of the PCA, allowing differentiation between the tested samples depending on genotype and the fruit by-product (e.g. peels, fibrous strands, and seeds). The corresponding scatterplots and loading plots are shown in Fig. A, B, and C of the supplementary material.

The scatterplot (Fig. A, of the supplementary material) shows a clear discrimination of the tested samples based on the fruit part where all the seed samples form a distinct group (group A). Moreover, five more distinct groups were identified, namely group B which included only fibrous strands (F) from genotypes “V1”, “V2” Turbinate fruit, “V3”, “V4” (both Round and Cylindrical fruit), “V5” (both Flattened and Round fruit), “V6”, “V7” Flattened fruit, “V8”, “V9” (both Round and Cylindrical fruit) and “V10”; group C which included only peel samples (P) from genotypes “V4” Round and “V9” Round; group D which included only peel samples from genotypes “V2” Cylindrical and “V9” Cylindrical; group E which included only peel samples from genotypes “V2” Turbinate, “V3”, “V6” and “V7” Pyriform; and group F which included peel samples from genotypes “V1”, “V4” Cylindrical, “V5” Flattened, “V7” Flattened and fibrous strands samples from genotype “V2” Cylindrical. Finally, peel samples from genotypes “V5” Round, “V8” and “V10” and fibrous strands samples from genotype “V7” Pyriform were clearly separated from the rest of the samples. These particular samples are distinguished for its high content of TPC (peel samples of “V5” R) and total tocopherols (peel samples of “V10” and fibrous strands samples of “V7” P), as well as the low content of total tocopherols and low antioxidant activity for TBARS assay (peel samples of “V8”) and high antihemolytic activity (fibrous strands of “V7” P). It also should be noted that samples from different fruit parts (except from group F) are clearly differentiated from each other (e.g. group A included only seeds samples, group B included only fibrous strands samples and groups C, D, E included only peel samples).

Moreover, the loading plot of the first two components also revealed groups of positively correlated variables (Fig. B, of the supplementary material). In particular, the upper left quadrant included quinic acid, malic acid, fumaric acid, shikimic acid, total organic acids, *alfa*-tocopherol, *beta*-tocopherol and peak 1 ((−)-epicatechin); the lower left quadrant included no variables; the upper right quadrant included citric acid, oxalic acid, TBARS, OxHLIA, *delta*-tocopherol, peak 2 (*cis*-chicoric acid), peak 3 (*trans*-chicoric acid), peak 4 (isorhamnetin-*O*-dideoxyhexosyl-hexoside), peak 5 (kaempferol-*O*-deoxyhexosyl-hexoside), total phenolic acids, total flavonoids, total flavan-3-ols, and total phenolic compounds; the lower right quadrant included *gamma*-tocopherol and total tocopherols. Similarly, the loading plot of PC1 and PC3 (Fig. C, of the supplementary material) revealed groups of positively correlated variables, namely the upper left quadrant included peak 1, quinic acid, shikimic acid, *alfa*-tocopherol and total organic acids; the lower left quadrant comprised malic acid, fumaric acid, and *beta*-tocopherol; the upper right quadrant included TBARS, *delta*-tocopherol, *gamma*-tocopherol, total tocopherols, peak 2, peak 3, total phenolic acids, total flavan-3-ols, and total phenolic compounds; and the lower right quadrant included oxalic acid, OxHLIA, peak 4, peak 5, and total flavonoids.

4. Conclusions

The characterization of the studied pumpkin fruit by-products revealed their potential as valuable sources of bioactive compounds. These by-products exhibited significant profiles of phenolic compounds, tocopherols, and organic acids, which not only may contribute to the organoleptic properties of the fruit but also hold important nutraceutical properties. Overall, our findings underscore the promising potential of using various pumpkin parts, including seeds, peels, and fibrous strands, as rich sources of bioactive compounds with significant industrial applications, while a significant genotypic variation was also recorded. The valorisation of these by-products not only offers economic benefits but also contributes to the appreciation of local landraces and sustainable agricultural practices. By capitalizing on these valuable genetic resources, we may not only promote sustainability but also foster a circular economy model, wherein waste is minimized, and natural resources are maximally utilized. Ultimately, our research highlights the importance of recognizing and harnessing the diverse benefits of pumpkin by-products, paving the way for innovative industrial applications and sustainable agricultural practices. However, further research is needed, especially regarding the evaluation of toxic effects of certain seed extracts, to ensure their safe utilization in food products.

CRediT authorship contribution statement

Maria G. Leichtweis: Writing – original draft, Investigation, Formal analysis, Data curation. **Adriana K. Molina:** Writing – original draft, Investigation, Formal analysis. **Maria Inês Dias:** Writing – review & editing, Methodology, Formal analysis. **Ricardo C. Calhelha:** Writing – review & editing, Methodology, Formal analysis. **Tânia C.S.P. Pires:** Writing – review & editing, Methodology, Formal analysis. **Ourania Pavli:** Methodology, Investigation, Formal analysis. **M. Beatriz P.P. Oliveira:** Writing – review & editing, Supervision. **Isabel C.F.R. Ferreira:** Writing – review & editing, Methodology, Investigation. **Spyridon A. Petropoulos:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Lillian Barros:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Carla Pereira:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was financially supported by FCT, P.I., within the scope of the Project PRIMA Section 2 - Multi-topic 2019: PulpIng (PRIMA/0007/2019). Also supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020)

and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); and national funding by FCT, P.I., through the institutional and individual scientific employment program contract for L. Barros (DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002), C. Pereira (DOI: 10.54499/CEECINST/00016/2018/CP1505/CT0010), and M.I. Dias contracts, and M.G. and A.K.M. PhD grants (2020.06706.BD and 2020.06231.BD, respectively).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.143306.

Data availability

Data will be made available on request.

References

Abbas, H. M. K., Huang, H.-X., Huang, W.-J., Xue, S.-D., Yan, S.-J., Wu, T.-Q., … Zhong, Y.-J. (2020). Evaluation of metabolites and antioxidant activity in pumpkin species. *Natural Product Communications, 15*(4). https://doi.org/10.1177/1934578X20920983, 1934578X2092098.

Añibarro-Ortega, M., Pinela, J., Barros, L., Ćirić, A., Silva, S. P., Coelho, E., … Ferreira, I. C. F. R. (2019). Compositional features and bioactive properties of Aloe vera leaf (fillet, mucilage, and rind) and flower. *Antioxidants, 8*(10), 444. https://doi.org/10.3390/ANTIOX8100444

AOAC. (2016). *Official methods of analysis of AOAC international* (20th ed.). Association of Official Analytical Chemists International: Arlington; USA.

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. *Journal of Functional Foods, 5*, 1732–1740. https://doi.org/10.1016/j.jff.2013.07.019

Benali, T., Bakrim, S., Ghchime, R., Benkhaira, N., El Omari, N., Balahbib, A., … Bouyahya, A. (2022). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews, 1–30*. https://doi.org/10.1080/02648725.2022.2122303

Boujemaa, I., El Bernoussi, S., Harhar, H., & Tabyaoui, M. (2020). The influence of the species on the quality, chemical composition and antioxidant activity of pumpkin seed oil. *OCL, 27*, 40. https://doi.org/10.1051/OCL/2020031

Busuioc, A. C., Botezatu, A. V. D., Furdui, B., Vinatoru, C., Maggi, F., Caprioli, G., & Dinica, R. M. (2020). Comparative study of the chemical compositions and antioxidant activities of fresh juices from Romanian Cucurbitaceae varieties. *Molecules, 25*(22), 5468. https://doi.org/10.3390/MOLECULES25225468

Chonoko, U., & Rufai, A. (2011). Phytochemical screening and antibacterial activity of *Cucurbita pepo* (pumpkin) against *Staphylococcus aureus* and *salmonella typhi. Bayero Journal of Pure and Applied Sciences, 4*(1), 145–147. https://doi.org/10.4314/bajopas.v4i1.30

Corrêa, R. C. G., De Souza, A. H. P., Calhelha, R. C., Barros, L., Glamoclija, J., Sokovic, M., … Ferreira, I. C. F. R. (2015). Bioactive formulations prepared from fruiting bodies and submerged culture mycelia of the Brazilian edible mushroom Pleurotus ostreatoroseus singer. *Food & Function, 6*(7), 2155–2164. https://doi.org/10.1039/C5FO00465A

Diop, A., Sarr, S. O., Sall, A. B., Niass, O., Ndiaye, B., & Diop, Y. M. (2020). Nutritional and antioxidant potential of seeds from two Cucurbitaceae species from Senegal. *European Journal of Chemistry, 11*(4), 364–369. https://doi.org/10.5155/eurjchem.11.4.364-369.2046

Dotto, J. M., & Chacha, J. S. (2020). The potential of pumpkin seeds as a functional food ingredient: A review. *Scientific African, 10*, Article e00575. https://doi.org/10.1016/J.SCIAF.2020.E00575

Dowidar, M. F., Ahmed, A. I., & Mohamed, H. R. (2020). The critical nutraceutical role of pumpkin seeds in human and animal health: An updated review. *Zagazig Veterinary Journal, 44511*(2), 199–212. https://doi.org/10.21608/zvjz.2020.22530.1097

Dragovic-Uzelac, V., Delonga, K., Levaj, B., Djakovic, S., & Pospisil, J. (2005). Phenolic profiles of raw apricots, pumpkins, and their purees in the evaluation of apricot nectar and jam authenticity. *Journal of Agricultural and Food Chemistry, 53*(12), 4836–4842. https://doi.org/10.1021/jf040494+

Dubey, A., Mishra, N., & Singh, N. (2010). Antimicrobial activity of some selected vegetables. *International Journal of Applied Biology and Pharmaceutical Technology, 1*(3), 994–999.

Eleiwa, N. Z. H., Bakr, R. O., & Mohamed, S. A. (2014). Phytochemical and pharmacological screening of seeds and fruits pulp of Cucurbita moschata Duchesne cultivated in Egypt. *International journal of Pharmacognosy and Phytochemistry*, 12261–1236. Doi:10.12817/20517858.29.1.27703371.

Fa-Sheng, L., Jing, X., De-Qiang, D., Xiao-Feng, C., Ting-Guo, K., & Xue, K. H. (2009). Structures of new phenolic glycosides from the seeds of Cucurbita Moschata. *Natural Product Communications, 4*(4), 511–512. https://doi.org/10.1177/1934578X0900400413

Gaweł-Bęben, K., Czech, K., Strzępek-Gomółka, M., Czop, M., Szczepanik, M., Lichtarska, A., & Kukula-Koch, W. (2022). Assessment of Cucurbita spp. Peel extracts as potential sources of active substances for skin care and dermatology. *Molecules, 27*(21), 7618. https://doi.org/10.3390/MOLECULES27217618/S1

Heleno, S. A., Ferreira, I. C. F. R., Esteves, A. P., Ćirić, A., Glamočlija, J., Martins, A., … Queiroz, M. J. R. P. (2013). Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. *Food and Chemical Toxicology, 58*, 95–100. https://doi.org/10.1016/J.FCT.2013.04.025

Hussain, A., Kausar, T., Din, A., Murtaza, A., Jamil, M. A., Noreen, S., & Iqbal, M. A. (2021). Antioxidant and antimicrobial properties of pumpkin (Cucurbita maxima) Peel, flesh and seeds powders. *Journal of Biology, Agriculture and Healthcare, 11*(6), 42–51. https://doi.org/10.7176/jbah/11-6-05

Iswaldi, I., Gómez-Caravaca, A. M., Lozano-Sánchez, J., Arráez-Román, D., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2013). Profiling of phenolic and other polar compounds in zucchini (Cucurbita pepo L.) by reverse-phase high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Food Research International, 50*(1), 77–84. https://doi.org/10.1016/J.FOODRES.2012.09.030

Kaleem, M. M., Nawaz, M. A., Ding, X., Wen, S., Shireen, F., Cheng, J., & Bie, Z. (2022). Comparative analysis of pumpkin rootstocks mediated impact on melon sensory fruit quality through integration of non-targeted metabolomics and sensory evaluation. *Plant Physiology and Biochemistry, 192*, 320–330. https://doi.org/10.1016/J.PLAPHY.2022.10.010

Kopczyńska, K., Średnicka-Tober, D., Hallmann, E., Wilczak, J., Wasiak-Zys, G., Wyszyński, Z., Kucińska, K., Perzanowska, A., Szacki, P., Barański, M., Gawron, P., Góralska-Walczak, R., Rembiałkowska, E., & Kazimierczak, R. (2021). Bioactive compounds, sugars, and sensory attributes of organic and conventionally produced courgette (Cucurbita pepo). *Foods, 10*(10), 2475. https://doi.org/10.3390/FOODS10102475/S1

Kostecka-Gugała, A., Kruczek, M., Ledwożyw-Smoleń, I., & Kaszycki, P. (2020). Antioxidants and health-beneficial nutrients in fruits of eighteen Cucurbita cultivars: Analysis of diversity and dietary implications. *Molecules, 25*(8), 1792. https://doi.org/10.3390/MOLECULES25081792

Krstić, S., Miljić, M., Antić-Stanković, J., Božić, D. D., Krivokuća, M. J., & Pirković, A. (2023). Pumpkin pulp extracts from a Serbian Cucurbita maxima breeding collection: Phenol profile and in vitro bioactivity. *Food Chemistry Advances, 3*, Article 100395. https://doi.org/10.1016/J.FOCHA.2023.100395

Kulczynski, B., & Gramza-Michałowska, A. (2019). The profile of secondary metabolites and other bioactive compounds in Cucurbita pepo L. and Cucurbita moschata pumpkin cultivars. *Molecules, 24*(16), 2945. https://doi.org/10.3390/MOLECULES24162945

Kulczyński, B., Gramza-Michałowska, A., & Królczyk, J. B. (2020). Optimization of extraction conditions for the antioxidant potential of different pumpkin varieties (Cucurbita maxima). *Sustainability, 12*(4), 1305. https://doi.org/10.3390/SU12041305

Leichtweis, M. G., Molina, A. K., Dias, M. I., Calhelha, R. C., Pires, T. C. S. P., Pavli, O., … Pereira, C. (2023). Variability in chemical profile and bioactivities of the flesh of Greek pumpkin landraces. *Horticulturae, 9*(11), 1–22. https://doi.org/10.3390/horticulturae9111232

Leichtweis, M. G., Molina, A. K., Petropoulos, S. A., Carocho, M., Pires, T. C. S. P., Dias, M. I., … Barros, L. (2023). Valorization of pumpkin Peel as a source of bioactive compounds: Optimization of heat- and ultrasound-assisted extraction. *Molecules, 28*(7). https://doi.org/10.3390/molecules28073168

Leichtweis, M. G., Molina, A. K., Pires, T. C. S., Dias, M. I., Calhelha, R., Bachari, K., … Barros, L. (2022). Biological activity of pumpkin byproducts: Antimicrobial and antioxidant properties. *Molecules, 27*(23). https://doi.org/10.3390/molecules27238366

Leichtweis, M. G., Oliveira, M. B. P. P., Ferreira, I. C. F. R., Pereira, C., & Barros, L. (2021). Sustainable recovery of preservative and bioactive compounds from food industry bioresidues. *Antioxidants, 10*(11), 1827. https://doi.org/10.3390/antiox10111827

Martínez, C., Valenzuela, J. L., & Jamilena, M. (2021). Genetic and pre- and postharvest factors influencing the content of antioxidants in cucurbit crops. *Antioxidants, 10*(6), 894. https://doi.org/10.3390/antiox10060894

Medjakovic, S., Hobiger, S., Ardjomand-Woelkart, K., Bucar, F., & Jungbauer, A. (2016). Pumpkin seed extract: Cell growth inhibition of hyperplastic and cancer cells, independent of steroid hormone receptors. *Fitoterapia, 110*, 150–156. https://doi.org/10.1016/J.FITOTE.2016.03.010

Nawirska-Olszańska, A., Biesiada, A., Sokół-Łętowska, A., & Kucharska, A. Z. (2014). Characteristics of organic acids in the fruit of different pumpkin species. *Food Chemistry, 148*, 415–419. https://doi.org/10.1016/J.FOODCHEM.2013.10.080

Noumedem, J. A. K., Mihasan, M., Lacmata, S. T., Stefan, M., Kuiate, J. R., & Kuete, V. (2013). Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against gram-negative multidrug-resistant bacteria. *BMC Complementary and Alternative Medicine, 13*, 26. https://doi.org/10.1186/1472-6882-13-26

Oliveira, T. C. G., Caleja, C., Oliveira, M. B. P. P., Pereira, E., & Barros, L. (2023). Reuse of fruits and vegetables biowaste for sustainable development of natural ingredients. *Food Bioscience, 53*, Article 102711. https://doi.org/10.1016/J.FBIO.2023.102711

Öztürk, T., & Turhan, S. (2020). Physicochemical properties of pumpkin (Cucurbita pepo L.) seed kernel flour and its utilization in beef meatballs as a fat replacer and functional ingredient. *Journal of Food Processing and Preservation, 44*(9), Article e14695. https://doi.org/10.1111/JFPP.14695

Palmeira, L., Pereira, C., Dias, M. I., Abreu, R. M. V., Corrêa, R. C. G., Pires, T. C. S. P., … Ferreira, I. C. F. R. (2019). Nutritional, chemical and bioactive profiles of different parts of a Portuguese common fig (Ficus carica L.) variety. *Food Research*

International, 126, Article 108572. https://doi.org/10.1016/J.FOODRES.2019.108572

Pereira, C., Barros, L., Carvalho, A. M., & Ferreira, I. C. F. R. (2011). Nutritional composition and bioactive properties of commonly consumed wild greens: Potential sources for new trends in modern diets. *Food Research International, 44*(9), 2634–2640. https://doi.org/10.1016/J.FOODRES.2011.05.012

Piccolella, S., Bianco, A., Crescente, G., Santillo, A., Baccari, G. C., & Pacifico, S. (2019). Recovering Cucurbita pepo cv. 'Lungo Fiorentino' wastes: UHPLC-HRMS/MS metabolic profile, the basis for establishing their Nutra- and cosmeceutical valorisation. *Molecules, 24*(8), 1479. https://doi.org/10.3390/MOLECULES24081479

Polyzos, N., Paschoalinotto, B. H., Compocholi, M., Pinela, J., Heleno, S. A., Calhelha, R. C., … Petropoulos, S. A. (2022). Fertilization of pot-grown Cichorium spinosum L.: How it can affect plant growth, chemical profile, and bioactivities of edible parts? *Horticulturae, 8*(10), 890. https://doi.org/10.3390/HORTICULTURAE8100890

Rodov, V., Paris, H. S., Friedman, H., Mihiret, M., Vinokur, Y., & Fennec, A. (2020). Chilling sensitivity of four near-isogenic fruit-color genotypes of summer squash (Cucurbita pepo, Cucurbitaceae) and its association with tocopherol content. *Postharvest Biology and Technology, 168*, Article 111279. https://doi.org/10.1016/J.POSTHARVBIO.2020.111279

Rozanowska, M., Edge, R., Land, E. J., Navaratnam, S., Sarna, T., & Truscott, T. G. (2019). Scavenging of retinoid cation radicals by urate, Trolox, and α-, β-, γ-, and δ-tocopherols. *International Journal of Molecular Sciences, 20*(11), 2799. https://doi.org/10.3390/IJMS20112799

Salami, A., Asefi, N., Kenari, R. E., & Gharekhani, M. (2020). Addition of pumpkin peel extract obtained by supercritical fluid and subcritical water as an effective strategy to retard canola oil oxidation. *Journal of Food Measurement and Characterization, 14*(5), 2433–2442. https://doi.org/10.1007/S11694-020-00491-4/FIGURES/4

Salehi, B., Quispe, C., Sharifi-Rad, J., Giri, L., Suyal, R., Jugran, A. K., … Zam, W. (2021). Antioxidant potential of family Cucurbitaceae with special emphasis on Cucurbita genus: A key to alleviate oxidative stress-mediated disorders. *Phytotherapy Research, 35*(7), 3533–3557. https://doi.org/10.1002/ptr.7045

Salehi, B., Sharifi-Rad, J., Capanoglu, E., Adrar, N., Catalkaya, G., Shaheen, S., … Cho, W. C. (2019). Cucurbita plants: From farm to industry. *Applied Sciences, 9*(16), 3387. https://doi.org/10.3390/APP9163387

Shokrzadeh, M., Azadbakht, M., Ahangar, N., Hashemi, A., & Saeedi Saravi, S. S. (2010). Cytotoxicity of hydro-alcoholic extracts of Cucurbita pepo and Solanum nigrum on HepG2 and CT26 cancer cell lines. *Pharmacognosy Magazine, 6*(23), 176–179. https://doi.org/10.4103/0973-1296.66931

Singh, A., & Kumar, V. (2022a). Nutritional, phytochemical, and antimicrobial attributes of seeds and kernels of different pumpkin cultivars. *Food Frontiers, 3*(1), 182–193. https://doi.org/10.1002/fft2.117

Singh, A., & Kumar, V. (2022b). Nutritional, phytochemical, and antimicrobial attributes of seeds and kernels of different pumpkin cultivars. *Food Frontiers, 3*(1), 182–193. https://doi.org/10.1002/FFT2.117

Singh, J., Singh, V., Shukla, S., Rai, K., & A.. (2016). Phenolic content and antioxidant capacity of selected cucurbit fruits extracted with different solvents. *Journal of Nutrition & Food Sciences, 06*(06). https://doi.org/10.4172/2155-9600.1000565

Slavova-Kazakova, A., Janiak, M. A., Sulewska, K., Kancheva, V. D., & Karamać, M. (2021). Synergistic, additive, and antagonistic antioxidant effects in the mixtures of curcumin with (−)-epicatechin and with a green tea fraction containing (−)-epicatechin. *Food Chemistry, 360*, Article 129994. https://doi.org/10.1016/J.FOODCHEM.2021.129994

Stryjecka, M., Krochmal-Marczak, B., Cebulak, T., & Kiełtyka-Dadasiewicz, A. (2023). Assessment of phenolic acid content and antioxidant properties of the pulp of five pumpkin species cultivated in southeastern Poland. *International Journal of Molecular Sciences, 24*(10), 8621. https://doi.org/10.3390/IJMS24108621

Yang, Z., Shi, L., Qi, Y., Xie, C., Zhao, W., Barrow, C. J., … Suleria, H. A. R. (2022). Effect of processing on polyphenols in butternut pumpkin (Cucurbita moschata). *Food Bioscience, 49*, Article 101925. https://doi.org/10.1016/J.FBIO.2022.101925

Yousefi, M., Ghafarifarsani, H., Raissy, M., Yilmaz, S., Vatnikov, Y. A., & Kulikov, E. V. (2023). Effects of dietary malic acid supplementation on growth performance, antioxidant and immunological parameters, and intestinal gene expressions in rainbow trout. *Oncorhynchus mykiss. Aquaculture, 563*, Article 738864. https://doi.org/10.1016/J.AQUACULTURE.2022.738864