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REPORT WITH THE TECHNICAL SPECIFICATIONS OF THE PRESERVING COMPOUNDS DEVELOPED

DELIVERABLE 2.2

PulpIng

Developing of **Pu**mpkin **Pulp** Formulation using a Sustainable **Integ**rated Strategy

Index

Document Information

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1. Summary

PulpIng project aims at the development of a high-quality pumpkin pulp product enriched and preserved by added-value compounds obtained from pumpkin by-products, fostering an integrative and sustainable strategy. Obtaining extracts with high preservative capacity from pumpkin by-products, more specifically the seeds, peel and fibers, is the main goal of the WP2 – "Sustainable recovery of compounds with preserving capacity from pumpkin by-products". This report regards the deliverable D 2.2 – "Report with the technical specifications of the preserving compounds developed" of the WP2, that comprises the chromatographic identification and identification of the possible preserving compounds (e.g. phenolic compounds) extracted from the pumpkin by-products

2. Description of work

The extracts obtained from the pumpkin by-products in Task 2.1 were characterized in terms of their preserving compounds composition. For that, the profile of phenolic compounds was identified through HPLC-DAD-ESI/MS. For the phenolic compounds tentative identification were used the comparison of the retention times, UV-Vis and mass spectra data of commercial standards and the correlation of the literature information. The quantification was given by the area of the peaks in comparison with the calibration curves of the most similar available commercial standards. In addition, the samples of pumpkin by-residues were also evaluated regarding the tocopherol and organic acids contents by HPLC-FD and UFLC-PDA, respectively. The compounds identification was based on the chromatography comparison with authentic standards, and the quantification on calibration curves obtained from internal standard method.

2.1. Goal

To describe the composition of molecules with antioxidant and antimicrobial properties present in the extracts obtained from the pumpkin by-products, for food application. A promising extract rich in preservative compounds will be use as a natural alternative additive to be incorporated in the produced pulp, promoting a more sustainable industrial process, with reduction of residue generation and replacement of synthetic additive use, and fostering a circular economy.

3. Results

The samples from different pumpkin varieties and its parts of fruit (peel, seeds, and fibers) were analysed regarding the composition of phenolic compounds, acids organics and tocopherols. The

pumpkins varieties were obtained from Portugal, Algeria, Tunisia, Greece, and Egypt. The Algerian samples were received by IPB already extracted, a condition that did not allow the analysis of their organic acids and tocopherol.

3.1 Tocopherols

The powder samples from Portugal, Greece, Tunisia, and Egypt, in a total of 95 samples, were assessed regarding their tocopherol content profile, as previously described [1]. The results are presented in **Tables 1** and **3-5**, divided by countries.

Trough the results obtained (**Table 1**), it is possible to determine that in the bioresidues (peel, fibers, and seeds) of the 3 varieties of Portuguese pumpkin, the alpha (α), beta (β), and delta (δ) tocopherols are present. The α-tocopherol was found in all the samples studied. Total tocopherols ranged from 0.061 to 88.3 mg/100g dw, depending on the variety, as well as the part of the pumpkin studied.

Table 1. Tocopherol content of different samples of pumpkin from Portugal (in mg/100g dw).

Part	Variety	Alfa	Gama	Beta	Delta	Total
Peel	Butternut Squash	4.9±0.1	n.d.	n.d.	n.d.	4.9±0.1
	Kabocha Squash	1.10±0.03	n.d.	15.9±0.4	0.59±0.02	17.6±0.4
	Common Pumpkin	12.05±0.02	n.d.	75.4±0.3	0.84±0.08	88.3±0.3
Fibers	Butternut Squash	3.09±0.02	n.d.	n.d.	n.d.	3.09±0.02
	Kabocha Squash	-	-	-	-	-
	Common Pumpkin	0.061±0.001	n.d.	n.d.	n.d.	0.061±0.001
Seeds	Butternut Squash	2.80±0.09	n.d.	9.4±0.3	n.d.	12.2±0.4
	Kabocha Squash	0.34±0.01	n.d.	11.1347±0.0008	0.333±0.009	11.803±0.001
	Common Pumpkin	2.650±0.003	n.d.	11.4±0.3	n.d.	14.1±0.3

"n.d.": not detected; "-": not evaluated due to insufficient sample quantity.

In **Table 2** and **Table 3**, the codes of the varieties from Greece and the results for these samples are presented, respectively. Regarding the tocopherols found in the seeds of the 10 varieties analyzed, α and β-tocopherol were detected in all samples, except in variety 9, which only presented the β-tocopherol. The β-tocopherol was the most abundant in almost all the seeds samples and the variety 8 was the only one that presented the δ-tocopherol.

Considering the peels, among the 17 varieties studied, only two of them (code 4 and 7) revealed the 4 isoforms of tocopherols. The varieties 6 and 9 presented α, β and δ isoforms and the remaining samples only revealed α and β with a higher concentration of β-tocopherol. On the other hand, δ-tocopherol was not detected in fibers and pulp samples and was detected in few seeds and peels samples. The seeds did not present the gamma (γ) tocopherol.

Table 2. Pumpkin variety name according to the code and part of the fruit.

Code	Seeds	Fibers	Peel	Pulp
1	V1 (Fytro FS-243)	V1 (Fytro FS-243) UTH	V1 (Fytro FS-243) GFV	V2 (Landrace from the region of Trikala) Turbinate
2	V2 (Landrace from the region of Trikala)	V1 (Fytro FS-243) GFV	V2 (Landrace from the region of Trikala) Turbinate	V5 (Local landrace “Leuka Melitis) Flattened
3	V3 (Big Max)	V2 (Landrace from the region of Trikala) Turbinate	V2 (Landrace from the region of Trikala) Cylindrical	V1 (Fytro FS-243) UTH
4	V4 (Local landrace “Nychaki”)	V2 (Landrace from the region of Trikala) Cylindrical	V3 (Big Max)	V2 (Landrace from the region of Trikala) Cylindrical
5	V5 (Local landrace “Leuka Melitis)	V3 (Big Max)	V4 (Local landrace “Nychaki”) Cylindrical	V4 (Local landrace “Nychaki”) Cylindrical
6	V6 (Local landrace from the region of Lakonia)	V4 (Local landrace “Nychaki”) Round	V1 (Fytro FS-243) UTH	V5 (Local landrace “Leuka Melitis) Round
7	V7 (Local landrace from the region of Lakonia)	V4 (Local landrace “Nychaki”) Cylindrical	V9 (Local landrace “Makedonika prasina") Cylindrical	V7 (Local landrace from the region of Lakonia) Pyriform
8	V8 (Local landrace from the region of Lakonia)	V5 (Local landrace “Leuka Melitis) Flattened	V7 (Local landrace from the region of Lakonia) Pyriform	V7 (Local landrace from the region of Lakonia) Flattened
9	V9 (Local landrace “Makedonika prasina")	V5 (Local landrace “Leuka Melitis) Round	V10 (Local landrace from the region of Laconia)	V9 (Local landrace “Makedonika prasina") Cylindrical
10	V10 (Local landrace from the region of Laconia)	V6 (Local landrace from the region of Lakonia)	V9 (Local landrace “Makedonika prasina") Round	Voutirato
11	-	V7 (Local landrace from the region of Lakonia) Pyriform	V6 (Local landrace from the region of Lakonia)	V9 (Local landrace “Makedonika prasina") Round
12	-	V7 (Local landrace from the region of Lakonia) Flattened	V8 (Local landrace from the region of Lakonia)	V10 (Local landrace from the region of Laconia)
13	-	V8 (Local landrace from the region of Lakonia)	V4 (Local landrace “Nychaki”) Round	V8 (Local landrace from the region of Lakonia)
14	-	V9 (Local landrace “Makedonika prasina") Cylindrical	V7 (Local landrace from the region of Lakonia) Flattened	V1 (Fytro FS-243) GFV
15	-	V9 (Local landrace “Makedonika prasina") Round	V5 (Local landrace “Leuka Melitis) Flattened	V3 (Big Max)
16	-	V10 (Local landrace from the region of Laconia)	Voutirato	V6 (Local landrace from the region of Lakonia)
17	-	Voutirato	V5 (Local landrace “Leuka Melitis) Round	V4 (Local landrace “Nychaki”) Round

Table 3. Tocopherol content of different samples of pumpkin from Greece (in mg/100g dw).

Part	Variety	Alfa	Gama	Beta	Delta	Total
Seeds	1	0.1028±0.0003	n.d.	6.5±0.1	n.d.	6.6±0.1
	2	2.03±0.04	n.d.	3.02±0.06	n.d.	5.0±0.1
	3	0.371±0.004	n.d.	12.3±0.2	n.d.	12.7±0.2
	4	2.10±0.06	n.d.	9.0±0.2	n.d.	11.0±0.3
	5	2.98±0.09	n.d.	0.86±0.02	n.d.	3.8±0.1
	6	0.116±0.002	n.d.	8.1±0.2	n.d.	8.2±0.2
	7	0.37±0.02	n.d.	9.89±0.07	n.d.	10.26±0.06
	8	0.212±0.009	n.d.	14.20±0.08	0.169±0.006	14.58±0.09
	9	n.d.	n.d.	1.75±0.03	n.d.	1.75±0.03
	10	2.29±0.03	n.d.	2.057±0.004	n.d.	4.35±0.03
Peel	1	0.320±0.009	n.d.	5.99±0.03	n.d.	6.31±0.02
	2	0.55±0.02	n.d.	24.1±0.2	n.d.	24.6±0.2
	3	1.4861±0.0008	n.d.	13.78±0.04	n.d.	15.27±0.04
	4	1.35±0.02	0.228±0.008	5.49±0.01	0.56±0.08	7.62±0.06
	5	0.236±0.009	n.d.	0.70±0.03	n.d.	0.94±0.04
	6	0.1099±0.0008	n.d.	4.44±0.09	0.343±0.005	4.89±0.09
	7	4.178±0.004	0.116±0.002	13.203±0.007	0.26±0.02	17.76 ±n.d. 0.02
	8	0.190±0.007	n.d.	5.98±0.05	n.d.	6.17±0.04
	9	5.9±0.1	n.d.	24.1±0.1	0.682±0.001	30.7±0.2
	10	4.71±0.02	n.d.	8.97±0.01	n.d.	13.68±0.03
	11	0.61±0.02	n.d.	19.0±0.2	n.d.	19.6±0.2
	12	1.02±0.02	n.d.	0.82±0.02	n.d.	1.83±0.03
	13	1.663±0.001	n.d.	3.40±0.03	n.d.	5.07±0.03
	14	0.2272±0.0006	n.d.	5.59±0.02	n.d.	5.81±0.03
	15	0.389±0.003	n.d.	3.959±0.008	n.d.	4.347±0.005
	16	0.3620±0.0008	n.d.	20.55±0.09	n.d.	20.91±0.09
	17	1.002±0.004	n.d.	5.03±0.1	n.d.	6.3±0.1
Fibers	1	4.29±0.06	0.303±0.006	n.d.	n.d.	4.59±0.07
	2	4.03±0.09	n.d.	n.d.	n.d.	4.03±0.09
	3	4.37±0.01	n.d.	n.d.	n.d.	4.37±0.01
	4	0.529±0.003	0.62±0.03	n.d.	n.d.	1.15±0.02
	5	6.03±0.01	n.d.	n.d.	n.d.	6.03±0.01
	6	2.176±0.007	n.d.	n.d.	n.d.	2.176±0.007
	7	1.61±0.01	n.d.	0.254±0.003	n.d.	1.861±0.009
	8	0.077±0.001	n.d.	n.d.	n.d.	0.077±0.001
	9	0.3913±0.0008	n.d.	n.d.	n.d.	0.3913±0.0008
	10	1.91±0.02	n.d.	0.76±0.01	n.d.	2.674±0.006
	11	6.83±0.03	n.d.	0.5535±0.0006	n.d.	7.38±0.03
	12	5.56±0.06	n.d.	n.d.	n.d.	5.56±0.06
	13	2.30±0.03	n.d.	1.18±0.04	n.d.	3.477±0.006
	14	1.87±0.06	n.d.	n.d.	n.d.	1.87±0.06
	15	2.696±0.005	n.d.	n.d.	n.d.	2.696±0.005
	16	1.245±0.005	n.d.	0.589±0.003	n.d.	1.834±0.008
	17	4.20±0.06	n.d.	n.d.	n.d.	4.20±0.06
Pulp	1	4.90 ± 0.08	6.59 ± 0.08	n.d.	n.d.	11.5 ± 0.2
	2	2.3 ± 0.1	0.85 ± 0.01	n.d.	n.d.	3.19 ± 0.08
	3	1.8 ± 0.1	0.1496 ± 0.0005	n.d.	n.d.	1.9 ± 0.1

Part	Variety	Alfa	Gama	Beta	Delta	Total
	4	0.81 ± 0.03	0.238 ± 0.003	n.d.	n.d.	1.05 ± 0.03
	5	1.15 ± 0.03	n.d.	0.1998 ± 0.0002	n.d.	1.35 ± 0.03
	6	1.03 ± 0.02	n.d.	1.02 ± 0.03	n.d.	2.05 ± 0.04
	7	0.70 ± 0.04	0.043 ± 0.001	0.185 ± 0.004	n.d.	0.93 ± 0.04
	8	0.218 ± 0.008	n.d.	0.2685 ± 0.0007	n.d.	0.486 ± 0.009
	9	1.70 ± 0.09	n.d.	0.572 ± 0.003	n.d.	2.3 ± 0.1
	10	0.55 ± 0.01	n.d.	4.52 ± 0.07	n.d.	5.07 ± 0.08
	11	2.91 ± 0.06	n.d.	1.24 ± 0.03	n.d.	4.15 ± 0.04
	12	1.97 ± 0.09	n.d.	0.28 ± 0.01	n.d.	2.3 ± 0.1
	13	1.16 ± 0.04	n.d.	0.58 ± 0.02	n.d.	1.73 ± 0.02
	14	5.29 ± 0.05	0.23 ± 0.01	0.78 ± 0.05	n.d.	6.30 ± 0.01
	15	1.57 ± 0.02	n.d.	1.05 ± 0.02	n.d.	2.62 ± 0.04
	16	2.47 ± 0.01	n.d.	4.5 ± 0.1	n.d.	7.0 ± 0.1
	17	2.68 ± 0.06	n.d.	1.619 ± 0.005	n.d.	4.30 ± 0.06

"n.d.": not detected.

In the different samples of bioresidues of Tunisian pumpkin (**Table 4**), β-tocopherol was found in greater quantity, mainly in the peel, which values ranged from 24.7 to 86 mg/100g, while in the seeds and fibers they ranged from 0.706 to 17.1 mg/100g. On the other hand, the peels of Batati presented the 4 isoforms and the peels of Karkoubi and Bejaoui, 3 isoforms (α, β, and γ), contrarily to the seeds and fibers, where only α and β-tocopherol were found.

Table 4. Tocopherol content of different samples of pumpkin from Tunisia (in mg/100g dw).

Part	Variety	Alfa	Gama	Beta	Delta	Total
Seeds+Fibers	Batati	4.9±0.2	n.d.	0.706±0.005	n.d.	5.6±0.2
	Karkoubi	3.71±0.07	n.d.	10.10±0.04	n.d.	13.81±0.03
	Bejaoui	2.6005±0.0003	n.d.	17.1±0.4	n.d.	19.7±0.4
Peel	Batati	11.21±0.05	0.3995±0,0002	25.4±0.7	1.06±0.04	38.0±0.6
	Karkoubi	0.207±0.003	n.d.	24.7±0.4	0.263±0.003	25.1±0.4
	Bejaoui	0.188±0.007	n.d.	86±1	1.6±0.1	88±1

"n.d.": not detected.

The tocopherols found in the Egyptian pumpkin samples (**Table 5**) were α, β, and δ isoforms, with the predominance of β-tocopherol in the pulp, peel and seeds, while in fibers the α-tocopherol was higher. The δ-tocopherol was only detected in the peels of these varieties.

Table 5. Tocopherol content of different samples of pumpkin from Egypt (in mg/100g dw).

Part	Variety	Alfa	Gama	Beta	Delta	Total
Pulp	Golden Cushaw	0.89±0.04	n.d.	2.45±0.08	n.d.	3.3±0.1
	Dickinson	0.714±0.006	n.d.	2.70±0.04	n.d.	3.41±0.03
	Butternut squash	1.74±0.01	n.d.	3.68±0.01	n.d.	5.41±0.03
	Halloween	0.588±0.007	n.d.	1.23±0.02	n.d.	1.82±0.01
	Honey Delite	0.4869±0.0006	n.d.	1.291±0.002	n.d.	1.778±0.001
Peel	Golden Cushaw	0.517±0.005	n.d.	15.6±0.3	0.323±0.003	16.4±0.3

Part	Variety	Alfa	Gama	Beta	Delta	Total
	Dickinson	0.53±0.02	n.d.	34.7±0.4	0.673±0.001	35.9±0.4
	Butternut squash	0.80±0.02	n.d.	18.3±0.1	0.179±0.006	19.3±0.1
	Halloween	0.499±0.003	n.d.	8.6±0.3	0.379±0.004	9.5±0.3
	Honey Delite	0.496±0.009	n.d.	18.4±0.2	0.4630±0.0008	19.3±0.2
Seeds	Golden Cushaw	2.9±0.1	n.d.	19.4±0.1	n.d.	22.31±0.03
	Dickinson	5.39±0.03	n.d.	30.8±0.4	n.d.	36.2±0.4
	Butternut squash	2.738±0.003	n.d.	13.3±0.3	n.d.	16.0±0.3
	Halloween	3.87±0.05	n.d.	25.74±0.04	n.d.	29.618±0.007
	Honey Delite	2.71±0.06	n.d.	31.7±0.3	n.d.	34.4±0.3
Fibers	Golden Cushaw	1.33±0.03	n.d.	n.d.	n.d.	1.33±0.03
	Dickinson	6.6±0.1	n.d.	0.6186±0.0006	n.d.	7.3±0.1
	Butternut squash	4.23±0.06	n.d.	0.33±0.01	n.d.	4.56±0.07
	Halloween	8.4±0.3	n.d.	n.d.	n.d.	8.4±0.3
	Honey Delite	2.42±0.07	n.d.	0.308±0.004	n.d.	2.72±0.07

"n.d.": not detected.

3.2 Organic acids

The samples from Portugal, Greece, Tunisia, and Egypt, in a total of 95 samples, were also assessed regarding their organic acids content profile, as previously described [1]. The results are presented in **Table 6** to **Table 9,** divided by country.

Regarding the sample from Portugal (**Table 6**), the profile of organic acids is almost the same between the samples, being oxalic, malic, and fumaric acids present in most of the samples. High contents of malic and citric acids were presented by the fibers of Butternut squash (17.7±0.8 and 11.7±0.4 g/100g dw, respectively), while the fibers of Common Pumpkin presented the highest content of fumaric acid. On the other hand, the seeds from this variety presented the lower content of oxalic acid (0.0643±0.0022 g/100g).

In **Table 7**, the results of Greek samples are shown. Oxalic, malic, and fumaric acids were predominant. In most of the peel samples, citric acid was also found, while in the fibers and the seeds quinic acid was detected in almost all varieties. Traces of shikimic and ascorbic acids were detected in pulp and peel samples.

The same was observed in the samples from Tunisia (**Table 8**): oxalic, malic, and fumaric acids were present in all samples. In the seeds and fibers of Batati, quinic acid was predominant, in a content of 2.6±0.1 dw. Citric acid was also found in three samples. Malic acid concentration was higher in the peel of Batati, which also presented high contents of fumaric and oxalic acid.

Regarding the samples from Egypt (**Table 9**), citric acid was detected, among oxalic, malic, and fumaric acids. Quinic acid was found in three seeds samples, as well shikimic acid and traces of ascorbic acid.

Table 6. Organic acid content of different samples of pumpkin from Portugal.

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
Peel	Butternut Squash	3.40±0.08	n.d.	6.2±0.1	n.d.	n.d.	2.4±0.1	0.0090±0.0004
	Kabocha Squash	5.16±0.05	n.d.	2.2±0.1	n.d.	n.d.	0.69±0.02	n.d.
	Common Pumpkin	5.5±0.1	n.d.	3.6±0.2	n.d.	n.d.	n.d.	0.0092±0.0003
Fibers	Butternut Squash	0.31±0.01	n.d.	17.7±0.8	n.d.	n.d.	11.7±0.4	0.065±0.001
	Kabocha Squash	-	-	-	-	-	-	-
	Common Pumpkin	3.50±0.09	n.d.	7.81±0.03	n.d.	n.d.	2.51±0.01	0.0223±0.0007
Seeds	Butternut	tr	n.d.	0.3799±0.0109	n.d.	n.d.	n.d.	0.0085±0.0004
	Kabocha Squash	tr	n.d.	1.29±0.06	n.d.	n.d.	n.d.	0.0073±0.0003
	Common Pumpkin	0.0643±0.0022	n.d.	0.2749±0.0106	n.d.	tr	0.24±0.01	0.0063±0.0003

"n.d.": not detected; "-": not evaluated due to insufficient sample quantity; "tr": traces.

Table 7. Organic acid content of different samples of pumpkin from Greece.

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
Seeds	1	0.051±0.002	1.76±0.08	0.34±0.01	n.d.	n.d.	n.d.	0.0170±0.0008
	2	0.029±0.001	0.33±0.01	n.d.	n.d.	tr	n.d.	0.01091±0.00005
	3	0.117±0.002	1.13±0.05	0.97±0.05	n.d.	tr	n.d.	0.0104±0.0005
	4	0.0167±0.0007	n.d.	n.d.	n.d.	tr	n.d.	0.0093±0.0003
	5	0.025±0.001	0.40±0.02	0.99±0.05	n.d.	n.d.	n.d.	0.00649±0.00003
	6	0.093±0.003	0.68±0.03	2.4±0.1	n.d.	0.063±0.003	n.d.	0.0137±0.0005
	7	0.129±0.005	1.32±0.05	1.08±0.05	n.d.	tr	n.d.	0.01315±0.00009
	8	0.032±0.001	0.53±0.02	0.64±0.02	n.d.	tr	0.183±0.009	0.0096±0.0004
	9	0.142±0.005	1.20±0.06	1.185±0.002	n.d.	n.d.	n.d.	0.01846±0.00003
	10	0.060±0.002	0.66±0.03	0.37±0.01	n.d.	tr	n.d.	0.011251±0.000007
Peel	1	4.61±0.01	n.d.	6.03±0.05	n.d.	tr	0.99±0.05	0.0204±0.0009
	2	5.08±0.02	n.d.	2.61±0.03	tr	tr	0.92±0.04	0.0119±0.0005
	3	4.9±0.1	n.d.	2.4±0.1	n.d.	n.d.	n.d.	0.0123±0.0005
	4	5.9±0.2	n.d.	3.0±0.1	tr	n.d.	1.03±0.05	0.0134±0.0006
	5	5.76±0.05	n.d.	3.3±0.2	tr	n.d.	1.13±0.05	n.d.

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
	6	4.89±0.07	n.d.	4.5±0.2	tr	n.d.	n.d.	n.d.
	7	5.48±0.04	n.d.	5.1±0.3	n.d.	n.d.	n.d.	0.384±0.005
	8	4.93±0.09	n.d.	5.5±0.2	tr	n.d.	0.62±0.03	0.0173±0.0008
	9	5.39±0.04	n.d.	2.40±0.09	tr	tr	n.d.	0.0121±0.0005
	10	4.99±0.02	n.d.	7.1±0.2	n.d.	n.d.	0.66±0.03	0.165±0.008
	11	5.43±0.04	n.d.	3.10±0.02	n.d.	tr	1.06±0.05	0.01064±0.00006
	12	5.51±0.06	n.d.	3.53±0.06	tr	0.00157±0.00007	n.d.	0.0135±0.0006
	13	5.7±0.3	n.d.	4.07±0.04	tr	tr	n.d.	0.026±0.001
	14	4.7±0.2	1.65±0.01	5.0±0.2	tr	tr	1.81±0.03	0.0163±0.0007
	15	4.06±0.02	2.15±0.09	3.6±0.2	tr	n.d.	0.571±0.005	0.012110±0.000008
	16	4.26±0.03	1.98±0.09	4.49±0.02	n.d.	tr	2.2±0.1	0.0147±0.0007
	17	2.93±0.01	n.d.	2.8±0.1	n.d.	n.d.	0.38±0.02	0.0144±0.0006
	1	2.9±0.1	8.2±0.4	5.8±0.3	n.d.	n.d.	n.d.	0.220±0.003
	2	3.671±0.003	8.9±0.3	6.0±0.1	n.d.	n.d.	1.55±0.04	0.270±0.001
	3	2.628±0.004	n.d.	15.2±0.6	n.d.	n.d.	n.d.	0.72±0.01
	4	4.44±0.09	n.d.	12.2±0.6	n.d.	n.d.	2.8±0.1	0.443±0.009
	5	4.75±0.08	9.3±0.3	8.6±0.4	n.d.	n.d.	n.d.	0.394±0.004
	6	4.455±0.001	9.6±0.5	11.83±0.07	n.d.	n.d.	n.d.	0.608±0.005
	7	3.95±0.09	7.8±0.4	10.4±0.4	n.d.	n.d.	n.d.	0.612±0.002
	8	3.12±0.09	7.4±0.1	7.9±0.4	n.d.	n.d.	n.d.	0.469±0.005
Fibers	9	2.519±0.001	7.5±0.4	9.2±0.4	n.d.	n.d.	n.d.	0.488±0.003
	10	2.73±0.04	4.9±0.2	7.8±0.4	n.d.	n.d.	n.d.	0.551±0.003
	11	2.79±0.04	7.0±0.3	6.40±0.07	n.d.	0.0249±0.0007	n.d.	0.2837±0.0005
	12	4.16±0.08	9.7±0.3	8.99±0.09	n.d.	n.d.	n.d.	0.478±0.007
	13	3.93±0.07	n.d.	7.6±0.1	n.d.	0.0210±0.0009	n.d.	0.479±0.002
	14	5.0±0.2	12.2±0.6	11.6±0.2	n.d.	n.d.	n.d.	0.457±0.004
	15	4.01±0.01	9.1±0.4	10.7±0.2	n.d.	n.d.	n.d.	0.608±0.002
	16	4.3±0.1	9.28±0.03	11.5±0.5	n.d.	n.d.	n.d.	0.433±0.004
	17	1.00±0.02	n.d.	9.00±0.04	n.d.	n.d.	n.d.	0.631±0.003
	1	4.45 ± 0.04	n.d.	3.96 ± 0.06	n.d.	tr	n.d.	0.02825 ± 0.00007
Pulp	2	5.301 ± 0.006	n.d.	4.28 ± 0.03	n.d.	tr	3.16 ± 0.09	0.0155 ± 0.0002
	3	3.61 ± 0.04	n.d.	2.7 ± 0.1	tr	tr	1.08 ± 0.02	0.0091 ± 0.0001

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
	4	4.8 ± 0.2	n.d.	4.1 ± 0.2	n.d.	tr	n.d.	0.034 ± 0.001
	5	4.5 ± 0.1	n.d.	4.9 ± 0.2	n.d.	n.d.	n.d.	0.0255 ± 0.0008
	6	1.80 ± 0.03	n.d.	3.14 ± 0.07	tr	tr	n.d.	0.031 ± 0.002
	7	3.75 ± 0.07	n.d.	4.2 ± 0.1	tr	tr	1.38 ± 0.06	0.021 ± 0.001
	8	3.64 ± 0.02	n.d.	4.3 ± 0.2	tr	tr	1.62 ± 0.01	0.0161 ± 0.0003
	9	6.4 ± 0.1	n.d.	3.7 ± 0.2	n.d.	n.d.	3.2 ± 0.1	n.d.
	10	4.36 ± 0.09	n.d.	4.50 ± 0.06	n.d.	n.d.	n.d.	0.061 ± 0.001
	11	3.40 ± 0.04	n.d.	4.3 ± 0.2	tr	n.d.	n.d.	0.0188 ± 0.0009
	12	4.79 ± 0.05	n.d.	5.2 ± 0.2	n.d.	n.d.	n.d.	0.0393 ± 0.0004
	13	4.77 ± 0.01	n.d.	3.48 ± 0.05	n.d.	tr	n.d.	0.069 ± 0.003
	14	2.16 ± 0.08	n.d.	2.09 ± 0.06	tr	n.d.	1.23 ± 0.06	0.0113 ± 0.0005
	15	5.50 ± 0.01	n.d.	4.0 ± 0.2	n.d.	n.d.	n.d.	0.0169 ± 0.0007
	16	3.641 ± 0.006	n.d.	5.16 ± 0.06	n.d.	n.d.	n.d.	0.0943 ± 0.0002
	17	5.17 ± 0.03	n.d.	5.38 ± 0.07	n.d.	n.d.	n.d.	0.0193 ± 0.0009

"n.d.": not detected; "tr": traces.

Table 8. Organic acid content of different samples of pumpkin from Tunisia.

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
Seeds + Fibers	Batati	0.102±0.005	2.6±0.1	1.35±0.05	n.d.	n.d.	n.d.	0.0255±0.0009
	Karkoubi	0.94±0.04	n.d.	1.66±0.03	n.d.	n.d.	n.d.	0.0173±0.0007
	Bejaoui	0.361±0.005	n.d.	0.73±0.02	n.d.	n.d.	2.21±0.03	0.02614±0.00002
Peel	Batati	4.496±0.002	n.d.	10.00±0.08	n.d.	n.d.	n.d.	0.223±0.006
	Karkoubi	0.539±0.005	n.d.	3.5±0.2	n.d.	n.d.	1.89±0.08	0.2503±0.0004
	Bejaoui	5.44±0.05	n.d.	2.96±0.03	n.d.	n.d.	1.11±0.03	0.0890±0.0009

"n.d.": not detected.

Table 9. Organic acid content of different samples of pumpkin from Egypt (in g/100g dw; "-": not detected; tr: traces).

Part	Variety	Oxalic g/100g dw	Quinic g/100g dw	Malic g/100g dw	Ascorbic g/100g dw	Shikimic g/100g dw	Citric g/100g dw	Fumaric mg/100g dw
Pulp	Golden Cushaw	4.92±0.03	n.d.	6.73±0.07	n.d.	n.d.	0.95±0.02	0.1229±0.0007
	Dickinson	4.466±0.007	n.d.	6.59±0.09	n.d.	n.d.	0.75±0.04	0.175±0.001
	Butternut squash	4.5±0.1	n.d.	4.5±0.2	n.d.	tr	n.d.	0.097±0.003
	Halloween	3.95±0.05	n.d.	6.0±0.3	n.d.	n.d.	0.90±0.04	0.1768±0.0004
	Honey Delite	4.12±0.02	n.d.	5.2±0.1	n.d.	tr	n.d.	0.356±0.002
Peel	Golden Cushaw	5.41±0.09	n.d.	5.1±0.2	tr	0.0041±0.0002	0.81±0.01	0.058±0.001
	Dickinson	4.5775±0.0009	n.d.	3.66±0.02	tr	n.d.	0.83±0.04	0.029±0.001
	Butternut squash	4.72±0.04	n.d.	4.3±0.2	n.d.	n.d.	0.96±0.04	0.084±0.003
	Halloween	4.95±0.05	n.d.	4.2±0.1	n.d.	0.00226±0.00005	0.93±0.04	0.062±0.002
	Honey Delite	5.4±0.1	n.d.	4.47±0.02	n.d.	n.d.	1.10±0.05	0.0137±0.0006
Seeds	Golden Cushaw	0.60±0.02	n.d.	2.76±0.09	tr	tr	n.d.	0.0188±0.0008
	Dickinson	1.56±0.04	n.d.	3.32±0.02	tr	n.d.	n.d.	0.0146±0.0007
	Butternut squash	n.d.	12.2±0.4	4.9±0.2	tr	tr	0.78±0.02	0.047±0.002
	Halloween	n.d.	10.6±0.4	3.91±0.08	tr	0.00602±0.00004	0.63±0.03	0.0309±0.0005
	Honey Delite	n.d.	11.61±0.02	4.0489±0.0006	n.d.	tr	n.d.	0.02648±0.00002
Fibers	Golden Cushaw	5.2±0.2	n.d.	10.7±0.4	n.d.	n.d.	3.5±0.2	0.114±0.001
	Dickinson	4.614±0.007	n.d.	11.2±0.5	n.d.	n.d.	4.2±0.2	0.187±0.001
	Butternut squash	0.33±0.01	n.d.	3.5±0.2	n.d.	tr	1.56±0.04	0.0617±0.0008
	Halloween	4.3±0.1	n.d.	8.1±0.4	n.d.	0.042±0.002	n.d.	0.127442±0.000005
	Honey Delite	3.73±0.09	n.d.	9.4±0.5	n.d.	n.d.	n.d.	0.2458±0.0004

"n.d.": not detected; "tr": traces.

3.4 Phenolic compounds

The hydroethanolic extracts of the samples from Portugal, Algeria, Tunisia, Greece, and Egypt, in a total of 108 samples, were dissolved in water:methanol (80:20, *v/v*) and assessed regarding their phenolic compounds, as previously described [1]. The characterization is presented in **Table 10** and the quantification in

Table 11 to

Table 16.

Identification

The phenolic composition chromatographic characterization, regarding UV-vis at the maximum absorption, deprotonated ion, mass fragmentation, and tentative identification, of the hydroethanolic extracts of the pumpkin samples is described in **Table 10**. Fourteen compounds were found, belonging to the phenolic acids (peak **3**), flavan-3-ols (peak **1**), and flavonoids (peaks **2**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14**) families.

Peak **1** ([M-H]$^-$ at *m/z* 289) was identified as (-)-epicatechin by comparing the retention time, UV-vis at the maximum absorption ($\lambda_{máx}$ 280 nm), and mass spectra with the available standard compound. This compounds was previously described in *Cucurbita moschata* samples from Australia [2].

Peak **3** presented a deprotonated ion [M-H]$^-$ at *m/z* 405 and a major MS^2 fragment at *m/z* 281 that corresponded to the loss of the 4-hydroxybenzyl alcohol moiety (124 Da); It also produced MS^2 fragment at *m/z* 137 (hydroxybenzoic acid) and *m/z* 93 (loss of glucosyl residue and CO_2). These chromatographic responses were in accordance with the previously described by Jaiswal & Kuknert [3] and for that manner the peak was tentatively identified as 7 4-*O*-(6′-*O*-glucosyl-4″-hydroxybenzoyl)-4-hydroxybenzyl alcohol. It is also important to state that this compound was found in *Lagenaria siceraria* Stand. (Bottle Gourd) [3] that belong to the *Cucurbitaceae* family, same as pumpkin.

The family of flavonoids were the most abundant in terms of number of compounds found, mainly *O*-glycosylated derivatives of quercetin, kaempferol, and isorhamnetin, as previously described by Iswaldi et al. [4] in *Cucurbita pepo* L. The compounds found could be divided in two groups, the first one presented two sugar moieties linked to the flavonoid aglycone (peaks **8** to **14**) and the second one three sugar moieties (peaks **2**, **6**, and **7**). Peaks **8/10/12** ([M-H]$^-$ at *m/z* 593), peak **9** ([M-H]$^-$ at *m/z* 609), and peaks **11/13/14** ([M-H]$^-$ at *m/z* 623), presented only one MS^2 fragment at *m/z* 285 (kaempherol aglycone), *m/z* 301 (quercetin aglycone), and *m/z* 315 (isorhamnetin aglycone), respectively, corresponding to the jointed loss of a deoxyhexosyl and hexosyl moiety (([M-H-146-162]$^-$), being tentatively identified as kaempferol-*O*-deoxyhexosyl-hexoside, quercetin-*O*-deoxyhexosyl-hexoside, and isorhamnetin-*O*-deoxyhexosyl-hexoside, respectively. Finally, peaks **2** ([M-H]$^-$ at *m/z* 775), **6** ([M-H]$^-$ at *m/z* 739), and **7** ([M-H]$^-$ at *m/z* 769) also presented a unique MS^2 fragment at *m/z* 301, *m/z* 285, and *m/z* 315, respectively, that corresponded to the joined loss of two deoxyhexosyl moieties and one hexosyl moiety ([M-H-146-146-162]$^-$), being tentatively identified as quercetin-*O*-dideoxyhexosyl-hexoside, kaempferol-*O*-dideoxyhexosyl-hexoside, and isorhamnetin-*O*-dideoxyhexosyl-hexoside, respectively. Also in this family, peaks **4** and **5** were tentatively identified as *cis*-Chicoric (Rt 14,93 min) and *trans*-Chicoric (Rt 15,01 min), respectively, as showed a deprotonated ion [M-H]$^-$ at *m/z* 473 and a major MS^2 fragment at *m/z* 331 that corresponds to the loss of the caffeoyl moiety, *m/z* 149, of a loss of a second caffeic acid moiety, *m/z* 293 and *m/z* 179. Chicoric acid was also reported in *Curcubita pepo* L. [4].

Quantification

As shown in

Table 11, in the Portuguese samples, the Common Pumpkin peel presented the higher total of phenolic compounds (9.4±0.3 mg/g of extract), followed by the fiber of Kabocha Squash (4.8±0.1 mg/g of extract) and the peel of Butternut Squash (4.73±0.01 mg/g of extract). These totals are mainly composed by the flavan-3-ols and flavonoids families, while phenolic acids are low representative or were not detected. The (-)-epicatechin (Peak 1) is the most abundant compound in all the samples evaluated.

Different profiles were seen in the samples from Algeria (

Table 12), where more expressive contents of phenolic acids were found in the Gold nugget pumpkin fibers (2.27±0.02 mg/g of extract) and flavonoids are the main composition of the total phenolic compounds in all the peels and in the seeds of Musquée de Provence. Peel of Gold nugget pumpkin presented the highest value of total phenolic compounds followed by the fibers of this variety (4.1±0.1 and 3.93±0.05 mg/g of extract, respectively). Furthermore, no peak was identified in the extracts of seeds of the varieties 'Gold nugget pumpkin' and 'Butternut squash'.

In **Table 13**, the quantification of the Tunisia samples is presented. The (-)-epicatechin (Peak 1) was also the most abundant compound in all these samples, and the only compound found in the mix of seeds and fibers of Batati and Karkoubi varieties. Peel of Bejaoui and Karkoubi presented the higher total of phenolic compounds (5,7±0,2 and 5,6±0,2 mg/g of extract) and more peaks tentatively identified.

In the samples from Greece, the seeds, fibers and pulp presented only one peak: the (-)-epicatechin (Peak 1). In general, the fibers showed the higher results, where variety 3 was the highest (7.30±0.05 mg/g of extract) followed by the variety 9 (7.6±0.3 mg/g of extract).

The samples of Greek peel (

Table 15) presented more diversity of compounds. Once again, the (-)-epicatechin was the predominant compound in all the samples. The flavonoids were also representative, composed by Isorhamnetin-*O*-dideoxyhexosyl-hexoside (peak 7) and Kaempferol-*O*-deoxyhexosyl-hexoside (peak 10). While the phenolic acids were found in low quantity or traces.

The profiles of phenolic compounds in the samples from Egypt are composed only of flavonoids. The totals ranged from 3.42±0.09 to 2.09±0.02 mg/g of extract. The peel of all varieties presented great contents of flavonoids, while just one variety of seeds (Dickinson) and one of fibers and pulp (Golden Cushaw) presented phenolic compounds.

Table 10. Phenolic compounds characterized by HPLC-DAD-ESI/MS in the different samples.

Peak	Rt (min)	λmax (nm)	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	7,71	280	289	245(100),205(45)	(-)-Epicatechin
2-	13,42	345	775	301(100)	Quercetin-*O*-dideoxyhexosyl-hexoside
3	14,73	263	405	281(100),137(12),93(5)	7 4-O-(6′-O-glucosyl-4″-hydroxybenzoyl)-4-hydroxybenzyl alcohol
4	14,93	328	473	331(100),293(76),179(5),149(5)	*cis*-Chicoric acid
5	15,01	328	473	331(100),293(89),179(6),149(7)	*trans*-Chicoric acid
6-	15,42	344	739	285(100)	Kaempferol-*O*-dideoxyhexosyl-hexoside
7-	15,85	354	769	315(100)	Isorhamnetin-*O*-dideoxyhexosyl-hexoside
8	16,71	348	593	285(100)	Kaempferol-*O*-deoxyhexosyl-hexoside
9	16,73	359	609	301(100)	Quercetin-*O*-deoxyhexosyl-hexoside
10-	17,12	348	593	285(100)	Kaempferol-*O*-deoxyhexosyl-hexoside
11-	17,6	365	623	315(100)	Isorhamnetin-*O*-deoxyhexosyl-hexoside
12	17,92	348	593	285(100)	Kaempferol-*O*-deoxyhexosyl-hexoside
13-	20,73	365	623	315(100)	Isorhamnetin-*O*-deoxyhexosyl-hexoside
14	20,85	**355**	623	315(100)	Isorhamnetin-*O*-deoxyhexosyl-hexoside

Table 11. Quantification of the phenolic compounds found in the samples from Portugal (in mg/g of extract).

Peak	Common Pumpkin			Butternut Squash			Kabocha Squash		
	Peel	Fibers	Seeds	Peel	Fibers	Seeds	Peel	Fibers	Seeds
1	4.58±0.08	3.04±0.05	1.74±0.03	2.56±0.03	2.47±0.07	2.63±0.02	1.50±0.07	2.7±0.1	1.29±0.05
2	0.50±0.02	0.484±0.006	0.49±0.02	n.d.	n.d.	n.d.	n.d.	0.533±0.003	0.474±0.001
3	0.214±0.009	n.d.	n.d.	n.d.	n.d.	n.d.	0.116±0.004	n.d.	n.d.
6	0.65±0.03	0.487±0.007	0.458±0.008	0.6096±0.0002	n.d.	0.457±0.008	0.58±0.02	0.5218±0.0004	0.461±0.003
7	1.60±0.08	n.d.	n.d.	0.543±0.004	n.d.	0.454±0.007	0.62±0.03	0.494±0.002	n.d.
10	0.59±0.03	n.d.	n.d.	0.519±0.005	n.d.	n.d.	0.58±0.03	0.493±0.003	n.d.
11	0.69±0.03	n.d.	n.d.	0.496±0.005	n.d.	n.d.	0.56±0.02	n.d.	n.d.
13	0.55±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	0.54±0.03	n.d.	n.d.
Total Flavan-3-ols	4.58±0.08	3.04±0.05	1.74±0.03	2.56±0.03	2.47±0.07	2.63±0.02	1.50±0.07	2.7±0.1	1.29±0.05
Total Phenolic Acids	0.214±0.009	n.d.	n.d.	n.d.	n.d.	n.d.	0.116±0.004	n.d.	n.d.
Total Flavonoids	4.6±0.2	0.97±0.01	0.95±0.03	2.17±0.01	n.d.	0.91±0.02	2.9±0.1	2.042±0.003	0.934±0.002
Total Phenolic Compounds	9.4±0.3	4.01±0.06	2.693±0.004	4.73±0.01	2.47±0.07	3.538±0.007	4.50±0.06	4.8±0.1	2.23±0.04

n.d. – not detected. Calibration curves used for quantification: (-)-catequin (y = 84,950x – 23,200, R^2 = 0.999, LOD = 0.17 µg/mL; LOQ = 0.68 µg/mL, peak 1); *p*-hydroxybenzoic acid (y = 208604x + 173056, R^2 = 0.9995, LOD = 1.37 µg/mL; LOQ = 4.15 µg/mL, peak 3); and quercetin 3-*O*-glucoside (y=34843x−160173; R^2=0.9998; LOD=0.21 µg/ml; LOQ=0.71 µg/mL, peaks 2, 6, 7, 10, 11, and 13).

Table 12. Quantification of the phenolic compounds found in the samples from Algeria (in mg/g of extract).

Peak	Gold nugget pumpkin				Butternut squash				Musquée de Provence			
	Pulp	Fibers	Seeds	Peel	Pulp	Fibers	Seeds	Peel	Pulp	Fibers	Seeds	Peel
1	n.d.	1.66±0.07	n.d.	0.51±0.02	0.524±0.002	0.97±0.03	n.d.	0.346±0.009	0.54±0.02	1.13±0.01	n.d.	0.577±0.008
3	tr.	2.27±0.02	n.d.	0.134±0.006	n.d.	0.176±0.006	n.d.	tr.	n.d.	0.207±0.007	0.0762±0.0002	n.d.
6	n.d.	n.d.	n.d.	0.94±0.05	n.d.	0.462±0.002	n.d.	0.4526±0.0003	n.d.	0.4790±0.0007	n.d.	0.476±0.003
7	n.d.	n.d.	n.d.	1.65±0.07	n.d.	n.d.	n.d.	0.44904±0.00006	n.d.	n.d.	0.460±0.005	0.474±0.002
11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4418±0.0001	n.d.	n.d.	n.d.	n.d.
13	n.d.	n.d.	n.d.	0.85±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total Flavan-3-ols	n.d.	1.66±0.07	n.d.	0.51±0.02	0.524±0.002	0.97±0.03	n.d.	0.346±0.009	0.54±0.02	1.13±0.01	n.d.	0.577±0.008
Total Phenolic Acids	tr.	2.27±0.02	n.d.	0.134±0.006	n.d.	0.176±0.006	n.d.	tr.	n.d.	0.207±0.007	0.0762±0.0002	n.d.
Total Flavonoids	n.d.	n.d.	n.d.	3.4±0.1	n.d.	0.462±0.002	n.d.	1.3434±0.0004	n.d.	0.4790±0.0007	0.460±0.005	0.951±0.005
Total Phenolic Compounds	tr.	3.93±0.05	n.d.	4.1±0.1	0.524±0.002	1.61±0.03	n.d.	1.689±0.008	0.54±0.02	1.818±0.007	0.536±0.005	1.53±0.01

n.d. – not detected. tr. – traces. Calibration curves used for quantification: (-)-catequin (y = 84,950x – 23,200, R^2 = 0.999, LOD = 0.17 µg/mL; LOQ = 0.68 µg/mL, peak 1); *p*-hydroxybenzoic acid (y = 208604x + 173056, R^2 = 0.9995, LOD = 1.37 µg/mL; LOQ = 4.15 µg/mL, peak 3); and quercetin 3-*O*-glucoside (y=34843x−160173; R^2=0.9998; LOD=0.21 µg/ml; LOQ=0.71 µg/mL, peaks 6, 7, 11, and 13).

Table 13. Quantification of the phenolic compounds found in the samples from Tunisia (in mg/g of extract).

Peak	Batati		Karkoubi		Bejaoui	
	Seeds+Fibers	Peel	Seeds+Fibers	Peel	Seeds+Fibers	Peel
1	4,35±0,05	1,16±0,06	3,02±0,05	2,10±0,06	2,78±0,02	2,6±0,1
6	n.d.	0,62±0,01	n.d.	0,57±0,02	0,483±0,002	0,56±0,02
7	n.d.	0,96±0,03	n.d.	0,51±0,02	n.d.	0,558±0,007
8	n.d.	n.d.	n.d.	0,52±0,02	n.d.	0,48±0,01
10	n.d.	n.d.	n.d.	0,85±0,04	n.d.	0,51±0,01
11	n.d.	0,495±0,008	n.d.	0,57±0,03	n.d.	0,5±0,01
13	n.d.	0,479±0,005	n.d.	0,4516±0,0003	n.d.	0,447±0,006
Total Flavan-3-ols	4,35±0,05	1,16±0,06	3,02±0,05	2,10±0,06	2,78±0,02	2,6±0,1
Total Flavonoids	n.d.	2,55±0,05	n.d.	3,5±0,1	0,483±0,002	3,06±0,06
Total Phenolic Compounds	4,35±0,05	3,7±0,1	3,02±0,05	5,6±0,2	3,27±0,02	5,7±0,2

n.d. – not detected. Calibration curves used for quantification: (-)-catequin (y = 84,950x – 23,200, R^2 = 0.999, LOD = 0.17 µg/mL; LOQ = 0.68 µg/mL, peak 1); and quercetin 3-*O*-glucoside (y=34843x−160173; R^2=0.9998; LOD=0.21 µg/ml; LOQ=0.71 µg/mL, peaks 6, 7, 8, 10, 11, and 13).

Table 14. Quantification of the phenolic compound (**Peak 1**) found in the samples of seeds of pumpkin from Greece (in mg/g of extract).

Greek variety	Seeds	Fibers	Pulp
1	1.54±0.07	3.7±0.1	0.471±0.008
2	6.3±0.3	1.64±0.09	0.909±0.002
3	3.7±0.2	7.6±0.3	0.49±0.02
4	1.00±0.05	4.39±0.03	1.156±0.004
5	1.12±0.06	3.974±0.006	0.590±0.008
6	4.1±0.2	4.25±0.07	1.58±0.03
7	1.52±0.02	3.14±0.02	1.44±0.06
8	n.d.	5.42±0.06	0.93±0.01
9	1.4±0.1	7.30±0.05	1.746±0.003
10	n.d.	4.46±0.07	0.59±0.01
11	-	3.33±0.09	1.850±0.006
12	-	3.0±0.1	0.495±0.009
13	-	4.09±0.07	1.04±0.02
14	-	3.7±0.1	0.50±0.01
15	-	4.875±0.005	1.17±0.03
16	-	4.11±0.04	1.090±0.001
17	-	1.532±0.008	1.03±0.01

n.d. – not detected. Calibration curves used for quantification: (-)-catequin ($y = 84{,}950x - 23{,}200$, $R^2 = 0.999$, LOD = 0.17 µg/mL; LOQ = 0.68 µg/mL).

Table 15. Quantification of the phenolic compounds found in the samples of peel of pumpkin from Greece (in mg/g of extract).

Peel samples	Peak 1	Peak 4	Peak 5	Peak 7	Peak 10	Total Flavan-3-ols	Total Phenolic Acids	Total Flavonoids	Total Phenolic Compounds
Ri 1	n.d.	0.033±0.002	0.0036±0.0002	0.511±0.002	0.560±0.004	n.d.	0.037±0.001	1.071±0.002	1.108±0.004
Ri 2	1.65±0.06	tr.	0.0236±0.0002	0.4712±0.0003	0.507±0.001	1.65±0.06	0.0236±0.0002	0.9786±0.0008	2.65±0.06
Ri 3	1.79±0.04	tr.	0.0109±0.0004	0.48±0.01	n.d.	1.79±0.04	0.0109±0.0004	0.48±0.01	2.29±0.05
Ri 4	1.37±0.04	tr.	tr.	0.577±0.004	0.472±0.001	1.37±0.04	tr.	1.048±0.003	2.41±0.04
Ri 5	n.d.	tr.	tr.	0.45969±0.00006	n.d.	n.d.	tr.	0.45969±0.00006	0.45969±0.00006
Ri 6	n.d.	tr.	0.0032±0.0001	0.4801±0.0001	0.4695±0.0003	n.d.	0.48333±0.00002	0.9496±0.0002	0.9528±0.0003
Ri 7	1.11±0.04	tr.	0.0057±0.0002	0.50084±0.00008	n.d.	1.11±0.04	0.0057±0.0002	0.50084±0.00008	1.62±0.04
Ri 8	n.d.	0.052±0.002	0.0116±0.0005	0.520±0.003	0.539±0.003	n.d.	0.064±0.002	1.0591±0.0001	1.123±0.003
Ri 9	1.71±0.08	0.0146±0.0004	0.101±0.005	0.58±0.01	0.519±0.002	1.71±0.08	0.115±0.005	1.10±0.01	2.92±0.07
Ri 10	1.922±0.002	tr.	tr.	n.d.	n.d.	1.922±0.002	tr.	n.d.	1.922±0.002
Ri 11	1.40±0.06	tr.	0.0147±0.0006	0.485±0.001	0.4964±0.0003	1.40±0.06	0.0147±0.0006	0.982±0.002	2.40±0.06
Ri 12	1.82±0.02	0.0112±0.0005	0.086±0.003	n.d.	n.d.	1.82±0.02	0.097±0.003	n.d.	1.92±0.02
Ri 13	1.260±0.006	tr.	tr.	n.d.	n.d.	1.260±0.006	tr.	n.d.	1.260±0.006
Ri 14	0.49±0.01	tr.	tr.	n.d.	0.4589±0.0002	0.49±0.01	tr.	0.4589±0.0002	0.95±0.01
Ri 15	1.141±0.007	n.d.	tr.	n.d.	0.4561±0.0005	1.141±0.007	tr.	0.4561±0.0005	1.597±0.006
Ri 16	0.861±0.009	n.d.	n.d.	0.468±0.002	0.493±0.006	0.861±0.009	n.d.	0.962±0.007	1.823±0.002
Ri 17	1.23±0.05	n.d.	n.d.	n.d.	n.d.	1.23±0.05	n.d.	n.d.	1.23±0.05

n.d. – not detected. tr. – traces. Calibration curves used for quantification: (-)-catequin (y = 84,950x – 23,200, R^2 = 0.999, LOD = 0.17 µg/mL; LOQ = 0.68 µg/mL, peak 1); *p*-caffeic acid (y = 388345x + 406369, R^2 = 0.9939, LOD=0.78 µg/mL and LOQ=1.97 µg/mL, peak 4 and 5); and quercetin 3-*O*-glucoside (y=34843x−160173; R^2=0.9998; LOD=0.21 µg/ml; LOQ=0.71 µg/mL, peaks 9 and 10).

Table 16. Quantification of the phenolic compounds found in the samples from Egypt (in mg/g of extract).

Samples		Peak 2	6	7	9	10	12	14	Total Phenolic Compounds (Total flavonoids)
Golden Cushaw	Fibers	0.507±0.004	0.497±0.001	0.463±0.001	0.491±0.002	0.469±0.004	0.4661±0.0008	0.475±0.001	3.36772±0.00002
	Peel	0.4632±0.0008	0.48540±0.00006	0.4863±0.0005	0.4746±0.0002	0.4989±0.0007	0.509±0.001	0.468±0.001	3.385±0.003
	Pulp	0.45299±0.00002	0.4519±0.0002	0.451917±0.000006	0.4599±0.0001	0.44809±0.00004	0.45033±0.00007	0.44728±0.00007	3.1623±0.0002
	Seeds	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dickinson	Fibers	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Peel	n.d.	0.5107±0.0007	0.5539±0.0007	0.4984±0.0005	n.d.	0.470±0.001	0.643±0.003	2.676±0.004
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Seeds	0.477±0.002	0.4826±0.0001	0.474±0.001	n.d.	0.4810±0.0002	0.480±0.001	0.4705±0.0004	2.865±0.005
Butternut Squash	Fibers	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Peel	n.d.	0.546±0.002	0.5341±0.0008	n.d.	n.d.	0.469±0.004	0.54±0.01	2.09±0.02
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Seeds	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Halloween	Fibers	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Peel	n.d.	0.51±0.01	0.51±0.01	0.49±0.01	0.59±0.01	0.84±0.03	0.499±0.008	3.42±0.09
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Seeds	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Honey Delite	Fibers	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Peel	0.450±0.001	0.4624±0.0002	0.47625±0.00002	0.456±0.002	0.4592±0.0006	0.5025±0.0008	0.4648±0.0009	3.272±0.005
	Pulp	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Seeds	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. – not detected. Calibration curves used for quantification: quercetin 3-*O*-glucoside (y=34843x−160173; R^2=0.9998; LOD=0.21 µg/ml; LOQ=0.71 µg/mL).

4. Prospection

Once defined the most bioactive extracts (Deliverable 2.1) and the present technical specifications of the preserving compounds (Deliverable 2.2), it will be possible to study the most suitable extraction conditions for obtaining the preserving compounds (Deliverable 2.3). In sequence, the mathematical models (by RSM) of the dependent variables used in the optimization of the extraction of the preserving compounds will also be obtained (Deliverable 2.4).

5. References

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