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REPORTS OF THE MOST BIOACTIVE EXTRACT OBTAINED FROM PUMPKIN BY-PRODUCTS

DELIVERABLE 2.1

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 D2.1 – "Reports of the most bioactive extract obtained from pumpkin by-products" of the WP2, that comprises the evaluation of the preservative capacity of pumpkin by-products extracts through *in vitro* assays of antioxidant and antimicrobial activity, in order to determine the most bioactive extract of the most promising pumpkin by-product.

2. Description of work

Following what was foreseen in Task 2.1, conventional extraction technologies, such as maceration, were applied to pumpkin by-products (seeds, peel, and fibers) using green solvents (water and ethanol). To assess the preserving capacity of the extracted compounds different *in vitro* chemical, biochemical, and cell-based assays were applied. The antioxidant activity was evaluated by measuring the extracts concentration able to inhibit by 50% the formation of thiobarbituric acid reactive substances (TBARS, from porcine brain, thus preventing the lipid peroxidation) and to protect 50% of erythrocytes population (from sheep blood) during a specific period of time (thus preventing the oxidative hemolysis). The antimicrobial activity was evaluated in foodborne pathogens (by the microdilution method and clinical isolates strains by the colorimetric microdilution method using the *p*-iodonitrotetrazolium chloride (INT) dye. To ensure the safety of the obtained bio-based ingredients, toxicological assays were also performed using *in vitro* cytotoxicity assays (cell primary cultures obtained from porcine liver) and *in vitro* genotoxicity assays.

2.1. Goal

Screening the preservative capacity of the pumpkin by-product extracts and determining the most promising by-product and its pumpkin genotypes in terms of natural preservative molecules. The residues generated in the fruit pulp production will be used as a source of compounds with preservative capacity to incorporate in the produced pulp, promoting a more sustainable industrial process, with the reduction of residue generation and replacement of synthetic additives, fostering a circular economy.

3. Results

Samples from different pumpkin varieties and parts of the fruit (peel, seeds, and fibers) were analyzed. Due to the pandemic condition, commercial pumpkins from Portugal and Algeria were evaluated to perform preliminary studies. Moreover, samples cultivated in Greece, Tunisia, and Egypt were assessed, as proposed.

3.1 Sample extraction

The first step was to obtain the extracts rich in preservative molecules. The extracts were obtained by maceration of 2 g of powder sample in 60 mL of ethanol solution (ethanol:water, 80:20) at room temperature and with agitation for 60 min (**Figure 1**). Samples were filtered and this procedure was repeated one more time. The extracts were lyophilized and stored for subsequent analysis. The results of the extraction yield can be seen in **Tables 1**, **3**, **4**, and **5**. In **Table 2**, the codification of the varieties from Greece is presented.

Figure 1. Maceration of pumpkin residues using ethanol/water 80:20 as extraction solvent.

Table 1. Extraction yield of peel, seeds, and fibers of different pumpkin varieties from Portugal (%).

Residue / Variety	Peel	Seeds	Fibers
Butternut squash	43.05	28.63	40.05
Common pumpkin	22.92	6.39	31.70
Kabocha squash	20.09	8.32	27.79

Table 2. Variety name of pumpkin cultivated in Greece according to the code and part of the fruit.

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

Table 3. Extraction yield of peel, seeds, and fibers of different pumpkin varieties from Greece (%).

Residue / Variety	Peel	Seeds	Fibers
1	41.23	8.93	50.02
2	40.73	7.01	56.99
3	36.53	15.66	52.28
4	45.28	9.86	59.44
5	31.77	7.52	46.80
6	31.97	9.57	50.62
7	22.01	7.55	62.90
8	32.81	6.49	36.32
9	34.84	8.76	43.19
10	45.54	7.85	63.05
11	53.63	-	58.04
12	35.70	-	85.57
13	39.29	-	66.06
14	33.65	-	55.87
15	28.47	-	55.64
16	40.00	-	52.45
17	26.89	-	59.43

Table 4. Extraction yield of peel, and the mix of seeds and fibers of 3 pumpkin varieties from Tunisia (%).

Residue / Variety	Peel	Seeds + Fibers
Batati	42.00	18.05
Karkoubi	37.28	15.67
Bejaoui	36.37	9.05

Table 5. Extraction yield of peel, seeds, and fibers of different pumpkin varieties from Egypt (%).

Residue / Variety	Peel	Seeds	Fibers
Golden Cushaw	47.70	9.43	69.07
Dickinson	41.18	12.60	65.12
Butternut squash	41.21	17.14	65.84
Halloween	49.59	14.39	63.88
Honey Delite	50.55	12.96	73.62

A good yield is evident in almost all matrices. Peels and fibers presented higher yield than the seeds, being that of the fibers, generally, slightly higher than that of the peels. The Algerian results are not presented once the samples were received already extracted.

3.2 Antioxidant activity

The samples from Portugal were evaluated in terms of antioxidant capacity, through 3 chemical methods: DPPH scavenging activity, reducing power, and β-carotene bleaching inhibition, as first screening, and all samples were evaluated through 2 chemical cell-based assays, namely the inhibition of lipid peroxidation capacity (TBARS) and the inhibition of oxidative hemolysis

(OxHLIA). The seeds were also assessed after being deffated by soxhlet, due to the fat content interfering in the OxHLIA assay in some samples (except in Algerian samples, which were received already extracted). The results were presented as IC_{50} values (µg/mL), which correspond to the concentration of extract required to achieve 50% inhibition of the oxidation activity. The lower the IC50 value, the higher the antioxidant activity of the extracts. The results are presented in **Tables 6** and **7**.

Table 6. Antioxidant activity obtained through chemical assays of the bio-residues of three different pumpkin varieties from Portugal (IC_{50}, µg/mL).

Pumpkin variety	Part	DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition
Butternut Squash	Peel	1979 ± 17	167 ± 7	89.1 ± 0.3
	Seeds	739 ± 30	1593 ± 39	15.54 ± 0.02
	Fibers	528 ± 15	487 ± 23	73.4 ± 0.2
Common Pumpkin	Peel	1292 ± 22	1033 ± 40	2.76 ± 0.06
	Seeds	1431 ± 28	1187 ± 39	25.85 ± 0.07
	Fibers	552 ± 23	527 ± 6	207.9 ± 0.4
Kabocha Squash	Peel	2117 ± 41	733 ± 35	35.66 ± 0.02
	Seeds	1868 ± 41	880 ± 20	1.30 ± 0.02
	Fibers	367 ± 10	378 ± 6	5.197 ± 0.002
Trolox		42 ± 1	41 ± 1	18± 1

Table 7. Antioxidant activity obtained through cell-based assays of the bio-residues of three different pumpkin varieties from Portugal (IC_{50}, µg/mL).

Pumpkin variety	Part	OXHLIA 60 min IC_{50}, µg/mL	TBARS IC_{50}, µg/mL
Butternut Squash	Peel	88 ± 3	7461 ± 315
	Seeds	59 ± 6	185 ± 7
	Deffated seeds	923 ± 26	170 ± 8
	Fibers	44 ± 4	6887 ± 53
Common Pumpkin	Peel	90 ± 3	3921 ± 33
	Seeds	43 ± 3	756 ± 27
	Deffated seeds	720 ± 52	483 ± 22
	Fibers	365 ± 13	6375 ± 68
Kabocha Squash	Peel	209 ± 10	7765 ± 31
	Seeds	46 ± 2	164 ± 8
	Deffated seeds	655 ± 31	1038 ± 31
	Fibers	96 ± 2	1568 ± 53
Trolox		21.8 ± 0.2	139 ± 5

As presented in **Tables 6** and **7**, all Portuguese samples presented great antioxidant capacity, showing IC_{50} values ranging from about 50 times higher than the positive control, Trolox, to about 14 times lower than Trolox. The fibers and seeds showed better antioxidant capacity than the peels, with the fibers revealing great antioxidant capacity in the employed chemical methods, and

the seeds in the cell-based ones. In terms of pumpkin varieties, the kabocha squash presented the best result in 3 of the 5 assays (DPPH, β-carotene, and TBARS).

Regarding samples from Algeria (**Table 8**), in the TBARS assay, the seeds stood out for all the varieties, with the best result being presented by the gold nugget pumpkin. The seeds of this variety showed an IC_{50} value about 1.5 times lower than that of the positive control, Trolox. On the other hand, in the OxHLIA assay, the results were quite similar between the type of bioresidues and between the varieties. Despite their strong lipid peroxidation inhibition capacity, the seeds of the gold nugget pumpkin did not present anti-hemolytic properties.

Table 8. Antioxidant activity obtained though cell-based assays of the bio-residues of three different pumpkin varieties from Algeria (IC_{50}, µg/mL).

Pumpkin variety	Part	OXHLIA 60 min IC_{50}, µg/mL	TBARS IC_{50}, µg/mL
Butternut Squash	Peel	588 ± 18	4569 ± 277
	Seeds	115 ± 6	573 ± 31
	Fibers	257 ± 13	3508 ± 91
Gold nugget Pumpkin	Peel	362 ± 8	3123 ± 136
	Seeds	n.a.	91 ± 4
	Fibers	566 ± 13	3659 ± 199
Musquée de Provence	Peel	335 ± 4	2123 ± 101
	Seeds	400 ± 34	549 ± 27
	Fibers	188 ± 2	4385 ± 242
Trolox		21.8 ± 0.2	139 ± 5

As shown in **Table 9**, all Greek pumpkin by-products revealed great antioxidant properties, where the seeds stood out presenting IC50 values of only about 1 to 10 times higher than that of the positive control, Trolox. Fibers followed the seeds and showed IC_{50} values ranging from about 5 to 28 times higher than Trolox. These results demonstrate their ability to inhibit lipid peroxidation and suggest their potential application as sources of preservative compounds.

Table 9. Lipid peroxidation inhibition capacity (TBARS) of different pumpkin varieties from Greece (IC_{50}, µg/mL).

Variety \ Residue	Seeds	Deffated seeds	Fibers	Peel
1	262 ± 11	1133 ± 54	1312 ± 51	3331 ± 163
2	677 ± 16	640 ± 28	2788 ± 112	825 ± 25
3	1476 ± 65	434 ± 7	1998 ± 97	850 ± 40
4	700 ± 29	468 ± 12	3054 ± 142	885 ± 18
5	478 ± 11	208 ± 9	3900 ± 165	1285 ± 13
6	607 ± 30	125 ± 1	1825 ± 84	1589 ± 63
7	974 ± 46	1007 ± 51	1723 ± 62	1796 ± 62
8	893 ± 38	405 ± 19	3023 ± 126	953 ± 21
9	372 ± 18	1771 ± 59	1739 ± 83	4216 ± 204
10	433 ± 17	640 ± 31	1911 ± 62	1710 ± 84
11	-	-	630 ± 25	4973 ± 84
12	-	-	2267 ± 90	5733 ± 260
13	-	-	3059 ± 141	4984 ± 221
14	-	-	1645 ± 39	2749 ± 127
15	-	-	1610 ± 59	1963 ± 94
16	-	-	1188 ± 53	3085 ± 135
17	-	-	3768 ± 190	985 ± 11
Trolox	139 ± 5			

The fibers and peel from Greek pumpkin (**Table 10**) showed great anti-hemolytic properties. The best result was presented by the peel of the variety 14. In addition, considering all the varieties, the fibers presented better range of anti-hemolytic capacity values: from 4.8 to 29.3 times higher than the control Trolox. To assess the anti-hemolytic capacity of the pumpkin seeds, it was necessary to perform new extractions in order to reduce their fat content. The defatted seeds presented great results, ranging from 52±2 to 795±31 µg/mL.

Table 10. Oxidative hemolysis inhibition capacity (OxHLIA) of different pumpkin varieties from Greece (IC_{50}; µg/mL; Δt = 60 min).

Sample / Variety	Seeds	Deffated seeds	Fibers	Peel
1	n.a	52 ± 2	210 ± 9	572 ± 23
2	n.a.	101 ± 3	327 ± 12	822 ± 34
3	n.a	171 ± 5	168 ± 6	555 ± 8
4	n.a.	431 ± 9	390 ± 5	294 ± 11
5	n.a	84 ± 3	638 ± 12	755 ± 33
6	n.a.	315 ± 14	106 ± 6	475 ± 24
7	n.a	795 ± 31	128 ± 5	122 ± 2
8	n.a.	289 ± 14	132 ± 9	612 ± 25
9	n.a	409 ± 23	123 ± 2	441 ± 18
10	n.a.	241 ± 10	393 ± 18	157 ± 7
11	-	-	104 ± 6	243 ± 17
12	-	-	348 ± 8	427 ± 8
13	-	-	140 ± 5	197 ± 12
14	-	-	359 ± 11	41 ± 2
15	-	-	444 ± 16	219 ± 8
16	-	-	371 ± 4	139 ± 13
17	-	-	485 ± 7	98 ± 4
Trolox	21.8 ± 0.2			

All the samples from Tunisia (**Table 11**) presented great antioxidant capacity. In the TBARS assay, the IC_{50} values rangered from about 6 to almost 36 times higher than the positive control, Trolox. The mix of grains and fibers from the Bejaoui variety presented the best result. Regarding the OxHLIA assay, the peel of Batati and Bejaoui presented the best results.

Table 11. Antioxidant activity obtained though cell-based assays of the bio-residues of three different pumpkin varieties from Tunisia (IC_{50}, µg/mL).

Pumpkin variety	Part	OXHLIA 60 min IC_{50}, µg/mL	TBARS IC_{50}, µg/mL
Batati	Seeds + Fibers	516 ± 40	2128 ± 85
	Deffated seeds+fibers	315 ± 26	967 ± 16
	Peel	113 ± 4	2134 ± 96
Karboubi	Seeds + Fibers	264 ± 13	287 ± 10
	Deffated seeds+fibers	149 ± 5	1865 ± 74
	Peel	232 ± 11	1874 ± 81
Bejaoui	Seeds + Fibers	491 ± 25	245 ± 11
	Deffated seeds+fibers	419 ± 12	602 ± 29
	Peel	145 ± 3	5107 ± 147
Trolox		21.8 ± 0.2	139 ± 5

The bio-residues of pumpkin from Egypt showed (**Table 12**) in the TBARS assay, in general, a good antioxidant activity even though the concentration values are higher than those of Trolox, mainly in 3 of the 5 varieties of seeds, with an IC_{50} value between 417 and 751 µg/mL; as well as in 2 of the 5 varieties of fibers with an IC_{50} of 749-992 µg/mL. Regarding the OxHLIA assay, the

samples presented great results, especially the defatted seeds of Dickinson and Butternut squash varieties, being the results just 2 to 3 times higher than the positive control.

Table 12. Antioxidant activity obtained though cell-based assays of the bio-residues of the different pumpkin varieties from Egypt (IC_{50}, µg/mL).

Pumpkin variety	Part	OXHLIA 60 min IC_{50}, µg/mL	TBARS IC_{50}, µg/mL
Golden Cushaw	Peel	149 ± 14	1144 ± 25
	Seeds	-	417 ± 13
	Deffated seeds	121 ± 4	404 ± 20
	Fibers	181 ± 7	1963 ± 53
Dickinson	Peel	186 ± 9	1609 ± 26
	Seeds	-	751 ± 28
	Deffated seeds	48 ± 3	308 ± 14
	Fibers	112 ± 7	1728 ± 77
Butternut squash	Peel	115 ± 8	2504 ± 121
	Seeds	-	1458 ± 73
	Deffated seeds	61 ± 2	315 ± 14
	Fibers	258 ± 17	992 ± 30
Halloween	Peel	117 ± 9	2477 ± 34
	Seeds	-	1765 ± 69
	Deffated seeds	222 ± 10	1839 ± 65
	Fibers	197 ± 8	2002 ± 23
Honey Delite	Peel	339 ± 10	3193 ± 148
	Seeds	-	459 ± 13
	Deffated seeds	177 ± 8	329 ± 16
	Fibers	106 ± 9	749 ± 33
Trolox		21.8 ± 0.2	139 ± 5

3.3 Antimicrobial activity

The samples from Portugal, Algeria, Greece, Tunisia and Egypt, were assessed regarding their antimicrobial activity. For that, the antibacterial capacity was tested against five strains of gram-negative bacteria (*Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica*, and *Yersinia enterocolitica*) and three gram-positive bacteria (*Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus*), and the antifungal capacity against *Aspergillus brasiliensis* and *Aspergillus fumigatus*, with interest in food matrices, in the maximum concentration of 10 mg/mL. The results are presented in **Tables 13** to **19**, divided by country.

Table 13. Antimicrobial and antifungal activity of the bio-residues of three different pumpkin varieties from Portugal (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	Butternut squash						Common pumpkin						Kabocha squash						Streptomicin 1 mg/mL		Methicilin 1 mg/mL		Ampicillin 10 mg/mL	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria																								
Enterobacter cloacae	10	>10	10	>10	2.5	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	>10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	10	>10	5	>10	5	>10	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																								
Bacillus cereus	>10	>10	>10	>10	5	>10	10	>10	2.5	>10	10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	>10	>10	5	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	10	>10	>10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	10	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	Butternut squash						Common pumpkin						Kabocha squash						Ketoconazole	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	10	>10	10	>10	5	>10	5	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.5	1

Table 14. Antimicrobial and antifungal activity of the bio-residues of three different pumpkin varieties from Algeria (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	Gold nugget pumpkin						Butternut squash						Musquée de Provence						Streptomicin 1 mg/mL		Methicilin 1 mg/mL		Ampicillin 10 mg/mL	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria																								
Enterobacter cloacae	5	>10	5	>10	>10	>10	5	>10	10	>10	10	>10	10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	>10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	5	>10	5	>10	10	>10	5	>10	10	>10	>10	>10	10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																								
Bacillus cereus	5	>10	>10	>10	>10	>10	2.5	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	2.5	>10	5	>10	10	>10	10	>10	>10	>10	5	>10	>10	>10	>10	>10	5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	2.5	>10	5	>10	10	>10	5	>10	5	>10	10	>10	10	>10	5	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	Gold nugget pumpkin						Butternut squash						Musquée de Provence						Ketoconazole	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	5	>10	5	>10	10	>10	5	>10	10	>10	10	>10	5	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	0.5	1

Table 15. Antimicrobial and antifungal activity of the seeds of different pumpkin varieties from Greece (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	S1		S2		S3		S4		S5		S6		S7		S8		S9		S10		Streptomicin 1 mg/mL		Methicilin 1 mg/mL		Ampicillin 10 mg/mL	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																										
Enterobacter cloacae	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	2.5	>10	2.5	>10	2.5	>10	2.5	>10	5	>10	10	>10	5	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																										
Bacillus cereus	10	>10	>10	>10	10	>10	10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	5	>10	10	>10	10	>10	5	>10	10	>10	5	>10	10	>10	5	>10	5	>10	5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	10	>10	10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	S1		S2		S3		S4		S5		S6		S7		S8		S9		S10		Ketoconazole	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.5	1

Table 16. Antimicrobial and antifungal activity of the peel of different pumpkin varieties from Greece (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	RI 1		RI 2		RI 3		RI 4		RI 5		RI 6		RI 7		RI 8		RI 9		RI 10	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																				
Enterobacter cloacae	>10	>10	2.5	>10	>10	>10	>10	>10	>10	>10	>10	>10	2.5	>10	>10	>10	>10	>10	>10	>10
Escherichia coli	10	>10	2.5	>10	10	>10	10	>10	10	>10	10	>10	2.5	>10	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10
Salmonella enterica	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10
Yersinia enterocolitica	>10	>10	5	>10	>10	>10	>10	>10	>10	>10	>10	>10	5	>10	>10	>10	>10	>10	>10	>10
Gram-positive bacteria																				
Bacillus cereus	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	5	>10	2.5	>10	>10	>10	>10	>10	>10	>10	5	>10	2.5	>10	>10	>10	>10	>10	>10	>10
Staphylococcus aureus	10	>10	2.5	>10	>10	>10	>10	>10	>10	>10	10	>10	2.5	>10	>10	>10	>10	>10	>10	>10

	RI 11		RI 12		RI 13		RI 14		RI 15		RI 16		RI 17		Streptomicin		Methicilin		Ampicillin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																				
Enterobacter cloacae	5	>10	10	>10	>10	>10	10	>10	>10	>10	5	>10	10	>10	>10	>10	10	>10	>10	>10
Escherichia coli	5	>10	10	>10	10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterica	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Yersinia enterocolitica	10	>10	5	>10	>10	>10	10	>10	>10	>10	10	>10	5	>10	>10	>10	10	>10	>10	>10
Gram-positive bacteria																				
Bacillus cereus	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	5	>10	>10	>10	>10	>10	>10	>10	>10	>10	5	>10	>10	>10	>10	>10	>10	>10	>10	>10
Staphylococcus aureus	5	>10	10	>10	10	>10	10	>10	10	>10	5	>10	10	>10	10	>10	10	>10	10	>10

	RI 1		RI 2		RI 3		RI 4		RI 5		RI 6		RI 7		RI 8		RI 9	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	>10	>10	10	>10	5	>10
Aspergillus fumigatus	>10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10

	RI10		RI 11		RI 12		RI 13		RI 14		RI 15		RI 16		RI 17		Ketoconazole	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	5	>10	10	>10	0.06	0.125
Aspergillus fumigatus	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	10	>10	0.5	1

Table 17. Antimicrobial and antifungal activity of the fibers of different pumpkin varieties from Greece (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	FI 1		FI 2		FI 3		FI 4		FI 5		FI 6		FI 7		FI 8		FI 9		FI 10	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																				
Enterobacter cloacae	10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10
Escherichia coli	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterica	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10
Yersinia enterocolitica	10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Gram-positive bacteria																				
Bacillus cereus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Staphylococcus aureus	>10	>10	>10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10

	FI 11		FI 12		FI 13		FI 14		FI 15		FI 16		FI 17		Streptomicin		Methicilin		Ampicillin	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria																				
Enterobacter cloacae	10	>10	10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	>10	>10
Escherichia coli	10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	5	>10	10	>10	10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterica	10	>10	10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10
Yersinia enterocolitica	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10	>10	>10
Gram-positive bacteria																				
Bacillus cereus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Staphylococcus aureus	10	>10	10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10

	FI 1		FI 2		FI 3		FI 4		FI 5		FI 6		FI 7		FI 8		FI 9	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10
Aspergillus fumigatus	>10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10

	FI 10		FI 11		FI 12		FI 13		FI 14		FI 15		FI 16		FI 17		Ketoconazole	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.5	1

Table 18. Antimicrobial and antifungal activity of the bio-residues of three different pumpkin varieties from Tunisia (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	Seeds+Fibers						Peel						Streptomicin 1 mg/mL		Methicilin 1 mg/mL		Ampicillin 10 mg/mL	
	Batati		Karkoubi		Bejaoui		Batati		Karkoubi		Bejaoui							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria																		
Enterobacter cloacae	10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	10	>10	10	>10	10	>10	10	>10	5	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	10	>10	>10	>10	10	>10	2.5	>10	10	>10	2.5	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																		
Bacillus cereus	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	Seeds+Fibers				Peel								Ketoconazole	
	Batati		Karkoubi		Batati		Karkoubi		Batati		Karkoubi			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	5	>10	5	>10	5	>10	10	>10	10	>10	10	>10	0.06	0.125
Aspergillus fumigatus	10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	0.5	1

Table 19. Antimicrobial and antifungal activity of the bio-residues of five different pumpkin varieties from Egypt (MIC: Minimum inhibitory concentration; MBC: Minimal bactericidal concentration; MFC: Minimal fungicidal concentration; n.t: not tested).

	Golden Cushaw						Dickinson						Butternut squash					
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria																		
Enterobacter cloacae	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10
Escherichia coli	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Salmonella enterica	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10
Yersinia enterocolitica	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Gram-positive bacteria																		
Bacillus cereus	>10	>10	10	>10	10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Listeria monocytogenes	10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10
Staphylococcus aureus	>10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10	10	>10

	Halloween						Honey Delite						Streptomicin 1 mg/mL		Methicilin 1 mg/mL		Ampicillin 10 mg/mL	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers							
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria																		
Enterobacter cloacae	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	>10	>10	10	>10	>10	>10	5	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria																		
Bacillus cereus	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	>10	>10	10	>10	>10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	10	>10	10	>10	10	>10	10	>10	5	>10	10	>10	0.007	0.007	0.007	0.007	0.15	0.15

	Golden Cushaw						Dickinson						Butternut squash					
	Seeds		Peel		Fibers		Seeds		Peel		Fibers		Seeds		Peel		Fibers	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus brasiliensis	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10	>10	>10	10	>10	10	>10
Aspergillus fumigatus	>10	>10	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	>10	10	>10

	Halloween						Honey Delite						Ketoconazole	
	Seeds		Peel		Fibers		Seeds		Peel		Fibers			
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC

Aspergillus brasiliensis	>10	>10	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	10	>10	>10	>10	10	>10	10	>10	>10	>10	0.5	1

The peel and the seeds stood out in the antibacterial activity, especially the peel of the variety 2 (Landrace from the region of Trikala - Turbinate) from Greece, which was capable to inhibit the growth of all bacteria and fungi tested. Many samples of peel showed protection against the two fungal strains assessed. Seven peel samples and six seed samples inhibited at least seven bacterial strains. The minimal inhibition concentration achieved was 2.5 mg/ml. None of the samples presented bactericidal nor fungicidal capacity at the tested concentrations.

3.4 Cytotoxicity

The potential safety for food application of the pumpkin by-product was verified, since none of the tested extracts showed toxicity in a primary culture of non-tumor porcine liver cells (PLP2), up to the maximum concentration tested of 400 µg/mL. This is an important first validation as, so far, no more studies have been found regarding toxic effects of pumpkins.

3.5 Most bioactive extract

With the data obtained from the antioxidant and antimicrobial activity of all samples tested, together with the yield information, it was possible to highlight the most promising bioresidues and varieties for obtaining extracts rich in preservative compounds. These results are presented in **Tables 20 and 21**. These selected samples are being used to proceed in the study of extraction optimization, namely: the peels of Butternut squash from Portugal (PT - Butternut) and the peels of variety 2 (Landrace from the region of Trikala - Turbinate), variety 16 (Voutirato), and variety 17 ((Local landrace "Leuka Melitis - Round), from Greece (GR - Ri 2, GR - Ri 16, and GR - Ri 17, respectively).

Table 20. Summary of the antioxidant activity of the selected samples.

Sample	OxHLIA	TBARS
PT - Butternut	88 ±3	7461 ± 315
GR - Ri 2	822 ± 34	825 ± 25
GR - Ri 16	139 ± 13	3085 ± 135
GR - Ri 17	98 ± 4	985 ± 11
Trolox	21.8 ± 0.2	139 ± 5

Table 21. Summary of the antimicrobial activity of the selected samples.

	Butternut Portugal		R2 Greece		R16 Greece		R17 Greece		Streptomicin 1mg/mL		Methicilin 1mg/mL		Ampicillin 10mg/mL	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Gram-negative bacteria														
Enterobacter cloacae	10	>10	2.5	>10	2.5	>10	5	>10	0.007	0.007	n.t.	n.t	0.15	0.15
Escherichia coli	10	>10	2.5	>10	2.5	>10	10	>10	0.01	0.01	n.t.	n.t.	0.15	0.15
Pseudomonas aeruginosa	>10	>10	10	>10	>10	>10	>10	>10	0.06	0.06	n.t.	n.t.	0.63	0.63
Salmonella enterica	10	>10	10	>10	10	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Yersinia enterocolitica	5	>10	5	>10	5	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Gram-positive bacteria														
Bacillus cereus	>10	>10	10	>10	2.5	>10	10	>10	0.007	0.007	n.t.	n.t.	n.t.	n.t.
Listeria monocytogenes	>10	>10	2.5	>10	2.5	>10	10	>10	0.007	0.007	n.t.	n.t.	0.15	0.15
Staphylococcus aureus	>10	>10	2.5	>10	2.5	>10	5	>10	0.007	0.007	0.007	0.007	0.15	0.15

	Butternut Portugal		R2 Greece		R16 Greece		R17 Greece		Ketoconazole	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus brasiliensis	10	>10	5	>10	5	>10	10	>10	0.06	0.125
Aspergillus fumigatus	>10	>10	10	>10	5	>10	10	>10	0.5	1

4. Prospection

Once defined the most bioactive extracts, the following studies are being developed: the technical specifications of the preserving compounds (Deliverable 2.2) and the most suitable extraction conditions for obtaining the preserving compounds (Deliverable 2.3). Subsequently, mathematical models (by RSM) of the dependent variables used in the optimization of the extraction of the preserving compounds will be obtained (Deliverable 2.4).