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IDENTIFICATION OF SEEDS OILS AND BIOCHEMICAL ANALYSIS

DELIVERABLE 1.3

PulpIng

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

Index

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1. Summary

The PulpIng project aims to create a premium pumpkin pulp product, enriched and preserved with value-added compounds derived from pumpkin by-products (discarded parts of fruit and leaves), while advocating for an integrated and sustainable approach. The main focus of WP1 involves evaluating selected pumpkin germplasm under in vitro, field, and greenhouse conditions, using different pumpkin bioresidues. This report refers to WP1 deliverable D1.3 "Seed oil identification and biochemical analysis". In this deliverable, various pumpkin seeds from different Mediterranean sites (Greece, Tunisia, and Egypt) were assessed to determine their crude fat content and fatty acid profiles. The objective was to identify the most suitable pumpkin seed oils for the development of the highest quality product based on their profile of fatty acids.

2. Description of work

Following Task 1.4, we undertook the evaluation and comparison of the quality of different pumpkin seeds sourced from local cultivars and varieties studied in Pulping project. This report specifically examines pumpkin seeds from Mediterranean regions, including Greece, Tunisia, and Egypt. The evaluation primarily focused on the proximal composition of crude fat and fatty acids. To determine the crude fat content, we employed the respective AOAC 920.85 method. Meanwhile, for the analysis of fatty acids, we utilized GC equipment coupled to a flame ionization detector (FID). These analytical techniques allowed us to assess and characterize the nutritional composition of the pumpkin seeds comprehensively according to their fatty acids profile.

2.1. Goal

The primary goal of this study was to conduct a comprehensive evaluation and comparison of the quality of diverse pumpkin seeds derived from local cultivars and varieties. Specifically, our focus lied on pumpkin seeds sourced from Mediterranean regions, including Greece, Tunisia, and Egypt. The main objectives were to determine the proximal composition of crude fat and assess the fatty acid profiles in these seeds. The findings of this study will contribute to identifying and selecting superior pumpkin seed varieties for potential utilization and further development in the production of high-qualityand functional food products.

3. Results

The seeds of various pumpkin genotypes cultivated in Greece, Egypt, and Tunisia underwent comprehensive characterization in terms of crude fat content using the Official Methods of Analysis of the international AOAC (AOAC 920.85). Additionally, the fatty acids contents of the

seeds are being analysed using GC coupled to FID. These analytical methods allowed us to obtain valuable data on the nutritional composition of the pumpkin seeds from different regions, providing essential insights for potential applications and further research.

3.1 Crude fat content

The assessed Greek genotypes are listed in Table 1, including the respective code, name of variety and total crude fat content. The seed samples exhibited a crude fat content from about 24 to almost 40 g/100g dw.

Table 1. Variety code and name of pumpkin cultivated in Greece, as well the respective crude fat content.

Code	Variety	Crude fat content (g/100 g dw)
	2020 (Stock seeds)	
1	V1 (Fytro FS-243)	33.44 ± 0.05
2	V2 (Landrace from the region of Trikala)	33 ± 1
3	V3 (Big Max)	29.0 ± 0.8
4	V4 (Local landrace "Nychaki")	35.7 ± 0.4
5	V5 (Local landrace "Leuka Melitis)	24 ± 1
6	V6 (Local landrace from the region of Lakonia)	25 ± 1
7	V7 (Local landrace from the region of Lakonia)	37.3 ± 0.2
8	V8 (Local landrace from the region of Lakonia)	37.5 ± 0.5
9	V9 (Local landrace "Makedonika prasina")	39.8 ± 0.5
10	V10 (Local landrace from the region of Laconia)	35.9 ± 0.2
	Growing period of 2021 (1st experimental year)	
11	V1 (Fytro FS-243)	35 ± 2
12	Local landrace "Voutirato"	26 ± 1
13	V3 (Big Max)	31 ± 1
14	V4 (Local landrace "Nychaki")	29 ± 1
15	V5 (Local landrace "Leuka Melitis)	33 ± 1
16	V6 (Local landrace from the region of Lakonia)	29 ± 1
17	V7 (Local landrace from the region of Lakonia)	36 ± 1
18	V8 (Local landrace from the region of Lakonia)	36 ± 1
19	V9 (Local landrace "Makedonika prasina")	41 ± 2
20	V10 (Local landrace from the region of Laconia)	31.1 ± 0.6
	Growing period of 2022 (2nd experimental year)	
21	V1 (Fytro FS-243) Without crop rotation	33 ± 2
22	V1 (Fytro FS-243) Crop rotation	31.0 ± 0.7
23	Local landrace "Voutirato"	27.3 ± 0.2
24	V3 (Big Max)	29 ± 1
25	V4 (Local landrace "Nychaki") Without crop rotation	34.7 ± 0.9
26	V4 (Local landrace "Nychaki") Crop rotation	34.2 ± 0.2
27	V5 (Local landrace "Leuka Melitis)	29.0 ± 0.5
28	V6 (Local landrace from the region of Lakonia)	28 ± 1
29	V7 (Local landrace from the region of Lakonia) Without crop rotation	35.1 ± 0.7
30	V7 (Local landrace from the region of Lakonia) Crop rotation	33.6 ± 0.7
31	V8 (Local landrace from the region of Lakonia)	36.9 ± 0.9
32	V9 (Local landrace "Makedonika prasina")	35.6 ± 0.9
33	V10 (Local landrace from the region of Laconia)	35 ± 1

Table 2 shows the results of the crude fat content for the seed samples obtained from Egypt. The seed samples demonstrated a crude fat content ranging from 14 to 34.5 g/100g dw.

Table 2. Variety code and name of pumpkin cultivated in Egypt, as well the respective crude fat content.

Code	Variety	Crude fat content (g/100 g dw)
1	Golden Cushaw	26 ± 1
2	Dickinson	14.0 ± 0.6
3	Butternut squash	16.3 ± 0.5
4	Halloween	23.3 ± 0.3
5	Honey Delite	34.5 ± 0.5

Batati was the Tunisian genotype subjected to evaluation. Table 3 presents the total crude fat content (11.8±0.6 g/100g dw) for this specific genotype.

Table 3. Variety code and name of pumpkin cultivated in Tunisia, as well the respective crude fat content.

Code	Variety	Crude fat content (g/100g dw)
1	Batati (NGB745)	11.8 ± 0.6

3.2 Fatty acids content

The fatty acid compositions of the seeds of the different pumpkin genotypes from Greece are present in Table 4. Five types of fatty acids were found in the samples, more specifically, the palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), and the arachidic acid (C20:0). The oleic acid and the linoleic acid were the predominant ones, varying from 11.08 ± 0.06 % to 58.77 ± 0.05 % and from 21.15 ± 0.01 % to 67.16 ± 0.04 %, respectively. In all the samples, the monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) represented from 73.60 % to 84.43 % of the total fatty acids, while the saturated fatty acids (SFA) content ranged between 15.57 % and 26.36 % of total fatty acids.

Table 4. Fatty acids of the seeds of the different pumpkin genotypes from Greece (relative %)

Code	C16:0	C18:0	C18:1n9c	C18:2n6c	C20:0	SFA	MUFA	PUFA
1	19.218 ± 0.003	6.65 ± 0.03	19.1 ± 0.1	54.5 ± 0.1	0.49 ± 0.03	26.36 ± 0.01	19.1 ± 0.1	54.5 ± 0.1
2	11.6 ± 0.1	6.02 ± 0.01	18.42 ± 0.03	63.5 ± 0.1	0.508 ± 0.004	18.1 ± 0.1	18.42 ± 0.03	63.5 ± 0.1
3	11.92 ± 0.06	5.87 ± 0.02	20.1 ± 0.04	61.54 ± 0.08	0.585 ± 0.002	18.37 ± 0.04	20.10 ± 0.04	61.54 ± 0.08
4	11.22 ± 0.02	5.42 ± 0.04	27.4 ± 0.1	55.41 ± 0.07	0.500 ± 0.001	17.14 ± 0.05	27.4 ± 0.1	55.41 ± 0.07
5	18.8 ± 0.4	5.43 ± 0.01	11.08 ± 0.06	64.6 ± 0.5	Nd	24.3 ± 0.4	11.08 ± 0.06	64.6 ± 0.5
6	14.13 ± 0.02	5.66 ± 0.01	12.60 ± 0.05	67.16 ± 0.04	0.45 ± 0	20.24 ± 0.01	12.6 ± 0.05	67.16 ± 0.04
7	19.6 ± 0.5	4.1 ± 0.1	11.3 ± 0.1	65 ± 0.3	Nd	23.7 ± 0.5	11.3 ± 0.1	65.0 ± 0.3
8	11.3 ± 0.5	5.4 ± 0.1	24.3 ± 0.4	59.08 ± 0.04	Nd	16.6 ± 0.4	24.3 ± 0.4	59.08 ± 0.04
9	10.62 ± 0.02	4.55 ± 0.01	18.53 ± 0.01	65.90 ± 0.04	0.40 ± 0.01	15.57 ± 0.04	18.53 ± 0.01	65.9 ± 0.04
10	10.1 ± 0.03	5.32 ± 0.02	23.2 ± 0.1	60.892 ± 0.001	0.52 ± 0.01	15.94 ± 0.06	23.2 ± 0.1	60.892 ± 0.001

Code	C16:0	C18:0	C18:1n9c	C18:2n6c	C20:0	SFA	MUFA	PUFA
11	18.3 ± 0.1	6.89 ± 0.08	29.9 ± 0.5	44.9 ± 0.5	Nd	25.22 ± 0.04	29.9 ± 0.5	44.9 ± 0.5
12	16.0 ± 0.2	7.36 ± 0.02	35.6 ± 0.2	41.0 ± 0.1	Nd	23.4 ± 0.1	35.6 ± 0.2	41.0 ± 0.1
13	17.04 ± 0.02	5.99 ± 0.06	31.76 ± 0.01	45.22 ± 0.04	Nd	23.02 ± 0.04	31.76 ± 0.01	45.22 ± 0.04
14	14.35 ± 0.07	8.00 ± 0.02	44.7 ± 0.2	32.6 ± 0.2	0.69 ± 0.01	23.05 ± 0.09	44.7 ± 0.2	32.6 ± 0.2
15	15.9 ± 0.06	6.23 ± 0.07	30.95 ± 0.02	46.92 ± 0.004	Nd	22.14 ± 0.02	30.95 ± 0.02	46.917 ± 0.004
16	17.94 ± 0.08	7.37 ± 0.06	37.2 ± 0.1	37.5 ± 0.3	Nd	25.3 ± 0.1	37.2 ± 0.1	37.5 ± 0.3
17	17.76 ± 0.03	8.13 ± 0.03	29.99 ± 0.07	44.13 ± 0.06	Nd	25.88 ± 0.01	29.99 ± 0.07	44.13 ± 0.06
18	13.7 ± 0.2	5.972 ± 0.003	19.5 ± 0.3	60.8 ± 0.6	Nd	19.7 ± 0.2	19.5 ± 0.3	60.8 ± 0.6
19	12.9 ± 0.01	6.42 ± 0.02	25.277 ± 0.005	54.9 ± 0.03	0.5 ± 0.01	19.82 ± 0.02	25.277 ± 0.005	54.9 ± 0.03
20	11.64 ± 0.01	6.76 ± 0.01	48.10 ± 0.06	32.86 ± 0.07	0.63 ± 0.01	19.03 ± 0.01	48.1 ± 0.06	32.86 ± 0.07
21	17.4 ± 0.1	7.64 ± 0.01	35.6 ± 0.1	38.7 ± 0.05	0.73 ± 0.04	25.8 ± 0.2	35.6 ± 0.1	38.7 ± 0.05
22	16.53 ± 0.02	7.4 ± 0.1	35.2 ± 0.1	40.072 ± 0.002	0.72 ± 0.02	24.7 ± 0.1	35.2 ± 0.1	40.072 ± 0.002
23	17.3 ± 0.02	6.778 ± 0.004	30.942 ± 0.001	44.3 ± 0.02	0.68 ± 0.001	24.75 ± 0.02	30.942 ± 0.001	44.3 ± 0.02
24	12.9 ± 0.02	10.05 ± 0.04	41.47 ± 0.01	34.627 ± 0.005	0.95 ± 0.01	23.9 ± 0.01	41.47 ± 0.01	34.627 ± 0.005
25	13.11 ± 0.04	7.40 ± 0.04	45.08 ± 0.07	33.81 ± 0.06	0.62 ± 0.02	21.12 ± 0.02	45.08 ± 0.07	33.81 ± 0.06
18	13.7 ± 0.2	5.972 ± 0.003	19.5 ± 0.3	60.8 ± 0.6	Nd	19.7 ± 0.2	19.5 ± 0.3	60.8 ± 0.6
19	12.9 ± 0.01	6.42 ± 0.02	25.277 ± 0.005	54.9 ± 0.03	0.5 ± 0.01	19.82 ± 0.02	25.277 ± 0.005	54.9 ± 0.03
20	11.64 ± 0.01	6.76 ± 0.01	48.10 ± 0.06	32.86 ± 0.07	0.63 ± 0.01	19.03 ± 0.01	48.1 ± 0.06	32.86 ± 0.07
21	17.4 ± 0.1	7.64 ± 0.01	35.6 ± 0.1	38.7 ± 0.05	0.73 ± 0.04	25.8 ± 0.2	35.6 ± 0.1	38.7 ± 0.05
22	16.53 ± 0.02	7.4 ± 0.1	35.2 ± 0.1	40.072 ± 0.002	0.72 ± 0.02	24.7 ± 0.1	35.2 ± 0.1	40.072 ± 0.002
23	17.3 ± 0.02	6.778 ± 0.004	30.942 ± 0.001	44.3 ± 0.02	0.68 ± 0.001	24.75 ± 0.02	30.942 ± 0.001	44.3 ± 0.02
24	12.9 ± 0.02	10.05 ± 0.04	41.47 ± 0.01	34.627 ± 0.005	0.95 ± 0.01	23.9 ± 0.01	41.47 ± 0.01	34.627 ± 0.005
25	13.11 ± 0.04	7.40 ± 0.04	45.08 ± 0.07	33.81 ± 0.06	0.62 ± 0.02	21.12 ± 0.02	45.08 ± 0.07	33.81 ± 0.06
26	12.95 ± 0.03	7.93 ± 0.01	49.8 ± 0.2	28.6 ± 0.2	0.7 ± 0.04	21.58 ± 0	49.8 ± 0.2	28.6 ± 0.2
27	15.7 ± 0.1	6.62 ± 0.09	36.1 ± 0.2	40.9 ± 0.2	0.71 ± 0.01	23.03 ± 0.02	36.1 ± 0.2	40.9 ± 0.2
28	16.59 ± 0.08	6.8 ± 0.03	32.48 ± 0.06	43.4 ± 0.2	0.7 ± 0.01	24.09 ± 0.1	32.48 ± 0.06	43.4 ± 0.2
29	16.86 ± 0.03	8.46 ± 0.01	31.44 ± 0.06	42.5 ± 0.05	0.75 ± 0.03	26.06 ± 0.01	31.44 ± 0.06	42.5 ± 0.05
30	15.89 ± 0.01	8.49 ± 0	33.7 ± 0.01	41.07 ± 0.02	0.85 ± 0.004	25.22 ± 0.01	33.7 ± 0.01	41.07 ± 0.02
31	13.345 ± 0.001	6.41 ± 0.02	35.972 ± 0.008	43.67 ± 0.05	0.61 ± 0.03	20.36 ± 0.05	35.972 ± 0.008	43.67 ± 0.05
32	11.529 ± 0.004	7.861 ± 0.008	48.59 ± 0.05	31.315 ± 0.04	0.702 ± 0.001	20.09 ± 0.01	48.59 ± 0.05	31.315 ± 0.04
33	11.98 ± 0.03	7.41 ± 0.01	58.77 ± 0.05	21.15 ± 0.01	0.701 ± 0.003	20.08 ± 0.04	58.77 ± 0.05	21.15 ± 0.01

Table 5 presents the fatty acid compositions of 5 distinct pumpkin varieties from Egypt. The same fatty acids were found in these samples, but linoleic acid was the predominant one, ranging from 46.9 ± 0.1% to 62.40 ± 0.04%, followed by the palmitic acid and the oleic acid, with ranges of 13.00 ± 0.02 - 20.15 ± 0.07% and 12.163 ± 0.005 - 26.0 ± 0.3%, respectively. The analysis also revealed that the seeds of these varieties are mostly abundant in polyunsaturated fatty acids, by contributing from 46.9 ± 0.1 to 62.4 ± 0.04% of the total fatty acids content.

Table 5. Fatty acids content of the seeds of different pumpkin varieties from Egypt (relative %).

Compound	C16:0	C18:0	C18:1n9c	C18:2n6c	C20:0	SFA	MUFA	PUFA
Golden Cushaw	19.3 ± 0.2	9.33 ± 0.02	23.8 ± 0.2	46.9 ± 0.1	0.63 ± 0.01	29.3 ± 0.2	23.8 ± 0.2	46.9 ± 0.1
Dickinson	13.00 ± 0.02	6.24 ± 0.02	17.86 ± 0.02	62.40 ± 0.04	0.50 ± 0.01	19.74 ± 0.01	17.86 ± 0.02	62.4 ± 0.04
Butternut squash	13.9 ± 0.3	5.77 ± 0.05	20.5 ± 0.9	59.8 ± 0.5	Nd	19.7 ± 0.3	20.5 ± 0.9	59.8 ± 0.5

Compound	C16:0	C18:0	C18:1n9c	C18:2n6c	C20:0	SFA	MUFA	PUFA
Halloween	14.22 ± 0.02	5.86 ± 0.01	26.0 ± 0.3	54.0 ± 0.4	Nd	20.08 ± 0.03	26.0 ± 0.3	54.0 ± 0.4
Honey Delite	20.15 ± 0.07	6.05 ± 0.03	12.163 ± 0.005	61.6 ± 0.1	Nd	26.2 ± 0.1	12.163 ± 0.005	61.6 ± 0.1

The fatty acid profile of the Tunisian seeds is displayed in Table 6, wherein linoleic acid was identified as the predominant component across all samples (62.5 ± 0.1%), and cosequently polyunsaturated fatty acids were the main class of fatty acids (63.9 ± 0.1%).

Table 6. Fatty acids of the Tunisian seeds (relative %)

Compound	C16:0	C18:0	C18:1n9c	C18:2n6c	C18:3n3	C20:0	SFA	MUFA	PUFA
Batati	15.1 ± 0.1	8.11 ± 0.02	12.27 ± 0.02	62.5 ± 0.1	1.40 ± 0.02	0.64 ± 0.02	23.9 ± 0.1	12.27 ± 0.02	63.9 ± 0.1