PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION
IN THE MEDITERRANEAN AREA

PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

ipb INSTITUTO POLITÉCNICO DE BRAGANÇA

CIMO Centro de Investigação de Montanha

montanhas de investigação

Decorgel

UNIVERSITY OF THESSALY

CBBC

CRAPC

ATB Leibniz-Institut für Agrartechnik und Bioökonomie

BENHA UNIVERSITY

https://pulping-prima.eu

PulpIng

Development of Pumpkin Pumpkin Pulp Formulation Using a Sustainable Integrated Strategie (PRIMA)

PRIMA – Section 2 - 2019
Global funding: 1.206.438,04 €

Objective: Stimulate a value chain, with innovative processes, that goes throughout all the developing stages of a pumpkin fruit pulp formulation functionalized with a bio-based preservative extracted from pumpkin by-products.

Expected impacts:

1. Promote sustainable production and valorization of pumpkins through integrated farming techniques based on innovative processes;
2. Recover bioactive ingredients with strong preservative capacity by sustainable and easy-to-perform techniques;
3. Develop a new functionalized pumpkin pulp formulation with natural preservatives isolated from by-products, with an optimized approach to increase product shelf-life;

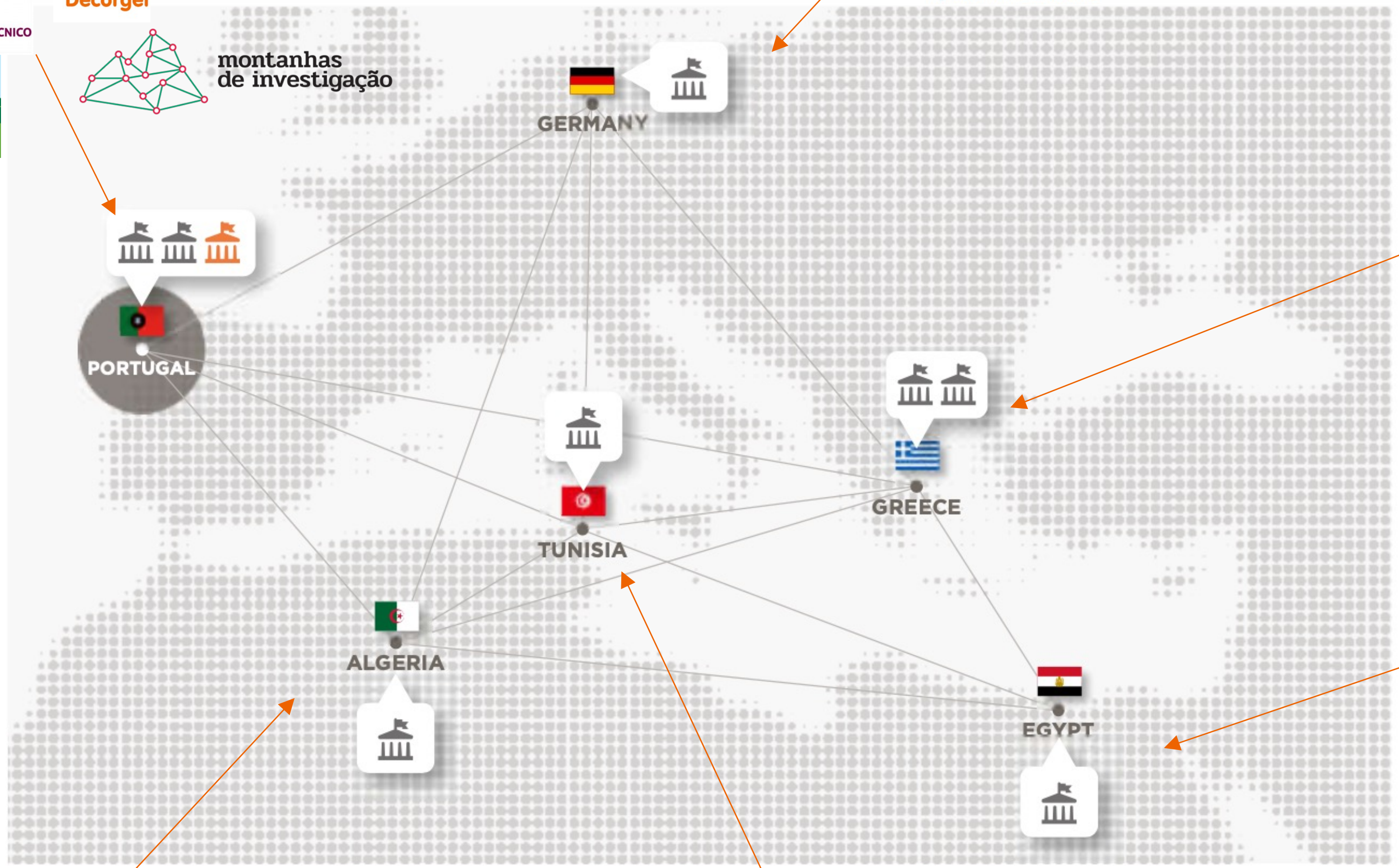
Consortium

Coordinating country

Portugal

Participating countries/ 6

Research Units/ 9



PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WORK PACKAGES

WP1 – Defining Agronomic Conditions for Pumpkin Production

- Selection of the best pumpkin genotypes
- Establishment of improved cultivation protocols

UNIVERSITY OF THESSALY
24 Months

WP2 – Sustainable Recovery of Compounds with Preserving Capacity from Pumpkin By-Products

- Identify natural compounds from pumpkin by-products
- Design of scalable extraction processes

ipb INSTITUTO POLITÉCNICO DE BRAGANÇA
12 Months

WP3 – Refinement and Stabilization of the Identified Preserving Compounds

- Selection of most promising fractions/extracts
- Development of stabilization procedures

CENTRE DE BIOTECHNOLOGIE DE BORJ CEDRIA CBBC
12 Months

WORK PACKAGES

WP4 – Pumpkin Fruit Formulation

- Development of a pumpkin fruit pulp incorporating natural preservatives

Decorgel
18 Months

WP5 – Preservation Studies and Quality Assessment During Shelf-Life

- Ensure the microbial quality of the pumpkin fruit pulp
- Promote accelerated shelf-life studies

ATB Leibniz-Institut für Agrartechnik und Bioökonomie
18 Months

WP6 – Waste and Wastewater Management and Life-Cycle Assessment

- Perform a complete waste and wastewater characterization
- Evaluate the environmental performance through life cycle assessment

montanhas de investigação
24 Months

WP7 – Management and Dissemination of Results

- Establish a management structure
- Coordinate and implement effective exploitation of results ough

ipb INSTITUTO POLITÉCNICO DE BRAGANÇA
36 Months

PRIMA

Objectives

- Improve cultivation and integrated farming techniques based on innovative processes
- Develop innovative, sustainable and industrially feasible methods to extract preserving ingredients
- Develop a novel pumpkin fruit pulp formulation functionalised with the isolated natural preservatives
- Develop/optimise non-thermal processing methods based on innovative technologies
- Carry out an optimised approach to enhance the shelf-life of pumpkin fruit pulp formulation
- Optimise waste management and perform a life-cycle assessment
- Promote dissemination and exploitation of the results of the participating countries

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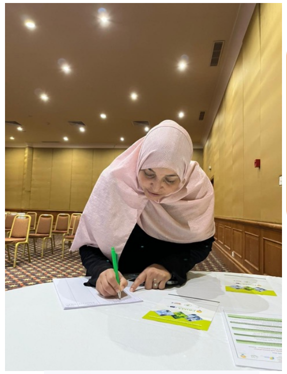
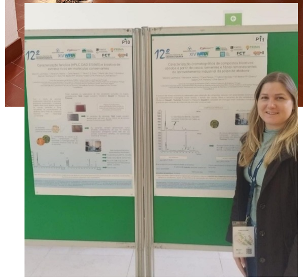
Productivity


TECNOALIMENTAR

PulpIng- Projeto inovador que procura desenvolver uma formulação de polpa de abóbora utilizando uma estratégia integrada sustentável

PulpIng é um projeto que visa a implementação de sistemas alimentares sustentáveis, capazes de produzir produtos mais saudáveis, obedecendo aos princípios da economia circular, de forma a fortalecer a cadeia agroalimentar; nomeadamente no que diz respeito aos canais de mercado associados ao desenvolvimento polpas de abóbora de elevada qualidade produzidas a partir de variedades locais da região mediterrânea.






PROJETO PULPING COMPLETA CADEIA DE VALOR DA ABÓBORA

CONFERÊNCIA INTERNACIONAL EM BRAGANÇA

Scientific Publications

Publicity Material

Patents

PulpIng

e-Newsletters

Seminars

Social Media

Workshops

molecules MDPI

Article

Valorization of Pumpkin Peel as a Source of Bioactive

horticulturae MDPI

Article

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

plants MDPI

Article

The Effects of Salt Stress on Germination, Seedling Growth and Biochemical Responses of Tunisian Squash (*Cucurbita maxima* Duchesne) Germplasm

Neji Tarchoun [1,*], Wassim Saadaoui [1], Najla Mezghani [1,2], Ourania I. Pavli [3], Hanen Falleh [4] and Spyridon A. Petropoulos [3,*]

https://www.facebook.com/pulping.project

https://www.instagram.com/pulping_project/

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PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WP 2: Sustainable recovery of compounds with preserving capacity from pumpkin by-products

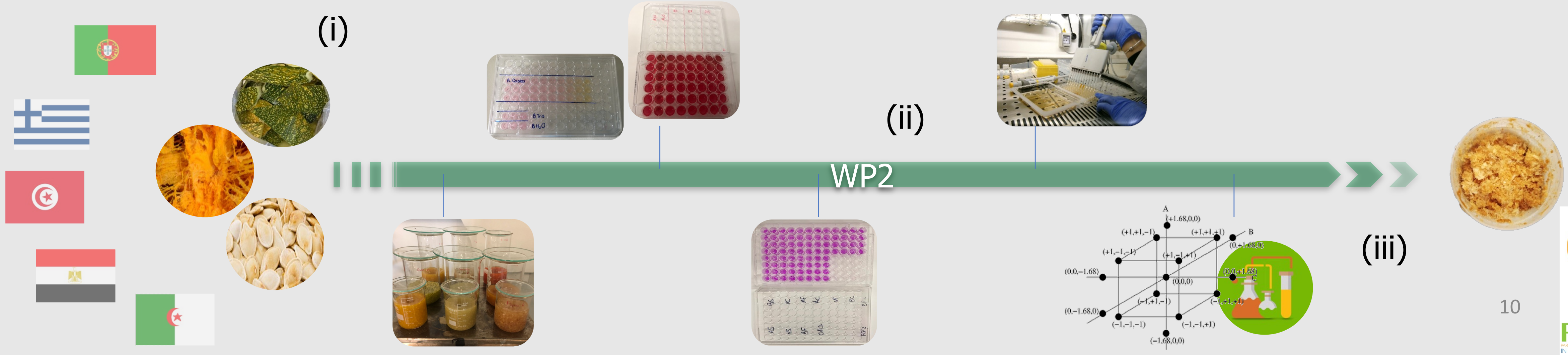
Maria Gabiela Leichtweis

Work Package 2

Lead partner: IPB
Participants: CBBC; MORE; CRAPC; UEM

- **Impact of the WP:**

The main achievements of this WP are: (i) the **selection of the most suitable parts** (seeds, peels, or fibers) **from the best pumpkin** (different varieties from Greece, Egypt, Tunisia, Portugal, and Algeria), based on their antioxidant and antimicrobial activities; (ii) the **optimization of sustainable and industrially** feasible extraction processes to obtain natural preservatives for food incorporation (iii).

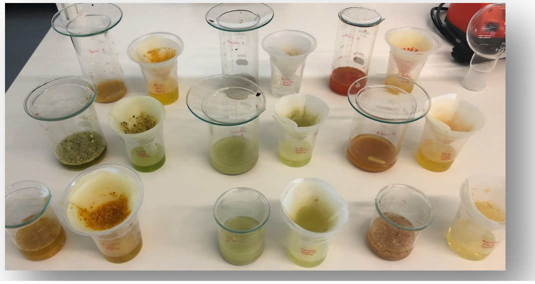


Work Package 2

- **RP activities & outcomes:**

Task 2.1. Prospection and identification of natural compounds with highest preserving capacity

- 108 samples from different pumpkin varieties and parts of the fruit were evaluate.
- Samples were extracted by hydroethanolic maceration for subsequent analyses.
- The extraction yield ranges were higher in the peels and fibers than in the seeds.

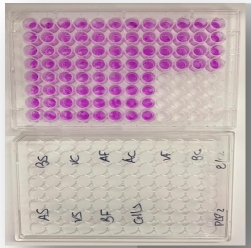


	Sample (unit)	Fibers	Seeds	Peel	Seeds+fibers
Portugal	9	42 ± 11	7 ± 2	36 ± 10	-
Algeria	12	49.6	17	49.5	-
Greece	61	56 ± 11	9 ± 3	36 ± 8	-
Tunisia	6	-	-	39 ± 3	14 ± 5
Egypt	20	68 ± 4	13 ± 3	46 ± 5	-

% Yield (average ± dp)

Samples

Portugal and Algeria were commercial obtained, due pandemic delay at pumpkin cultivation!



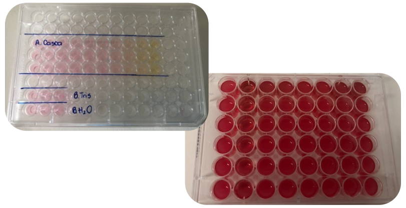
- Toxicity capacity
  - None of the tested extracts showed toxicity up to the maximum concentration tested (400 μg/mL) in a primary culture of non-tumor porcine liver cells (PLP2).
  - Except some varieties of seeds from Greece

Work Package 2

- **RP activities & outcomes:**

Task 2.1. Prospection and identification of natural compounds with highest preserving capacity *(cont.)*

- Antioxidant activity though two cell-based assays
  - TBARS: Thiobarbituric acid reactive substance assay
  - OxHLIA: Oxidative hemolysis inhibition assay



- In TBARS assay all the samples presented great results, especially the seeds.

TBARS		Fibers	Seeds	Peel	Seeds+fibers
Portugal	Min	1568 ± 53	164 ± 8	3921 ± 33	-
	Max	6887 ± 53	756 ± 27	7765 ± 31	
Algeria	Min	3508 ± 91	91 ± 4	2123 ± 101	-
	Max	4385 ± 242	573 ± 31	4569 ± 227	
Greece	Min	630 ± 25	262 ± 11	825 ± 25	-
	Max	3900 ± 165	1476 ± 65	5733 ± 260	
Tunisia	Min	-	-	1874 ± 81	245 ± 11
	Max			5107 ± 147	2128 ± 85
Egypt	Min	749 ± 33	417 ± 13	1144 ± 25	-
	Max	2002 ± 23	1765 ± 69	3193 ± 148	

IC50 in ug/mL

OxHLIA

- In OxHLIA assay the seeds needed to be previously defatted, once the high fat content in the sample interfered in the analysis.

- All samples presented great activity, being these results more heterogeneous through the varieties and fruit.

Work Package 2

- **RP activities & outcomes:**

Task 2.1. Prospection and identification of natural compounds with highest preserving capacity *(cont.)*

- Antimicrobial and antifungal activity
  - Against 8 bacterial and 2 fungal strains.
  - Maximum tested concentration (10 mg/mL).

✓ Minimum Inhibitory Concentration (MIC) achieved where of 2.5 mg/mL against bacteria and 5 mg/mL against fungi.

Minimum Bactericidal Concentration (MBC)/

Minimum Fungicidal Concentration (MFC) None of the samples presented bactericidal/fungicidal capacity, at the maximum tested concentration.

Antimicrobial activity

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

	Aspergillus brasiliensis		*Aspergillus fumigatus*	
	MIC	MBC	MIC	MBC
P - Butternut	10	>10	>10	>10
G - Ri 2	5	>10	10	>10
G - Ri 16	5	>10	5	>10
G - Ri 17	10	>10	10	>10
Ketoconazole	0.06	0.125	0.5	1

Deliverable D2.1.
Reports of the most bioactive extract obtained from pumpkin by-products

Greece	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 enterocolitica	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

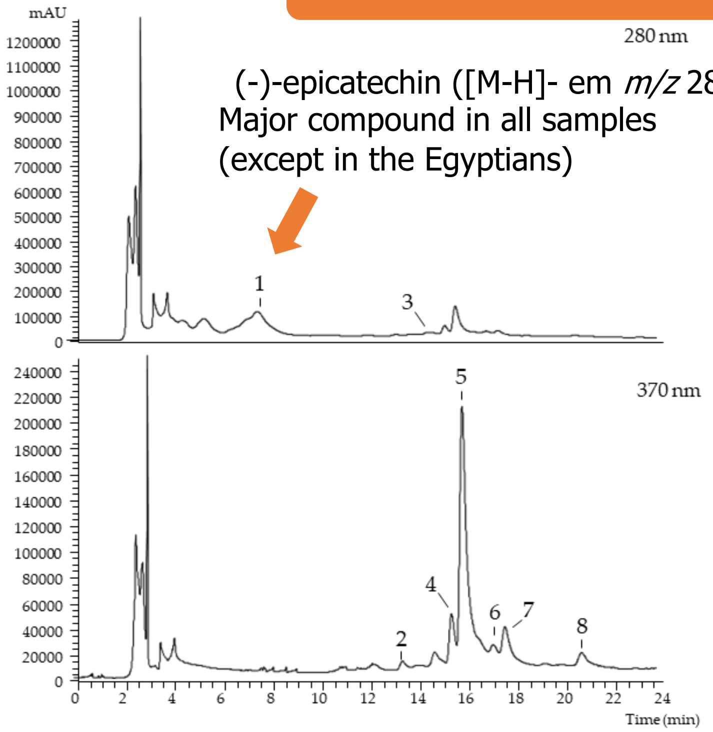
Work Package 2

- **RP activities & outcomes:**

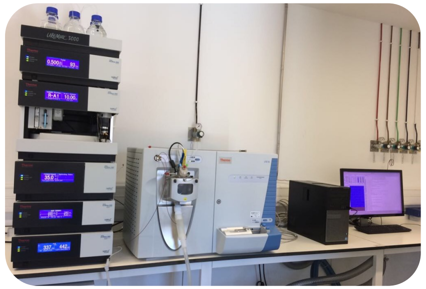
Task 2.1. Prospection and identification of natural compounds with highest preserving capacity

- Technical specifications of the preserving compounds developed

PHENOLIC COMPOUNDS



(-)-epicatechin ([M-H]- em *m/z* 289)
Major compound in all samples
(except in the Egyptians)



The family of flavonoids were the most abundant in terms of number of compounds found, mainly *O*-glycosylated derivatives of quercetin, kaempferol, and isorhamnetin

→ There were also found the phenolic acid 7 4-O-(6′-O-glucosyl-4″-hydroxybenzoyl)-4-hydroxybenzyl alcohol ([M-H]- em *m/z* 405) and chicoric acid ([M-H]- em *m/z* 473)

TOCOPHEROLS

The alpha and beta tocopherols were predominant in almost all the samples
The isoforms gamma and delta were also found in some samples, especially in the peels

ORGANIC ACIDS

The oxalic and malic acids were found in almost all the samples
When present, quinic acid was in significant concentrations
Contents of ascorbic, shikimic, citric and fumaric acids were also reported.

Work Package 2

- **RP activities & outcomes:**

Task 2.1. Prospection and identification of natural compounds with highest preserving capacity

Not under study in this Task

Great bioactive results
Great extraction yields
Unexplored matrix

Excellent bioactive results
The lowest yields
Toxicity in some samples
Is already valued in some regions

- Butternut squash (PT)
- Local landrace "Trikala – Turbinate (GR)
- Voutirato (GR)
- Local landrace "Leuka Melitis – Round (GR)

Good extraction yields
Considerable bioactivities
Difficulty obtaining the matrix

Work Package 2

- **RP activities & outcomes:**

Task 2.2. Optimization of sustainable and industrially feasible extraction processes of natural preservatives

- The selected samples in Task 2.1 were performed

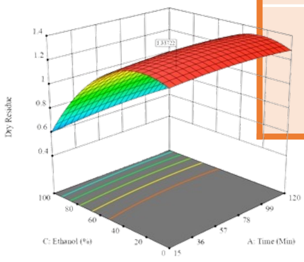
Heat assisted extraction (HAE)

Time	15 - 67.5 - 120 min
Temp.	30-55-80 °C
% EtOH	0-50-100

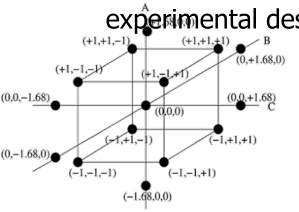
Ultrasound-assisted extraction (UAE)

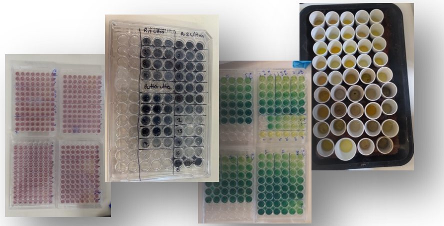
Time	5 - 32.5 - 60 min
Power	100-250-400 W
% EtOH	0-50-100

RSM



Box-Behnken experimental design





Responses
- DPPH
- Total phenol (Folin–Ciocalteu)
- Reduction power
- Yield 105 °C

Samples	Method	Response	t (min)	T (ºC)	EtHO (%)	Optimazed response
Ri2	HAE	Dry residue Reducing Power Total phenol	75	30	24	1.28 g/100g dw 158 ug/mL 136 mg/g dw
Ri16	HAE	Dry residue Reducing Power Total phenol	15	30	10	1.4 g/100g dw 112 ug/mL - mg/g dw
Ri17	UAE	Dry residue Reducing Power Total phenol	80	5	0	1.12 g/100g dw - ug/mL 120 mg/g dw
BS	HAE	Dry residue Reducing Power Total phenol	84	30	0	1.49 g/100g dw - ug/mL 169 mg/g dw

Global effects

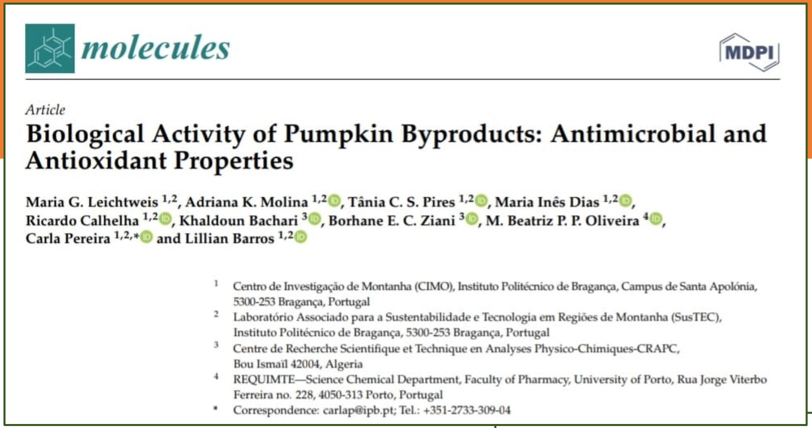
- The amount of ethanol showed higher influence in the yields of dry residue.
- Temperature and time also showed some influence when obtaining better EC_{50} results for reduction power.
- Considering total phenols, the influence of total phenols is quite case specific.
- It was not possible to optimize the DPPH responses.

Work Package 2

- **RP activities & outcomes:**

Final Achievements

- A screening of more than 100 samples of more than 30 varieties of pumpkins was obtained, in terms of their bioactivities and chemical composition
- The optimal extraction conditions, using eco-friendly and easy-to-perform methodologies, and green extraction solvents, were obtained for the selected samples
- Extracts rich in preservative compounds were obtained from pumpkin peels for food application


molecules MDPI

Article

Biological Activity of Pumpkin Byproducts: Antimicrobial and Antioxidant Properties

Maria G. Leichtweis [1,2], Adriana K. Molina [1,2], Tânia C. S. Pires [1,2], Maria Inês Dias [1,2], Ricardo Calhelha [1,2], Khaldoun Bachari [3], Borhane E. C. Ziani [3], M. Beatriz P. P. Oliveira [4], Carla Pereira [1,2,*] and Lillian Barros [1,2]

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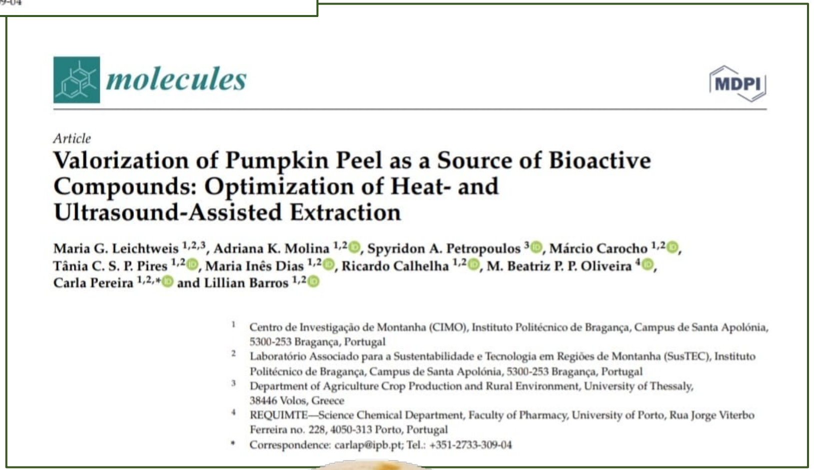
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Article

Valorization of Pumpkin Peel as a Source of Bioactive Compounds: Optimization of Heat- and Ultrasound-Assisted Extraction

Maria G. Leichtweis [1,2,3], Adriana K. Molina [1,2], Spyridon A. Petropoulos [3], Márcio Carocho [1,2], Tânia C. S. P. Pires [1,2], Maria Inês Dias [1,2], Ricardo Calhelha [1,2], M. Beatriz P. P. Oliveira [4], Carla Pereira [1,2,*] and Lillian Barros [1,2]

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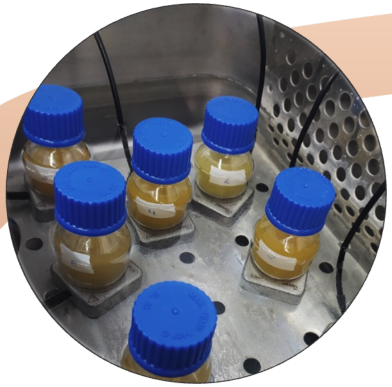

B. squash peel extract to be incorporated into a pumpkin pulp formulation in WP 4

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WP 3: Refinement and stabilization of the identified preserving compounds

Pr KSOURI RIADH, Tunisian Coordinator
Laboratory of Aromatic and Medicinal Plants (LPAM), Biotechnology Center of Borj Cedria

Implication of Tunisien teams on Pulping Project

Laboratory of Vegetable Crops (LVC)

Prof. TARCHOUN Neji
Dr Najla Mezgani (MA)

Post-Doc
Wassin Saadaoui
Khawla Hamdi

Laboratory of Aromatic and Medicinal Plants (LPAM)

Pr. Riadh Ksouri
Dr. Hanen Falleh (scientific Leader)
Pr Wided Megdiche
Pr Raja Serairi
Post-Doc
Dr. Rim Ben Mansour
Dr. Walid Yeddes
Dr Feten Zar Kalai

WP1. Defining agronomic conditions for pumpkin production
(ISA-CM as members)

WP2. Sustainable recovery of compounds with preserving capacity from pumpkin by-products
(CBBC as members)

WP3. Refinement and stabilization of the identified preserving compounds
(CBBC as leaders)

WP4. Pumpkin fruit pulp formulation
(ISA-CM and CBBC as members)

Work Package 3

Lead partner: CBBC
Participants: IPB; MORE; CRAPC

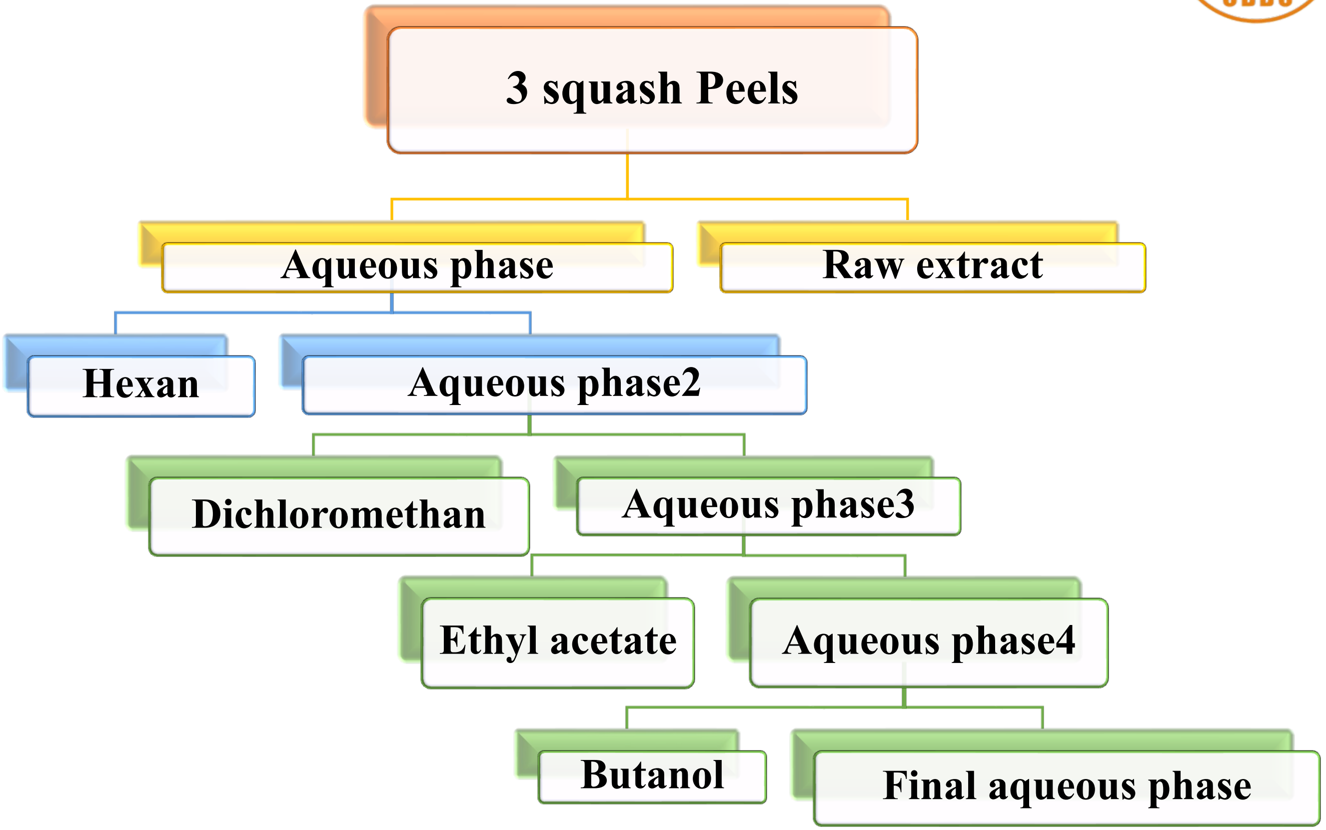
Task 3.1. Refinement of natural preservatives

Task 3.2. Stabilization of natural preservatives

- Batati (NGBTUN 746), Karkoubi (NGBTUN 748) and Bejaoui (NGBTUN 751) peels were selected.

- Samples were extracted by hydroethanolic maceration for subsequent analyses.

- The extraction were fractionated using a Separatory funnel protocol.



The main results will be presented only for Batati genotype
(same methodology was adopted for the other genotypes)

3.1. Refinement

The three most active fractions (MeOH, ethylacetate, and water) of the genotype **Batati peels** were combined and further analyzed by realizing the mixture design method, aimed at suggesting the best ratio

Mixture Optimization

Y = b1 * X1 + b2 * X2 + b3 * X3 + b12 * (X1*X2) + b13 * (X1*X3) + b23 * (X2*X3)

Caractéristiques du problème

Etude dans un domaine expérimental: mixture design matrix

Nombre de variables 3: **Methanol, Ethyl acetate, water**

Nombre d'expériences 13

Nombre de réponses 2: **PI & PPT**

Experimental Domain

	Composant	Contrainte Inf	Contrainte Sup
Z1	méthanol	0.0000	1.0000
Z2	acétate d'éthyl	0.0000	1.0000
Z3	eau	0.0000	1.0000
	Somme des proportions	1.0000	

Experiment Matrix

N° Exp	X1	X2	X3
1	1.0000	0.0000	0.0000
2	0.0000	1.0000	0.0000
3	0.0000	0.0000	1.0000
4	0.6667	0.3333	0.0000
5	0.3333	0.6667	0.0000
6	0.6667	0.0000	0.3333
7	0.3333	0.3333	0.3333
8	0.0000	0.6667	0.3333
9	0.3333	0.0000	0.6667
10	0.0000	0.3333	0.6667
11	0.6667	0.1667	0.1667
12	0.1667	0.6667	0.1667
13	0.1667	0.1667	0.6667

Results

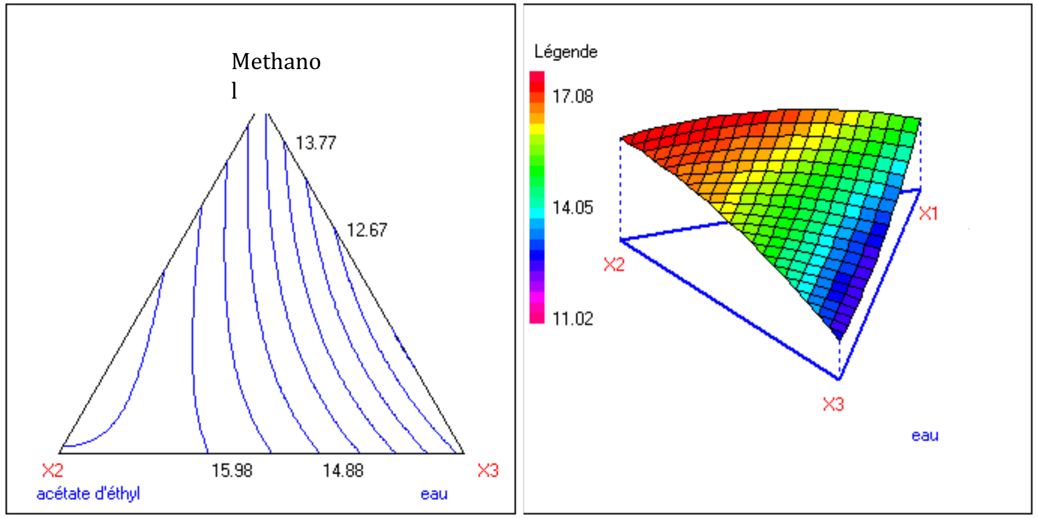
Batati genotype (NGBTUN 746)

Results obtained were interesting, since these values were higher than those obtained during the preliminary fractionation

N° Exp	MeOH	Ethyl Acetate	Water	PTT (mg GAE/g DR)	DPPH (inhibition %)
1	1.0000	0.0000	0.0000	14.44	53.32
2	0.0000	1.0000	0.0000	17.08	29.57
3	0.0000	0.0000	1.0000	12.64	37.98
4	0.6667	0.3333	0.0000	15.92	66.09
5	0.3333	0.6667	0.0000	16.10	55.23
6	0.6667	0.0000	0.3333	12.53	27.89
7	0.3333	0.3333	0.3333	15.85	49.62
8	0.0000	0.6667	0.3333	15.30	44.93
9	0.3333	0.0000	0.6667	11.02	31.59
10	0.0000	0.3333	0.6667	14.50	47.61
11	0.6667	0.1667	0.1667	15.20	58.95
12	0.1667	0.6667	0.1667	16.20	50.88
13	0.1667	0.1667	0.6667	14.64	35.40

So, an optimizer of response was used following the next formulas, these equations were transposed into isoprenic curves.

TPC



Isoresponses and mixture plot

(Batati)

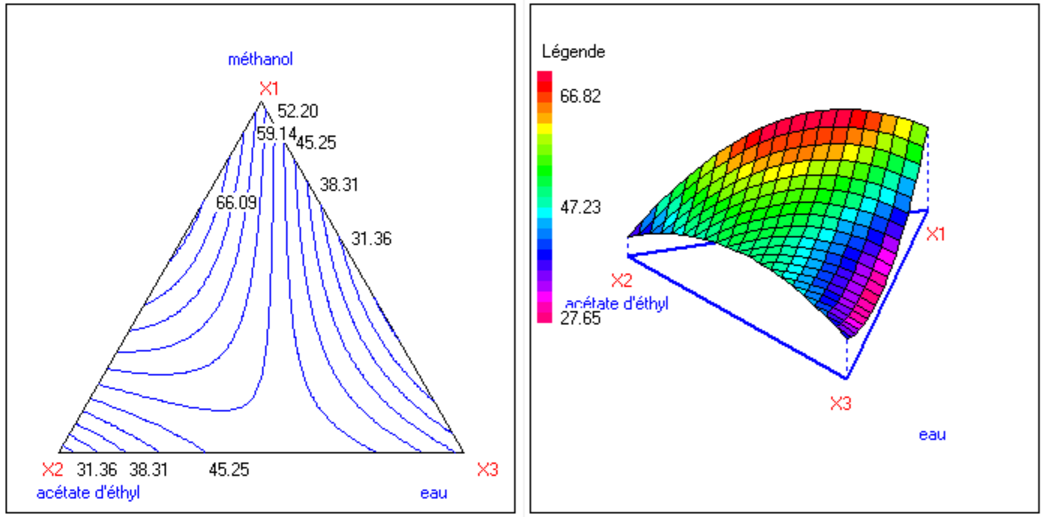
Y TPC = 14,47 * X1 + 16,48 * X2 + 12,52 * X3

DPPH test

Isoresponses and mixture plot

(Batati)

Y DPPH = 53,91 * X1 + 28,97 * X2 + 37,40 * X3 + 95,07 * (X1*X2) -68,16* (X1*X3) + 57,22 * (X2*X3)



Results

***

Coordonnées de l'optimum

Variable	Valeur	Facteur	Valeur
X1	0.534	X1	0.534
X2	0.458	X2	0.458
X3	0.008	X3	0.008

Caractéristiques de l'optimum

Réponse	Nom	Valeur	d (i) %	Poids	di min %	di max %
Y1	TPC	16.44	90.73	1	0.00	99.42
Y2	DPPH	65.54	98.78	1	0.00	98.78
	DESIRABILITE		94.67		0.00	99.10

The used software concluded that the targeted limit could be achieved with a 99% desirability for the Batati genotype using this solvent mixture as follow:

53.4% Methanol+ 45.8% Ethyl acetate + 0.8% water

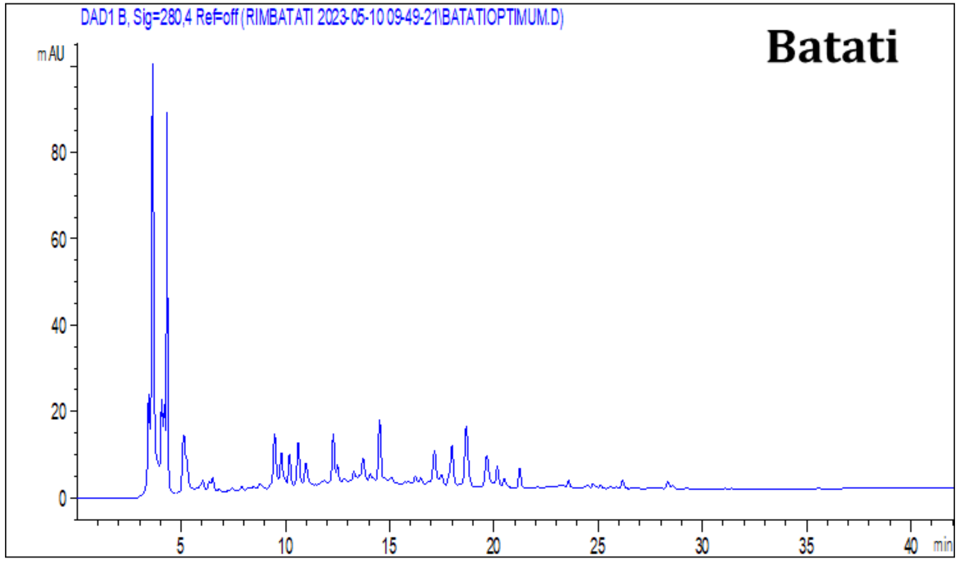
The experimental validation of these formulas is detailed in this Table

	Predicted values	Experimental values
TPC	16,44	15,60
DPPH	65,54	64,14

Once the experimental validation confirmed the mathematical model, all the obtained refined extracts were assessed for their phenolic composition (using HPLC), along with an assessment of their biological activities, such as antibacterial activity and cytotoxic effects.

HPLC Analysis of Peel-Refined Extracts

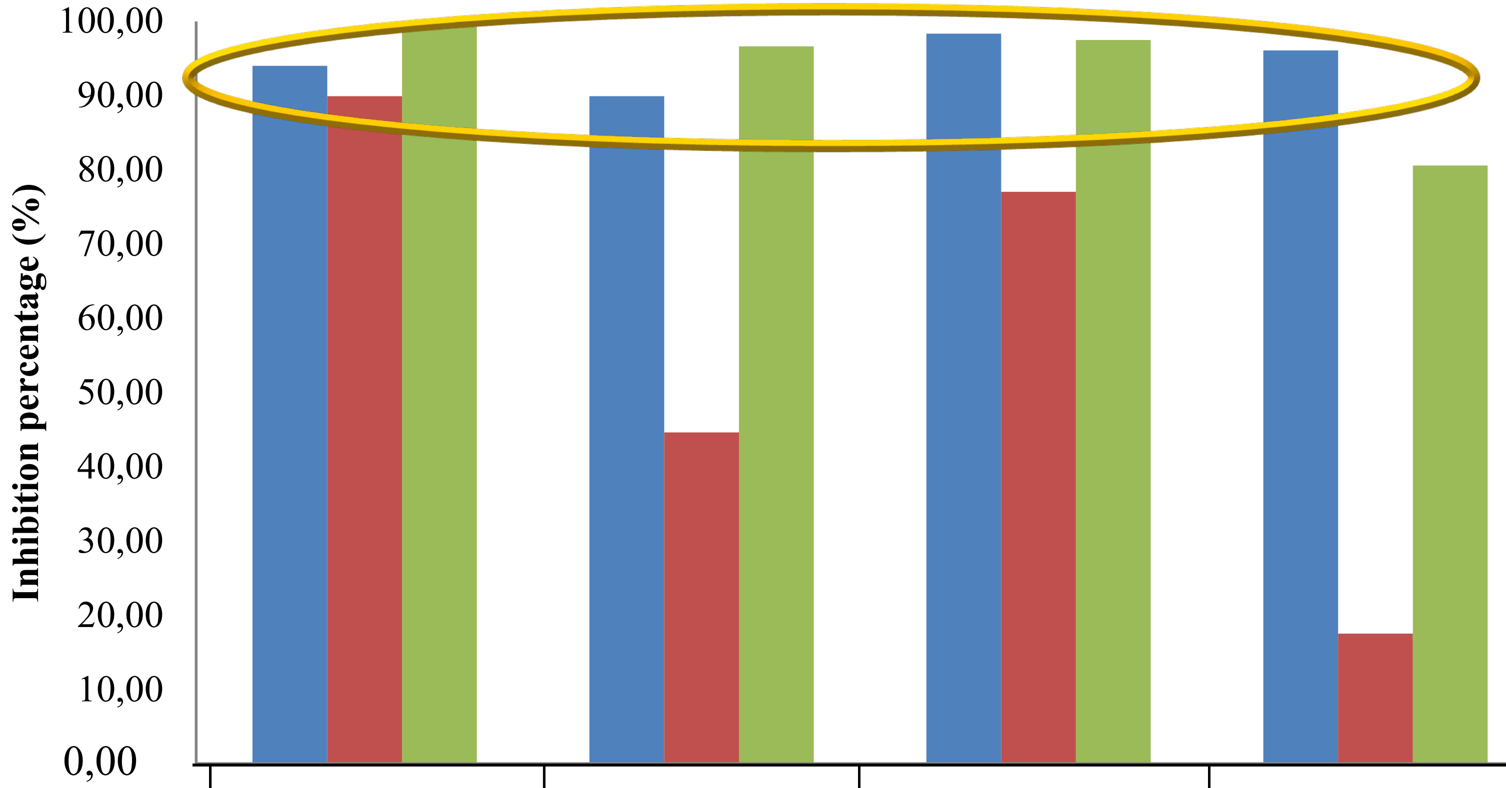
Compounds	RT (min)	Content (mg/gE)
		Batati
gallic acid	6.1	0.01
catechin gallate	7.34	-
hydroxytyrosol	9.15	0.25
epigallocatechin	10.68	**0.48**
chlorogenic acid	11.6	-
catechin	12.18	**0.50**
epicatechin	13.82	0.36
caffeic acid	14.25	-
sinapic acid	14.47	0.12
myrecitin 3-O-galactoside	15.37	-
rutin	16.44	0.04
ellagic acid	17.42	0.01
vanillin	17.79	-
kaempferol	18.28	0.31
myrecitin	22.54	-
resveratrol	24.5	0.03
quercetin	26.16	0.04
apigenin	28.31	-
Total		2.39



The most abundant compounds were catechin and epigallocatechin

Antibacterial Activity of Peel-Refined Extracts

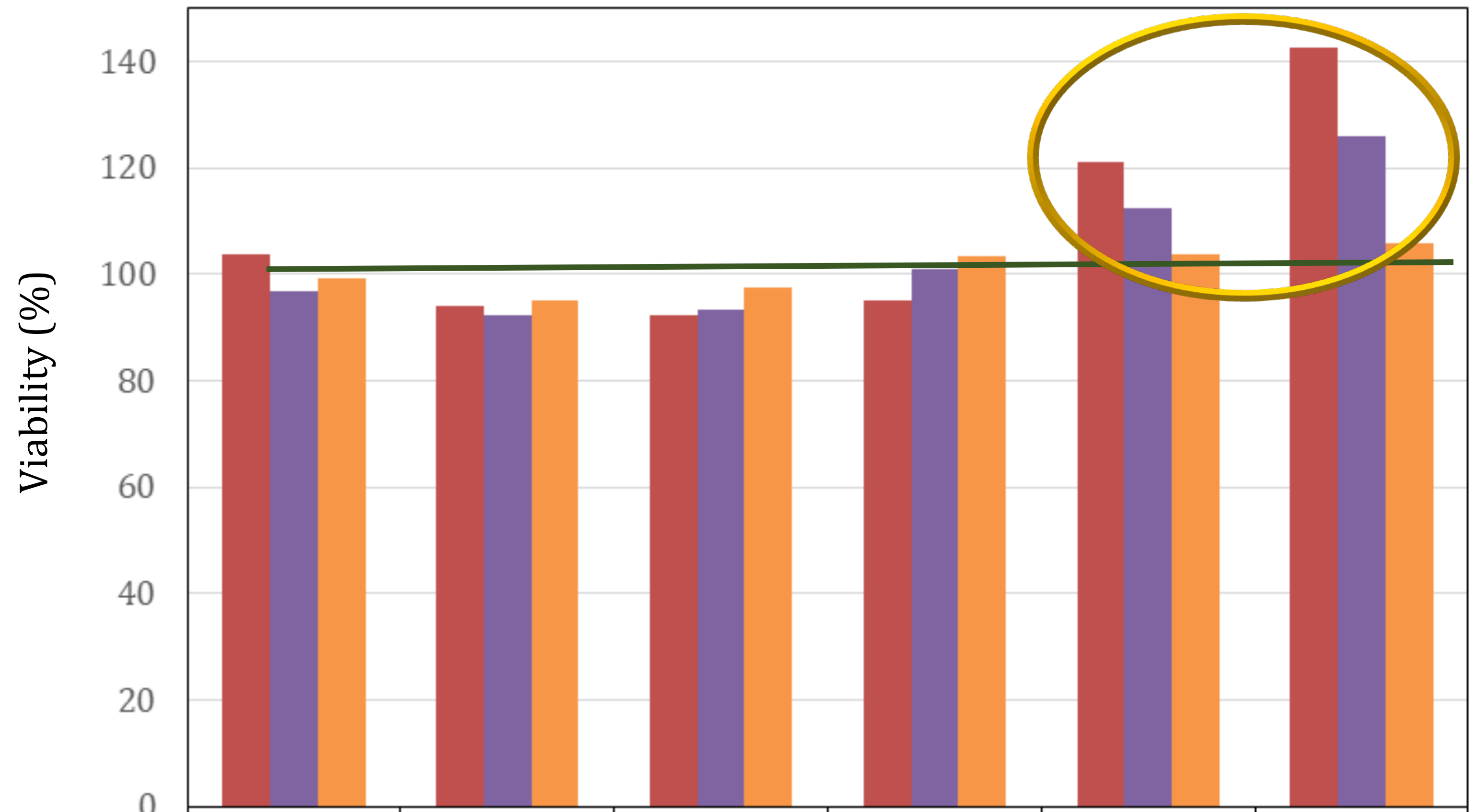
Batati peel-refined extracts exhibited the best inhibitory effects against all pathogens about 90 %: *E. feacalis, P. aeruginosa, S. typhimurium, and S. aureus.*



	E. feacalis	*P. aeruginosa*	*S. thyphimirium*	*S. aureus*
Batati	94.01±1.2b	89.88±1.1b	98.29±2.2a	96.03±2.1a
Bejaoui	89.88±2.1c	44.64±2.0c	77.00±2.7b	17.55±0.7c
Karkoubi	99.26±0.9 a	96.60±1.4a	97.49±1.8a	80.53±3.2b

Cell Viability of Peel-Refined Extracts

The cell viability for all the tested extracts was over 92%, indicating no significant toxicity for the studied samples. Moreover, result may suggest a potential stimulatory effect of the refined extracts on cell growth.



	12,5	25	50	100	200	400
Batati	103,818	94,016	92,299	95,080	121,014	142,442
Bejaoui	96,841	92,338	93,355	101,011	112,311	126,076
Karkoubi	99,321	95,145	97,442	103,396	103,659	105,797

3.2. Stabilization

The encapsulation of refined phenolic extracts using maltodextrin, gum arabic, and concentrated phenolic extract as coating materials, was made.

Response surface methodology (RSM) was employed to optimize the process, considering total phenolic content (TPC), antiradical activity, particle size, and polydispersity index (PDI) as key responses.

Batati (NGBTUN 746)

Batati peels

Model: **Mixture design**

$$Y = b1 * X1 + b2 * X2 + b3 * X3 + b12 * (X1*X2) + b13 * (X1*X3) + b23 * (X2*X3)$$

Characteristics

- Study in an experimental domain: Response Surface Methodology (RSM)
- Number of variables 3:
  Maltodextrin, Gum arabic & concentrated phenolic extract
- Number of experiences 13
- Coefficients number 6
- Response number 4: **IP , TPC, Size & Pdi**

Encapsulation protocol

Coating materials (10g) → 90 g of hot distilled water (40°C)

Maltodextrin (X1) Gum arabic (X2)

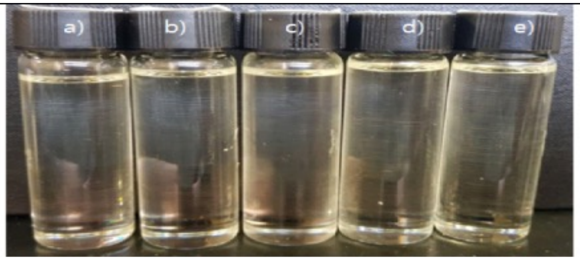
Mixing under magnetic stirring (1 hour)

↓

Stored at 4°C for 24 hours

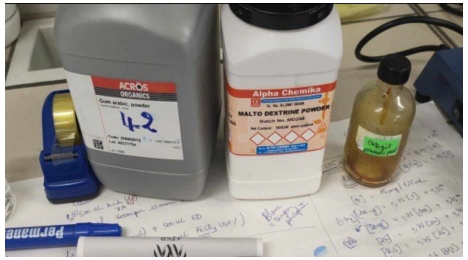
↓

Coating solution + **concentrated phenolic extract (X3)**



Homogenization
(magnetic stirring for 60 minutes at 60°C)
Ultra-turax stirring
(5 minutes at 11.000 rpm)
Sonication
(5 min)

The adopted software concluded that the targeted limit can be achieved with 91% desirability using a mixture as follow:



23.8% Maltodextrin+ 27.7% Arabic gum+48.5% refined extract

Results revealed that the experimental values of TPC, DPPH test, particle size and Pdi were in good agreement with predicted ones.

Experimental validation of the obtained formula

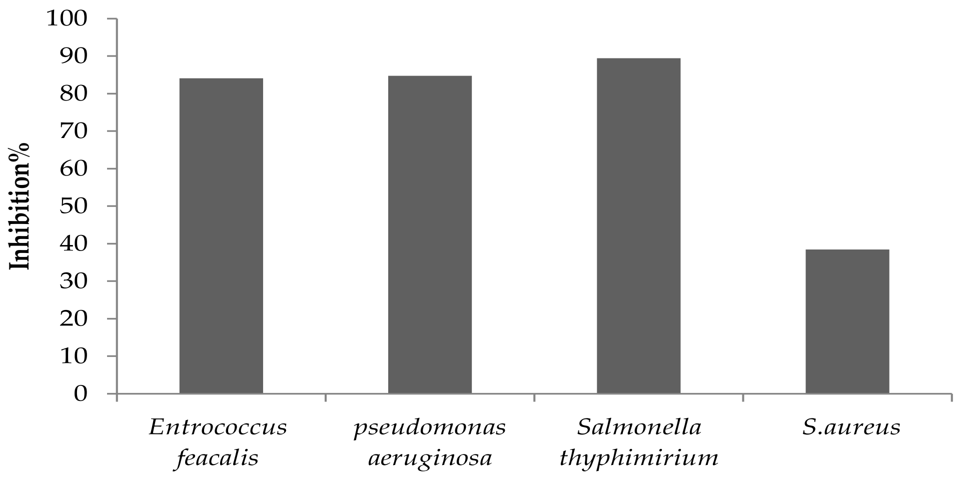
	Predicted values	Experimental values
TPC	47.46	46.01
DPPH test	66.54	64.85
Size	620.31	571.22
Pdi	0.52	0.49

Physicochemical parameters of optimized formula

The nanoemulsion displayed favorable physicochemical properties, including low viscosity, a slightly acidic pH, and good turbidity, making it suitable for incorporation into food or beverage products.

Physicochemical (pH, viscosity, turbidity, color)
Biological capacities (antioxidant and antibacterial activities)

Parameter (unit)	Result
DPPH Test (Inhibition%)	64.2± 2.04
ABTS Test (Inhibition%)	53.89± 0.07
Viscosity (mPa/s)	17± 0.01
pH	4,2± 0.23
Solubility (%)	93
Turbidity	0.412
Color	
L*	102.4
a*	3.5
b*	7.8



Growth Inhibition Percent (PI) of optimized emulsion against various bacterial strains

The optimized nanoemulsion exhibited promising antioxidant (DPPH and ABTS tests) and antibacterial activity against various bacteria, with the highest inhibition observed against *Salmonella typhimurium* (89%).

PulpIng – Development of Pumpkin Pulp Formulation Using a Sustainable Integrated Strategy

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processes

MDPI

Article

The Use of Response Surface Methodology to Optimize Assisted Extraction of Bioactive Compounds from *Cucurbita maxima* Fruit By-Products

Rim Ben Mansour [1], Hanen Falleh [1,*], Majdi Hammami [1], Lillian Barros [2,3], Spyridon A. Petropoulos [4,*], Neji Tarchoun [5] and Riadh Ksouri [1]

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Abstract: This work aimed to optimize the extraction conditions of bioactive compounds obtained from three squash by-products (e.g., peel, endocarp, and seeds) using the response surface methodology (RSM). The selected independent variables were ethanol concentration, extraction time, and extraction temperature. Squash by-products' bioactive molecules were extracted according to the matrix proposed by the experimental plan. Significant variability in total phenolic compound content (TPC) and antioxidant activity, depending on the extraction time, the solvent concentration, and the extraction temperature, was recorded for the tested by-products. The experimental results adequately fitted with second-order polynomial models and showed significant linear, quadratic, and interaction effects of the independent variables. Data analysis suggested that the optimal extraction conditions were 12.2% ethanol for 11.2 min at 55 °C for peels; 28.5% ethanol for 10.5 min at 37 °C for endocarp; and 20% ethanol for 10.5 min at 60 °C for seeds. The results obtained showed that the experimental and predicted values of TPC and antioxidant activities as an indicator of a successful extraction fit with each other, thus indicating the optimal extraction conditions. Under these conditions, the obtained extracts exhibited high, although variable, TPC with epicatechin and epigallocatechin as major compounds, as well significant antimicrobial potency, which reached 100% and 80% inhibition of the tested bacteria and fungi.

Keywords: *Cucurbita maxima*; by-products; response surface methodology; antioxidant activity; total phenolic compound content; squash

check for updates

Citation: Mansour, R.B.; Falleh, H.; Hammami, M.; Barros, L.; Petropoulos, S.A.; Tarchoun, N.; Ksouri, R. The Use of Response Surface Methodology to Optimize Assisted Extraction of Bioactive Compounds from *Cucurbita maxima* Fruit By-Products. *Processes* **2023**, *11*, 1726. https://doi.org/10.3390/pr11061726

Academic Editors: Carla Silva and Dariusz Dziki

Received: 18 April 2023
Revised: 25 May 2023
Accepted: 31 May 2023
Published: 5 June 2023

1. Introduction

Fruit and vegetable processing industries create enormous amounts of under-utilized by-products with an inordinate economic potential and high environmental burden [1]. Processing by-products account for 25% up to 60% of the weight of the fruit and principally consist of skin (peels) and lower percentages of pulp and seeds. All of these fractions present a remarkable chemical composition and, therefore, could be considered as raw ingredients for the development of integrated biorefinery methodologies and as sources of natural bioactive agents. Subsequently, the need to obtain nutritious foods from new sources and lower waste in industry has created a great interest in studying different

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horticulturae

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Article

Improved Recovery of Antioxidant Compounds from Refined Pumpkin Peel Extract: A Mixture Design Method Approach

Rim Ben Mansour [1], Hanen Falleh [1,*], Nermine Nefzi [1], Sarra Dakhlaoui [1], Sawssen Selmi [1], Majdi Hammami [1], Lillian Barros [2,3], Spyridon A. Petropoulos [4,*], Neji Tarchoun [5] and Riadh Ksouri [1,*]

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Abstract: This study employed the mixture design method to determine optimal solvent combinations, aiming to obtain refined extracts from squash peels with enhanced antioxidant properties. We optimized extraction solvents, focusing on recovering the total phenolic compounds (TPC) and increased antioxidant properties using a second-order polynomial equation through the response surface methodology (RSM). Six solvents (MeOH, Hexane, DCM, EtOAc, BuOH, and water) were assessed for their effects on TPC and antioxidant activity in preliminary experiments. The refined extracts underwent a HPLC analysis for a phenolic composition determination and were further evaluated for their antibacterial activity and cytotoxicity. The results revealed a rich phenolic content in the refined extract from peels of Bejaoui landrace, primarily catechin (8.06 mg/g dry extract (DE)), followed by epicatechin and kaempferol (5 mg/g DE). Antibacterial tests against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Staphylococcus aureus* showed significant antimicrobial activities, especially for Karkoubi and batati landraces, where the growth inhibitions were 99%, 96%, 97%, and 80% and 94%, 89%, 98%, and 96% for the respective bacteria. The peel extracts exhibited a negligible cytotoxicity on the RAW264.7 cell line, even at high concentrations. Our findings emphasize the potential antioxidant and antibacterial properties of peel extracts due to diverse phenolic compounds, suggesting the potential use of squash peels in the food and nutraceuticals industries as sources of natural antimicrobial agents.

Keywords: *Cucurbita maxima* Duchesne; phenolic compounds; antioxidant activity; antimicrobial properties; squash by-products; response surface methodology

check for updates

Citation: Mansour, R.B.; Falleh, H.; Nefzi, N.; Dakhlaoui, S.; Selmi, S.; Hammami, M.; Barros, L.; Petropoulos, S.A.; Tarchoun, N.; Ksouri, R. Improved Recovery of Antioxidant Compounds from Refined Pumpkin Peel Extract: A Mixture Design Method Approach. *Horticulturae* **2023**, *9*, 1111. https://doi.org/10.3390/horticulturae9101111

Academic Editor: Zi Teng

Received: 15 September 2023
Revised: 29 September 2023
Accepted: 6 October 2023
Published: 8 October 2023

1. Introduction

Currently, two pivotal strategies are employed within the environmental protection curricula, namely the circular economy and Zero Waste. The first refers to a non-linear economic system that recovers energy and raw materials as much as possible, aiming at the sustainable use of natural resources. The second strategy stresses the importance of the rational use of products and the reduction in the amount of waste produced [1]. The handling of crop waste is one of the most important problems that the agricultural and food sector has to address nowadays [2]. Processing fruits and vegetables into commercial products generates significant quantities of bulk waste, depending on the species (peels,

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Article

Enhancing Antioxidant Activity of Squash By-Products: Insights from Response Surface Methodology

Walid Yeddes [1], Feten Zar Kalai [1], Majdi Hammami [1], Neji Tarchoun [2], Lilian Barros [3,4], Spyridon A. Petropoulos [5,*], Hanen Falleh [1], Riadh Ksouri [1,*]

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Abstract: This study optimized the extraction of phenolic compounds from two Tunisian squash landraces (Bejaoui and Karkoubi) by-products through response surface methodology aiming to enhance their antioxidant capacity. The tested extraction parameters were ethanol concentration, time, and temperature. HPLC analysis revealed various phenolic compounds, such as vanillic acid, catechin gallate, hydroxytyrosol, epigallocatechin, chlorogenic acid, and epicatechin. The experimental and predicted values of total phenolic content and antiradical activity closely matched, confirming the success of the extraction process under the optimal conditions. For Bejaoui peels, the optimal extraction parameters were 51.5% ethanol at 40.8 °C, during 50.5 minutes. Bejaoui strands showed optimal conditions with 50.4% ethanol at 37.1 °C for 36.3 minutes, while Bejaoui seeds had optimal results with 30% ethanol, 36.4 °C, and 8 minutes. On the other hand, Karkoubi peels, fibrous strands, and seeds had optimal extraction parameters of 13.2% ethanol, 43.4 °C, and 47.2 minutes; 33.4% ethanol, 46.6 °C, and 10.8 minutes; and 10.65% ethanol, 55.34 °C, and 23.16 minutes, respec-

Cellular and Molecular Biology, accepted

Optimizing Encapsulation of Squash-Refined Extract for Functional Food Applications: A Sustainable Approach to Reduce Food Waste

Rim Ben Mansour [1], Hanen Falleh [*1], Majdi Hammami [1], Lillian Barros [2,3], Neji Tarchoun [4], Riadh Ksouri [*1]

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Abstract

This study explored the encapsulation of refined phenolic extracts using maltodextrin, gum arabic, and concentrated phenolic extract as coating materials. Response surface methodology (RSM) was employed to optimize the process, considering total phenolic content (TPC), antiradical activity, particle size, and polydispersity index (PDI) as key responses. The results revealed significant interactions between the coating materials, influencing all studied parameters. A desirability function approach identified an optimal formulation containing 23.8% maltodextrin, 27.7% gum arabic, and 48.5% refined extract. This formulation achieved a TPC of 46.01 mg GAE/gDR, DPPH radical scavenging activity of 64.2%, and a particle size below 500 nm with a narrow size distribution (PDI < 0.5). The optimized nanoemulsion exhibited promising antibacterial activity against various bacteria, with the highest inhibition observed against *Salmonella typhimurium* (89%). Furthermore, the nanoemulsion displayed favorable physicochemical properties, including low viscosity, a slightly acidic pH, and good turbidity, making it suitable for incorporation into food or beverage products. This research demonstrates the successful development of stable and bioactive nanoemulsions loaded with phenolic extracts using RSM optimization. The achieved characteristics suggest potential applications for these nanoemulsions in functional food or nutraceutical development.

Keywords: RSM experimental design, Pumpkin peels, refined-extract, encapsulation, physical characterization.

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ATSB-2024

PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WP 4: Pumpkin fruit pulp formulation

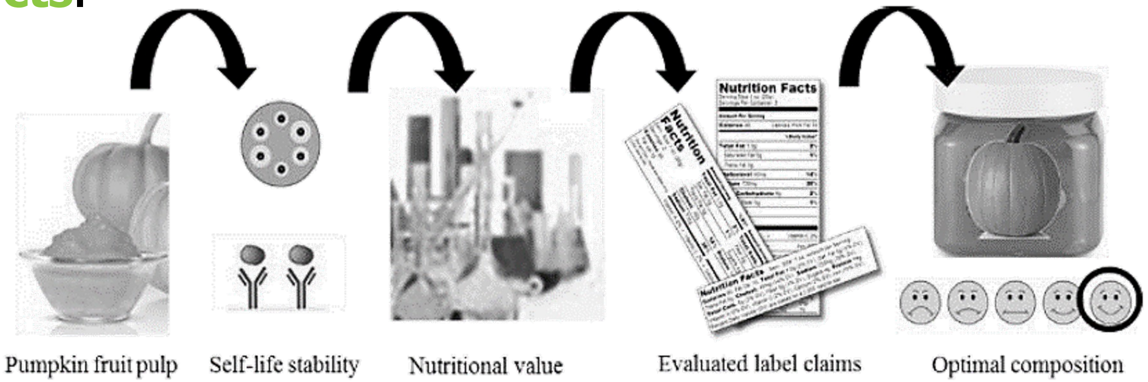
Isabel Oliveira

Work Package 4

Lead partner: DECORGEL
Participants: IPB; MORE; CBBC; ATB

- **Impact of the WP:**

The main achievements of this WP were the development of a **pumpkin fruit pulp** incorporating **preservatives** extracted from fruit and plant **by-products**.



- **Link with other WPs:**

The **WP4** was developed in **close collaboration** with the partners responsible for **WP2** (IPB) and **WP3** (CBBC), being **fully dependent** on the data and results obtained by them.

Work Package 4

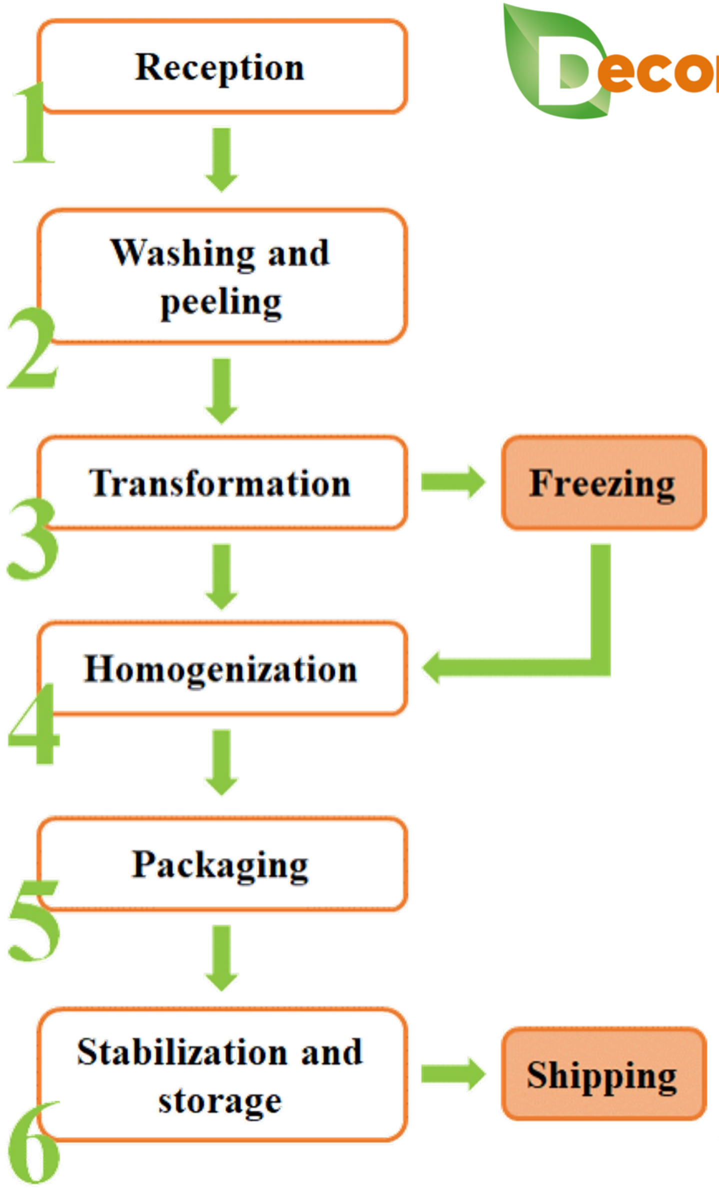
- **Task 4.1.** Production of pumpkin fruit pulp

<u>Definition of best practices and production line:</u>

The **optimal solutions** for pumpkin fruit pulps preparation match product requirements with equipment, which Decorgel configure based on the **specific recipes and needs**, ensuring the **right composition** of ingredients and **processing parameters**.

The pumpkin fruit pulp production line at Decorgel comprises

6 main stages:



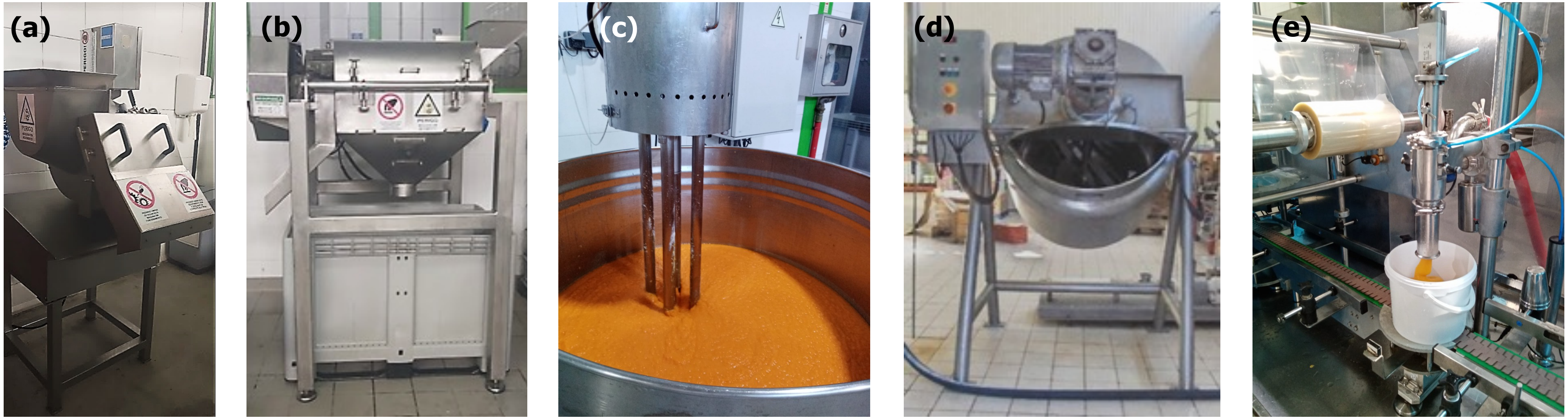
Work Package 4

- **Task 4.1.** Production of pumpkin fruit pulp

Equipment used for pumpkin fruit pulp production:

Dicing machine **(a)** | Pulp making machine **(b)** | Vertical mixer **(c)** | Tilting jacketed steam kettle with agitator **(d)**

Packaging system with metal detector **(e)**



Transformation | Homogenization | Packaging

Work Package 4

- **Task 4.1.** Production of pumpkin fruit pulp

Decorgel Innovating together

Fruit formulation datasheet of the pumpkin preparation produced at Decorgel for commercial purposes:

Ingredients:

- Pumpkin pulp (91%)
- modified corn starch
- acidity regulators (citric acid, sodium citrate)
- preservative (potassium sorbate)
- dye (natural beta-coratene)

Organoleptic properties

- Color: orange
- Flavor: pumpkin
- Texture: compact

Nutritional value (per 100 g)	
Energy	244 kJ / 58 kcal
Lipids	0.17 g
of which saturated	0.00 g
Carbohydrates	12 g
of which sugars	4.3 g
Proteins	1.1 g
Salt	0.00 g

Physical chemical properties

- ºBrix between 10 and 16
- pH between 4.3 and 4.7
- Exogenous foreign bodies: should tend to zero
- Endogenous foreign bodies: < 10 un/ 10 kg

Microbiological properties

(Reg. (CE) No. 2073/2005)

- Microorganisms (at 30 ºC) <10^3 CFU/g
- Coliforms (at 30 ºC) <10^2 CFU/g
- Molds and yeasts < 5 x 10^2 CFU/g
- *E. coli* < 10 CFU/g
- *L. monocytogenes* absent in 25 g

PRIMA

Work Package 4

- **Task 4.2.** Incorporation of pumpkin by-products preservative in pumpkin fruit pulp

Decorgel Innovating together

To achieve a healthier product without losing any properties in terms of product quality, stability and market/consumer demands

1st step: Extraction of pumpkin by-products under optimal global conditions, as described in Task 2.2

2nd step: Incorporation of the extracts into the pumpkin pulp formulations:

- Pulp + **peel extract** (10 g/kg) + potassium sorbate (at 50% of the standard formulation)
- Pulp + **seed extract**
- Standard formulation with **potassium sorbate** (positive control)
- Pulp **without preservative** (negative control)

3rd step: Evaluation of the quality of the formulations during the shelf-life

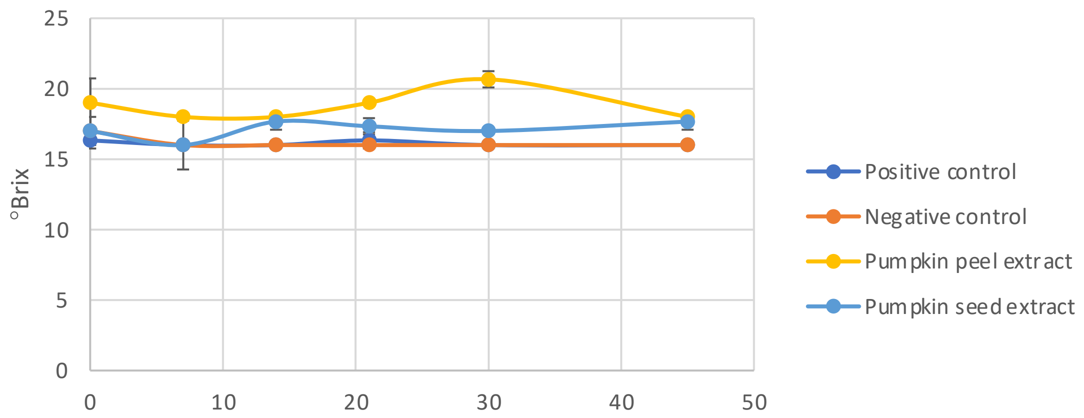
At time points 0 (day of production), 7, 14, 21, 30, and 45 days of storage

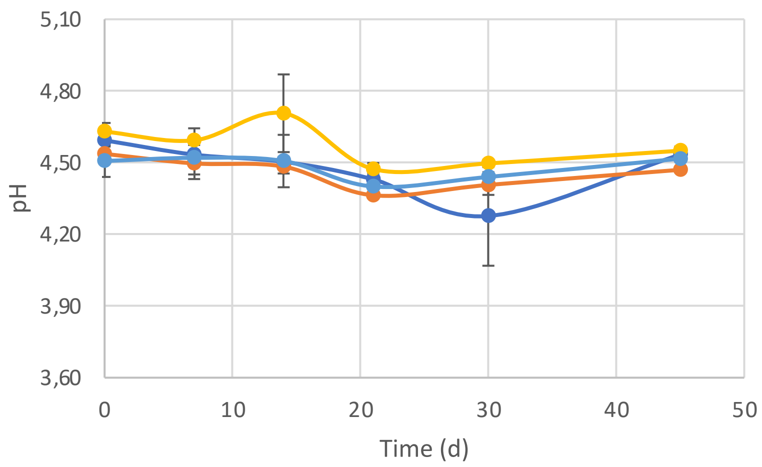
- **Decorgel analysis:** ºBrix, pH, Consistency (cm/30 s), and Water activity
- **CIMO/IPB analysis:** Nutritional value (moisture, ash, protein, fat, fatty acids, and carbohydrates), Color, Microbial load, and Toxicity

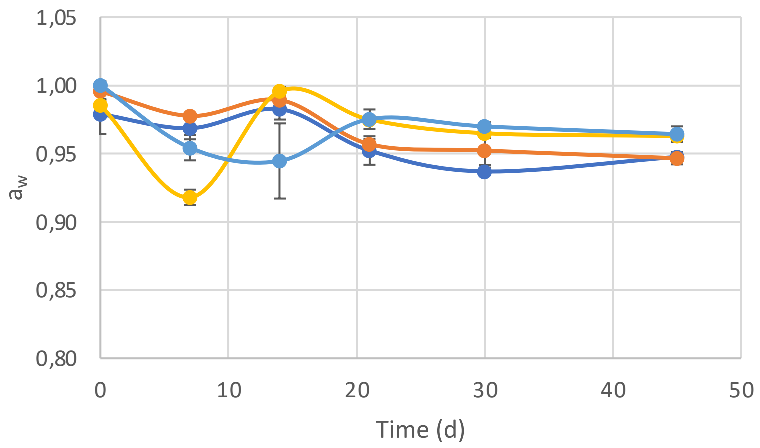
PRIMA

- **Task 4.2.** Incorporation of pumpkin by-products preservative in pumpkin fruit pulp

Evaluation of the quality of the formulations during the shelf-life







- The consistency of the products remained constant over time at 0 cm/30 s
- ºBrix values between 16 and 21
- pH variation between 4.3 and 4.7
- Water activity presented a very high value ≥0.92

Work Package 4

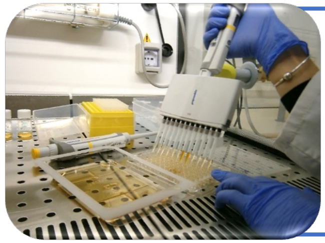
- **Task 4.2.** Incorporation of pumpkin by-products preservative in pumpkin fruit pulp

Decorgel Innovating together

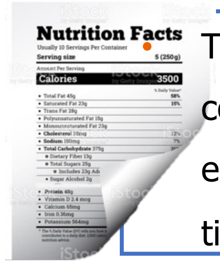
Evaluation of the quality of the formulations during the shelf-life

Formulations	Days of storage					
	0	7	14	21	30	45
Peel extract						
Seed extract						
Positive control						
Negative control						

- At time 0, the color of the peel extract is slightly more orange, and the seed extract is slightly more yellowish than the controls.
- At time 21, the peel extract formulation begins to lose color, matching the tone of the other formulations.
- After 30 days, the color of all formulations is affected, except for the seed extract, which lasts up to 45 days.



Regarding the microbial load, <u>no growth</u> was evidenced in all formulations until the 45 days of storage evaluated.



The minimal differences observed in nutritional composition will be statistically evaluated to explain the significance and influence of storage time and preservatives in the formulations.

PRIMA

Work Package 4

- **Task 4.2.** Incorporation of pumpkin by-products preservative in pumpkin fruit pulp

Assessment of consumer preference and acceptability of final formulations

106 evaluators participated

Final formulations for sensory evaluation:

- Traditional formulation with potassium sorbate.

- Pumpkin peel extract at a concentration of 10g/kg + 50% of the amount of potassium sorbate concentration in the traditional formulation

The results suggest that despite small differences, both formulations are comparable and the partial replacement of potassium sorbate with peel extract is acceptable to consumers.

We could conclude that there are no substantial differences in the perceptions of colour, texture, aroma, taste, and overall acceptability perceptions between the formulations by the participants.

Work Package 4

- **Conclusions**

The incorporation of the optimized extracts in the pumpkin pulp formulation was achieved

During the shelf-life of the product the values of consistency, pH, and water activity remained similar to the standard formulation and between the defined range to the product

Formulations meet microbiological safety and nutritional parameters were kept.

The color particularities observed in different storage times require adapting the product's applicability

There are no substantial differences in the perceptions of colour, texture, aroma, taste, and overall acceptability perceptions between the formulations by the 106 participants.

THE GREAT POTENTIAL OF THE PUMPKIN PULP FORMULATION DEVELOPED HERE IS NOTABLE. THE REPLACEMENT, EVEN PARTIAL, OF THE ARTIFICIAL PRESERVATIVE POTASSIUM SORBATE WITH NATURAL PUMPKIN PEEL EXTRACT, ALIGNS THE MAJOR ASPECTS OF FOOD SAFETY AND ENVIRONMENTAL SUSTAINABILITY.

PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WP 5: Preservation studies and quality assessment during shelf-life

Luma Rossi Ribeiro

Work Package 5

- **Summary of activities performed:**

- **<u>Pumpkin pulp production in Task 5.1, 5.2 and 5.3:</u>**

Raw pumpkin → Washing → Cut → Crushing → Packing → Raw pulp

HPP

Thermal Process

→ Shelf life 45 days

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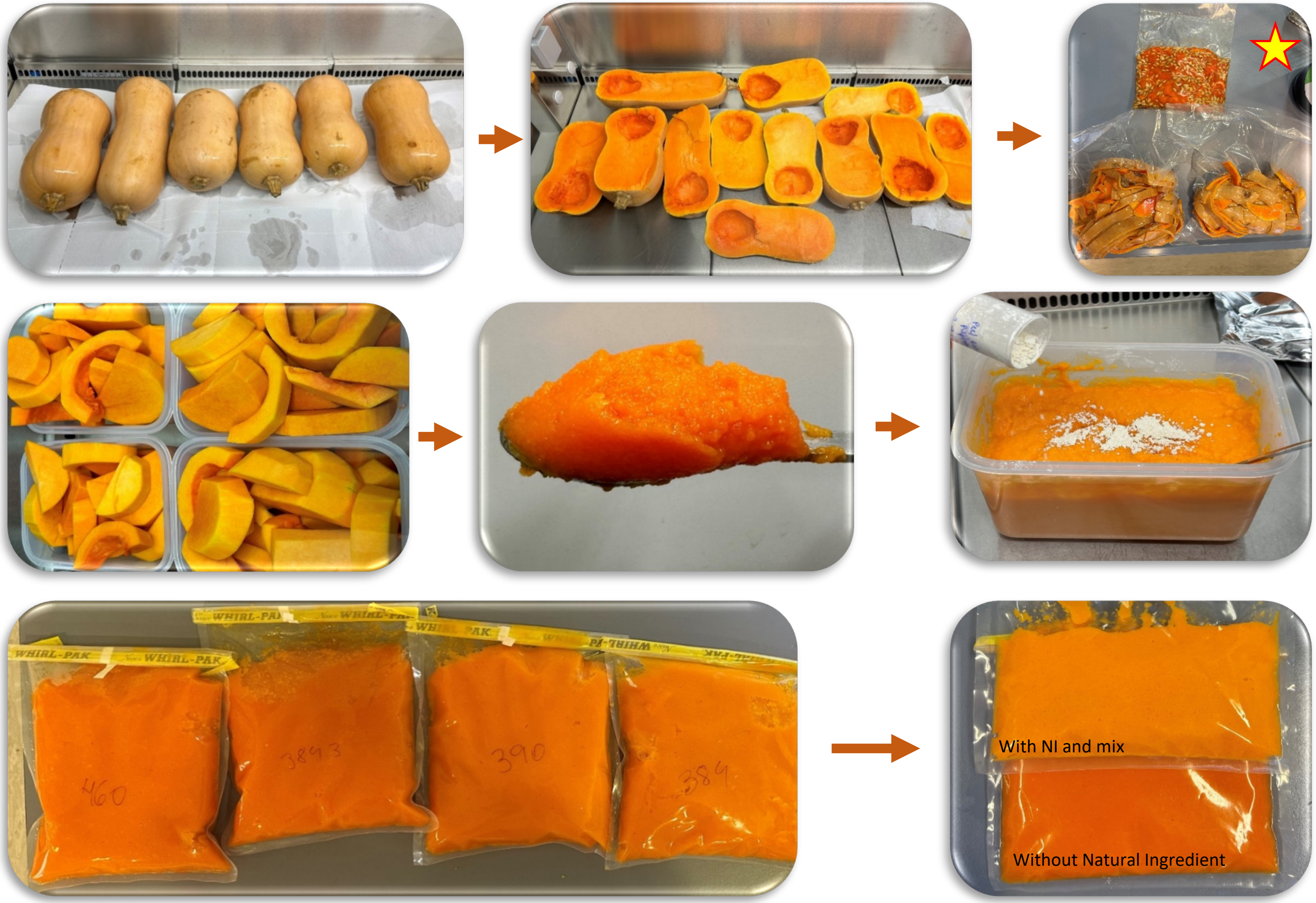
Work Package 5

- **RP activities & outcomes: <u>Pumpkin pulp production</u>**



➢ **5 Sample conditions:**

- HPP
- Pasteurization
- Natural Ingredient
- Potassium sorbate

PS.: *all samples were added with industrial mix: natural ß-carotene, starch, citric acid and citrate*

Final yield: 67%

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Work Package 5

- **RP activities & outcomes: Samples conditions**

5 Sample conditions:

A1. Pumpkin Pulp (1186,26 g) + mix of ingredients (natural ß-carotene, starch, citric acid and citrate - 113,74 g) + **HPP treatment.**

A2. Pumpkin Pulp (1185,61 g) + mix of ingredients (natural ß-carotene, starch, citric acid, citrate and potassium sorbate - 114,39 g) + **Pasteurization treatment.**

A3. Pumpkin Pulp (1185,61 g) + mix of ingredients (natural ß-carotene, starch, citric acid, citrate and potassium sorbate - 114,39 g) + **HPP treatment.**

A4. Pumpkin Pulp (1170,27 g) + mix of ingredients (natural ß-carotene, starch, citric acid and citrate - 113,74 g) + **Natural ingredient** (15,99 g) + **HPP treatment.**

A5. Pumpkin Pulp (1169,94 g) + mix of ingredients (natural ß-carotene, starch, citric acid, citrate and 50% potassium sorbate - 114,07 g) + **Natural ingredient** (15,99 g) + **HPP treatment.**

Work Package 5

- **RP activities & outcomes: <u>Experimental design</u>**

High Pressure Processing
➢ 600 MPa / 4 min

X

Thermal Process
➢ 90 °C / 3 min

➢ **Shelf life:** 1, 21 and 45 days at **5 °C**:

➢ **Microbiology tests:**
- Total mesophilic bacteria (PCA)
- Molds and Yeast (DRBC-agar)
- *Enterobacteriaceae* (Endo-agar)

➢ **Quality parameters:**
- pH
- Color
- TPA
- IPB

➢ **Oxidative stability:**
- Lutein, β-carotene
- α-Tocopherol
- Vitamin K1
- Phenolic /Antioxidant activity

Pulping

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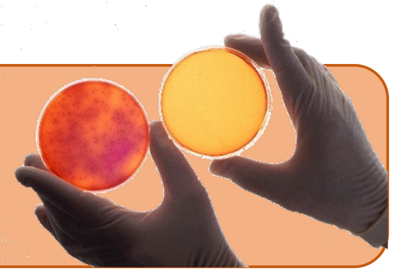
Work Package 5

- **Results: Microbiology**

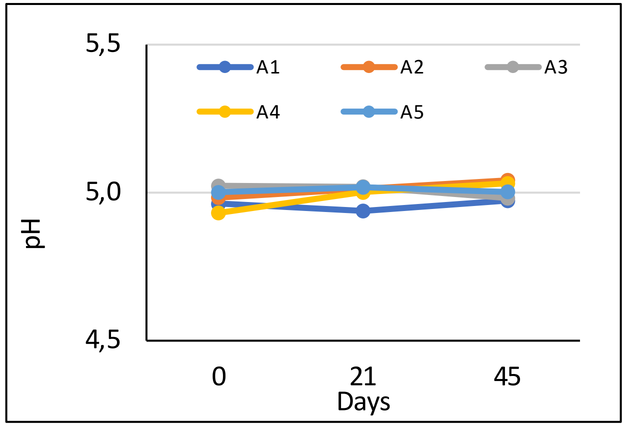
- 45 days of shelf life → no microbial growth. This can be attributed not only for the treatments, but also for the industrial mix and the natural ingredient (samples 4 and 5) added to the pulp.



- Sample 1 (HPP / no PS or NI)
- Sample 2 (Pasteurized + PS)
- Sample 3 (HPP + PS)

- Sample 4 (HPP + NI / no PS)
- Sample 5 (HPP + NI + 50% PS)

- Sample 4 same good results → positive impact of NI as a natural preservative in combination with HPP.
- Sample 5 (HPP + NI + 50% PS) no growing was observed, so either in this product the use of potassium sorbate could be reduced by 50% or even totally replaced by the NI, since in sample 4 no microbial growing was observed.



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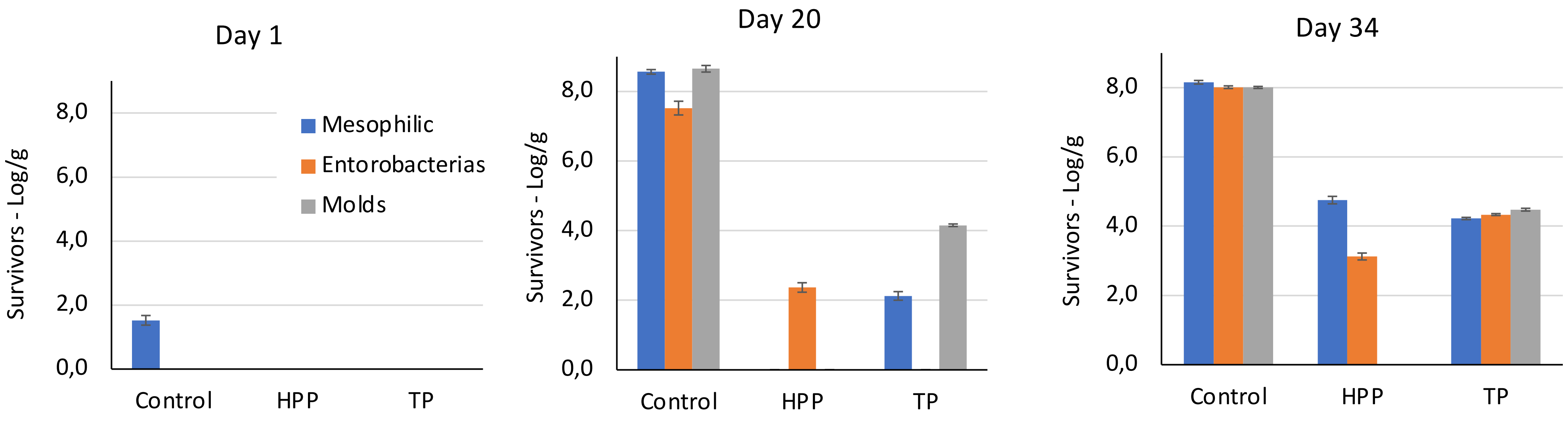
Work Package 5

- **Results: Microbiology**

  - Previous results (process optimization), same HPP and Pasteurization conditions, 100% pulp.



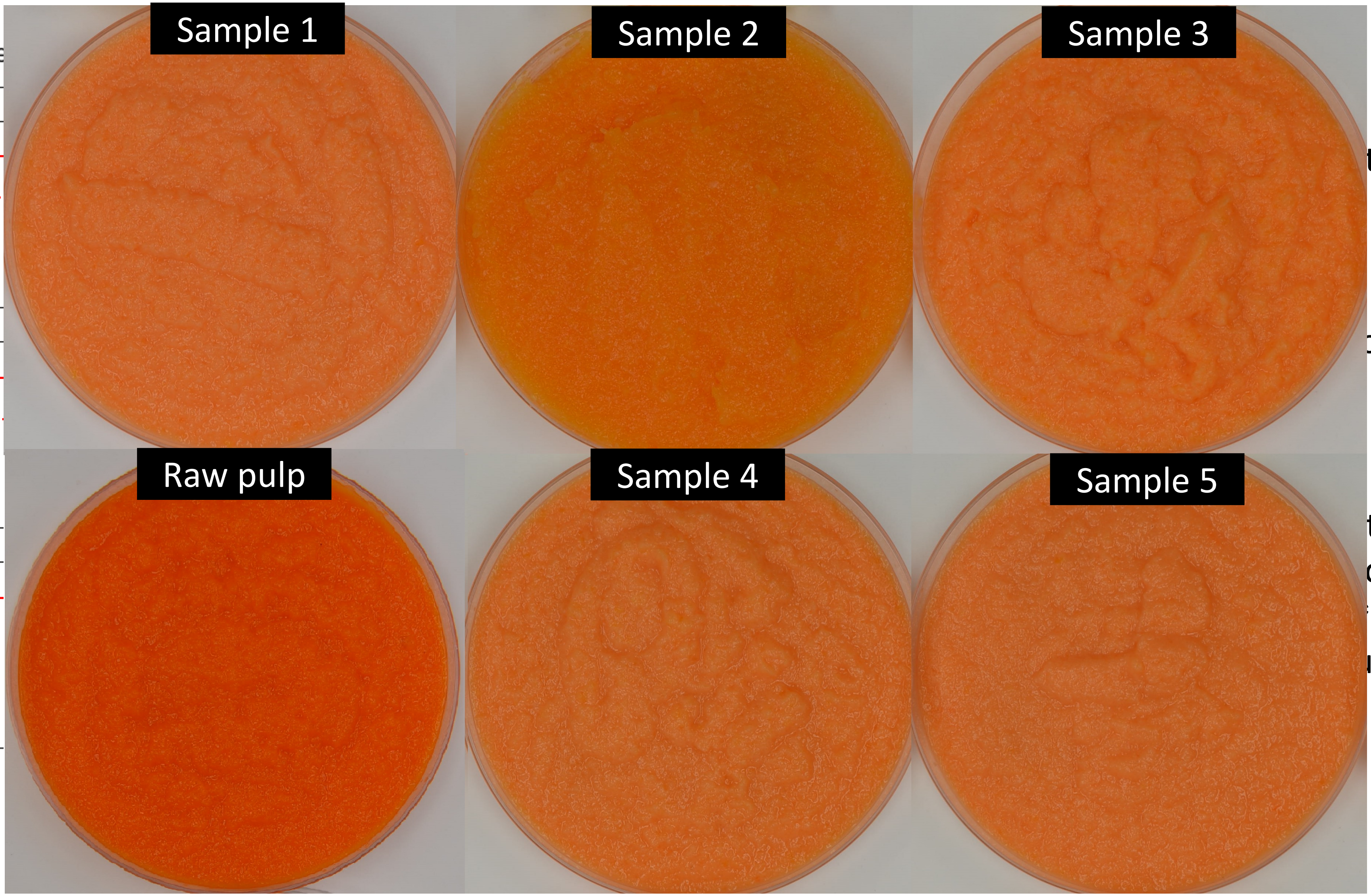
- 45 days of shelf life → no microbial growth.

Work Package 5

- **Results: <u>Color</u>**

Table 4. Color e

Days	
1	A1
	A2
	A3
	A4
	A5
21	A1
	A2
	A3
	A4
	A5
45	A1
	A2
	A3
	A4
	A5



t color changes was

processed by
olor darker, as it can
lower value in L*

the same trend was
days of shelf life, a
value, representing
ulps.

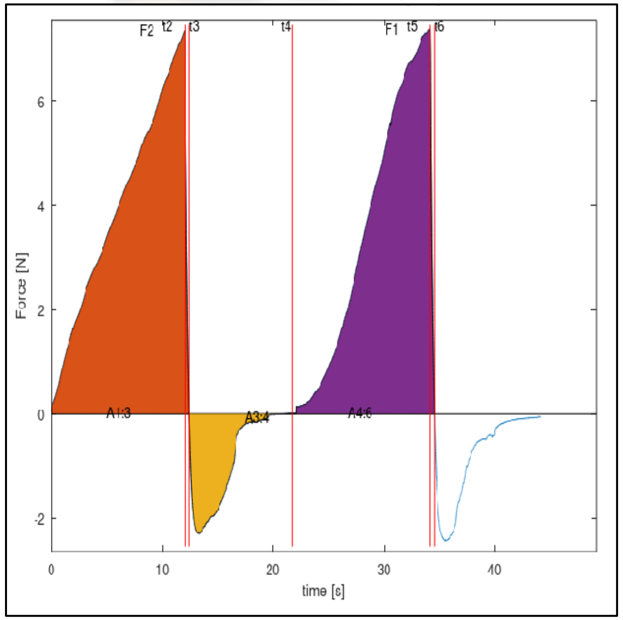
Work Package 5

- **Results: TPA**

D1	A1	A2	A3	A4	A5
Firmness	2.59 ± 0.4 ^{Cab}	8.78 ± 0.73 ^{Aa}	4.53 ± 1.28 ^{Ba}	2.3 ± 0.11 ^{Ca}	2.3 ± 0.14 ^{Ca}
Elasticity	0.98 ± 0.06 ^{Aa}	0.93 ± 0.24 ^{Aa}	1.1 ± 0.12 ^{Aa}	1.04 ± 0.04 ^{Aa}	1.04 ± 0.05 ^{Aa}
Cohesion	0.23 ± 0.26 ^{Ab}	0.38 ± 0.14 ^{Aa}	0.14 ± 0.55 ^{Aa}	0.48 ± 0.19 ^{Aab}	0.48 ± 0.25 ^{Aab}
Gumminess	0.60 ± 0.64 ^{Ba}	3.38 ± 1.46 ^{Aa}	0.67 ± 0.93 ^{Ba}	1.09 ± 0.4 ^{ABa}	1.09 ± 0.48 ^{ABa}
Chewiness	0.58 ± 0.73 ^{Aa}	3.27 ± 1.93 ^{Aa}	0.71 ± 1.05 ^{Aa}	1.14 ± 0.43 ^{Aa}	1.14 ± 0.52 ^{Aa}
Stickiness	4.28 ± 1.38 ^{Ba}	29.57 ± 1.45 ^{Aa}	4.50 ± 2.00 ^{Ba}	3.79 ± 0.74 ^{Ba}	3.79 ± 1.08 ^{Ba}

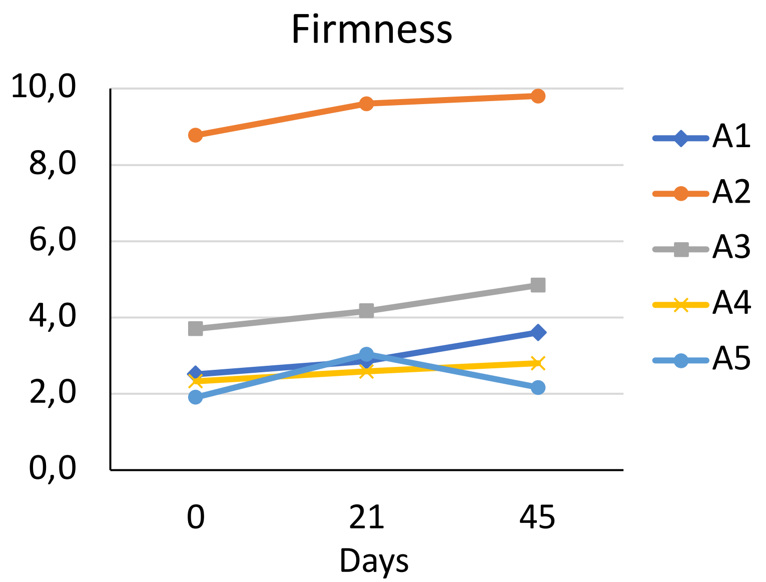
D21	A1	A2	A3	A4	A5
Firmness	2.82 ± 0.3 ^{Cb}	9.33 ± 0.58 ^{Aa}	4.21 ± 0.41 ^{Ba}	2.47 ± 0.2 ^{Ca}	3.06 ± 0.19 ^{BCa}
Elasticity	1.06 ± 0.04 ^{Aa}	1.11 ± 0.12 ^{Aa}	1.01 ± 0.11 ^{Aa}	1.01 ± 0.09 ^{Aa}	0.88 ± 0.15 ^{Aa}
Cohesion	0.27 ± 0.15 ^{Ab}	0.38 ± 0.10 ^{Aa}	0.29 ± 0.08 ^{Aa}	0.35 ± 0.13 ^{Ab}	0.19 ± 0.09 ^{Ab}
Gumminess	0.76 ± 0.41 ^{Ba}	3.49 ± 1.05 ^{Aa}	1.23 ± 0.3 ^{Ba}	0.89 ± 0.39 ^{Ba}	0.58 ± 0.30 ^{Ba}
Chewiness	0.80 ± 0.40 ^{Ba}	3.90 ± 1.38 ^{Aa}	1.24 ± 0.33 ^{Ba}	0.98 ± 0.42 ^{Ba}	1.96 ± 0.38 ^{Ba}
Stickiness	3.15 ± 0.58 ^{Ba}	22.63 ± 4.8 ^{Ab}	3.85 ± 0.56 ^{Ba}	3.03 ± 0.88 ^{Ba}	3.2 ± 1.47 ^{Ba}

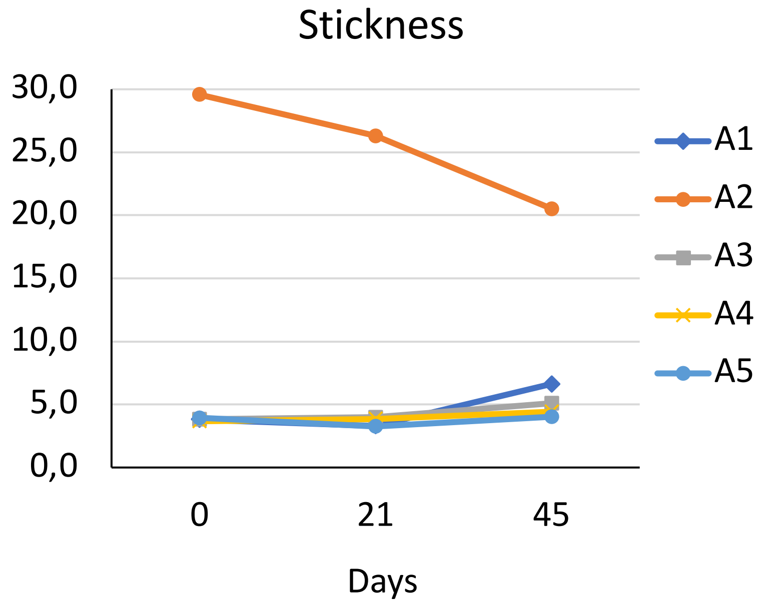
D45	A1	A2	A3	A4	A5
Firmness	3.76 ± 0.42 ^{Cb}	9.81 ± 0.24 ^{Aa}	4.68 ± 0.28 ^{Ba}	2.71 ± 0.14 ^{Da}	2.13 ± 0.09 ^{Da}
Elasticity	1.34 ± 0.19 ^{Aa}	1.10 ± 0.00 ^{Ba}	1.03 ± 0.03 ^{Ba}	0.98 ± 0.05 ^{Ba}	1.06 ± 0.05 ^{Ba}
Cohesion	0.75 ± 0.17 ^{ABa}	0.59 ± 0.25 ^{ABCa}	0.24 ± 0.08 ^{Ca}	0.36 ± 0.21 ^{BCa}	0.87 ± 0.18 ^{Aa}
Gumminess	2.81 ± 0.72 ^{ABa}	5.87 ± 2.54 ^{Aa}	1.17 ± 0.42 ^{Ba}	0.99 ± 0.66 ^{Ba}	1.85 ± 0.39 ^{Ba}
Chewiness	3.76 ± 1.1 ^{ABa}	6.42 ± 2.78 ^{Aa}	1.22 ± 0.46 ^{Ba}	0.91 ± 0.69 ^{Ba}	0.52 ± 0.40 ^{Ba}
Stickiness	5.22 ± 2.52 ^{Ba}	20.53 ± 3.97 ^{Ab}	4.89 ± 0.51 ^{Ba}	3.64 ± 1.02 ^{Ba}	3.57 ± 0.66 ^{Ba}

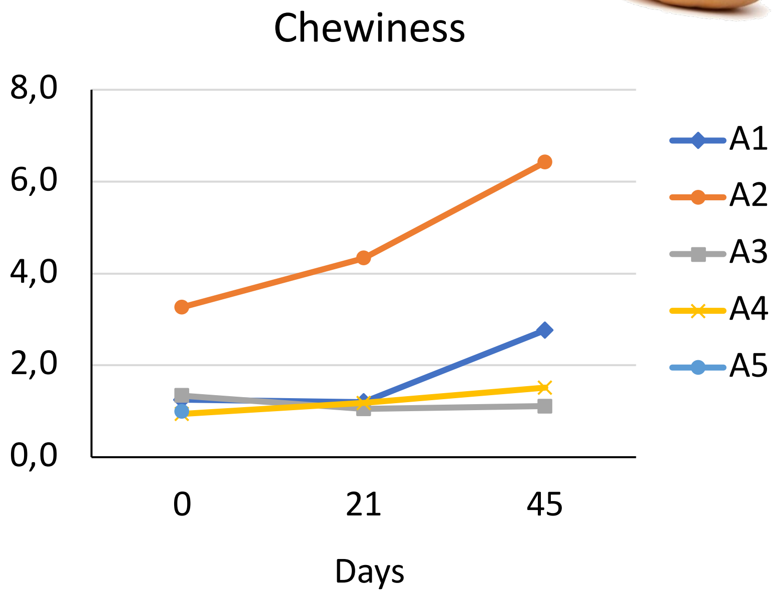


Work Package 5

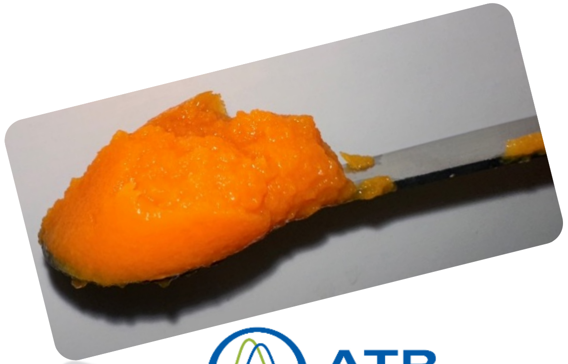
- **Results: <u>TPA</u>**







A2 > A3 > A1 > A4 > A5



Pulping

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▪ Results: <u>Oxidative Stability</u>

Changes in β-carotene, lutein and γ-tocopherol content in freeze-dried pumpkin pulp samples after different treatments. Analysis was performed using HPLC-MS/MS.

	Storage time (d)	Treatment				
		A1	A2	A3	A4	A5
β-carotene (mg/100 g dw)	0	150.01 ± 7.45^{a}	54.98 ± 7.95^{b}	138.01 ± 10.05^{ac}	123.32 ± 5.33^{c}	91.1 ± 5.33^{d}
	21	111.08 ± 27.65^{a}	48.88 ± 9.38^{b}	100.58 ± 5.70^{ac}	102.47 ± 4.39^{ac}	84.46 ± 3.49^{c}
	45	52.06 ± 1.65^{a}	ND	47.28 ± 6.75^{a}	41.74 ± 9.68^{a}	52.17 ± 17.32^{a}
	D%	65.30		65.74	66.16	42.73
Lutein (mg/100 g dw)	0	1.57 ± 0.07^{a}	1.21 ± 0.32^{b}	3.74 ± 0.11^{c}	1.46 ± 0.01^{ab}	0.87 ± 0.10^{d}
	21	1.17 ± 0.12^{ad}	1.53 ± 0.11^{b}	2.52 ± 0.16^{c}	1.40 ± 0.31^{ab}	0.99 ± 0.15^{d}
	45	0.54 ± 0.05^{a}	0.58 ± 0.09^{a}	1.05 ± 0.02^{b}	0.90 ± 0.07^{c}	0.63 ± 0.07^{a}
	D%	65.81	51.87	71.98	38.09	27.35
γ-tocopherol (mg/100 g dw)	0	0.131 ± 0.008^{a}	0.132 ± 0.003^{a}	0.186 ± 0.002^{b}	0.137 ± 0.02^{a}	0.080 ± 0.020^{c}
	21	0.084 ± 0.004^{a}	0.093 ± 0.003^{b}	0.077 ± 0.003^{c}	0.062 ± 0.005^{d}	0.064 ± 0.002^{d}
	45	0.031 ± 0.002^{a}	0.037 ± 0.001^{b}	0.029 ± 0.001^{c}	0.030 ± 0.002^{ac}	0.030 ± 0.001^{ac}
	D%	76.13	72.27	84.42	78.10	62.26

ND: not detectable, dw: dry weight, *D*%: degradation percentage
Different letters indicate significant differences within a row (ANOVA, $p < 0.05$, Tukey-HSD)

**A2; lower values;
A5; best stabilization during shelf life**

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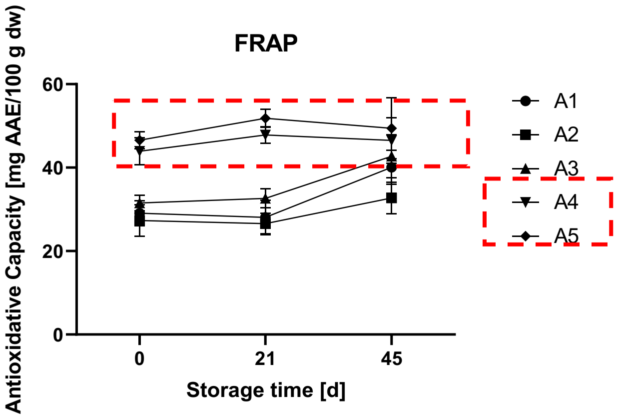
Work Package 5

Results: Oxidative Stability

Changes in total phenolic content (TPC) in freeze-dried pumpkin pulp samples after different treatments

	Storage time (d)	Treatment				
		A1	A2	A3	A4	A5
TPC (mg GAE/100 g dw)	0	48.86 ± 1.13^{Aa}	42.52 ± 2.02^{Aa}	38.01 ± 2.61^{Ab}	52.49 ± 4.20^{Ac}	52.55 ± 1.69^{Ac}
	21	42.89 ± 1.78^{Ba}	40.54 ± 2.66^{Aa}	35.48 ± 1.12^{Ab}	48.75 ± 2.58^{ABc}	52.48 ± 1.87^{Ad}
	45	41.95 ± 1.55^{Bac}	38.91 ± 3.00^{Aab}	35.05 ± 0.77^{Ab}	45.87 ± 2.97^{Bc}	50.49 ± 2.73^{Ad}
	D%	14,14	8,49	7,79	12,61	3,92

TPC: Total phenolic content, GAE: Gallic Acid Equivalent, dw: dry weight, *D%*: degradation percentage
Different small letters indicate significant differences within a row, different capital letters indicate significant differences within a column (ANOVA, $p < 0.05$, Tukey-HSD)



Changes of Antioxidative Capacity in freeze-dried pumpkin pulp samples after different treatments. Dw: dry weight, AAE: Ascorbic Acid Equivalent

- Samples 4 and 5 exhibit significantly higher TPC compared to the other samples, probably due to the addition of natural ingredients extracted from peels and seeds, which are rich sources of phenolic compounds.

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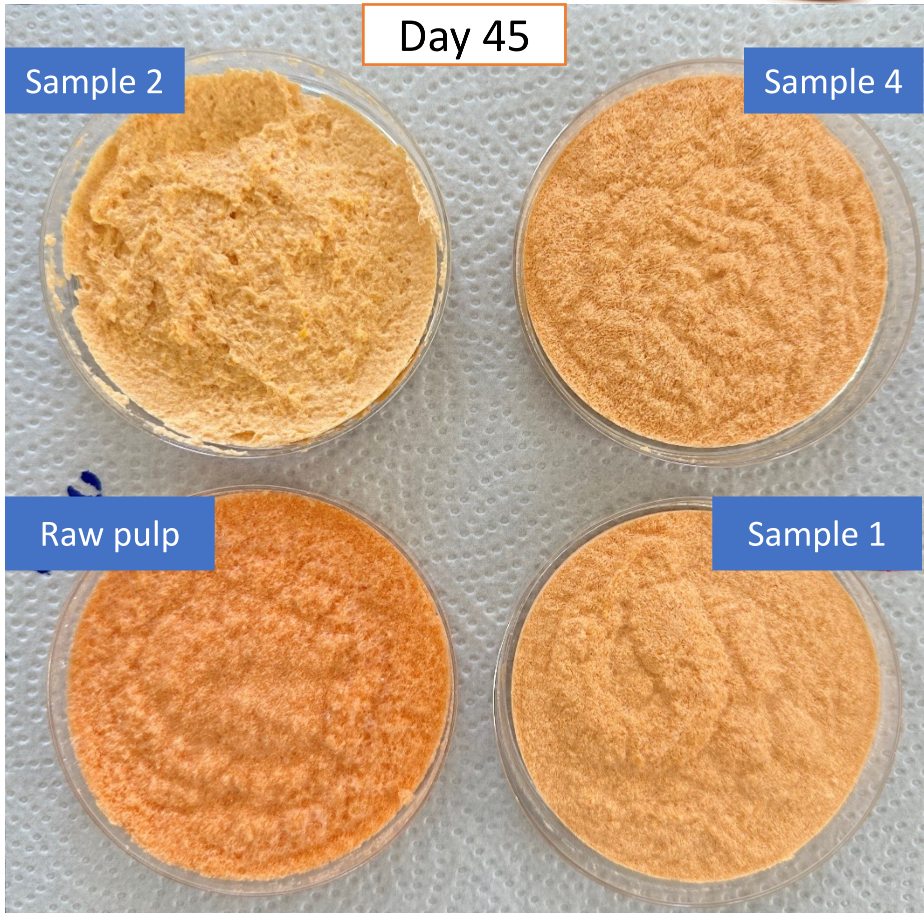
Work Package 5

- **Ongoing….**
- **Results: <u>IPB analysis</u>**

  - Nutritional analysis;
  - Sugar content (fructose, glucose, sucrose, trehalose, total free sugar);
  - Fatty acids

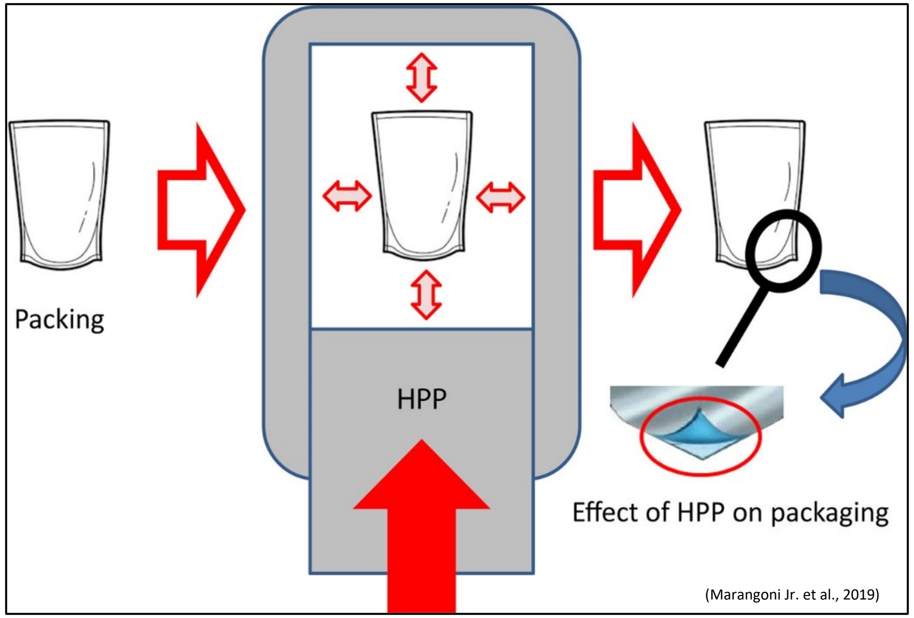
		In g/100g DW				kcal
Formulation	Storage time	Ash	Protein	Fat	Carbohydrates	Energy value
A1	0	3.5 ± 0.2	4.65 ± 0.03	0.42 ± 0.02	91.4 ± 0.1	388.1 ± 0.5
	45	3.6 ± 0.2	5.01 ± 0.02	0.335 ± 0.003	91.0 ± 0.1	387.2 ± 0.6
A2	0	4.0 ± 0.2	4.99 ± 0.01	0.37 ± 0.01	90.7 ± 0.2	386.0 ± 0.6
	45	4.0 ± 0.1	4.41 ± 0.09	0.34 ± 0.01	91.3 ± 0.2	385.8 ± 0.5
A3	0	4.6 ± 0.2	4.03 ± 0.03	0.426 ± 0.009	90.9 ± 0.2	384 ± 1
	45	4.74 ± 0.01	3.91 ± 0.07	0.43 ± 0.02	90.92 ± 0.09	383.15 ± 0.03
A4	0	4.4 ± 0.2	5.58 ± 0.02	0.39 ± 0.02	89.6 ± 0.3	384.3 ± 0.8
	45	4.4 ± 0.2	5.5 ± 0.2	0.35 ± 0.02	89.7 ± 0.4	384.1 ± 0.9
A5	0	4.2 ± 0.2	6.045 ± 0.001	0.46 ± 0.02	89.3 ± 0.2	385.3 ± 0.9
	45	4.1 ± 0.1	6.10 ± 0.03	0.302 ± 0.004	89.5 ± 0.1	385.3 ± 0.5



Work Package 5

- **Next steps:**
  - **Task 5.4: Accelerated shelf life test**
  - **Task 5.5: Packaging recommendations**

(Medallion Labs, 2017)



(Marangoni Jr. et al., 2019)

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2024 EFFoST/IFT-NPD Workshop on nonthermal processing of foods
Nonthermal processes to foster
diversity, sustainability and resilience of future food systems
7-9 October 2024
Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
https://effost-ift-npw2024.atb-potsdam.de

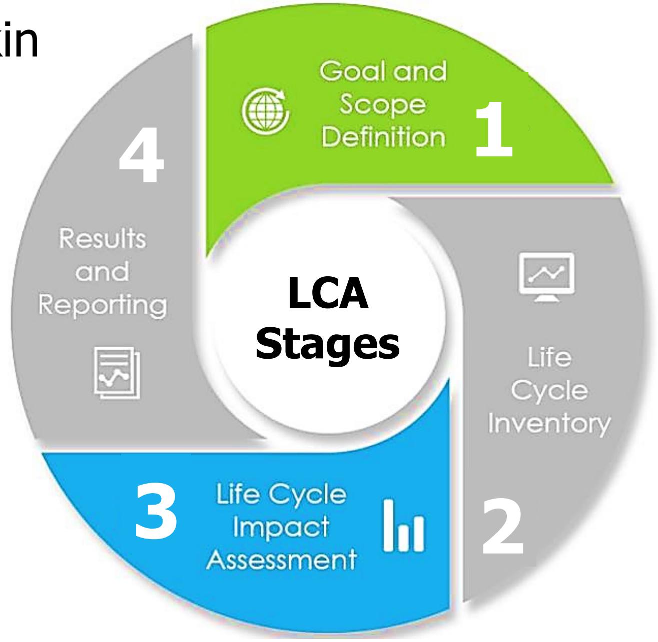
PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WP 6: Waste and Wastewater Management and Life-Cycle Assessment

Joana Pesqueira

Work Package 6

The **goal** of the present study consists of the **assessment of the environmental impacts of pumpkin fruit pulp production** traditionally and with a novel formulation (i.e., with a natural preservative extracted from pumpkin by-products), according to a **cradle-to-grave approach**.



Three central phases:

1. Agriculture phase (pumpkin cultivation);
2. Industrial phase (pulp processing and packaging);
3. Use and end-of-life.

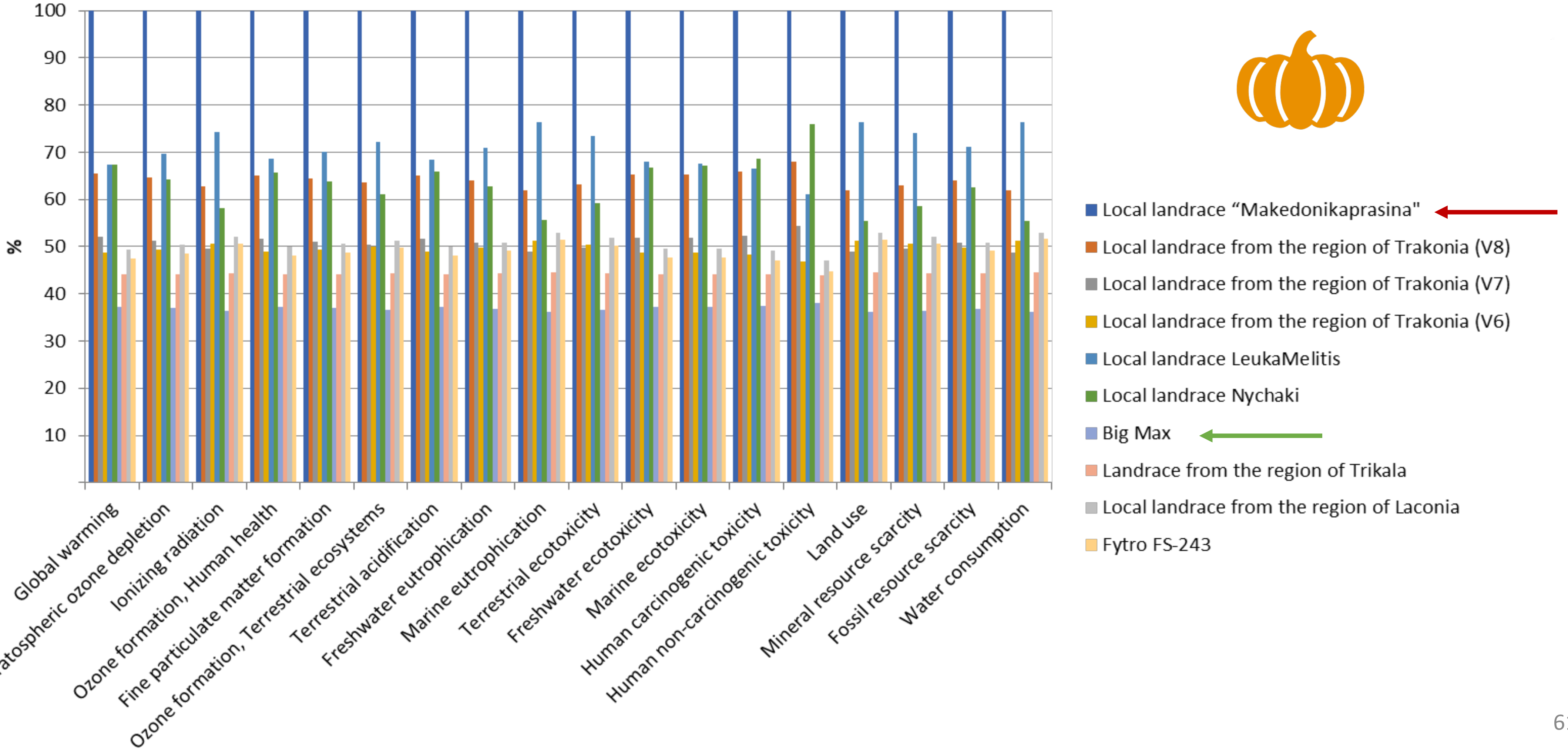
5.5 kg of packaged pumpkin fruit pulp

18 impact categories

Work Package 6

Agriculture phase

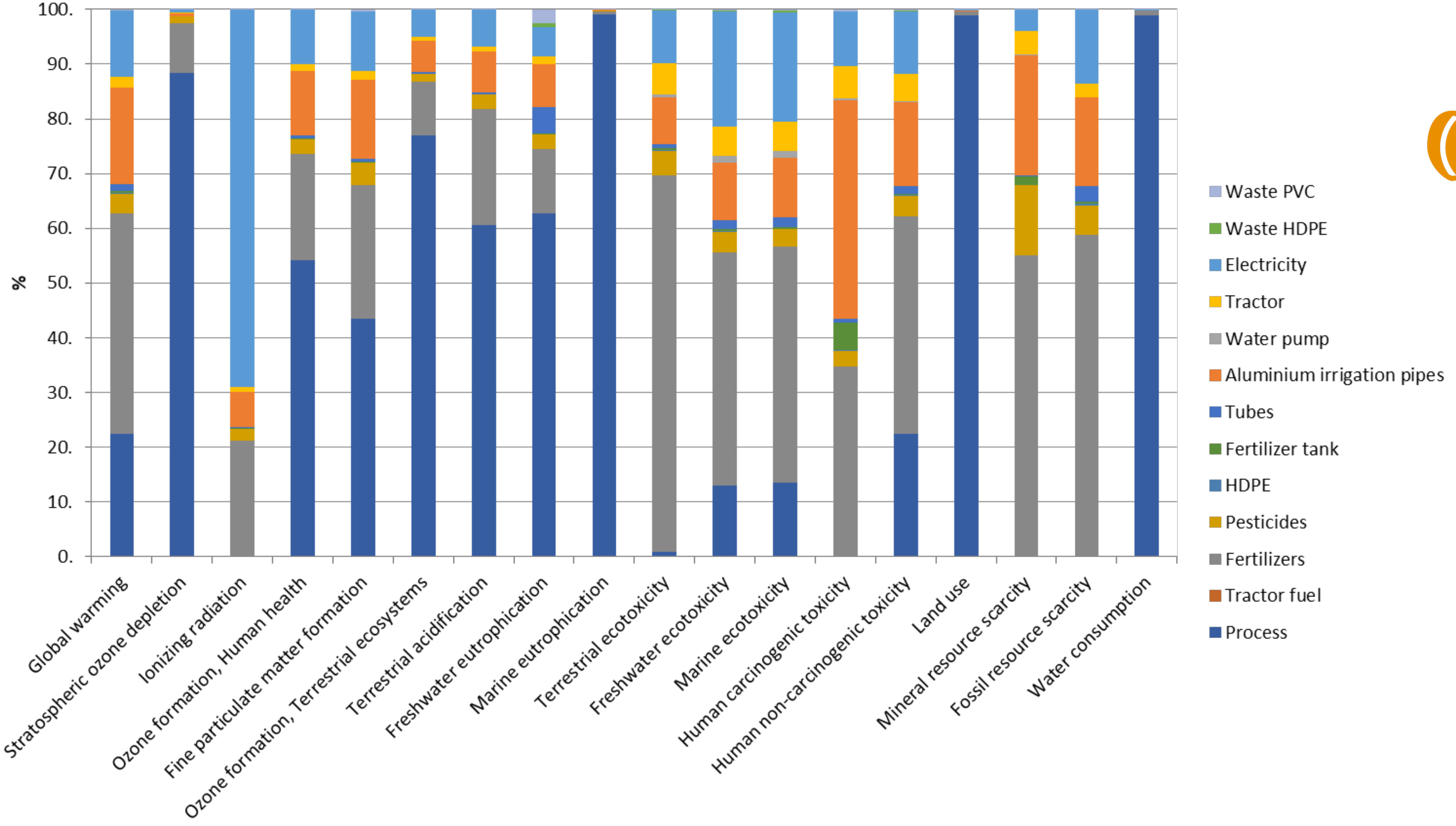
- Comparison between varieties



Work Package 6

Agriculture phase

- Contributions to pumpkin cultivation impacts



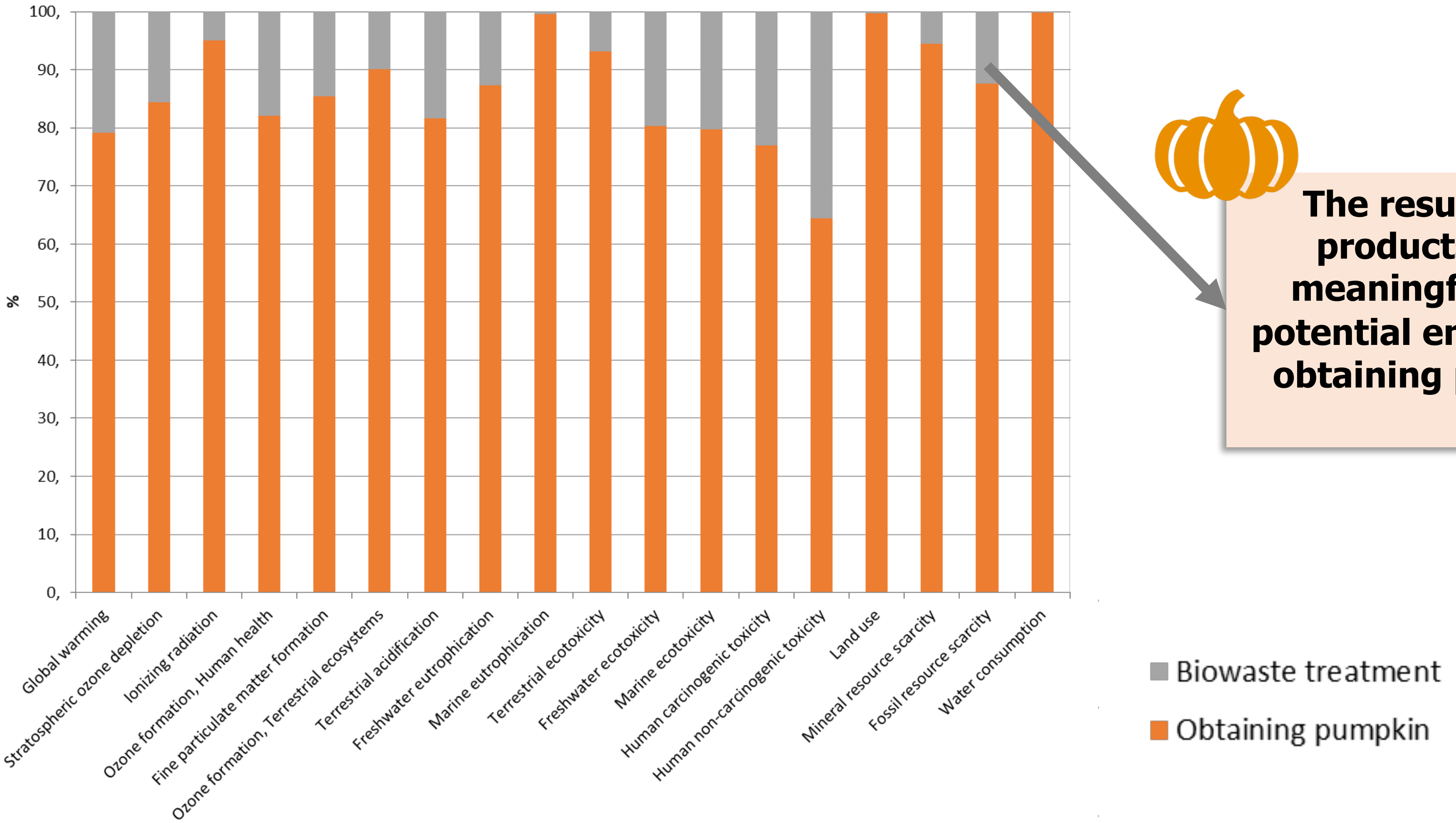
Main contributors per impact category

- Process emissions (9)
- Production of fertilizers (7)
- Infrastructure (1)
- Electricity (1)

Work Package 6

Agriculture phase

- Accounting for waste on the obtaining of pumpkin flesh

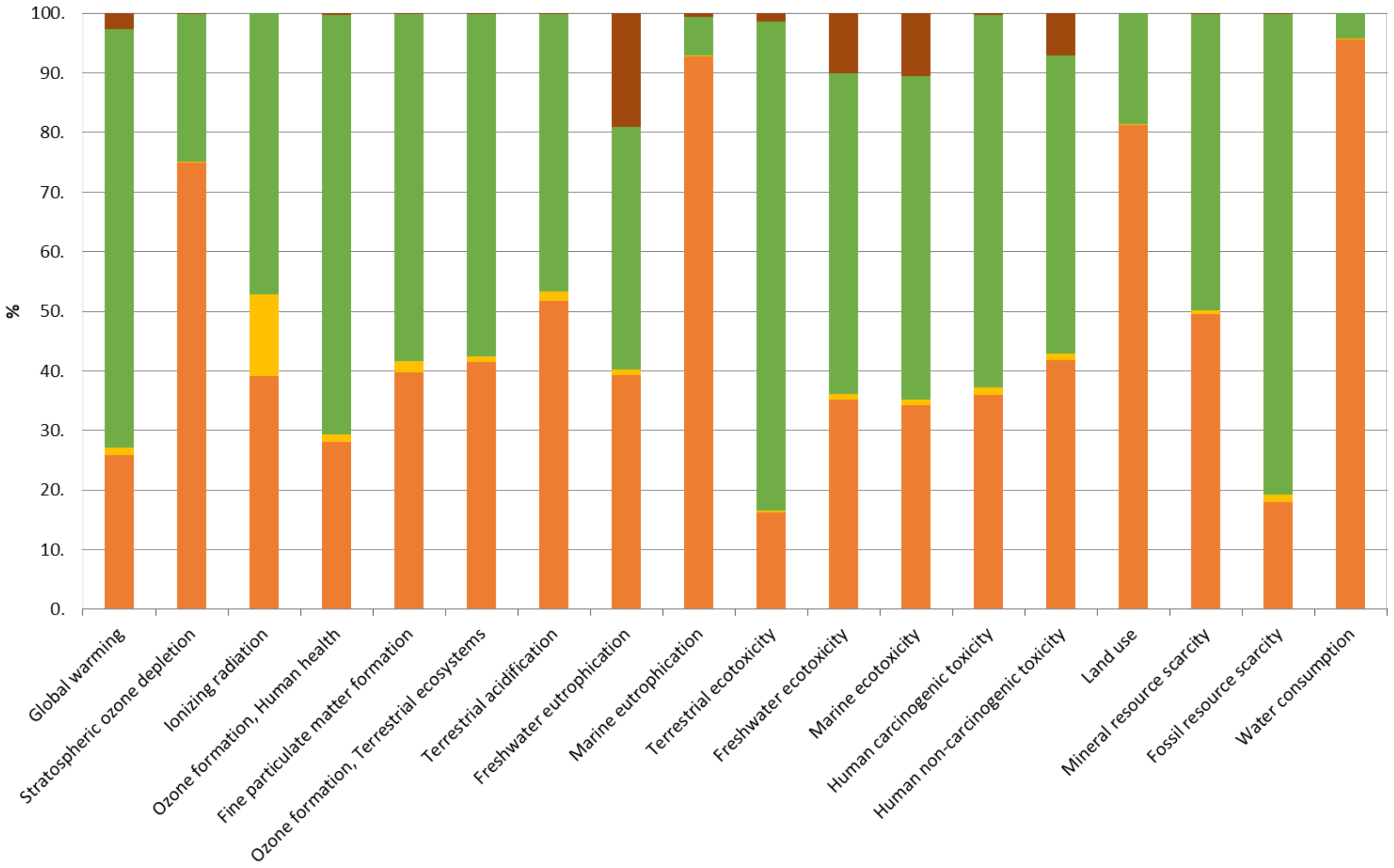


The results show that the by-products of pumpkin have a meaningful contribution to the potential environmental impacts of obtaining pumpkin flesh for pulp production.

Work Package 6

Traditional life cycle

- Main contributors



- Pumpkin Cultivation
- **Pumpkin pulp production**

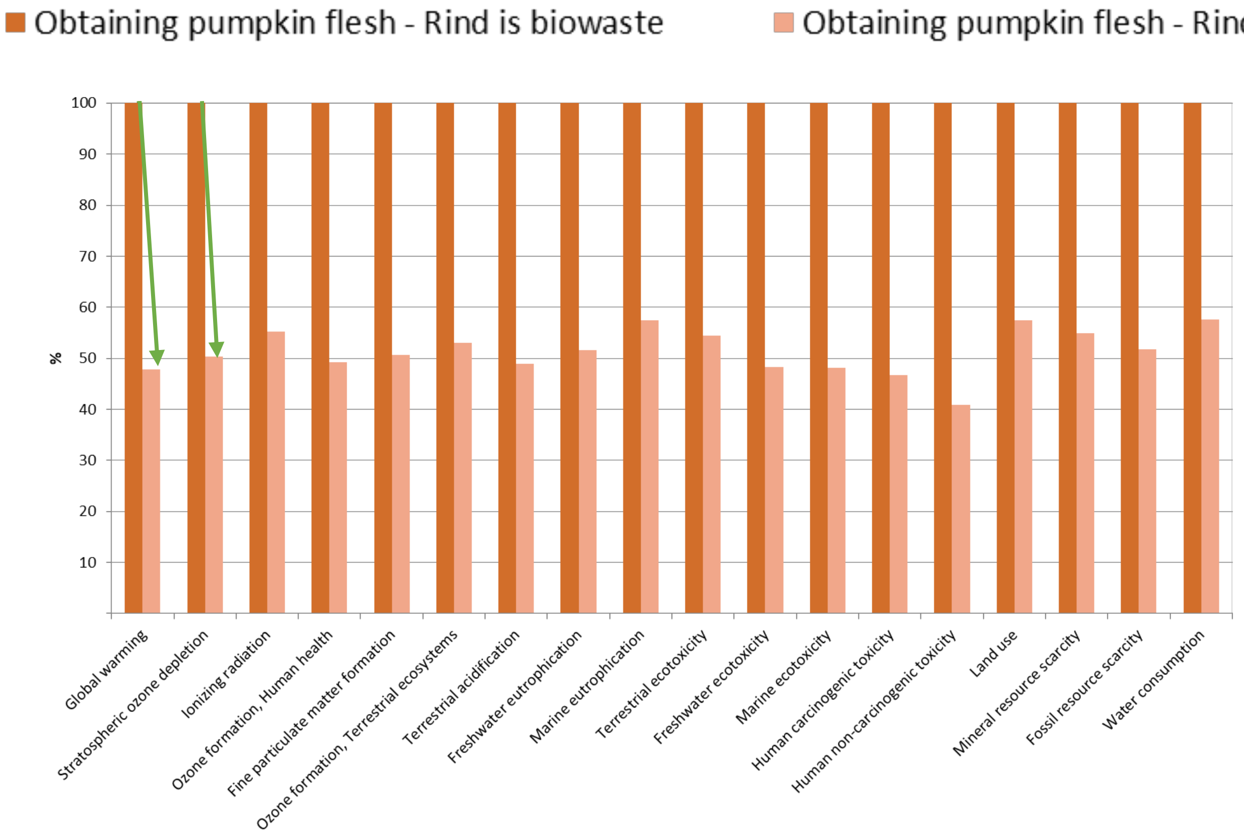
Mixing and Homogenization

Additives Electricity

Work Package 6

Using the New Extract

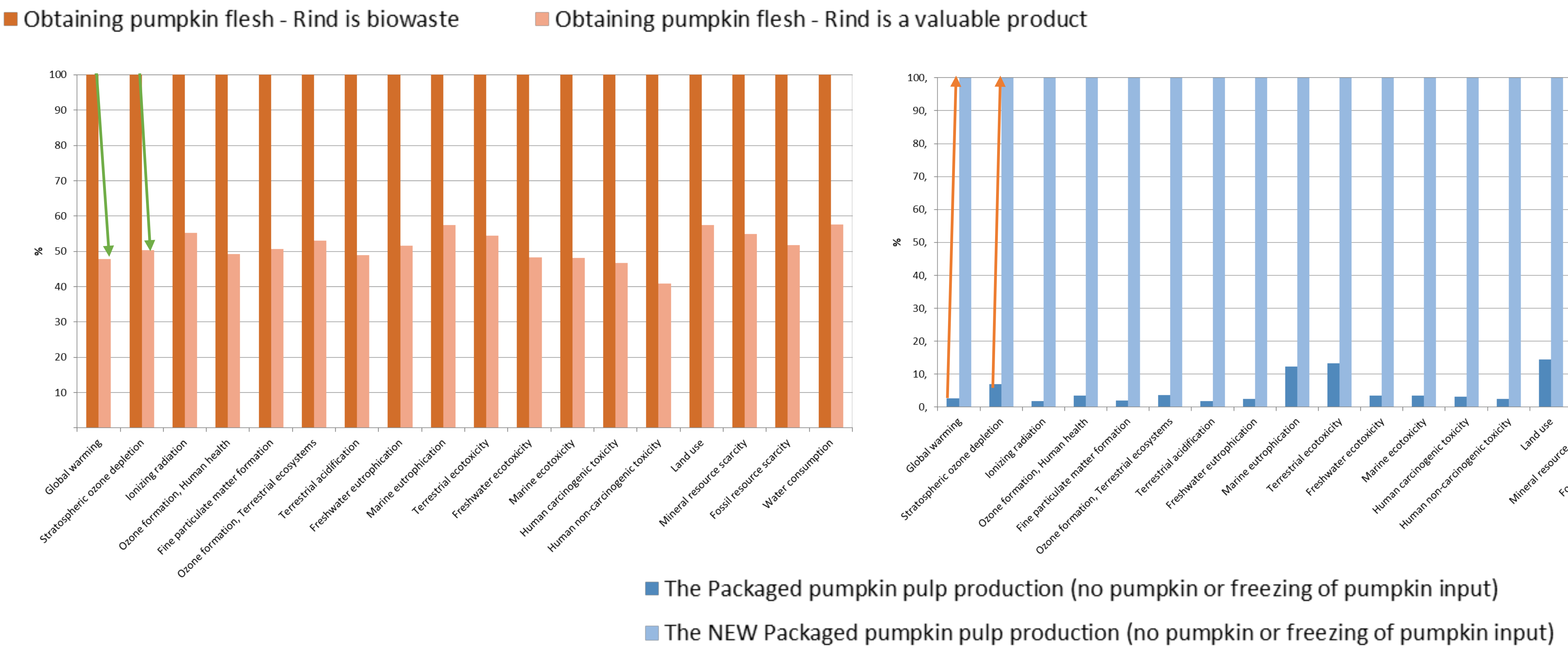
- Influence on the impacts of obtaining pumpkin flesh and on the production



Work Package 6

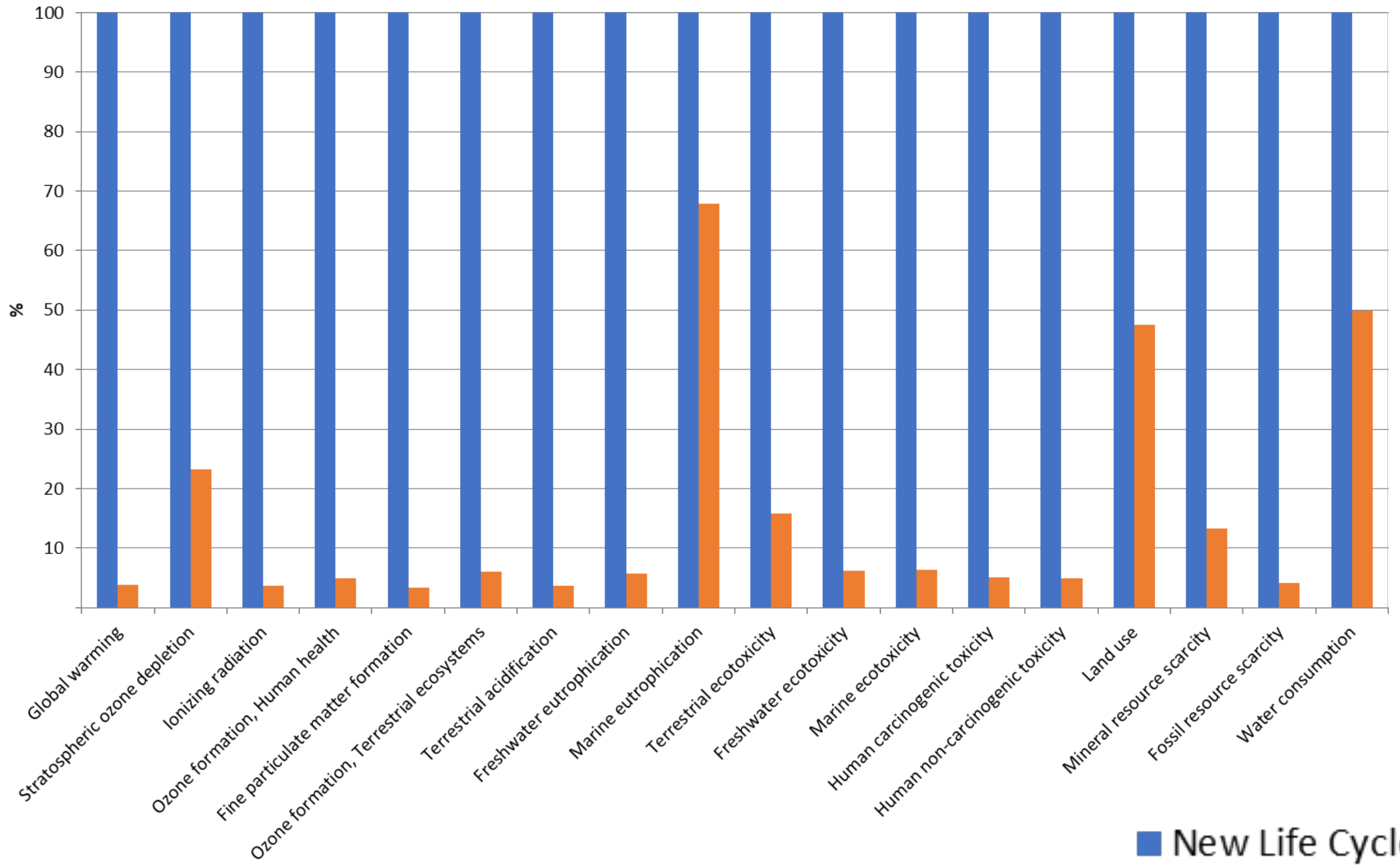
Using the New Extract

- Influence on the impacts of obtaining pumpkin flesh and on the production



Work Package 6

Traditional *vs* New life cycle



mountains of research

- Considering the whole life cycle, the use of the extract may increase the environmental impacts (uncertainty is high due to scale)
- However, considering the potential of waste reduction, and of the extract as a valorisation route, it is worth optimizing the extract production process
- The high energy use associated to **lyophilisation** – particularly before extraction – is the **hotspot**

PRIMA

Work Package 6

Traditional *vs* New life cycle

- These are not discouraging results – optimization is needed.
- For example, using photovoltaic energy in the extract production can reduce the potential environmental impacts in several categories.
- Osmotic dehydration combination with lyophilisation has shown good results.
- Possible lifetime increase was not considered.

PulpIng – Development of **Pu**mpkin Pu**lp** Formulation Using a Sustainable **In**te**g**rated Strategy

WP 1: Defining agronomic conditions for pumpkin production

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Work Package 1

Lead partner: UTH
Participants: IPB; MORE; CBBC; CRAPC; BU

- **Objectives of the WP:**

- Selection of genotypes with abiotic stress tolerance
- Morpho-agronomic characterization of pumpkin genotypes (cultivars and local landraces)
- Selection of cultivars and local landraces with improved agronomic performance
- Estimation of genetic diversity in most promising genotypes
- Establishment of cultivation protocols for optimized agronomic performance and high quality
- Establishment of organic farming protocols for pumpkin cultivation
- Quality assessment of pumpkin raw materials

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- **Summary of activities performed during this WP:**
  - In this WP, we have cultivated for two growing periods 10 pumpkin genotypes (Greece), 5 pumpkin varieties (Egypt) and 4 local squash landraces (Tunisia) and identified those with the best performance.
  - We have also evaluated the response of the genotypes of Greece and Tunisia under stress conditions (salinity, temperature, water stress) at germination and seedling phase.
  - We performed the molecular characterization of Greek genotypes, as well as the quality evaluation of fruit for two growing periods.
  - The results of the project have been disseminated in national and international conferences and have been published in peer-reviewed scientific papers in open access mode (see presentation of WP7).
  - Data for LCA analysis have been collected from both growing periods (see presentation of WP6)

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- **Tasks of the WP:**

Task 1.1. Germplasm evaluation for abiotic stress tolerance **(M1-24)**

Task 1.2. Domestication and improvement of crop productivity and resource use efficiency **(M5-36)**

Task 1.3. Molecular characterization of selected genotypes **(M12-36)**

Task 1.4. Quality evaluation of raw materials **(M8-36)**

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- **RP activities & outcomes: Task 1.1**

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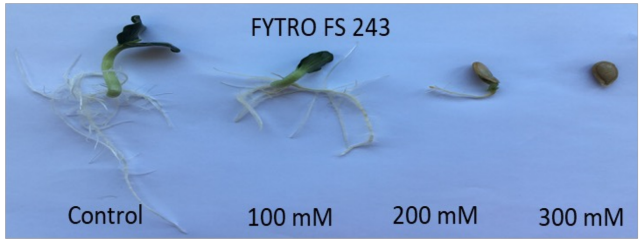
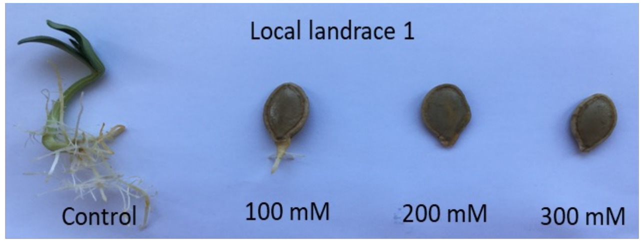
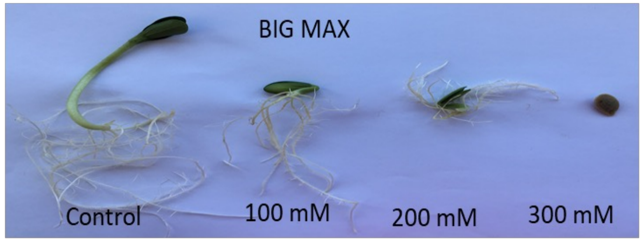
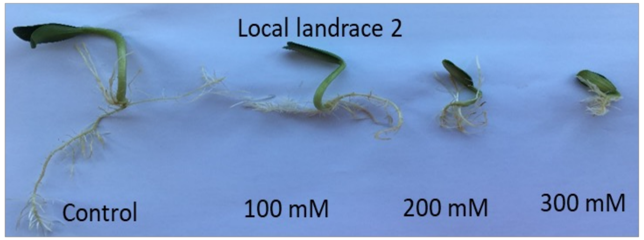
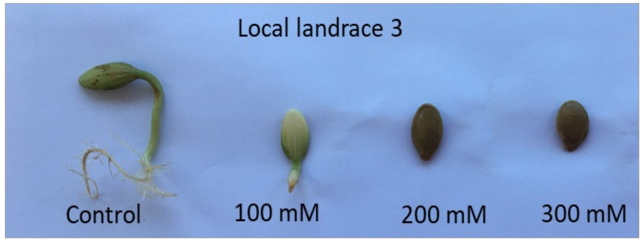
- **RP activities & outcomes: Task 1.1**

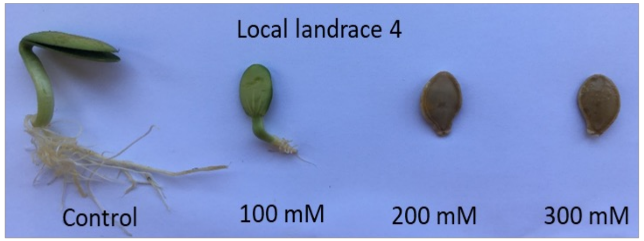


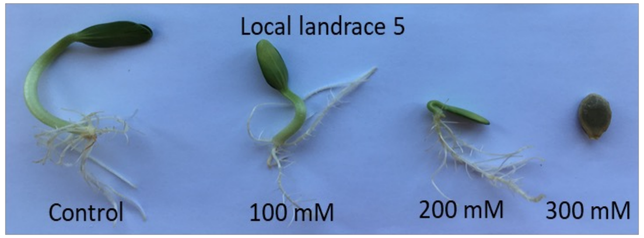












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PRIMA

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- Drought stress severely affected all traits associated with germination and seedling growth in a genotype dependent manner. Overall findings suggest the superiority of local landraces 2 and 5, while landraces 1 and 4 proved the most sensitive genotypes.
- Salinity stress affected all traits associated with germination and seedling growth, with varied effects depending on the stress level applied.
- Genotypes differed significantly in their response to the varying salinity levels, thus indicating the existence of considerable genetic variation related to salt tolerance at germination stage.
- Local landrace 2 proved the most salt-tolerant genotype.
- Temperature stress hindered germination in most of the tested genotypes, except for Big Max which had the best overall performance both under very high (37 °C) or very low temperatures (4 °C).

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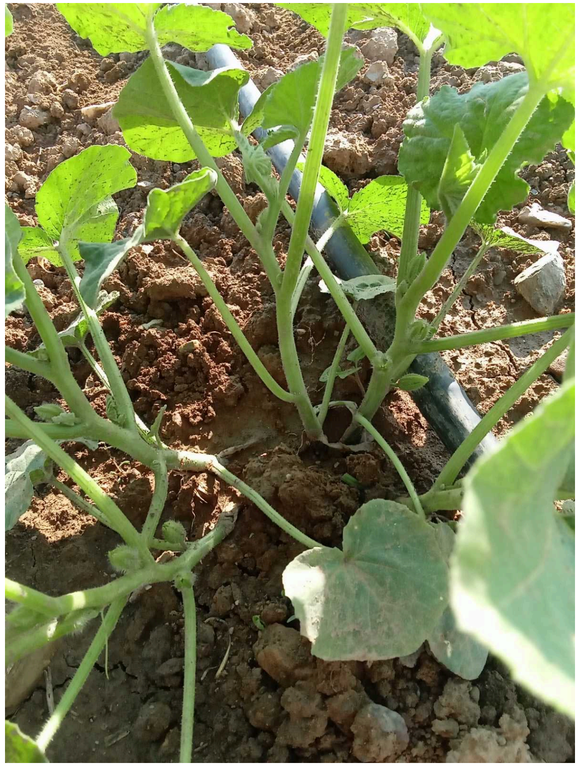
- **RP activities & outcomes: Task 1.2**



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- The results from the experiments in Greece showed the great variability in pumpking agronomic performance among the studied local landraces and the commercially available genotypes.
- V1 (Fytro FS-243), V4 (Local landrace “Nychaki”) and V7 (Local landrace from the region of Lakonia) had the best crop performance based on the yield parameters and the quality of fruit as determinded in other tasks of WP1 and in WP2 where the recovery of bioactive compounds from fruit by-products was evaluated.

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- The results from the experiments in Tunisia showed that plant yield, fruit weight and flesh thickness were the most variable traits in conventional farming for the tested landraces.
- For conventional farming, we selected Galaoui landrace based on the yield parameters with high fertilization rate and Karkoubi pink based on the fruit weight parameters with standard fertilization rate.
- For organic farming, we selected Galaoui small seeds based on the fruit size.

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- **RP activities & outcomes: Task 1.3**

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- The findings underline the suitability of RAPD markers for determining the genetic diversity both at the intra- and inter-populational level.
- RAPD analysis of the germplasm under study revealed a significant level of genetic diversity both within and among populations, while it is interesting both from an agronomic and breeding perspective that the Greek local landraces derived from different geographic regions under study exhibit a considerable genetic variation.

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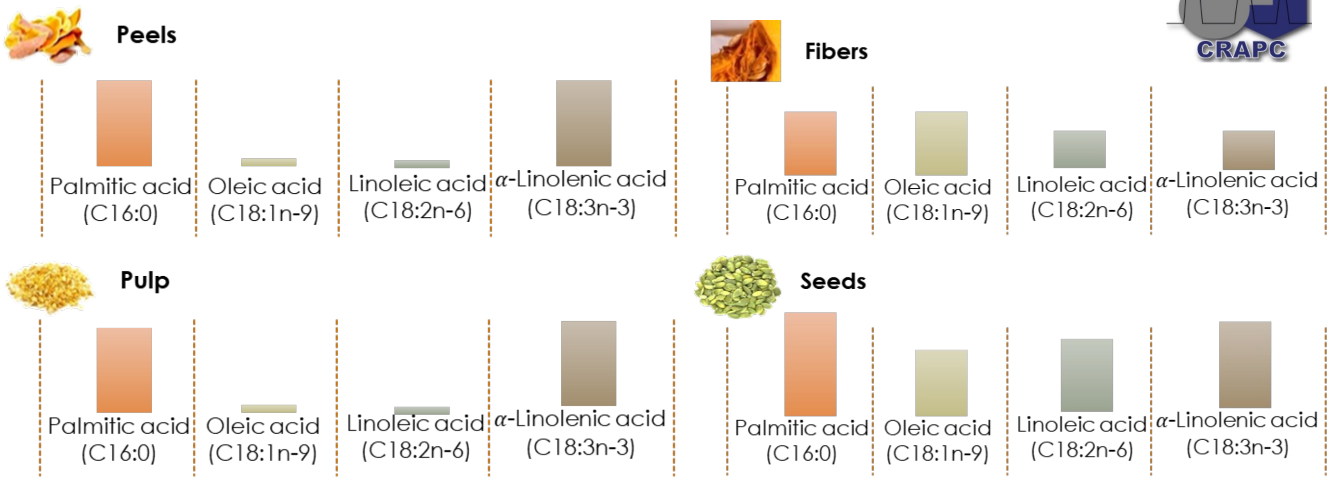
- **RP activities & outcomes: Task 1.4.**

The proximate composition evaluation of the pulp pumpkin of varieties cultivated in Greece and in Egypt, revealed carbohydrates as the major macronutrients, followed by protein, and fat.

Sample		Fat	Proteins	Ash	Fibers	Carbohydrates content	Energy
Greece (17 pulp samples)	Min	0.38 ± 0.02	8.0 ± 0.3	3.5 ± 0.1	12.8 ± 0.2	41.1± 0.4	307.7 ± 1.5
	Max	1.17 ± 0.02	21.4 ± 0.6	10.95 ± 0.05	27.4 ± 0.8	72.2 ± 0.6	361.0 ± 0.1
	Average	0.8 ± 0.2	13 ± 4	7 ± 2	20 ± 5	59 ± 9	336 ± 17
Egypt (5 pulp samples)	Min	1.27 ± 0.04	12.9 ± 0.1	8.29 ± 0.02	15.2 ± 0.4	50,8 ± 0,8	325.0 ± 0.8
	Max	1.81 ± 0.01	19.3 ± 0.3	11.259 ± 0.009	19.4 ± 0.2	61,0 ± 0,9	342.8 ± 0.8
	Average	1.5 ± 0.2	15 ± 2	9 ± 1	18 ± 2	56 ± 4	334 ± 6

g/100g dw (average ± dp)

The fatty acid analysis results of the samples from Algeria showed that the saturated fatty acid such the palmitic acid and stearic were higher in the peels, pulp, and fibers. Interestingly, the monounsaturated fatty acid (oleic acid) and polyunsaturated fatty acids (LA and ALA) were higher in seeds.



The physico-chemical parameters of colour and pH of Greece (G), Egypt (E) and Algeria (A) pulp samples.

Sample	pH	Color (RGB)
G 1	6.29 ± 0.01	
G 2	6.364 ± 0.004	
G 3	6.4033 ± 0.0006	
G 4	6.235 ± 0.004	
G 5	6.34 ± 0.01	
G 6	6.924 ± 0.005	
G 7	6.144 ± 0.004	
G 8	6.03 ± 0.03	
G 9	5.997 ± 0.004	
G 10	6.522 ± 0.004	
G 11	5.822 ± 0.001	
G 12	6.251 ± 0.005	
G 13	6.49 ± 0.02	
G 14	6.35 ± 0.01	
G 15	5.93 ± 0.02	
G 16	6.345 ± 0.009	
G 17	6.06 ± 0.02	
E 1	6.30 ± 0.04	
E 2	6.225 ± 0.009	
E 3	6.278 ± 0.095	
E 4	6.040 ± 0.007	
E 5	6.13 ± 0.06	
A 1		
A 2		
A 3		

Thank You!