Agro-food Wastes as a Source of Antioxidants, Lipids and Cutin by Microwave-assisted and Ultrasound-assisted extraction

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NBP
Grupo de análisis de polímeros y nanomateriales

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Horizon 2020
European Union Funding
for Research & Innovation

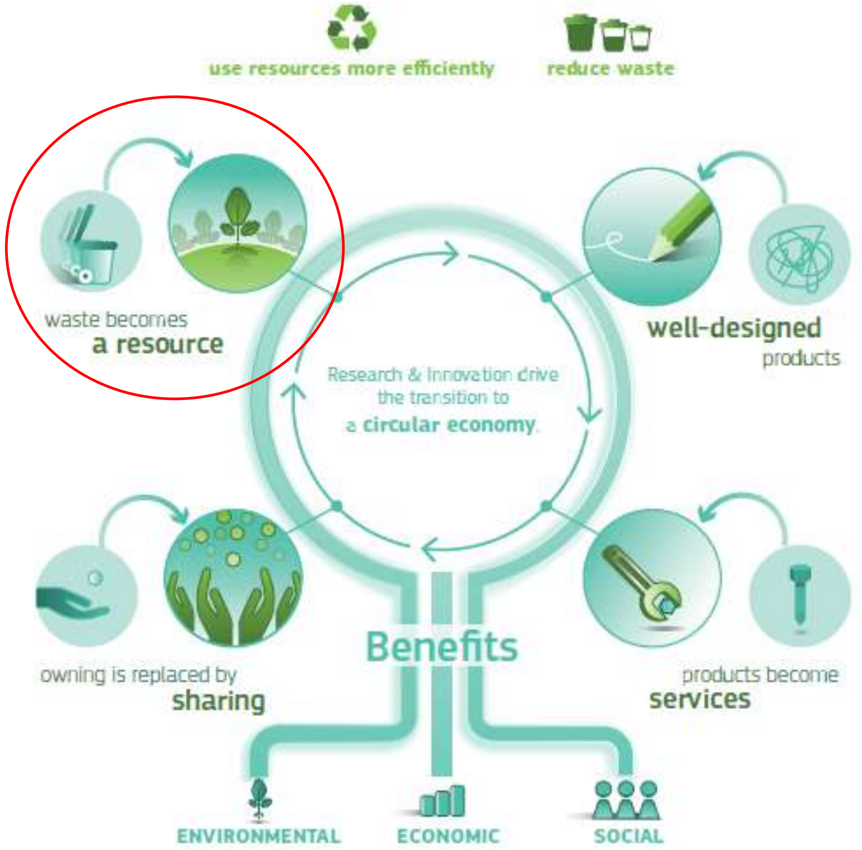
BBI JU

Bio-based Industries Consortium

Agro-food wastes and circular economy

- ✓ Low cost
- ✓ High availability
- ✓ Renewable

Circular economy is based on the efficient and sustainable use of the planet's resources to prevent irreversible environmental degradation and resource depletion



European Union

88 million tons → 143 billion €

- ✓ High environmental and economic impacts

Minimize Waste

Generate Increase Income

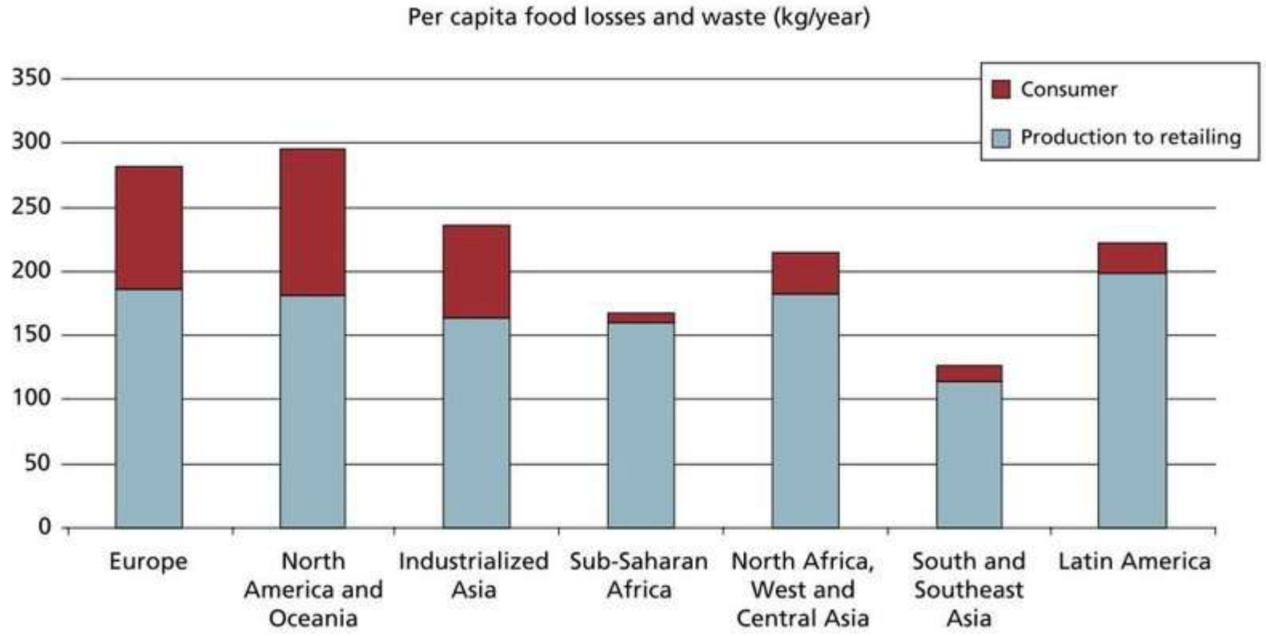
Reduce Environmental Footprint

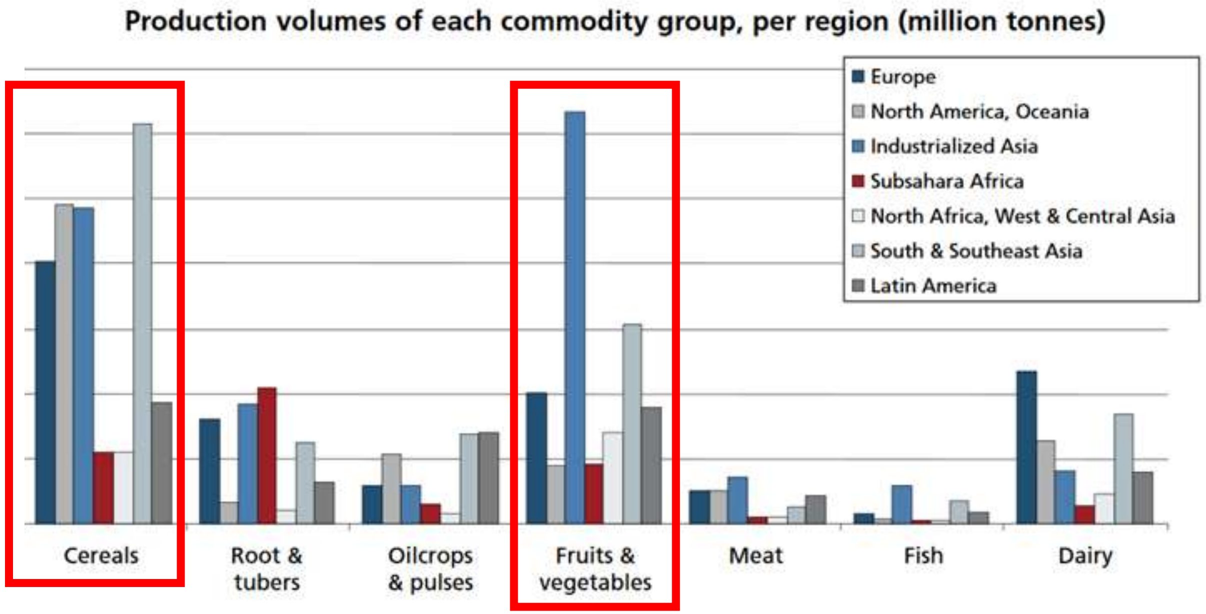
Reduce Resources Dependency

Advanced valorization alternatives should be developed to maximize the added value of waste source

Food waste

(FAO, 2020)



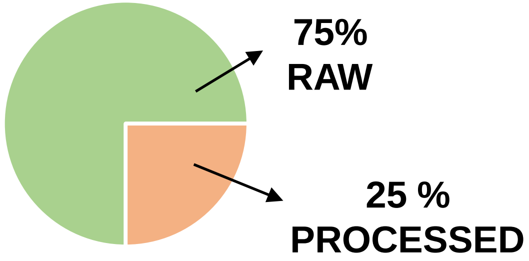


The production of food waste by producers and consumers has increased dramatically in industrialized countries

Cereals, fruit and vegetables are the main food waste produced around the world

TOMATO ANNUAL GLOBAL PRODUCTION (2020)

≈ 187 million tons (Faostat)



8.5 million tons of TOMATO WASTE

ANIMAL FEEDING or COMPOSTING
DISCARDED in LANDFILLS

SERIOUS ENVIRONMENTAL AND ECONOMIC PROBLEMS

TOMATO WASTE and by-PRODUCTS

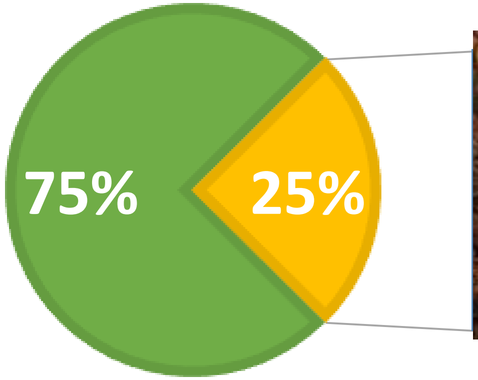
SOURCE of NATURAL BIOACTIVE COMPOUNDS

- ✓ **CUTIN**
- ✓ **POLYPHENOLS**
- ✓ **FATTY ACIDS**
- ✓ **TOCOPHEROLS**
- ✓ **CAROTENOIDS**

(OTHERS)

WASTE VALORIZATION

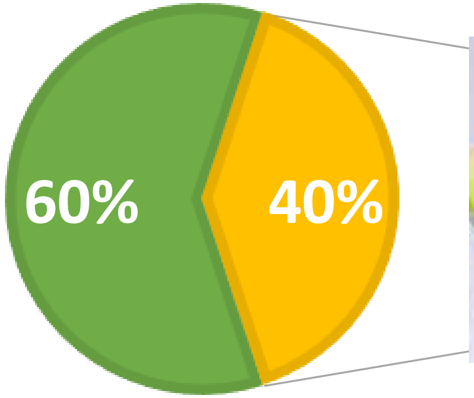
10 million tons in EU



Juice and apple by-products

Apple peels

215,000 tons in EU



Watermelon by-products

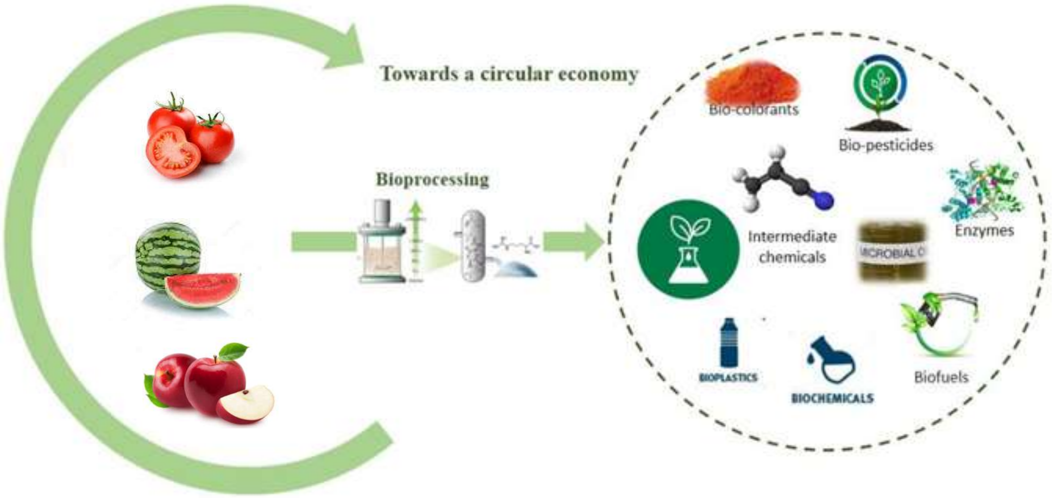
Watermelon peels

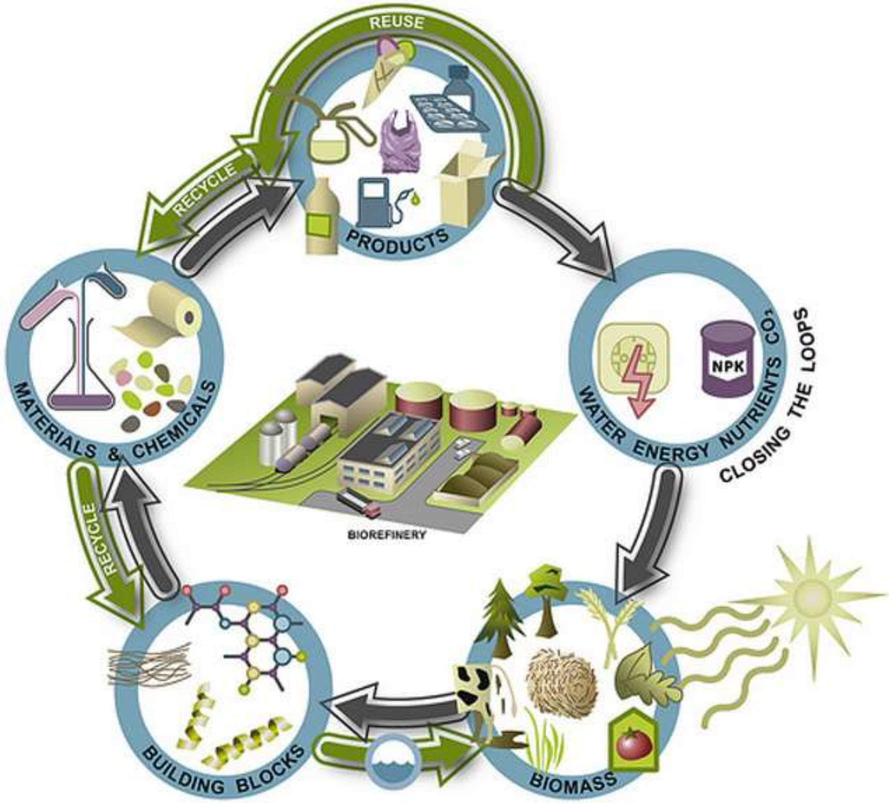
SOURCE of NATURAL BIOACTIVE COMPOUNDS

- ✓ Pectin
- ✓ Protein
- ✓ Polyphenols
- ✓ Cutin

Food waste

Conversion of food waste into valuable functional additives and building blocks will permit their whole valorization





Basic building blocks for chemicals, materials, coatings and advanced biofuels are derived from renewable biological resources

Extraction methods

Main challenges for valorization of food residues and by-products lies on the optimization of efficient and sustainable extraction methods and techniques: cascade approach

Conventional extraction

Solid-liquid extraction:
- High temperatures and long times
- Maceration at room T for days

→

PROBLEMS
Long extraction times
Thermal degradation
High amount of organic solvents

Alternative green extraction techniques

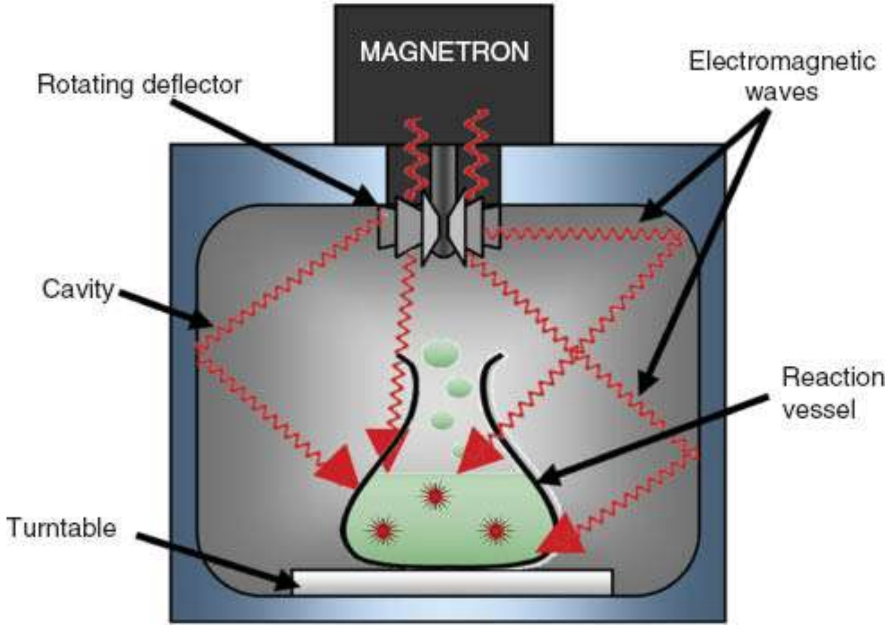
Supercritical fluid extraction (SFE)
Pressurized liquid extraction (PLE)
Microwave-assisted extraction (MAE)
Ultrasound-assisted extraction (UAE)
Subcritical water extraction (SWE)

→

Short extraction times
Low amount of organic solvents
Higher selectivity and extraction yields

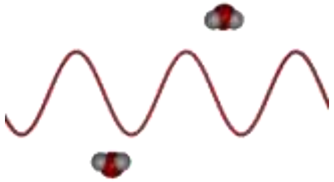
Green extraction methods

Microwave-assisted extraction (MAE)



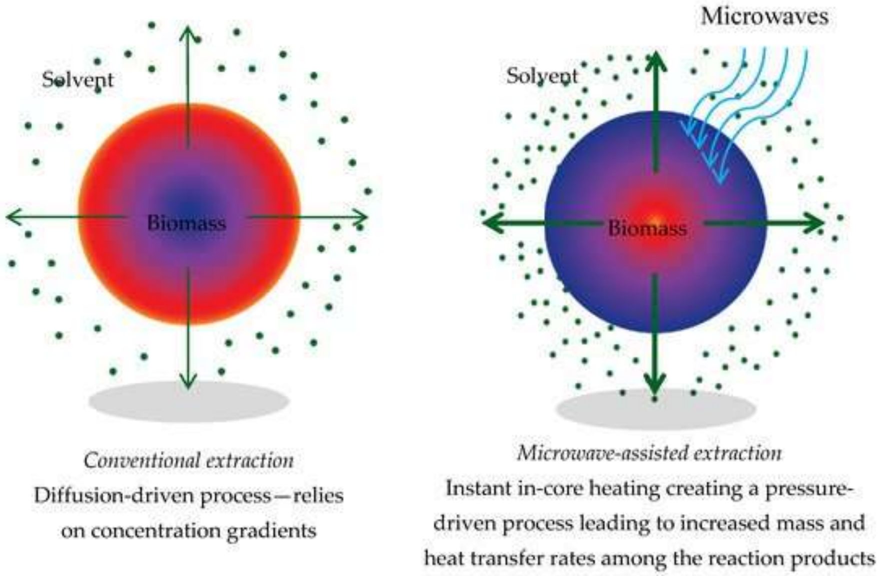
MW: ionizing radiation causing molecular movement by ions migration and dipolar rotation

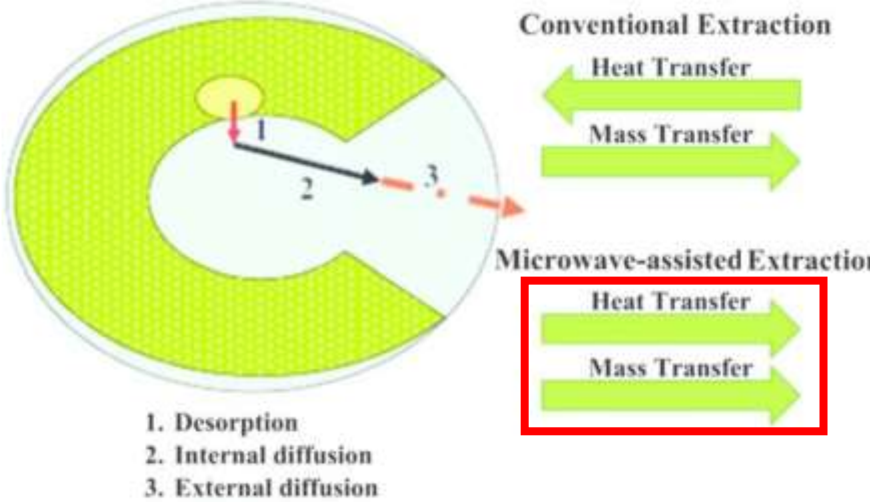
Solvent should absorb MW radiation



Heat release = Rupture of hydrogen bonds in dipolar molecules

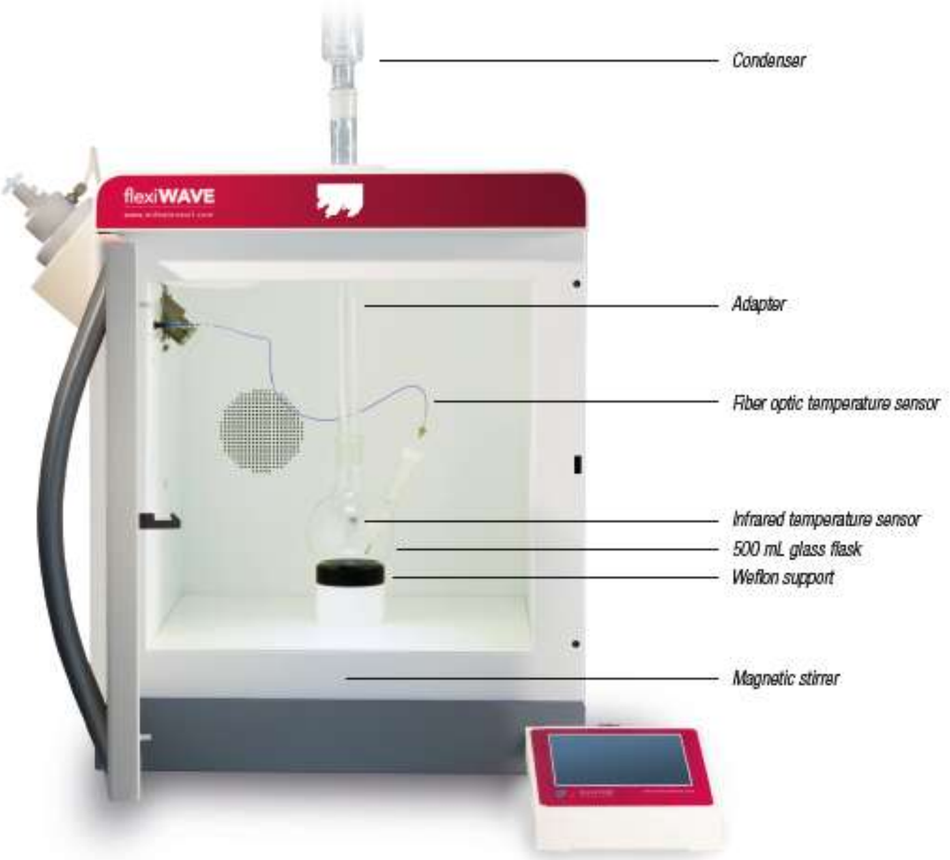
Pressure = physical modification; Good penetration of solvents through the molecular structure





Green extraction methods

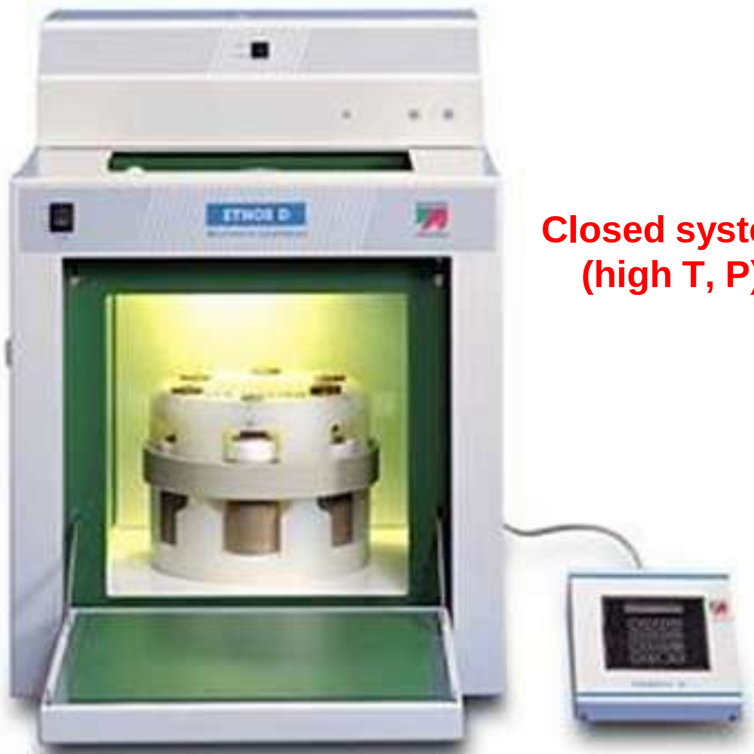
Microwave-assisted extraction (MAE)



Open system (atmospheric pressure)

Microwave assisted hydrodistillation MAHD (without solvent)

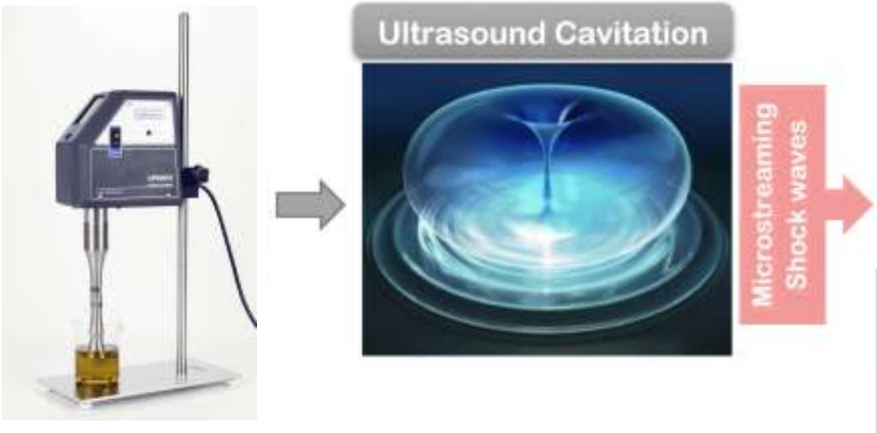
- ✓ Uniform heating
- ✓ Reduce losses of heating energy
- ✓ High efficiency
- ✓ Short extraction time
- ✓ High reproducibility

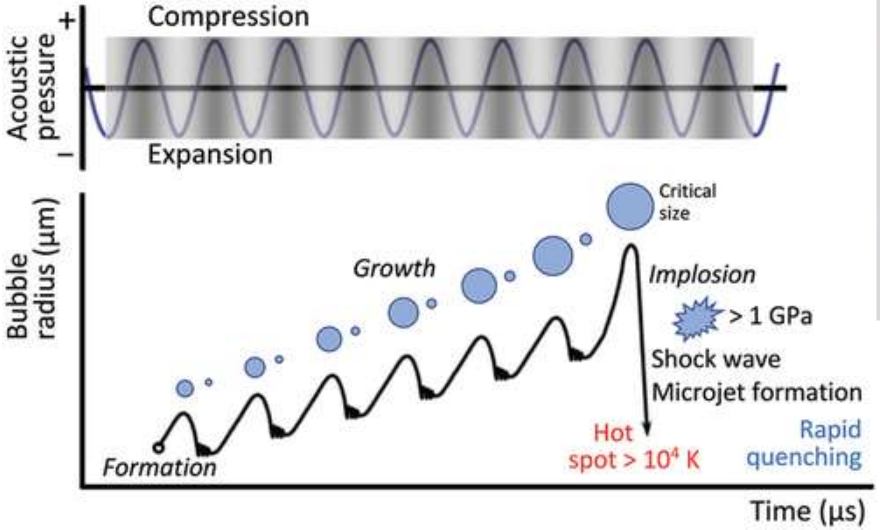


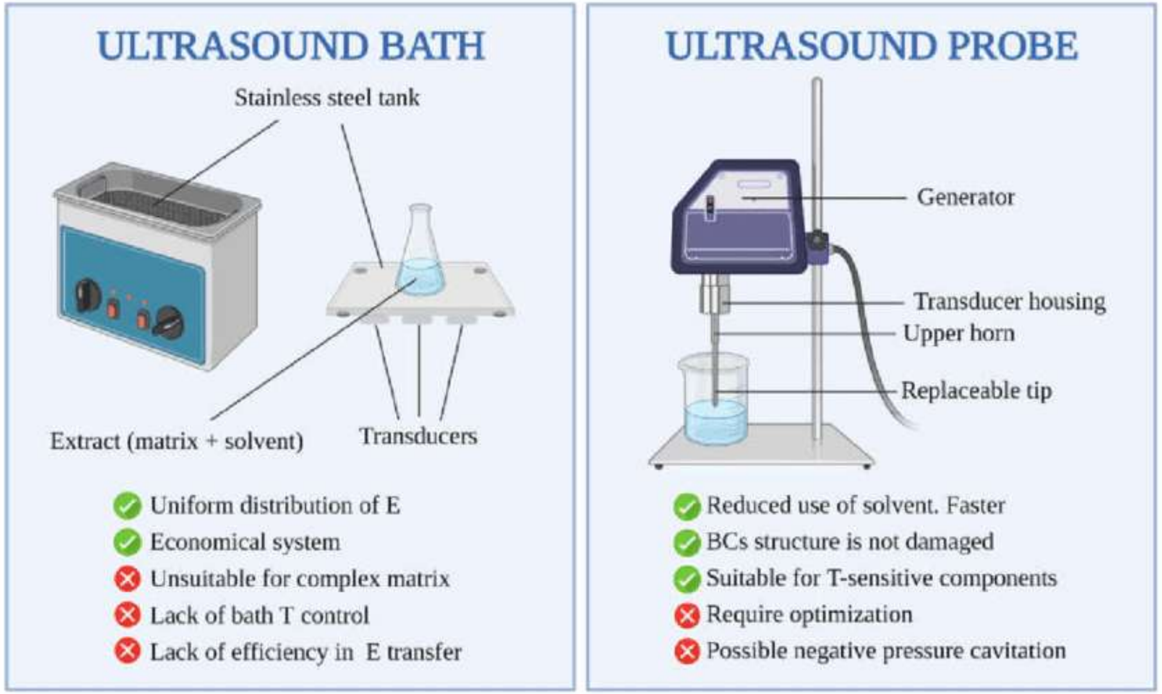
Closed system (high T, P)

Green extraction methods

Ultrasound-assisted extraction (MAE)

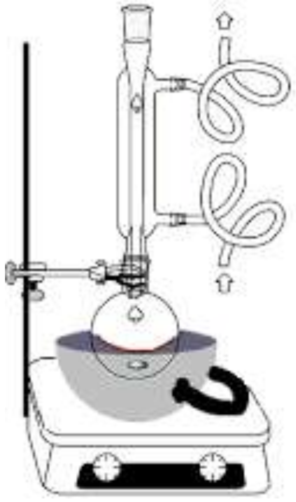




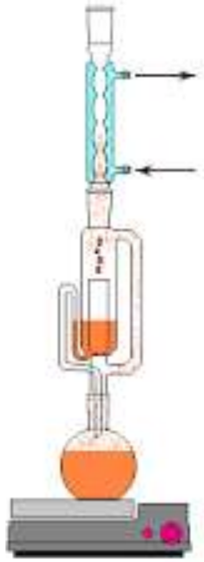


Formation, growth and implosive collapse of bubbles in a liquid irradiated with high intensity ultrasound

CONVENTIONAL EXTRACTION TECHNIQUES



Reflux



Soxhlet

TIME AND SOLVENT CONSUMING

GREENER ALTERNATIVES

MAE

SYNERGIC HEAT AND MASS TRANSFER GRADIENTS

UAE

MECHANICAL IMPACT OF US WAVES AND CAVITATION

Enhance

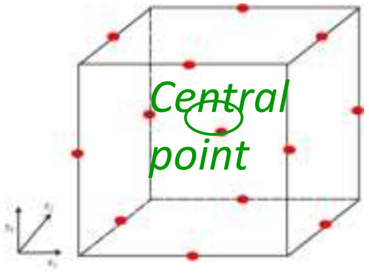
- ✓ SOLVENT PENETRATION
- ✓ CELL WALL DESTRUCTION

- ✓ ENHANCED EXTRACTION YIELDS
- ✓ REDUCED EXTRACTION TIME AND SOLVENT CONSUMPTION
- ✓ MORE ENVIRONMENTALLY FRIENDLY

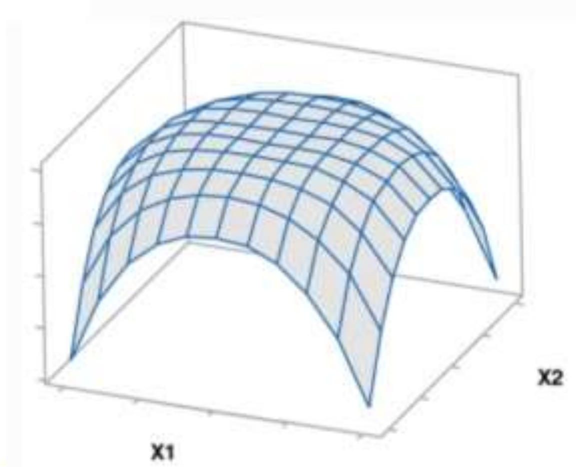
Green extraction methods

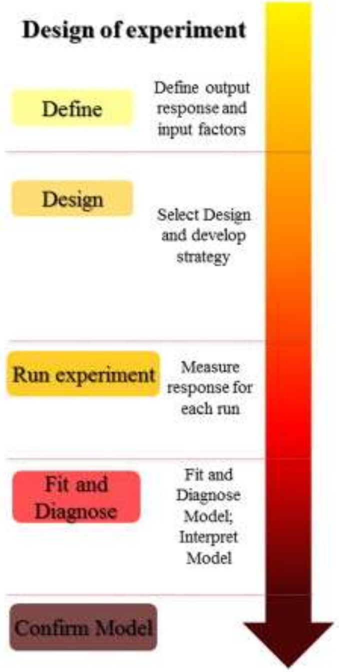
OPTIMIZATION

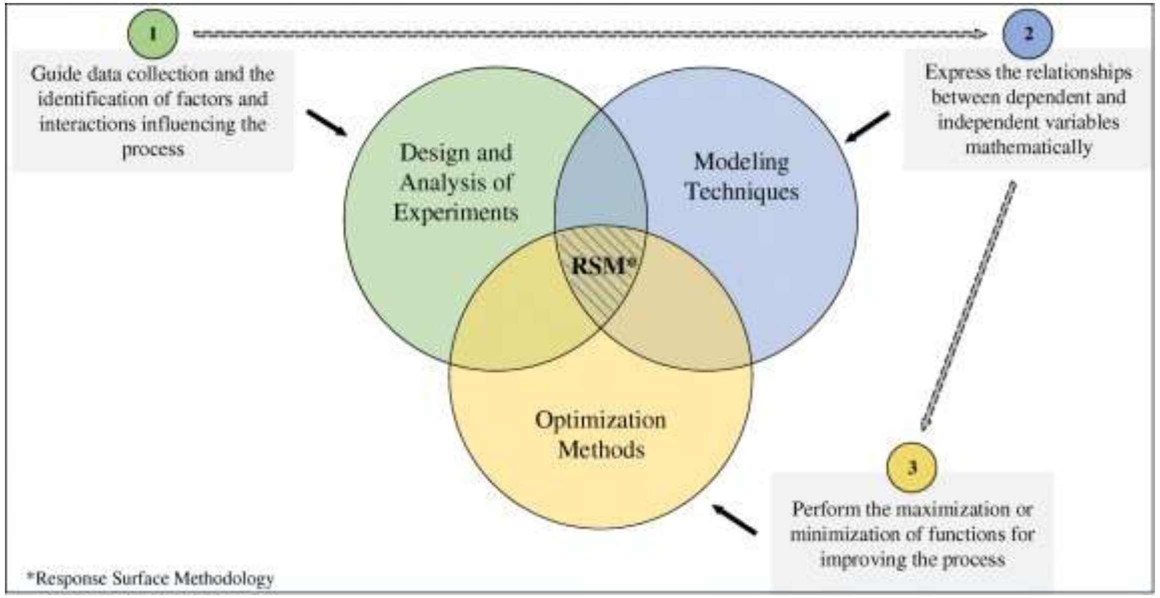
Response Surface Methodology (RSM)



$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{\substack{i=1 \\ j=1}}^{k} \beta_{ij} x_i x_j$$







MAE/UAE parameters to be considered

- Solvent composition
- Extraction Time
- Temperature
- Irradiation power
- US amplitude
- L/S ratio

- Extraction yield
- TPC
- Antioxidant activity
- Bioactives profile (GC-MS, HPLC-MS)

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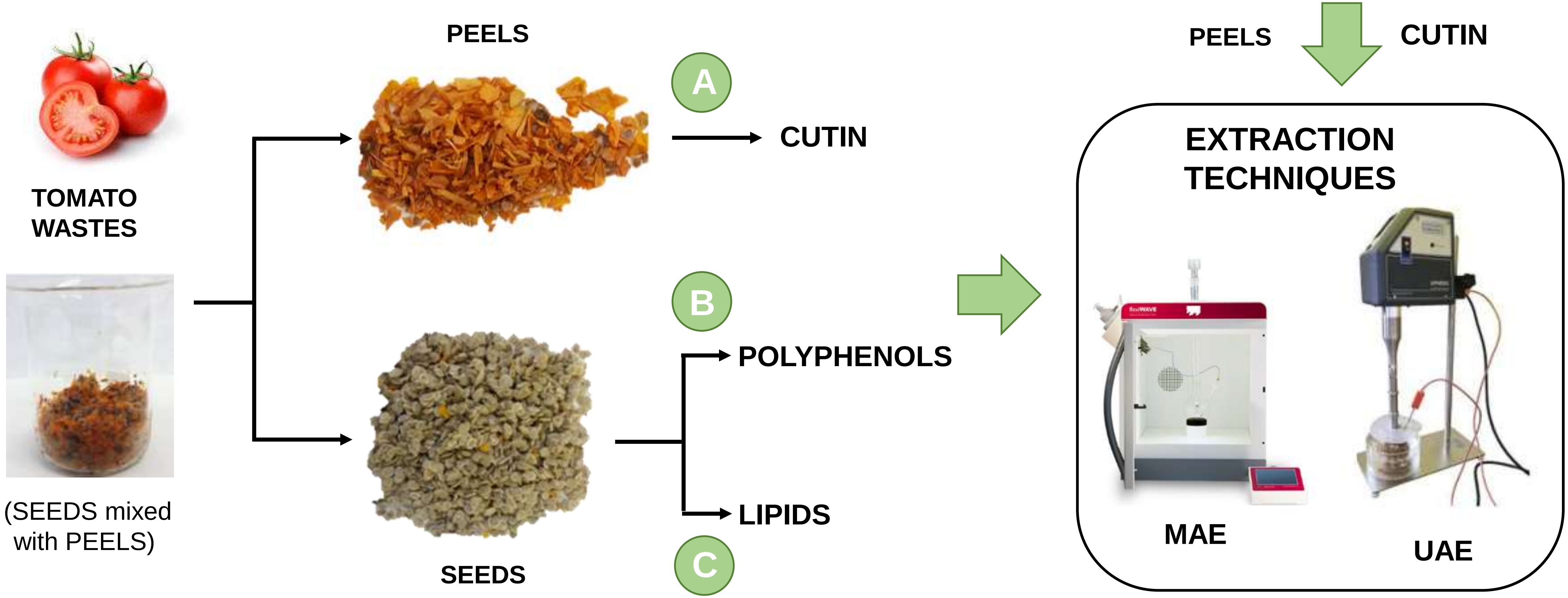
TOMATO WASTE AS A SOURCE OF ANTIOXIDANTS, LIPIDS and CUTIN



A Optimization of cutin extraction

Cutin extraction using advances techniques

MAE

Microwave heating

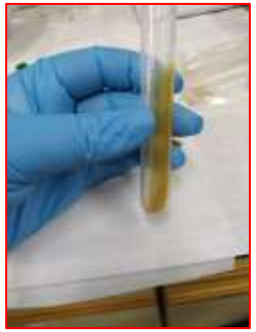
Filter

Solid discarded

Acid

Centrifuge
30 min, 5000 rpm

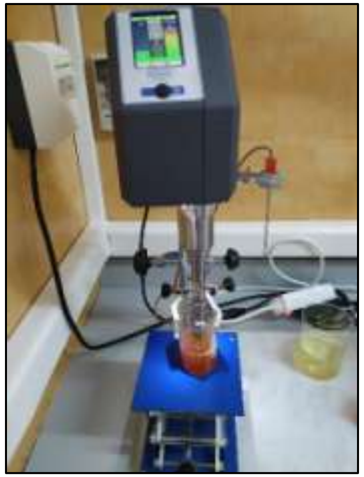
Supernatant discarded



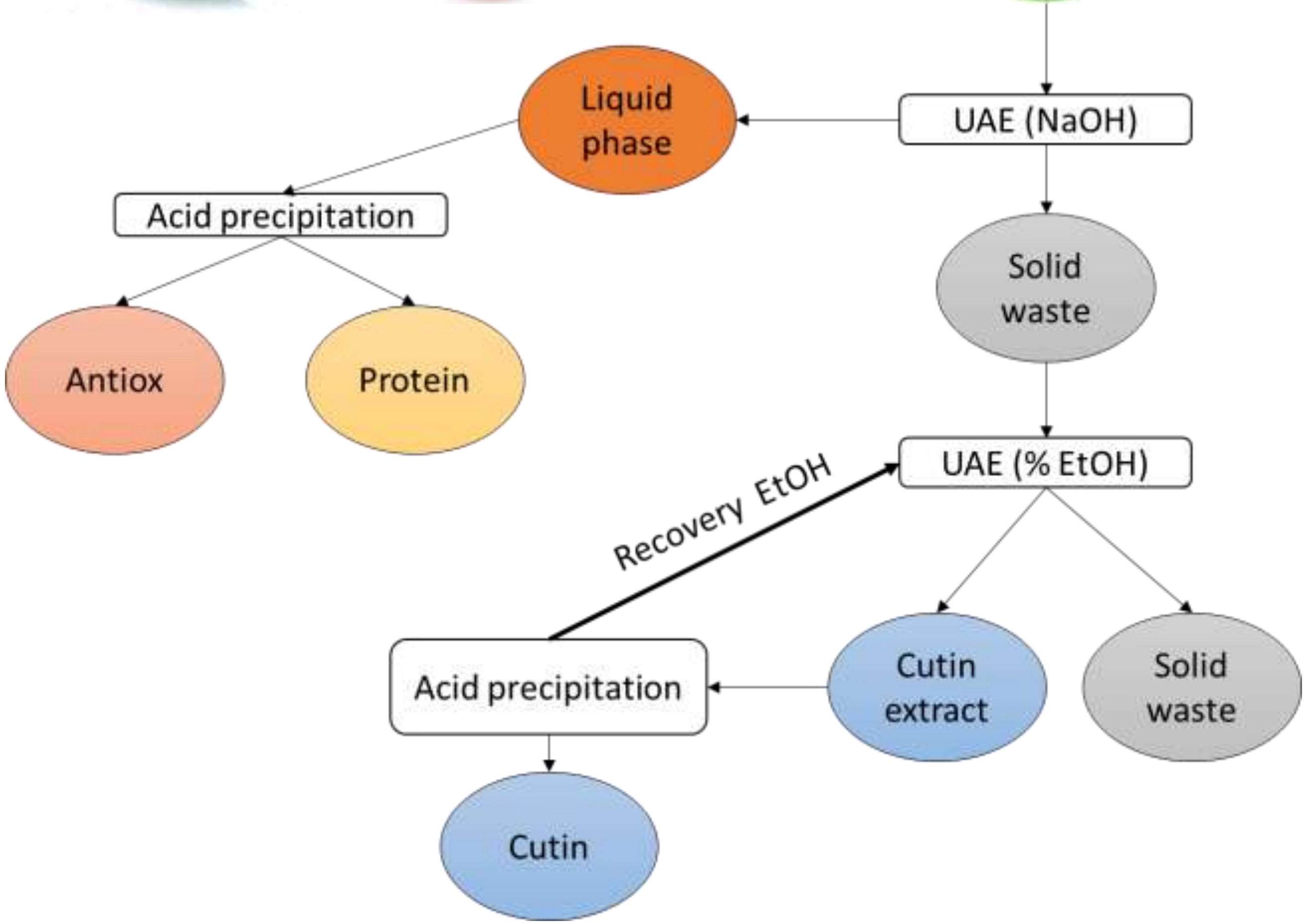
Freeze drying

Conditions	Solvent	Solid/liquid Ratio	pH
105 minutes, 95 ºC	NaOH 3%	1/50	4.5

UAE



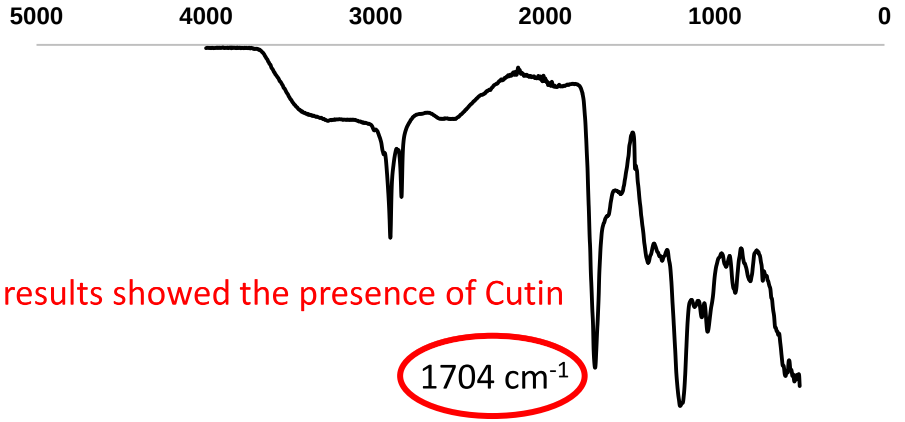
Cascade approach



Conditions	Solvent	Solid/liquid Ratio	pH
(2 steps) - 435 Ws/mL, 100% Ampl. - 750 Ws/mL, 100% Ampl.	- NaOH 3% wt - EtOH 40% (v/v)	1:80	2.5

Comparison between different agro-waste sources

Source	Technique	Extraction yield (%)
Tomato peels	UAE	13 ± 2[a]
Apple peels		19 ± 1[b]
Watermelon peels		6 ± 1[c]
Tomato peels	MAE	17 ± 1[b]
Apple peels		16 ± 2[ab]
Watermelon peels		9 ± 1[d]



✓ FTIR results showed the presence of Cutin

MAE

Sample	T_{max} 1 (ºC)	T_{max} 2 (ºC)	T_{max} 3 (ºC)
Tomato	207 ± 6[ab]	379 ± 2[a]	479 ± 1[a]
Watermelon	202 ± 1[a]	284 ± 1[b]	464 ± 1[b]
Apple	215 ± 2[b]	288 ± 1[c]	473 ± 2[c]

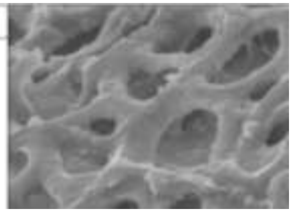
UAE

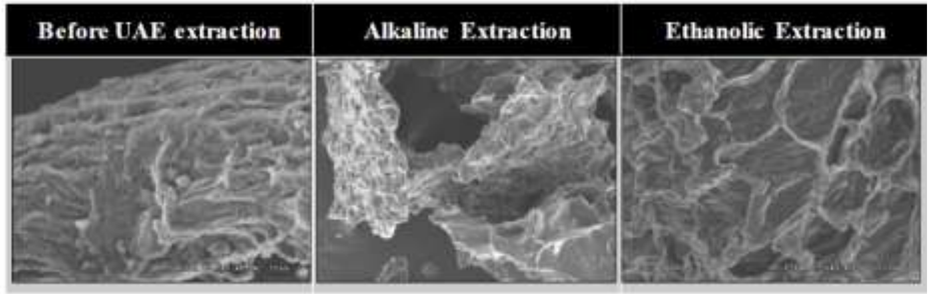
Sample	T_{max} 1 (ºC)	T_{max} 2 (ºC)	T_{max} 3 (ºC)
Tomato	201 ± 2[a]	310 ± 1[a]	470 ± 1[a]
Watermelon	199 ± 2[a]	306 ± 2[a]	447 ± 1[b]
Apple	174 ± 5[c]	288 ± 2[b]	473 ± 1[a]

✓ Higher thermal stability for MAE and UAE extracts

Raw Material

After MAE





✓ Great effect in structure properties

✓ **Fatty acid composition**

cutin monomers (percentage of total peak areas in GC/MS chromatogram)

Compound	**UAE**	**MAE**
Hexadecanoic acid	13 ± 2 ^{a}	16 ± 2 ^{a}
9,12-Octadecadienoic acid (Z,Z)	9 ± 2 ^{a}	16 ± 2 ^{b}
Oleic acid	5 ± 1 ^{a}	8 ± 1 ^{b}
Octadecanoic acid	3 ± 1 ^{a}	4 ± 1 ^{a}
10,16-dihydroxyhexadecanoic acid	59 ± 7 ^{a}	28 ± 6 ^{b}

Compound	UAE	MAE
Hexadecanoic acid	8.4 ± 0.2^{a}	8.5 ± 0.6^{a}
9,12-Octadecadienoic acid (Z,Z)	20.0 ± 1.0^{a}	20.0 ± 3.0^{a}
Trans-9-octadecenoic acid	12.2 ± 0.5^{a}	8.9 ± 0.3^{b}
Octadecanoic acid	4.5 ± 0.3^{a}	4.8 ± 0.3^{a}
10,16-dihydroxyhexadecanoic acid	6.6 ± 0.3^{a}	2.0 ± 0.1^{b}
Alpha.-linolenic acid	-	23.7 ± 0.5
9,10,18-trihydroxyoctadecanoic acid	10.5 ± 0.6^{a}	0.9 ± 0.1^{b}

Compound	**UAE**	**MAE**
Hexadecanoic acid	43.0 ± 1.0^{a}	21.7 ± 0.1^{b}
9,12-Octadecadienoic acid (Z,Z)	11.1 ± 0.4^{a}	17.0 ± 0.1^{b}
Alpha.-linolenic acid	16.0 ± 0.5^{a}	18.5 ± 0.1^{b}
Octadecanoic acid	12.0 ± 1.0^{a}	9.0 ± 0.1^{b}
9,10-Dihydroxyoctadecanedioic acid	2.5 ± 0.6	-

Other compounds obtained from the cascade approach by UAE

Sample	Yield (%)	Protein content (mg eq BSA/g extract)
Tomato	9 ± 1[a]	590 ± 3[a]
Watermelon	7 ± 2[ab]	857 ± 1[b]
Apple	5 ± 1[b]	625 ± 2[c]

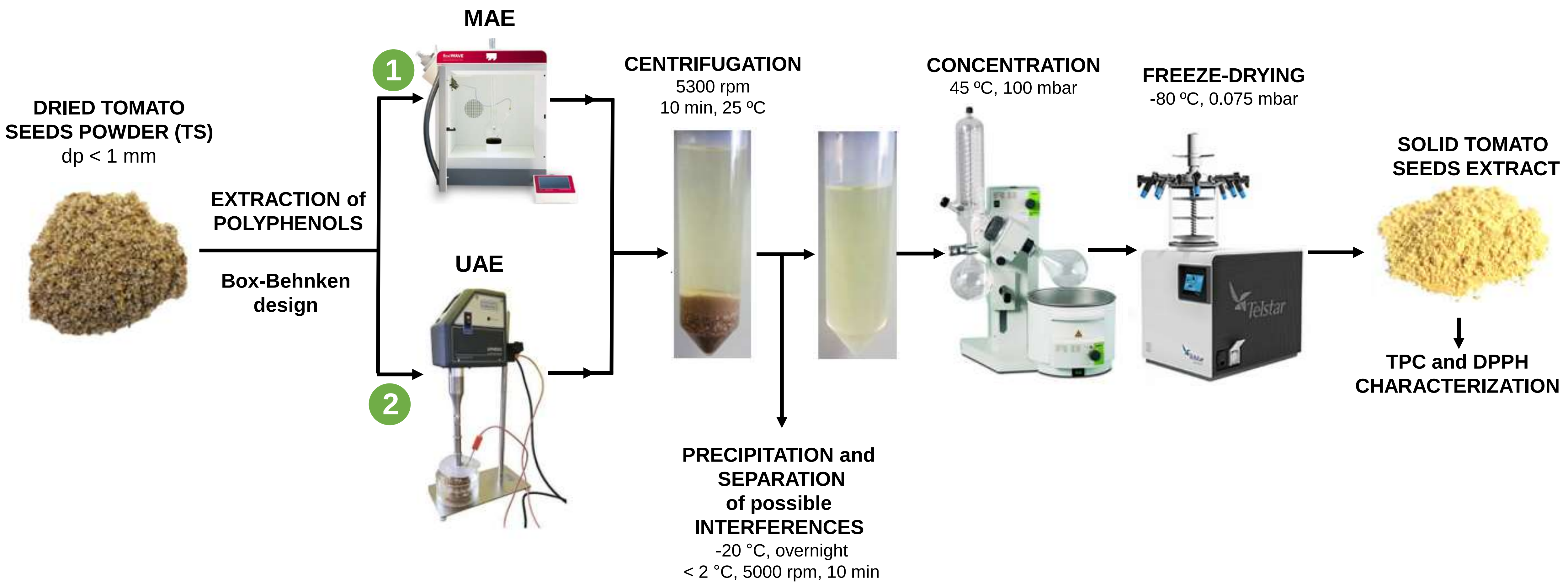
Soluble proteins

Sample	Extraction yield (%)	TPC (mgGAE/100g dm)	ABTS (µmolTE/100g dm)	FRAP (µmolTE/100g dm)	DPPH (µmolTE/100 g dm)
Tomato	36 ± 5[a]	103.5 ± 0.9[a]	279 ± 3[a]	264 ± 2[a]	159 ± 1[a]
Watermelon	25 ± 6[b]	107.2 ± 0.2[b]	356 ± 1[b]	507 ± 4[b]	158 ± 1[a]
Apple	32 ± 3[ab]	61.4 ± 0.1[c]	1559 ± 20[c]	1767 ± 5[c]	902 ± 16[b]

Antioxidant compounds

B POLYPHENOLS from TOMATO SEEDS

B PHENOLIC COMPOUNDS

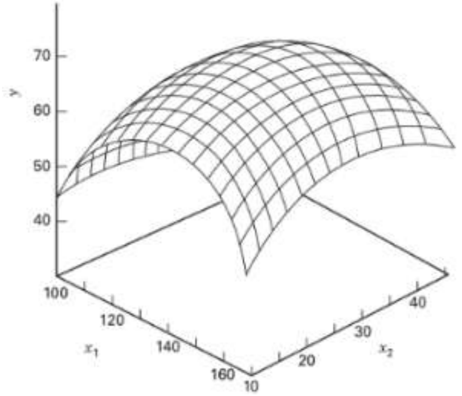


Box-Behnken design (BBD)

EXPERIMENTAL FACTORS

MAE	UAE
t (5 – 30 min)	t (5 – 15 min)
T (40 – 80 ºC)	A% (40 – 100%)
%EtOH (40 – 80% v/v)	%EtOH (40 – 80% v/v)
V (50 – 80 mL)	

RESPONSE VARIABLES *TPC and DPPH*



$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_i X_i^2 + \sum \sum \beta_{ij} X_i X_j$$

SOLID TOMATO SEEDS EXTRACT

TPC and DPPH CHARACTERIZATION

DATA REGRESSION
Statgraphics®

OPTIMUM EXTRACTION CONDITIONS

VERIFICATION

OPTIMUM EXTRACTION CONDITIONS

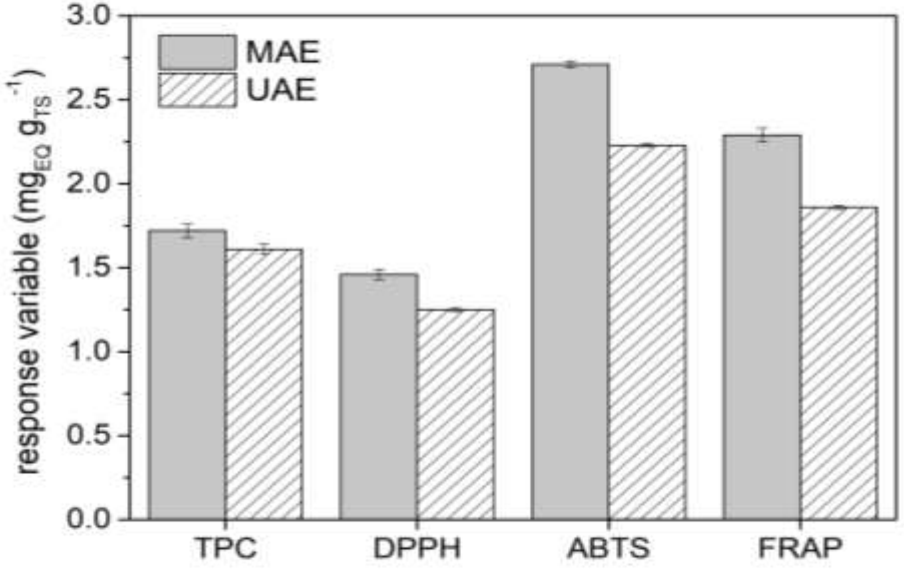
1 **MAE**

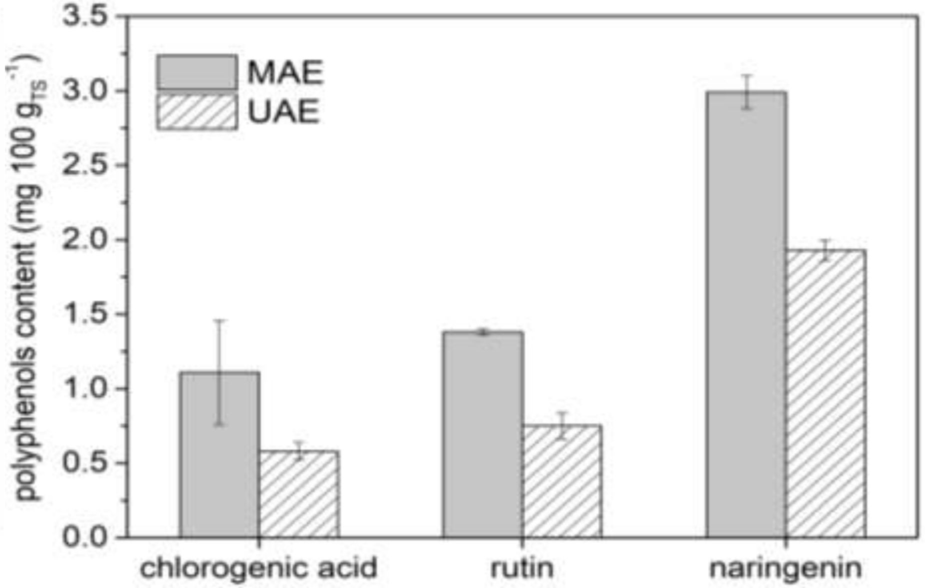
T = 80 ºC
t = 15 min
V = 80 mL
%EtOH = 63% v/v
Desirability = 0.914

2 **UAE**

t = 15 min
Amplitude = 80%
%EtOH = 61% v/v
Desirability = 0.952

OPTIMIZED EXTRACTS CHARACTERIZATION





MAE vs UAE

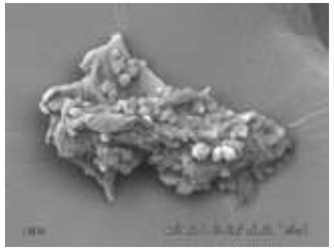
1) MAE optimized extract showed HIGHER RESPONSES:

- ✓ **TPC**
- ✓ **DPPH**
- ✓ **FRAP**
- ✓ **ABTS**

2) PHENOLIC PROFILE determination by HPLC-MS

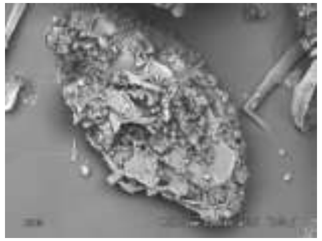
MAE optimized extract showed HIGHER contents in

- ✓ **CHLOROGENIC ACID**
- ✓ **RUTIN**
- ✓ **NARINGENIN**



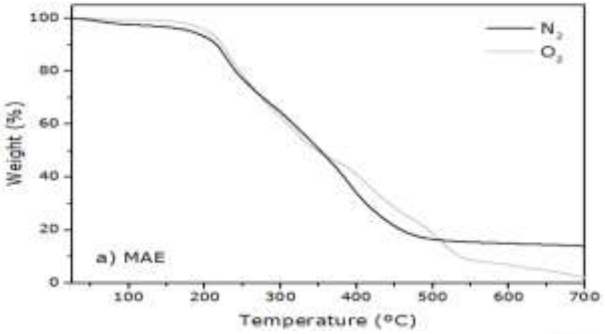
Raw Material

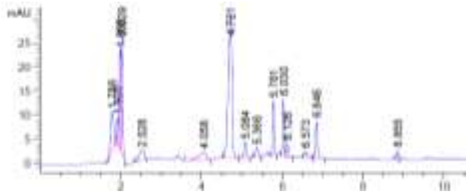
After MAE



After UAE

✓ Good thermal stability for MAE and UAE extracts (200 ºC)

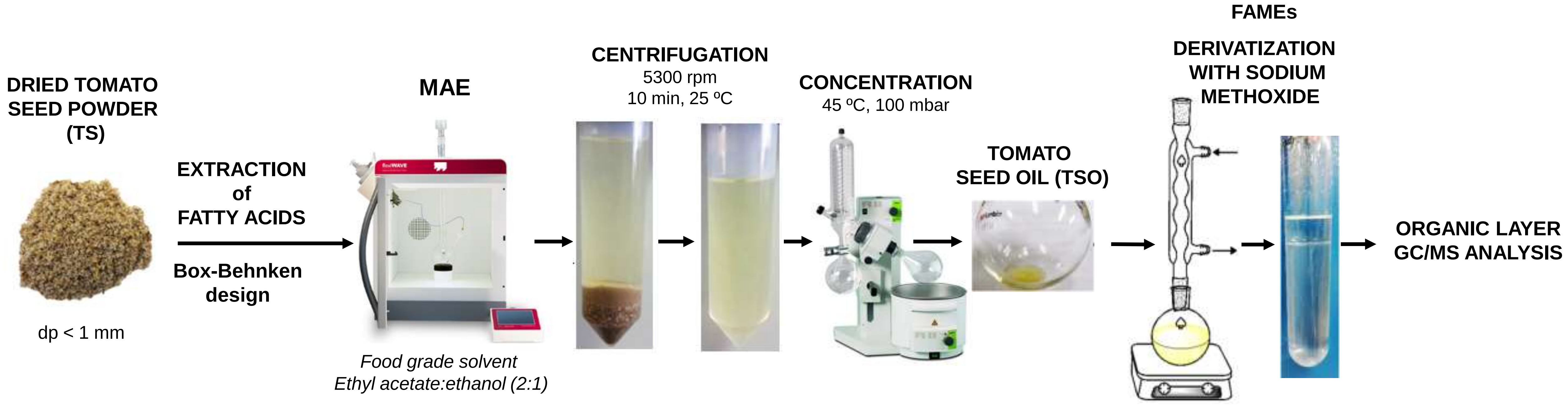




C

LIPIDS from TOMATO SEEDS

C FATTY ACIDS

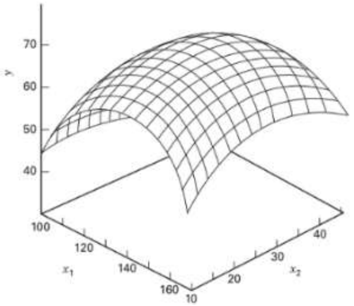


Box-Behnken design (BBD)

EXPERIMENTAL FACTORS

MAE
t (10 – 30 min) T (40 – 70 ºC) V (50 – 80 mL)

RESPONSE VARIABLES
Palmitic acid (C16:0)
Stearic acid (C18:0)
Oleic acid (C18:1)
Linoleic acid (C18:2)



$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_i X_i^2 + \sum \sum \beta_{ij} X_i X_j$$

DERIVATIZATION WITH SODIUM METHOXIDE

FAMEs

ORGANIC LAYER GC/MS ANALYSIS

FAMEs QUANTIFICATION

DATA REGRESSION
Statgraphics®

OPTIMUM EXTRACTION CONDITIONS

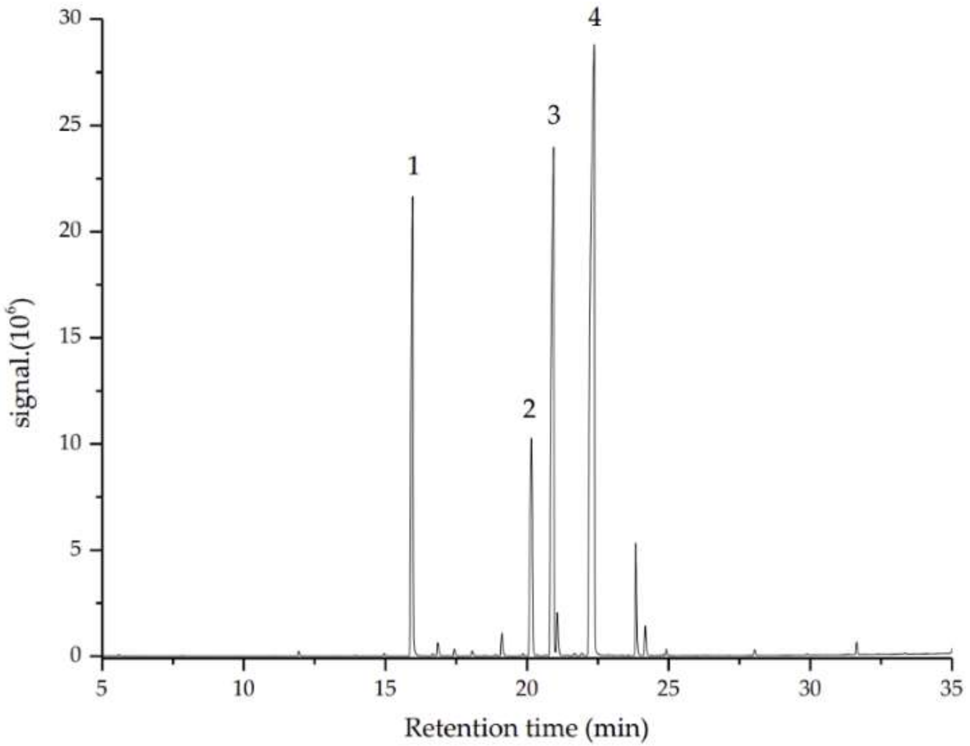
VERIFICATION

MAE
OPTIMUM EXTRACTION CONDITIONS

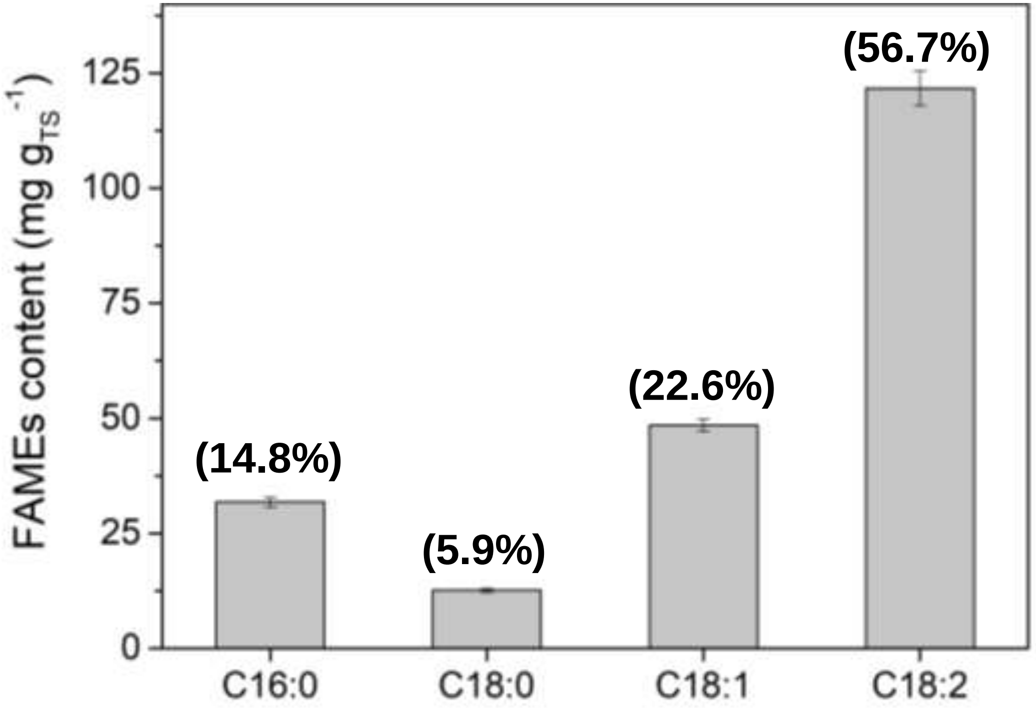
SV = ethyl acetate:ethanol (2:1, v/v)

t = 10 min
T = 70 ºC
V = 80 mL

FAMEs PROFILE by GC/MS



MAJOR FAMEs COMPOSITION in OPTIMIZED TSO



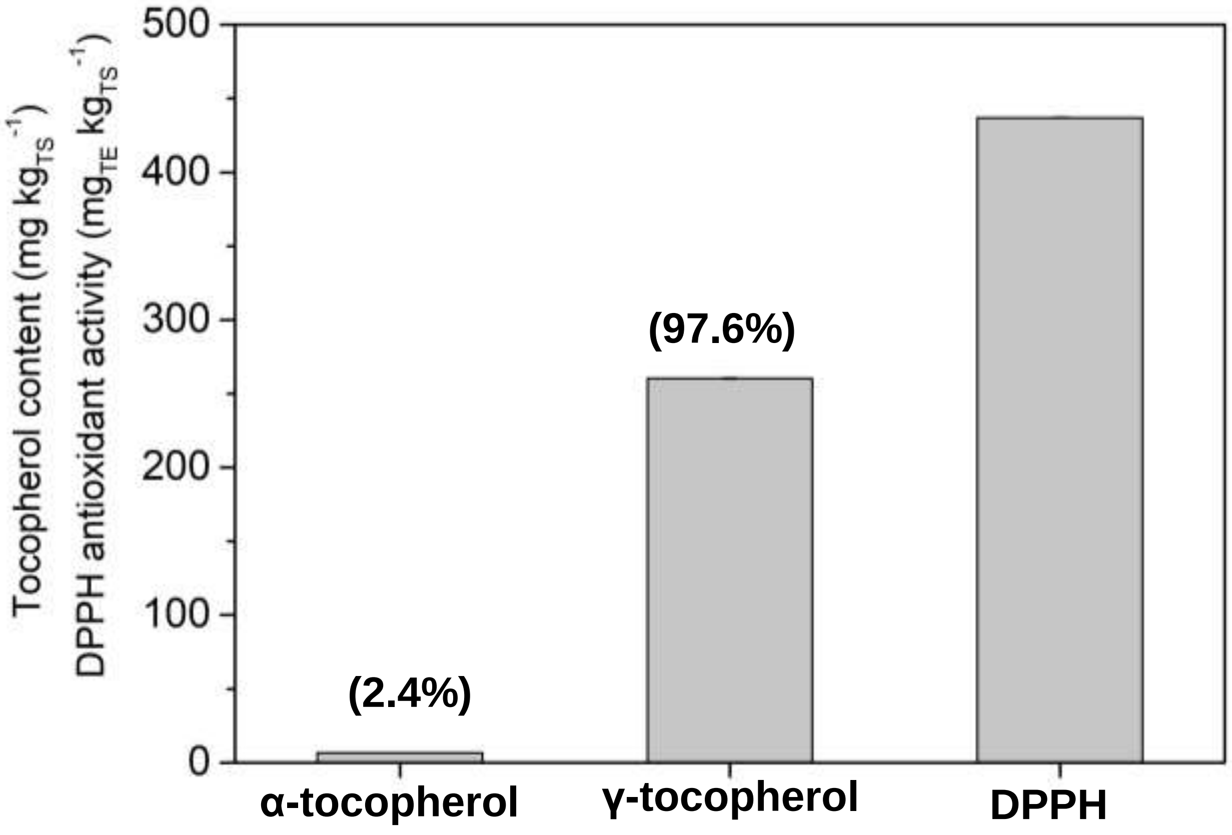
FOUR MAJOR FAMEs

1: Methyl palmitate (C16:0)
2: Methyl stearate (C18:0)
3: Methyl oleate (C18:1)
4: Methyl linoleate (C18:2)

- ✓ **Other FAMEs combined peak area accounted only up to 4.6%**

- ✓ **Enhanced TSO extraction yield compared to conventional extraction techniques**
- ✓ **TSO FAMEs relative composition in close agreement with other authors**
- ✓ **Approx. 80% of unsaturated fatty acids.**

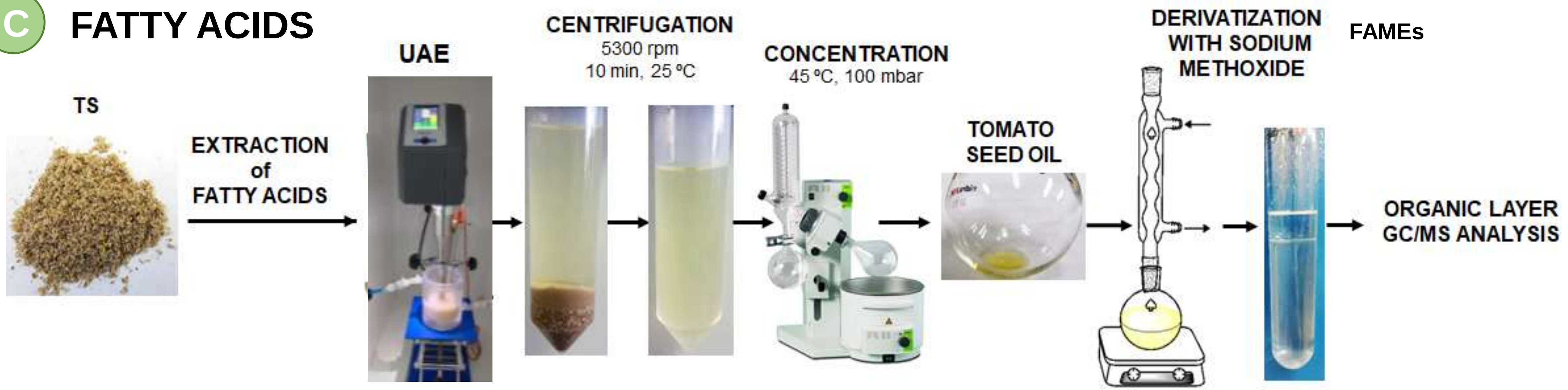
TOCOPHEROLs CONTENT AND ANTIOXIDANT ACTIVITY



tomato seed oil

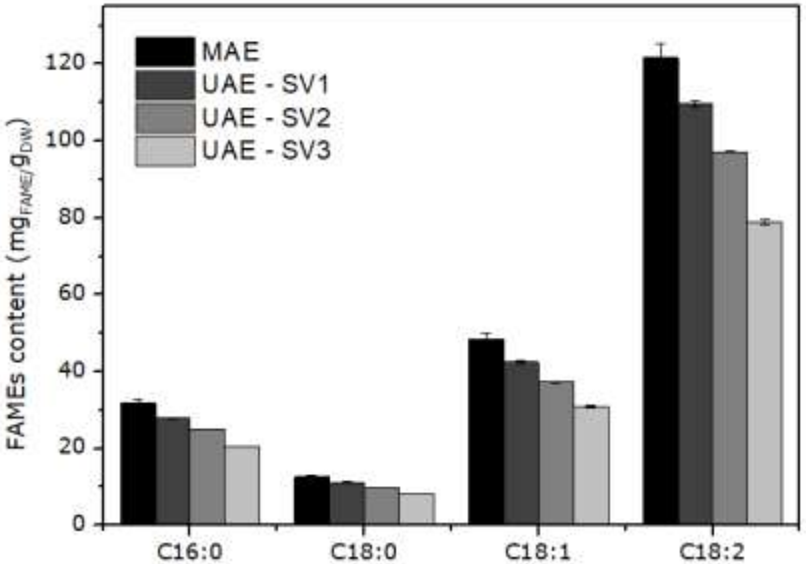
- ✓ γ-tocopherol major homologue
- ✓ TSO showed antioxidant activity

C FATTY ACIDS



t = 5 min; A = 100 %

UAE	
SV_1	ethyl acetate:ethanol (2:1, v/v)
SV_2	n-hexane:isopropanol (3:2, v/v)
SV_3	n-hexane:acetone:methanol (2:1:1, v/v/v)



MAE vs UAE

✓ **MAE showed better results compared to UAE for fatty acids extraction**

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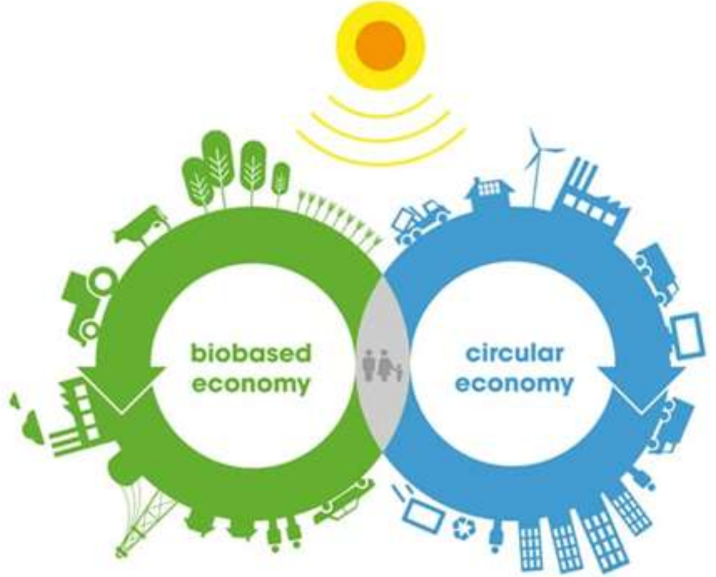
CONCLUSIONS

The development of innovative, fast and efficient methods based on MAE and UAE for the extraction of high value chemicals from agro-food residues is an interesting approach to obtain high added value compounds, such as polyphenols, lipids and cutin for biopolymers and coating applications.

Circular economy represents challenges and opportunities for companies and citizens to improve life quality



THANKS FOR YOUR ATTENTION

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Agro-food Wastes as a Source of Antioxidants, Lipids and Cutin by Microwave-assisted and Ultrasound-assisted extraction

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