Subcritical water extraction of quercetin and derivatives from onion skin wastes (*Allium cepa* cv. Horcal): effect of temperature and solvent properties

Coimbra (Portugal), 1 March 2022

EIFS2022
Coimbra- Portugal

UNIVERSIDAD DE BURGOS

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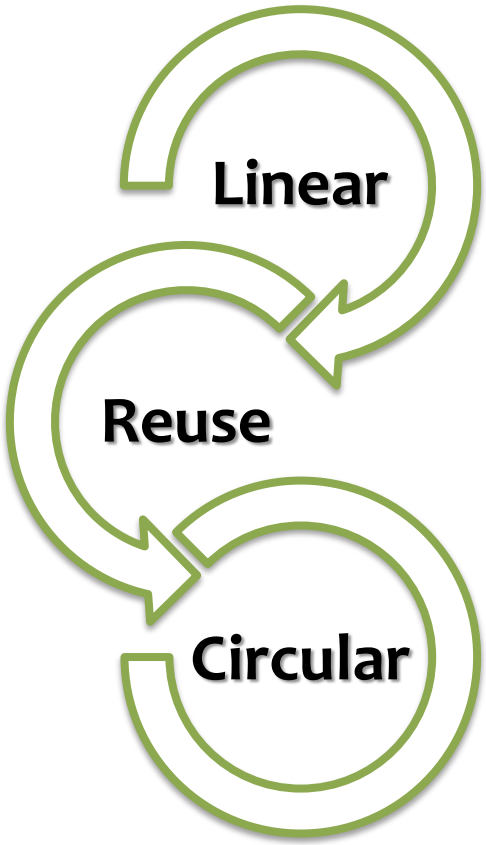
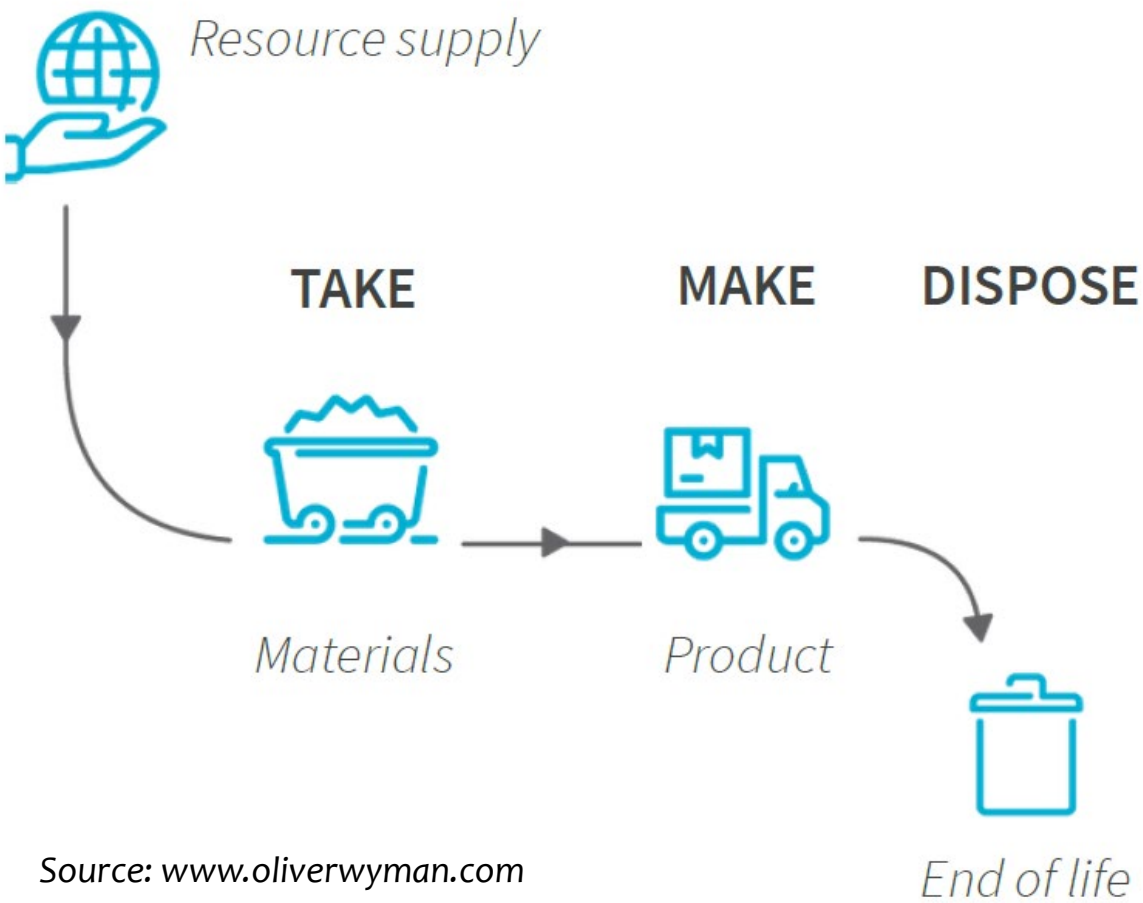
1. Introduction

Our journey: economy is changing



1. Introduction: From Linear to Circular Economy

What is the Linear Economy?



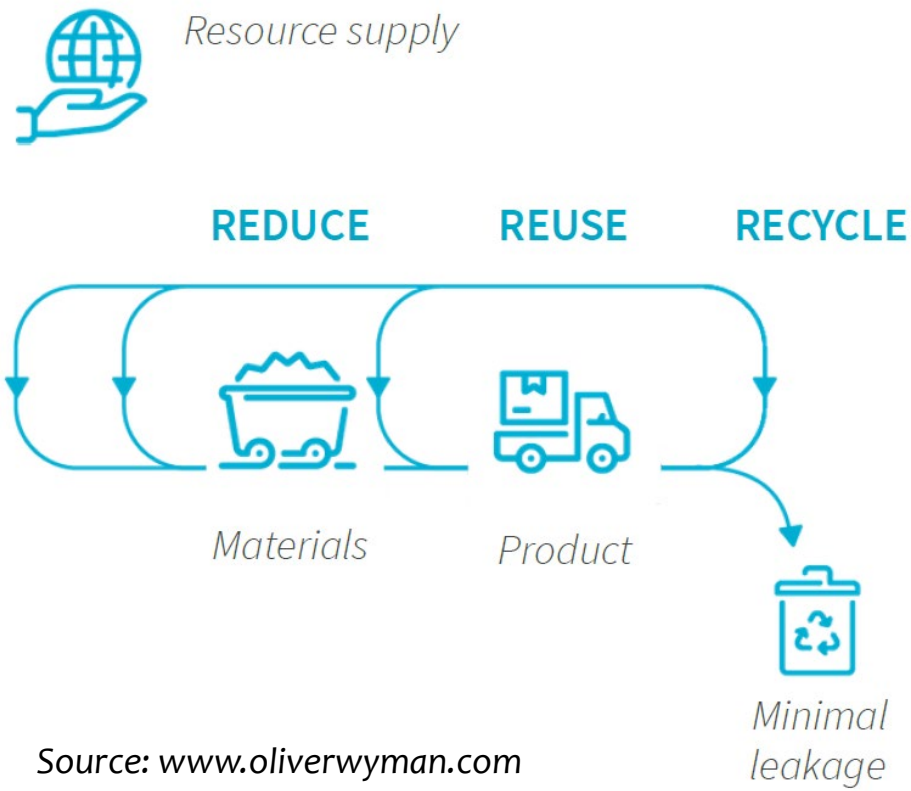
Source: www.oliverwyman.com

1. Introduction: From Linear to Circular Economy

What is the Circular Economy?

A new paradigm

- "closing the loop"



Source: www.oliverwyman.com

1.1 Circular Economy: Background

December 2015

✓ **Circular Economy Action Plan COM(2015) 614 final**

- **Purposes**
  - new boost to jobs, growth and investment
  - to develop a carbon neutral, resource-efficient and competitive economy
- **54 actions**

1.2 Circular Economy: biomass valorization

✓ **Circular Economy Action Plan COM(2015) 614 final**

➢ **Two key aspects:**

3. Turning waste into resources (Recycling)
4. Closing loops of recovered materials (Secondary raw material)

➢ **Growing concern in the EU**

- Food industry: huge amount of food wastes
- Change of paradigm:
  - ✓ Not residues anymore
  - ✓ By-products

New opportunities

1.3 Onion Skin Wastes: an opportunity

Problems

- ✘ Worldwide production: 96.8 Mt (2018), 0.5% wastes
- ✘ Not to be used for animal feeding or fertilizing

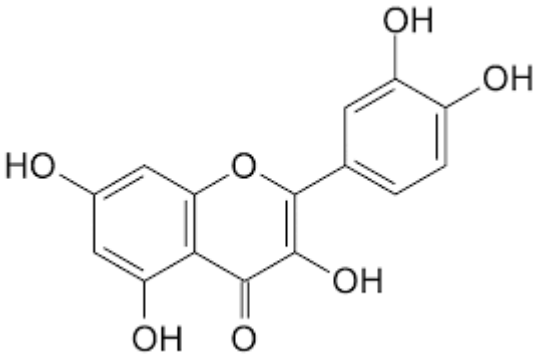
Opportunities

- ✓ Rich quercetin, quercetin derivatives and other flavonoids
- ✓ New extraction processes

1.3 Onion Skin Wastes

OPPORTUNITIES

- ✓ Quercetin derivatives:
  - ➢ Quercetin aglycone (QC)
  - ➢ Quercetin-4'-O-glycoside (QC4')
  - ➢ Quercetin-3,4'-O-diglycoside (QC3,4')
  - ➢ Quercetin-3-O-glycoside (QC3)



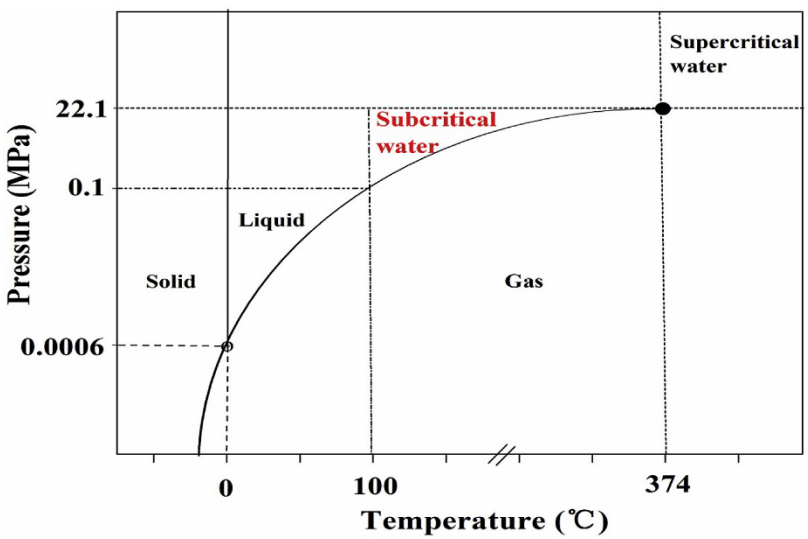
- ✓ Low solubility in water
- ✓ Strongly linked to the structural compounds of onion

2. Flavonoids recovery procedures

2.1 Conventional

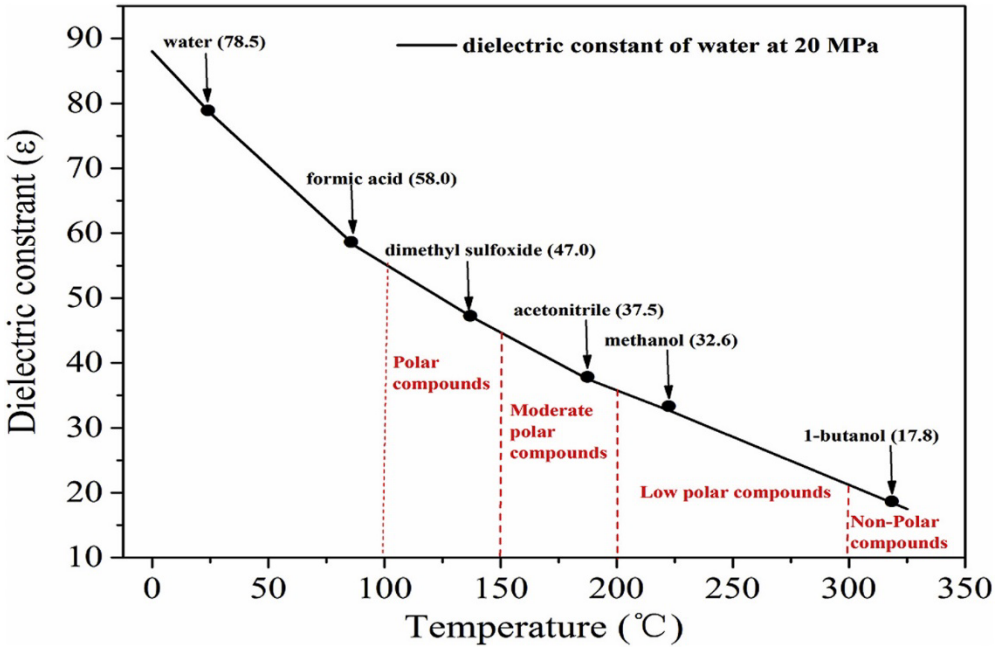
- Based on organic solvents: EtOH, MeOH, aqueous mixtures
- Complex downstream processing to remove them

2.2 Alternative: Subcritical Water (SubW)



Water at temperature ranging from 100 °C (boiling point) to 374 °C (critical point); remains in a liquid state due to the application of pressure

2.2 Alternative: Subcritical Water (SubW)



Zhang et al. Trends in Food Science & Technology 95, 183-195 (2020)

1. Change in physical properties:
   - viscosity, surface tension and dielectric constant
2. Mass transfers enhancement
3. Promotion of the hydrolysis reactions

2.2 Subcritical Water: literature review

Batch Extractors

- 110 to 230 °C
- Extraction times <30 min
- Significant increase in the extraction yield
- Difficulty to control the heating/cooling times

Semicontinuous Extractors

- continuous pumping of fresh solvent through the raw material

- kinetic modelling of the extraction experiments
- discussion about how the change in the physical properties of water when heated and cooled after the extraction affects the solubility of the target compounds

3. Materials & Methods

3.1 Raw Material: Onion skin wastes (OSW) *Horcal* cultivar

- Key Ingredient for blood sausage production
- Local Company: Embutidos Cardeña (http://www.morcilladeburgos.com/)
- Uses 350 t/year of onion, producing 11 t/year of external skin
- 11-12% extractives
- Particle size < 1mm

3.2 Total Flavonoids Content (TFC)

Spectrophotometric Method: absorbance at 415 nm after incubation with 0.5 mL of the sample were mixed with 1.5 mL of absolute ethanol, 0.1 mL of CH_3COOK solution (0.1 M), 0.1 mL of $AlCl_3$ solution (10%, w/v) and 2.8 mL of distilled water.

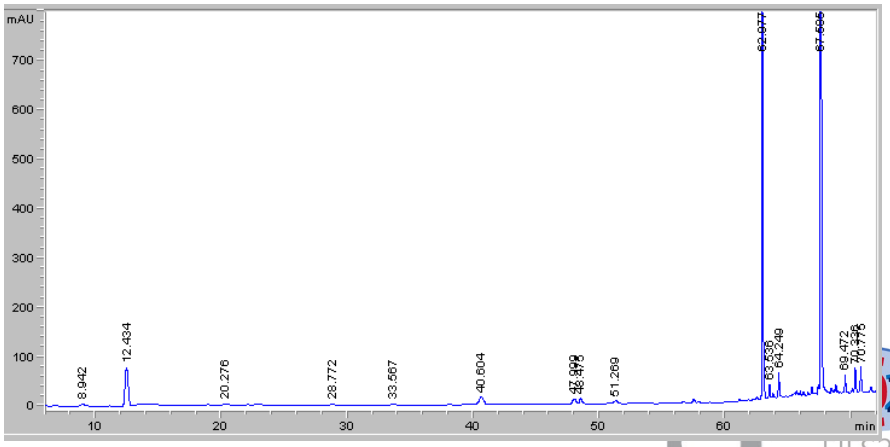
Standard Curve: Based on quercetin. Results expressed as milligrams of Quercetin Equivalent per gram of dry OSW (mg QE/g OSW).

3.3 Quercetin and derivatives identification

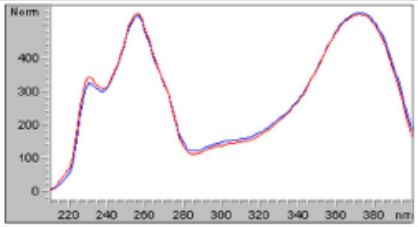
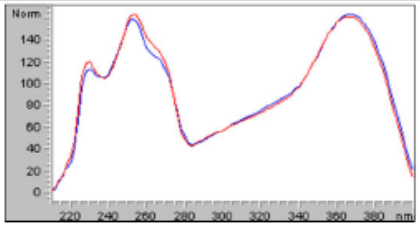
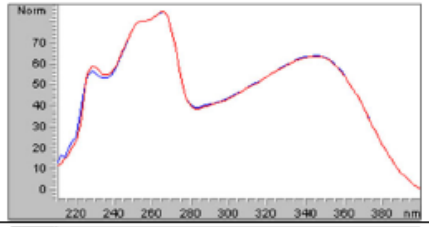
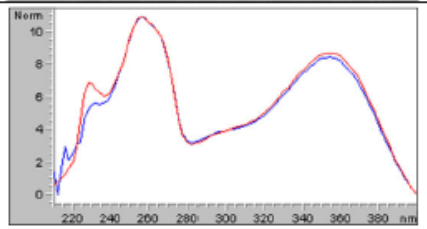
HPLC

Double comparison:

- peak retention time
- UV spectra



3. Materials & Methods. QC and derivatives identification.

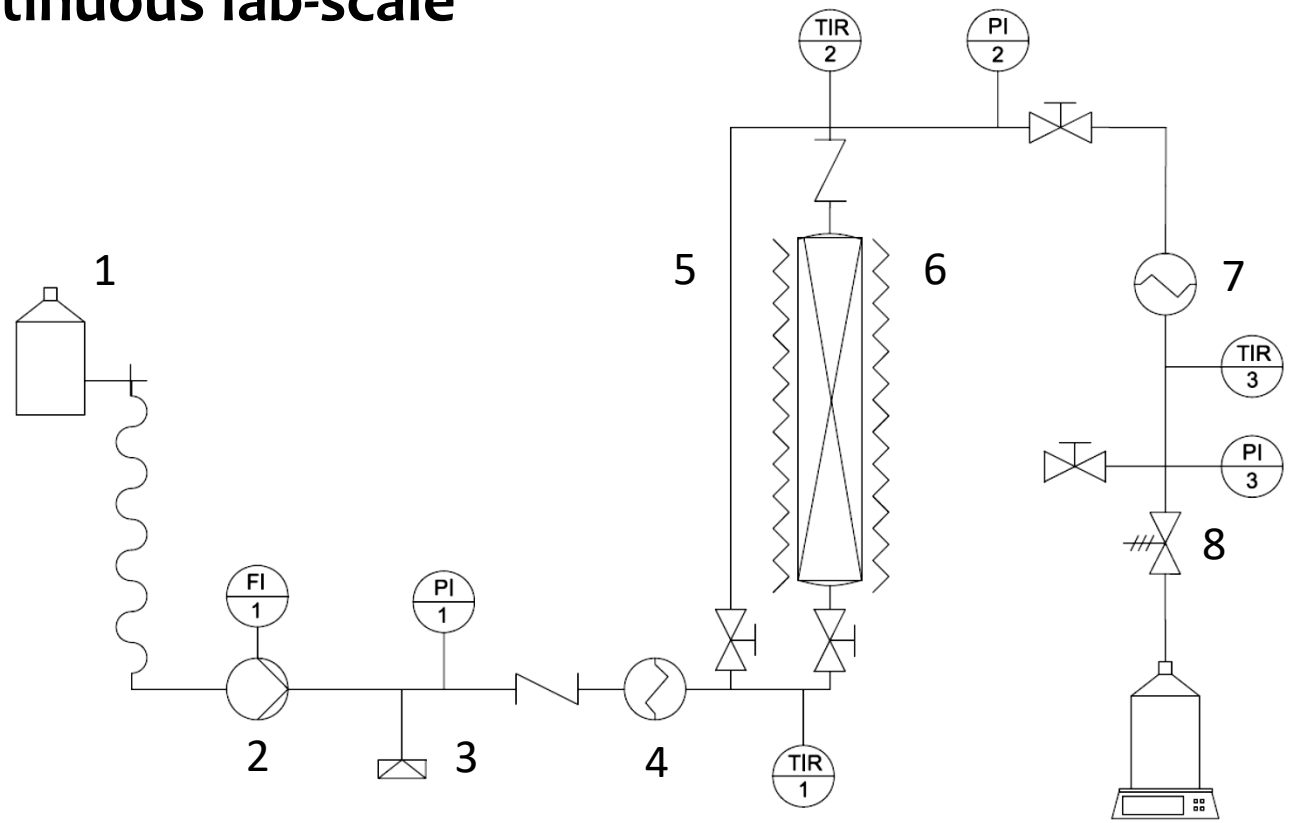
Compound	Retention time (min)	UV spectra	Match (%)
QC	Standard: 67.37 Peak: 67.55		98.8±0.7
QC4'	Standard: 62.8 Peak: 62.77		99.2±0.4
QC3,4'	Standard: 47.39 Peak: 47.56		99.7±0.1
QC3	Standard: 56.40 Peak: 56.56		97.4±0.9

3. Materials & Methods. QC and derivatives identification.

Column	Kinetex® Biphenyl column (250 × 4.6 mm, particle size 5 µm, pore size 100 Å)
Detector	DAD - Agilent 1100
Mobile phase	(A) ammonium acetate 5 mM with acetic acid (1%, *v/v*) in water (B) ammonium acetate 5 mM with acetic acid (1%, *v/v*) in acetonitrile.
Flow rate	0.8 mL/min
Temperature	25˚C
Wavelenghts	280, 330, 370 nm
Reference	Alonso-Riaño, P.; Diez, M.T.S.; Blanco, B.; Beltrán, S.; Trigueros, E.; Benito-Román, O. Water ultrasound assisted extraction of polyphenol compounds from brewer's spent grain: Kinetic study, extract characterization, and concentration. Antioxidants **2020**, 9, 265.

3. Materials & Methods

3.4 SubW semicontinuous lab-scale plant



1) solvent bottle; 2) pump; 3) bursting disk; 4) heater; 5) by-pass pipe; 6) 26 mL extractor; 7) chiller; 8) backpressure regulator.

3.4 SubW semicontinuous plant. Working conditions.

Working Conditions

- 4 g of OSW + 8 g of 5 mm glass beads (avoid packaging and channeling through the bed)
- Flow rate: 2.5 mL/min
- Residence time: 10-11 min
- Extraction time: 180 min
- Temperature range: 105 to 180 °C.
- Analysis of TFC and individual flavonoids
- Modelling

4. Experimental results

4.1 Conventional Extraction

- 37 °C for 60 min, under stirring (275 rpm)
- solvent ethanol:water mixture (70%, v/v)

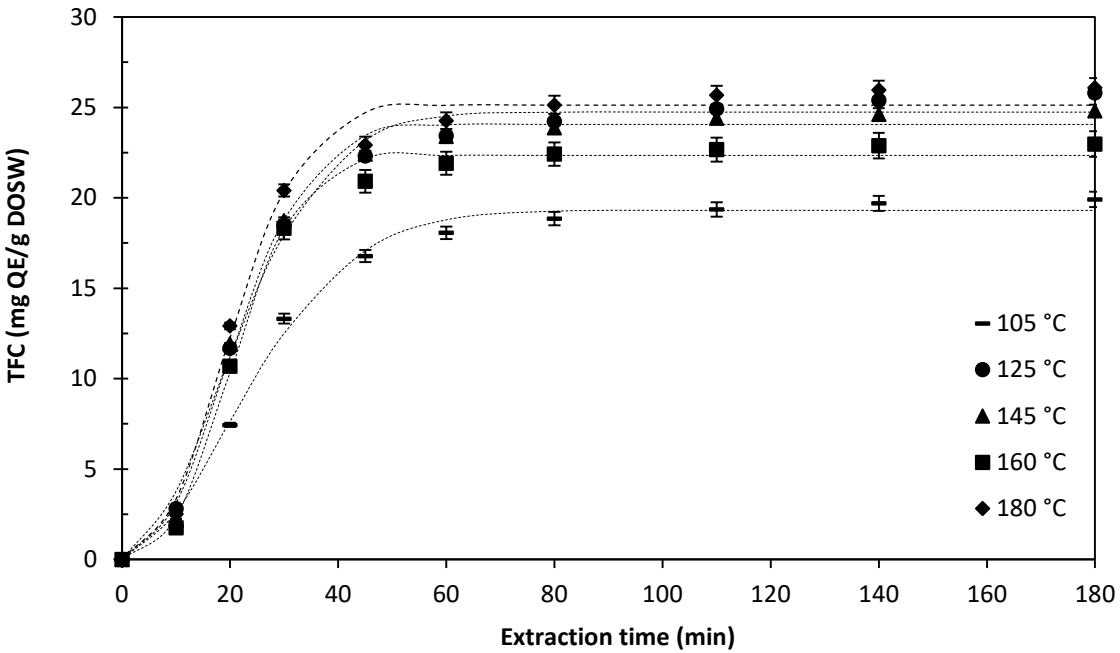
Dielectric constant: 43 (aprox)

Maximum TFC

20.4±0.2 mg QE/g DOSW

4.2 SubW extraction: Flavonoids extraction kinetics.

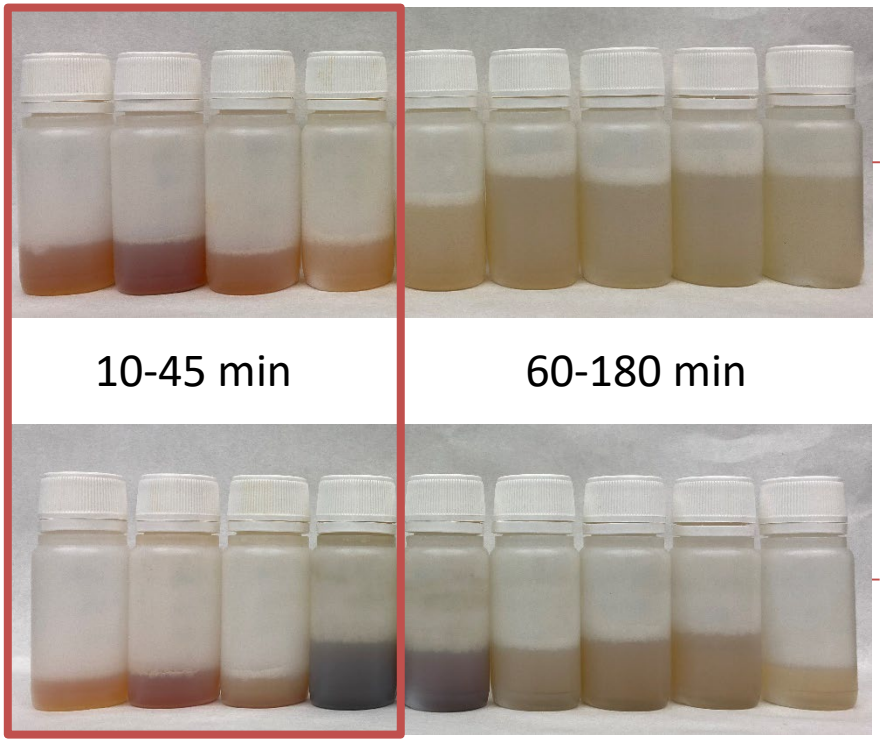
Effect of temperature



- **Fast extraction**
  - 95% of the total flavonoids <45 min
- **Effect of T:**
  - 105 °C: 19.9±0.4 mg QE/g OSW
  - 145 °C: 25 mg QE/g OSW

4. Experimental results

4.2 SubW extraction: Flavonoids extraction kinetics.

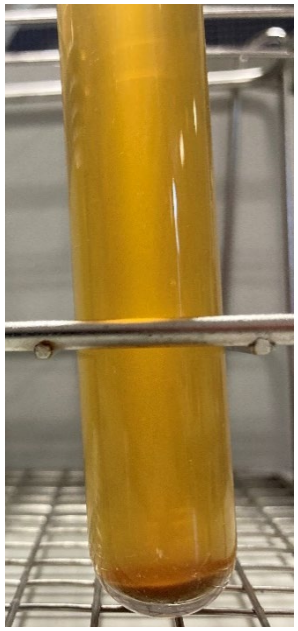


125 ºC

145 ºC

4. Experimental results

4.2 SubW extraction: Effect of solvent polarity.



- **Extract at room temperature**
  - A precipitate is formed
- **How to proceed?**
  - Centrifugate it, and HPLC
  - Resuspend and HPLC

4. Experimental results.

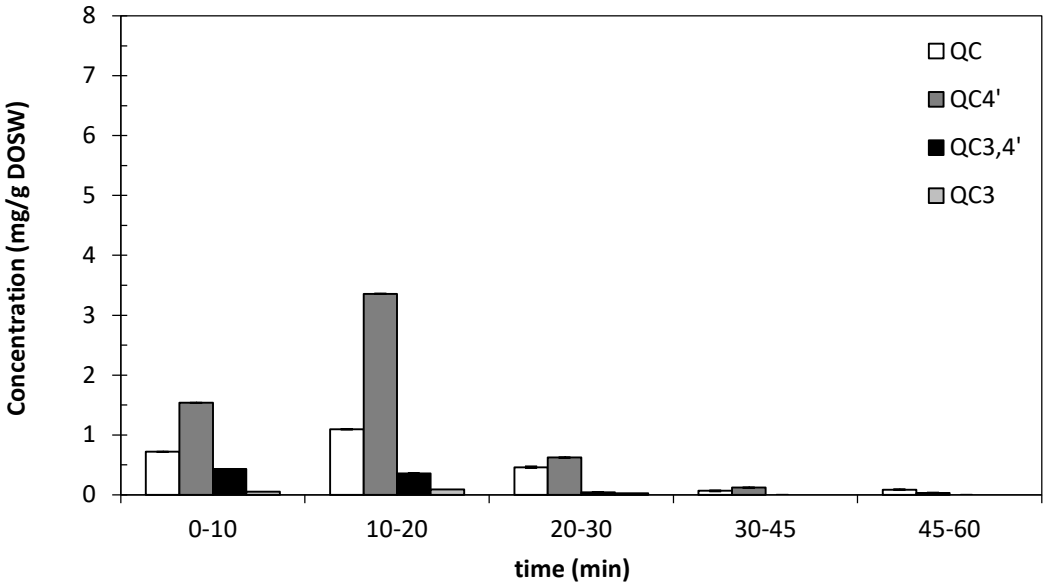
CENTRIFUGED

QC: x6.4

QC4': x1.5

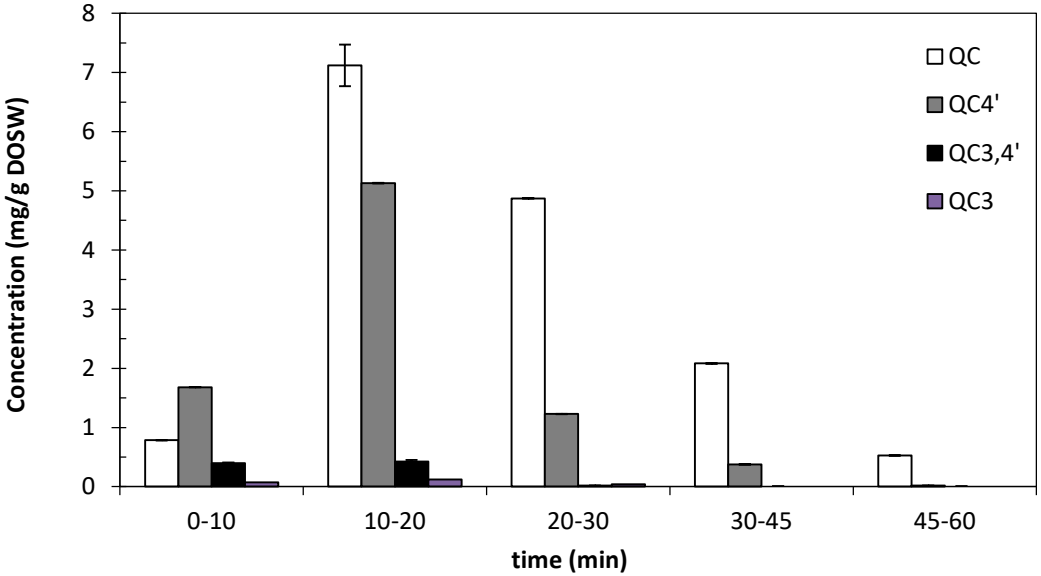
QC3: x1.4

QC3,4: no changes



RESUSPENDED

Ethanol, final concentration 70%, v/v



Extracts obtained at 145 °C

4. Experimental results.

CENTRIFUGED

TOTAL QCS SOLUBLE FRACTION

105 °C: 40.6%

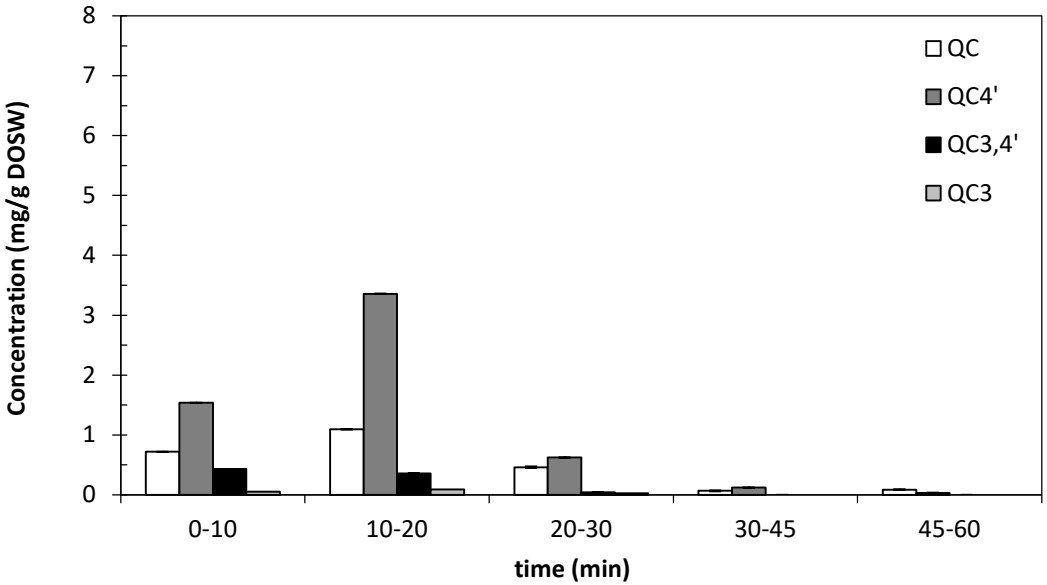
145 °C: 36.5%

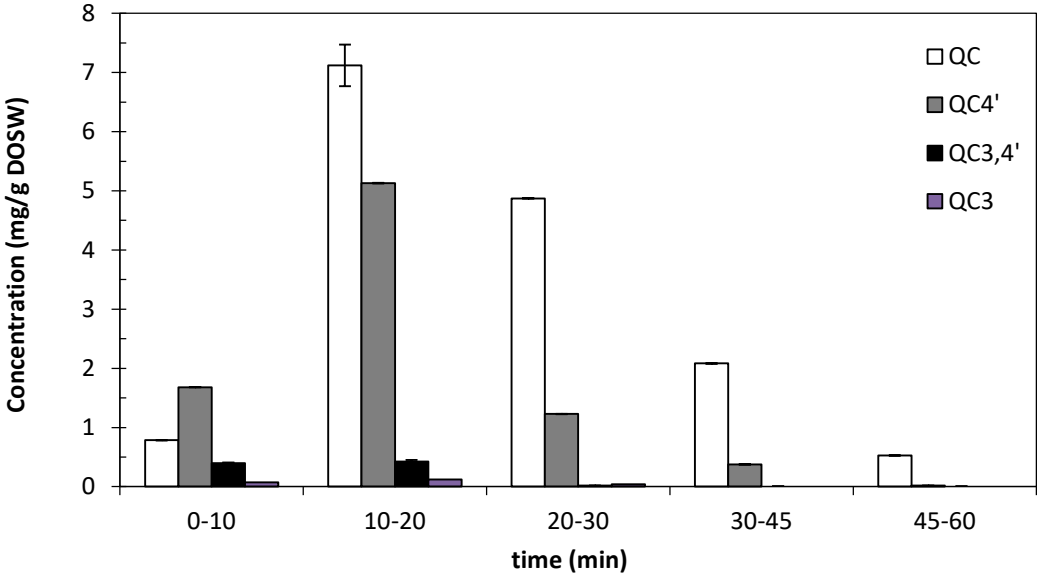
180 °C: 28.9%

RESUSPENDED

Ethanol, final concentration 70%, v/v

Extracts obtained at 145 °C





4. Experimental results. Effect of solvent polarity

4.2 SubW extraction: Effect of solvent polarity.

T (°C)	ε
25	78.6
105	53.9
145	44.7
180	37.9

Dielectric constant → compounds solubility

Irreversible precipitation at room temperature of compounds that were dissolved during the extraction at high temperatures

↓

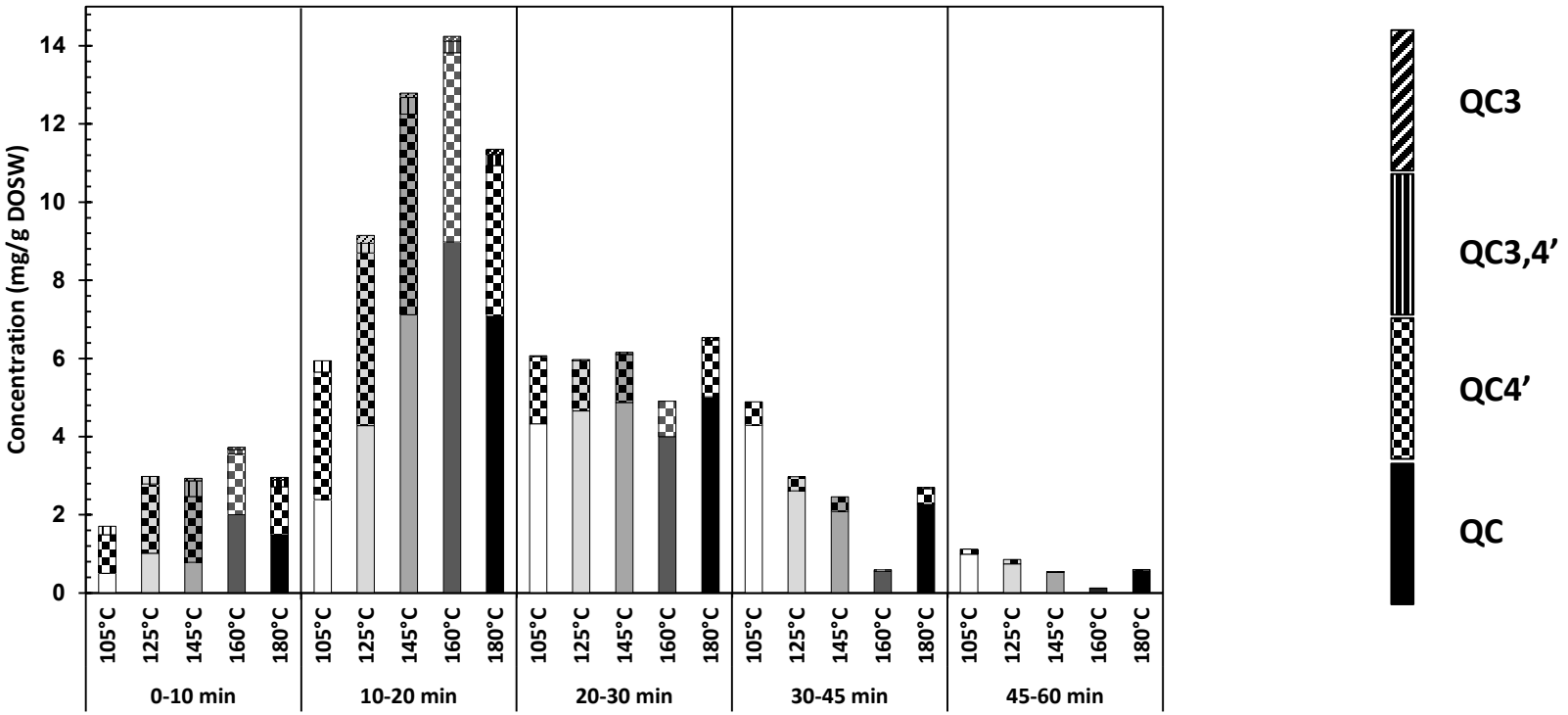
QC has a solubility in water of 2.15 ppm at 25 °C, which is increased up to 665 ppm at 140 °C [1].

An ethanol/water mixture (70%, *v/v*) has a dielectric constant around 43

[1]Srinivas et al. Solubility and solution thermodynamic properties of quercetin and quercetin dihydrate in subcritical water. *J. Food Eng.* **2010**, *100*, 208–218, doi:10.1016/j.jfoodeng.2010.04.001.

4. Experimental results.

4.2 SubW: effect of temperature of individual flavonoids.



Total quercetins extracted as a function of the extraction time and the temperature.

4. Experimental results. Effect of solvent polarity

4.2 SubW: effect of temperature of individual flavonoids.

	Total Extracted (mg/g OSW)					
	Conventional*	105 °C	125 °C	145 °C	160 °C	180 °C
QC	6.6±0.2	12.5±0.2	13.3±0.1	15.4±0.4	16.0±0.2	16.4±0.6
QC4'	9.8±0.3	6.7±0.1	8.0±0.2	8.4±0.1	7.8±0.1	7.0±0.1
QC3	0.21±0.03	ND	0.23±0.01	0.29±0.03	0.245±0.012	0.27±0.01
QC3,4'	2.04±0.03	0.54±0.04	0.44±0.02	0.84±0.04	0.453±0.015	0.45±0.02
Total QCs*	18.7±0.6^{A}	19.7±0.3^{B}	22.0±0.3^{C}	24.9±0.6^{D}	24.5±0.3^{D}	24.1±0.7^{D}
Ratio QC4'/QC	1.48±0.04^{E}	0.54±0.01^{C}	0.60±0.02^{D}	0.55±0.02^{C}	0.49±0.01^{B}	0.43±0.02^{A}

* EtOH 70% (v/v), 37 °C, 60 min

4. Experimental results. Effect of solvent polarity

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QC

- SubW favors the extraction
- Up to 2.5 times more

4. Experimental results. Effect of solvent polarity

4.2 SubW: effect of temperature of individual flavonoids.

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QC4'

- SubW decreases the extraction
- Possible hydrolysis?

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SubW increases the extraction yield of quercetin and derivatives by 30% at 145 °C

5. Conclusions

✓Onion Skin Wastes

- Potential source of flavonoids, specially QC and QC4'
- Subcritical Water:
  - Useful to recover the highly insoluble QC (2.5 times increase)
  - Fast extraction (<45 min)
  - Important to re-dissolve the precipitate formed as a consequence of the temperature decrease: dielectric constant increases and the solubility of some compounds drops
  - Temperature effect: best results at 145 °C

6. Acknowledgements

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Coimbra (Portugal), 1 March 2022

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