Valorization of onion skin wastes using subcritical water: flavonoids and pectin recovery

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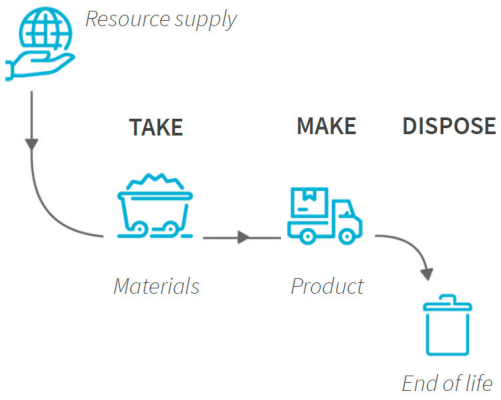
Contents

Background: Circular Economy



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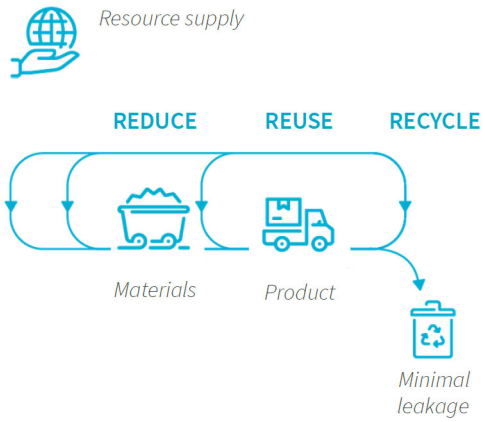


Background: Circular Economy

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**

➢**Food industry:** huge amount of food wastes

Onion Skin Wastes: from global to local

- 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: high antioxidant capacity
- >20% Galacturonic Acid (pectin)

Onion Skin Wastes: opportunities

PROBLEMS

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

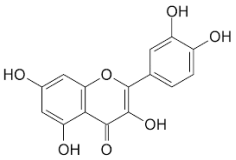
OPPORTUNITIES

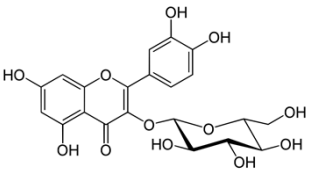
- ✓ Bioactive compounds:
  - **Flavonoids (**quercetin & derivatives)
  - **Pectin** growing demand: 40,000 t/y (+5% annual)

Onion Skin Wastes: opportunities

FLAVONOIDS

- Quercetin aglycone (QC)
- Quercetin-4'-O-glycoside (QC4')
- Quercetin-3,4'-O-diglycoside (QC3,4')
- Quercetin-3-O-glycoside (QC3)





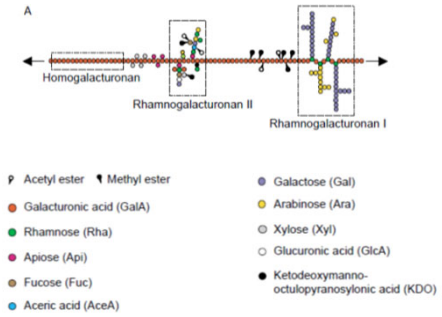
✓ Low solubility in water

✓ Strongly linked to the structural compounds of onion

Onion Skin Wastes: opportunities

PECTIN

- Set of complex heteropolysaccharides present in plant cell walls



1) ***Homogalacturonan* (HG)**, linear polymer of 1,4-D-GalA

2) ***Rhamnogalacturonan I* (RGI)**, repeating disaccharide (GalA – Rha) to which a variety of different glycan chains (principally arabinan and galactan) are attached to the rhamnose residues

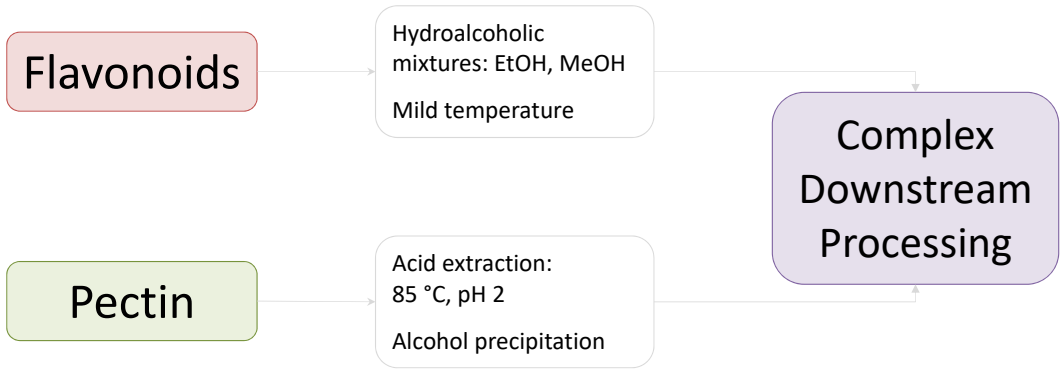
3) ***Rhamnogalacturonan II* (RGII)**, backbone of HG rather than RG, with complex side chains attached to the to the GalA residues.

Onion Skin Wastes: opportunities

PECTIN

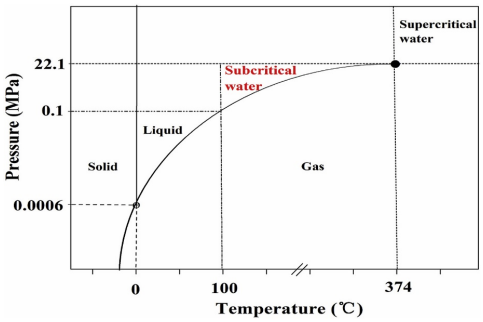
- E440, when used as additive (>65% GalA) to improve some technological aspects in formulations
- Actual source
  - 85%, citrus peels
  - 14%, apple pomace
  - Minor fraction from sugar beet
- Low molecular weight pectin: pectoligosaccharides (POS)
  - prebiotic, increasing demand

OSW valorization: conventional approach



OSW valorization: Subcritical Water approach

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



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

OSW valorization: Subcritical Water approach

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

OSW valorization: Subcritical Water approach

Working Conditions

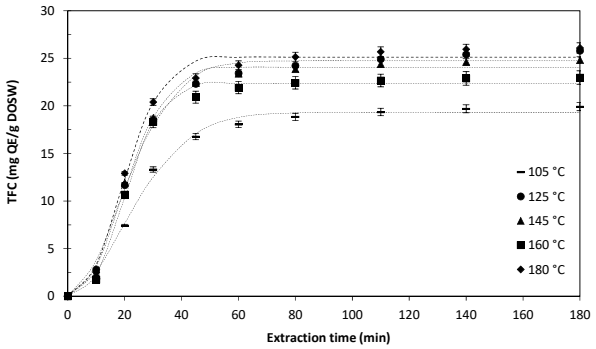
- 4 g of OSW + 8 g of 5 mm glass beads (avoid packaging and channeling through the bed)
- Flow rate: 2.5 mL/min
- Residence time: 10-11 min
- Extraction time: 180 min
- Temperature range: 105 to 180 °C
- Analysis of TFC and individual flavonoids
- Pectin isolation

In a typical experiment, around 4 g of OSW were placed in the extractor mixed with around 8 g of 5 mm glass beads in order to avoid packaging and channeling through the bed. Subsequently, the heating band was put around it; finally, the extractor was placed in the extraction plant. Then, the whole system was filled up with water and pressurized. After that, water was heated by means of the first band heater: it was continuously pumped through it at the working flow rate, but bypassing the extractor. Once water reached the working temperature, bypass valve was closed, so water was continuously pumped through the extractor. The band heater around the extractor helped to keep the desired working temperature along the experiment. The extract was cooled down in a chilled water bath right after exiting the extractor and kept at 4 °C until analysis. All the extraction experiments lasted 180 min, and the subcritical water experiments were done in a temperature range from 105 to 180 °C. Some initial experiments were done in order to select the suitable flow rate. This working procedure, in which only water at the extraction temperature is passed through the extractor, minimizes the time the raw material is exposed to the high temperature, preserving the integrity of the bioactive compounds.
The total extraction time was split in nine intervals (0-10 min; 10-20 min; 20-30 min; 30-45 min; 45-60 min; 60-80 min; 80-110 min; 110-140 min and 140-

180 min). The extracts collected in each interval were analyzed and then, the accumulated extraction curve calculated.

Results. Flavonoids recovery

SubW: effect of temperature of individual flavonoids

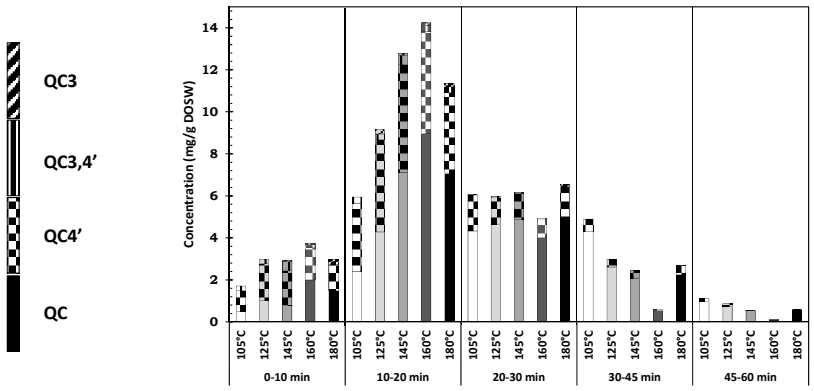


- **Fast extraction**
  - 95% of the total flavonoids <45 min
- **Effect of T:**
  - 105 °C: 19.9±0.4 mg QE/g OSW
  - 145 °C: 25 mg QE/g OSW

According to the results shown in Table 3, it is possible to see that temperature affects the extraction of flavonoids and the best results in terms of total quercetins extracted (the sum of QC+QC4'+QC3,4'+QC3), were obtained at 145 °C: 24.9±0.6 mg/g DOSW, which accounts for the 92% of the total flavonoids detected. Figure 5 presents the total amount of quercetins extracted within the first hour of extraction at each of the studied temperatures. It can be seen that the extraction is fast, happening mainly in the period from 10 to 20 min of extraction time, decreasing the extraction rate from that moment. It is at 160 °C that the highest recovery happens in this period, but in total at 145 °C slightly more quercetins are recovered after 60 min of extraction. In all the conditions QC is the main compound extracted followed by QC4'. The extraction of QC3,4' happens mainly within the first 20 min, whereas QC3 is barely extracted.
More specifically, according to Table 3, at 145 °C, QC aglycone concentration was 15.4±0.4 mg/g, which represents 62% of the total quercetins identified, whereas QC4' concentration was 8.4±0.12mg/g onion. Further increases of temperature involved higher share of quercetin aglycone of the total quercetins detected: at 180 °C QC aglycone represented 68% of the total.

Results. Flavonoids recovery

SubW: effect of temperature of individual flavonoids



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

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Results. Flavonoids recovery

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

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Results. Flavonoids recovery

SubW: effect of temperature of individual flavonoids

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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*	$18.7±0.6^{A}$	$19.7±0.3^{B}$	$22.0±0.3^{C}$	$24.9±0.6^{D}$	$24.5±0.3^{D}$	$24.1±0.7^{D}$
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QC

- SubW favors the extraction
- Up to 2.5 times more

QC4'

- SubW decreases the extraction
- Possible hydrolysis?

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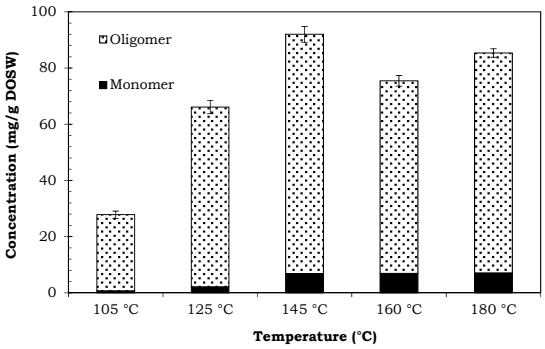
SubW increases the extraction yield of quercetin and derivatives by 30% at 145 °C

More specifically, according to Table 3, at 145 °C, QC aglycone concentration was 15.4±0.4 mg/g, which represents 62% of the total quercetins identified, whereas QC4' concentration was 8.4±0.12mg/g onion. Further increases of temperature involved higher share of quercetin aglycone of the total quercetins detected: at 180 °C QC aglycone represented 68% of the total. These results are in agreement with other authors who reported that, in the onion skin, QC glucosides are barely found [32], since 67-86% of the total QC is in the aglycone form [21]. QC is the main flavonoid in the external layers of the onion, because it derives from the deglycosilation of the QC glucosides as a consequence on the intrinsic skin formation process [33]. It has the very specific role of protecting the onion from the UV radiation, given the fact that quercetin aglycone has higher antioxidant activity than the glycoside derivatives [34]. If the whole onion is used as a source of flavonoids, then QC4' and QC3,4' account for the 80% of the total flavonoids extracted [11,15]. The remaining 20% are 17 different components, among them, quercetin-3-o-glucoside and isorhamnetin-3-glucose are the most abundant [35]

Results. Pectin recovery

SubW: effect on pectin extraction & composition



Total GalA concentration as a function of temperature after 180 min of extraction. Both the oligomeric fraction and the monomeric fraction are presented

Results. Pectin recovery

SubW: effect on pectin extraction & composition

	Conventional	Subcritical Water		
	85 °C, pH 2 (HCl)	125 °C	145 °C	180 °C
Yield (%)	2.6 ± 0.1^{A}	5.2 ± 0.2^{B}	9.0 ± 0.4^{C}	8.6 ± 0.3^{C}
GalA (%)	45.4 ± 0.4^{A}	72.6 ± 1.1^{C}	67.0 ± 0.1^{B}	67.7 ± 0.8^{B}
Ara (%)	0.32 ± 0.03^{A}	0.82 ± 0.06^{B}	0.41 ± 0.02^{A}	1.19 ± 0.03^{C}
Gal (%)	3.8 ± 0.3^{A}	9.3 ± 0.5^{C}	6.6 ± 0.4^{B}	9.2 ± 0.4^{C}
Rha (%)	0.65 ± 0.12^{A}	2.29 ± 0.11^{B}	4.5 ± 0.3^{C}	6.6 ± 0.3^{C}
HG (mol %)	80.3 ± 1.6^{C}	75.4 ± 2.7^{B}	71.7 ± 2.4^{B}	64.2 ± 2.2^{A}
RG-I (mol %)	10.8 ± 0.9^{A}	17.8 ± 0.9^{B}	21.1 ± 1.1^{C}	29.1 ± 1.8^{C}
Rha/GalA	0.02	0.04	0.08	0.13
(Gal+Ara)/Rha	5.9	4.1	1.4	1.5
UA:NS ratio	7.8 ± 0.5^{C}	4.7 ± 0.4^{B}	4.8 ± 0.6^{B}	3.4 ± 0.3^{A}

- SubW
  - Increases extraction yield
  - Increases GalA content
  - Changes domain composition

It can be observed that SubW extraction provided higher pectin extraction yields than conventional extraction. Pectin extracted from OSW using water acidified with HCl had a relatively high GalA content (45.4%), but low concentration of neutral sugars (ratio uronic acids:neutral sugars (UA:NS), 7.8) which indicates that the pectin extracted under acidic conditions was mainly composed by the HG domain.

Regarding the SubW extracted pectins composition, these showed much higher GalA content (around 67% at 145 and 180°C) and even higher at 125°C (72.6±1.1%) than that extracted using acid extraction process. Galactose was the most abundant neutral sugar, which has also been detected in other raw materials, such as potato pulp [38], followed by rhamnose. The ratio Rha/GalA increased with the temperature, up to 0.13 at 180°C, which is indicating the increased presence of the RG-I domain in the SubW extracted pectin.

aime et al. [23] indicated that the Gal, Ara and Xyl content decreased in the onion brown skin, in comparison with inner layers. This was translated in a loss of the galactan and arabinan side chains, which makes the ratio UA:NS to increase in the outer layers of the onion. Pectins obtained in the present work using SubW presented a ratio UA:NS very low compared to other authors. Pectin extracted using the conventional procedure had a ratio UA:NS equal to 7.8, whereas this value was lower for pectins extracted using SubW. Therefore, various types of oligosaccharides can be released in the SubW hydrolysis processes: rhamnogalacturonan-oligosaccharides, galacturonan-oligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides and arabino-galactan oligosaccharides.

Results. Pectin recovery

SubW: effect on pectin extraction & composition

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Yield (%)	2.6±0.1^{A}	5.2±0.2^{B}	9.0±0.4^{C}	8.6±0.3^{C}
GalA (%)	45.4±0.4^{A}	72.6±1.1^{C}	67.0±0.1^{B}	67.7±0.8^{B}
Ara (%)	0.32±0.03^{A}	0.82±0.06^{B}	0.41±0.02^{A}	1.19±0.03^{C}
Gal (%)	3.8±0.3^{A}	9.3±0.5^{C}	6.6±0.4^{B}	9.2±0.4^{C}
Rha (%)	0.65±0.12^{A}	2.29±0.11^{B}	4.5±0.3^{C}	6.6±0.3^{C}

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Results. Pectin recovery

SubW: effect on pectin structural features

	Conventional	Subcritical Water		
	85 °C, pH 2 (HCl)	125 °C	145 °C	180 °C
Rha/GalA	0.02	0.04	0.08	0.13
(Gal+Ara)/Rha	5.9	4.1	1.4	1.5

Rha/GalA

- contribution of the RG-I domain in pectin
- ranges from 0.05 to 1, then the main constituent of the pectin is considered to be the RG-I region

(Gal + Ara)/Rha

- Tells if RG-I is highly branched

It can be observed that SubW extraction provided higher pectin extraction yields than conventional extraction. Pectin extracted from OSW using water acidified with HCl had a relatively high GalA content (45.4%), but low concentration of neutral sugars (ratio uronic acids:neutral sugars (UA:NS), 7.8) which indicates that the pectin extracted under acidic conditions was mainly composed by the HG domain.
The decrease in the (Gal + Ara)/Rha molar ratio indicates that SubW pectin is not highly branched (it seems that these are linear chains formed by Gal) or at least the branches are short, probably due to hydrolysis reactions happening during the extraction process. Arabinose and xylose were also present in the structure of pectins, which might be indicating the presence of arabinogalactan and xylogalacturonan chains [40].

Results. Pectin recovery

SubW: effect on pectin structural features

	Conventional	Subcritical Water		
	85 °C, pH 2 (HCl)	125 °C	145 °C	180 °C
HG (mol %)	80.3 ± 1.6^{C}	75.4 ± 2.7^{B}	71.7 ± 2.4^{B}	64.2 ± 2.2^{A}
RG-I (mol %)	10.8 ± 0.9^{A}	17.8 ± 0.9^{B}	21.1 ± 1.1^{C}	29.1 ± 1.8^{C}

$$HG\ (\%, mol) = GalA\ - Rha$$

$$RG - I\ (\%, mol) = [GalA\ - HG\ ] + Rha + Gal + Ara$$

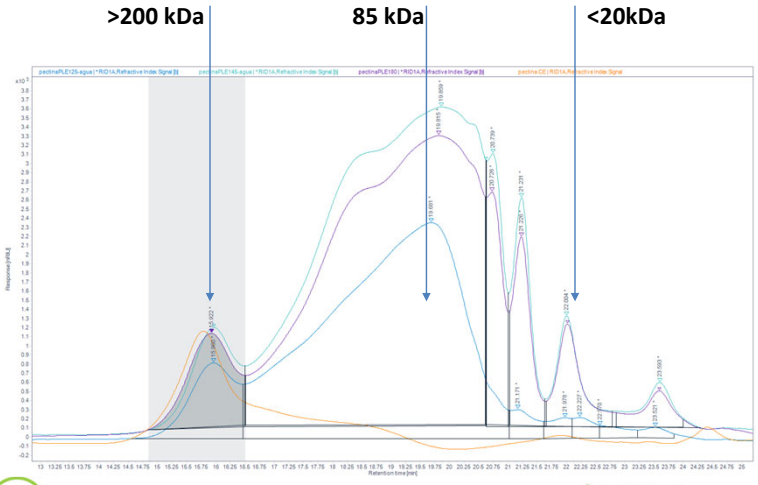
N.H. M'sakni et al. Eur. Polym. J. 42 (2006) 786–795. https://doi.org/10.1016/j.eurpolymj.2005.09.014.

- SubW:
  - Increases the RG-I domain
  - Bioactivity and ability to form gels of these molecules is related to their molecular weight, composition and structure:
    - RG-I: Prebiotic function
    - HG: Gelling ability

The calculation of the HG and RG-I domains confirmed the increased content in the RG-I domain, which opens a wide range of opportunities for the hairy pectins (RG-I) extracted from OSW using SubW. Recent studies have indicated that there is an increased interest in the RG-I-rich pectins (known as pectin-derived oligosaccharides –POS-) as they have prebiotic functions [35], [39]. The bioactivity and ability to form gels of these molecules is related to their molecular weight, composition and structure. The decrease in the (Gal + Ara)/Rha molar ratio indicates that SubW pectin is not highly branched (it seems that these are linear chains formed by Gal) or at least the branches are short, probably due to hydrolysis reactions happening during the extraction process. Arabinose and xylose were also present in the structure of pectins, which might be indicating the presence of arabinogalactan and xylogalacturonan chains [40].

Results. Pectin recovery

SubW: effect on pectin molecular weight



- Conventional Extraction
  - One single peak (>200 kDa)
  - HG domain
- SubW Extraction
  - Multimodal distribution
  - Most abundant 85 kDa
  - T promotes the appearance of lower MW populations
- Separation and purification based on MW: membrane technology

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Pectin after acid extraction only presented one peak at 16 min (corresponding to a MW > 200 kDa), whereas in the SubW pectins, besides this peak, up to 5 more peaks appeared, indicating the presence of lower molecular weight populations. Considering the composition of pectins reported in Table 4, in which acid extraction pectin was mainly composed by HG structures, it can be concluded that the peak that eluted at 16 min corresponds to HG domain, which is in agreement with the results reported by Dias et al. [41]. In SubW extracted pectins, HG domain represented from 6.7% at 145 ºC to 10% at 125 ºC. In these pectins, the main population had a MW in the range 52-56 kDa, with a polydispersity index from 1 (125 ºC) to 1.2. Pectins obtained at 145 and 180 ºC presented monodisperse populations with smaller MW. This can be explained by hydrolysis reactions happening due to the high temperature.

Conclusions

SubW is an efficient reaction medium to promote the solubilization of high added value components from OSW:

➢ **Flavonoids**

- Potential source of flavonoids (QC and QC4')
- Dramatic increase in the extraction yield of the highly insoluble QC (2.5 times increase)
- Fast extraction (<45 min) at 145 °C

Conclusions

SubW is an efficient reaction medium to promote the solubilization of high added value components from OSW:

➢ **Pectin**

- RG-I pectin (up to 21.1±1.1 mol %), compared to the conventional pectin isolation process that yields mainly the linear HG pectin (10.8±0.9 mol %).
- High temperatures promote pectin hydrolysis (decrease in MW and shorter branches): careful selection of the extraction conditions
- Future: downstream processing by membrane technology

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Valorization of onion skin wastes using subcritical water: flavonoids and pectin recovery

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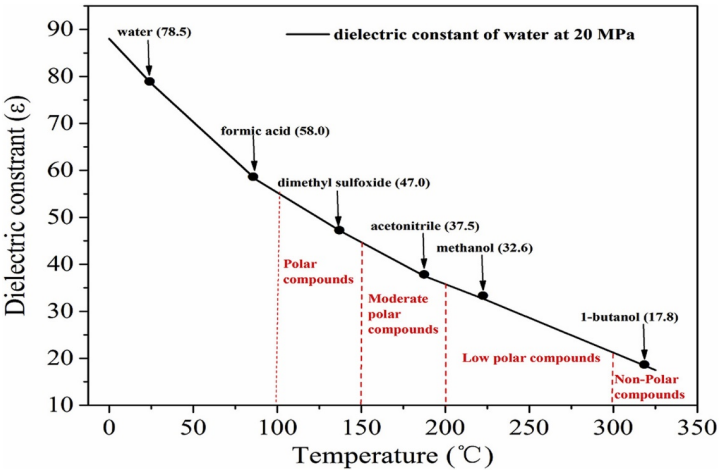
2. Alternative: subcritical water (SubW)



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