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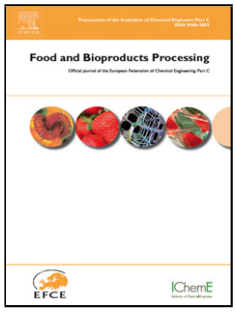

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Freeze-dried extract from onion (*Allium cepa* cv. Horcal) skin wastes: extraction intensification and flavonoids identification

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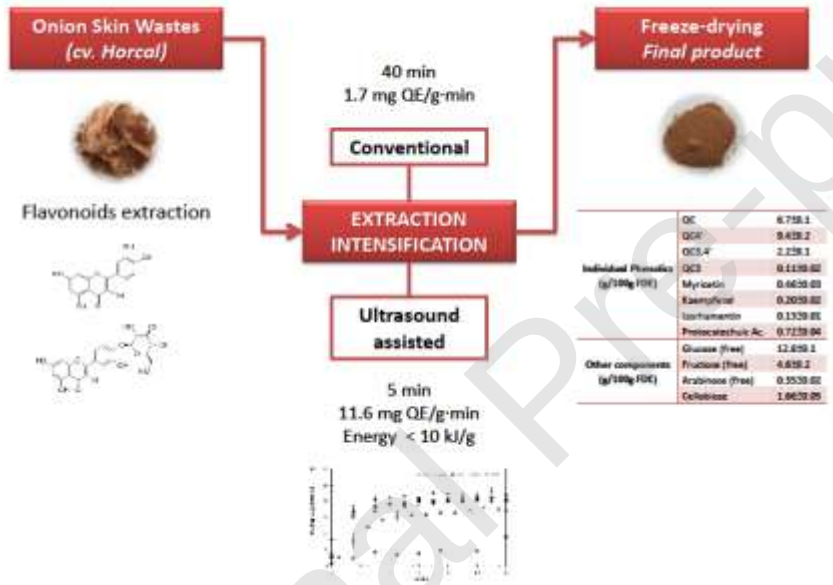
Graphical abstract



Highlights

- Ultrasound intensified extraction of flavonoids from onion skin waste
- Extraction time and energy input were reduced to <5 min and 10 kJ/g DOSW
- Ultrasounds intensified the extraction: productivity was 11.6 mg QE/(g dry OSW·min)
- An extract containing21% flavonoids was obtained at optimum conditions
- Bioactive compounds and antioxidant capacity were preserved for 3 months at 4 °C

Abstract

In this work, the intensification of the extraction of flavonoids from dry onion skin wastes (DOSW) by Ultrasound Assisted Extraction (UAE) was investigated. Conventional stirred tank extraction was used to find the optimal temperature (37 ºC) and solvent (ethanol 70%, v/v), providing 20.7±0.4 mg QE/g DOSW after 1 h. In the UAE process, the amplitude of oscillation and the solvent-to-solid ratio were key parameters to control the specific energy used. Energies above 10 kJ/g OSW did not increase the extraction yield (maximum flavonoids content was 23.9±0.2 mg QE/g DOSW), which could be obtained using amplitudes of 40%, high solvent-to-solid ratios (30 mL/g DOSW) and extraction times below 5 min. Although both extraction techniques provided similar profiles of phenolic compounds, being quercetin and quercetin-4'-O-glucoside the main flavonoids (7.4 mg/g DOSW and 10.4 mg/g DOSW, respectively), UAE increased the productivity up to 11.6 mg QE/(g DOSW · min), seven times higher than conventional extraction. The extraction kinetics was modeled and studied. The optimum extract was freeze-dried resulting in a solid powder rich in flavonoids (21%) which kept 90% of the antioxidant activity after 180 days of storage at temperatures below 4 ºC.

Keywords: *onion; extraction; ultrasounds; quercetin; flavonoids; process intensification*

Abbreviations

QC: Quercetin

QC4': Quercetin-4'-O-glucoside

QC3: Quercetin-3-O-glucoside

QC3,4': Quercetin-3,4'-O-diglucoside

QE: Quercetin equivalent

OSW: Onion Skin Waste

UAE: Ultrasound Assisted Extraction

1. Introduction

Onion (*Allium cepa* L.) is extensively produced worldwide. According to FAO (FAO. Food and Agriculture Organzation of the United Nations, 2020), in 2018 around 96.8 Mt of onion were harvested, generating around 0.5% of the total production as non-edible wastes (skins, bulbs or roots) (Munir et al., 2018). Onion is well known for having a distinctive smell which advises against the use for cattle feeding or as fertilizer (Roldan et al., 2008). However, onion, and specially the outermost onion skins are one of the richest sources of flavonoids (Pérez-Gregorio et al., 2014), such as quercetin and its glycosylated derivatives.

The extraction of flavonoids from onion skin wastes is usually carried out using aqueous mixtures of organic solvents such as ethanol (Jin et al., 2011; Khiari and Makris, 2012; Kiassos et al., 2009; Lee et al., 2014; Sharma et al., 2015) or methanol (Piechowiak et al., 2020; Price and Rhodes, 1997; Søltoft et al., 2009). In the case of extraction of quercetin for human consumption, the preferred solvent is aqueous ethanol (in the range from 50 to 75%) due to health and safety issues. Besides solvent, temperature, extraction time and solvent to raw material ratio are known to affect the extraction of flavonoids from onion skin wastes. As summarized in Table 1, times vary from a few minutes to 24 h; temperatures are used in the range 4 ºC to 60 ºC and solvent-to-solid ratio used are in the range from 6 to 250 mL/g onion. Previous works have demonstrated that the extraction of quercetin from onion skin wastes is time and energy consuming; therefore, it is necessary to have efficient extraction methods that reduce both time and energy consumed. In this sense, Ultrasound Assisted Extraction (UAE) is an excellent tool to intensify the extraction processes.

UAE consists in the application of ultrasounds to a suspension of the raw material in an appropriate solvent (Chemat et al., 2011). The principle of action of the ultrasound in the extraction process is found in the way the sound waves propagate into the liquid, creating alternating high pressure (*compression*) and low pressure (*rarefaction*) cycles. These cycles lead to the formation and collapse of bubbles, in the so-called cavitation process. The cavitation process produces increases in temperature and changes of pressure, affecting the mass transfer properties of the solvent (Ran et al., 2019). As pointed out by (Benito-Román et al., 2013), the extraction performance in a UAE process strongly depends on the amount of energy used, which is affected by the amplitude of oscillation of the ultrasonic probe, the amount of solvent and raw material used and their nature. The effect of the solvent to raw material ratio is not often studied, although it is expected to play a key role in the extraction process: it affects the extraction driving force and determines how the ultrasonic waves propagate in the solvent. In order to have a successful UAE process it is necessary to study how the process parameters affect the energy used, trying to find an optimal combination to maximize the extraction yield and minimize the energy used.

UAE is a versatile technique widely used to extract bioactive molecules from a number of natural matrices (Vilkhu et al., 2008), such as pectin from passion fruit peel, lycopene from tomatoes, antioxidants and carotenoids from pomegranate, polyphenols and anthocyanins from plum, grape peels, jabuticaba peel or grape pomace, as summarized by (Ran et al., 2019). It is possible to find some attempts of the use of UAE to recover bioactive compounds from onion skin wastes, summarized in Table 1. There is a wide range of experimental conditions used, strongly dependent on the ultrasonic device used in the extraction process, being classified in ultrasonic baths (Jang et al., 2013; Kumar et al., 2014; Søltoft et al., 2009) and ultrasonic probes (Jin et al., 2011). In general, these research works report an increase in the extraction yield compared to the conventional extraction process. The comparison of the results among researchers is hard because the onion genotype affects the content of phenolic compounds (Pérez-Gregorio et al., 2014) and because the energy used to carry out the extraction is not reported.

In the present work, both the conventional and the UAE processes were studied, in order to compare the two processes and evaluate the extent of intensification produced by the UAE. As raw material skin wastes obtained from the onion cultivar *Horcal* was used. This variety has not been previously used as a source of flavonoids, as far as the authors' knowledge. In the first stage, a conventional extraction process was used to select the solvent and temperature that maximize the extraction of flavonoids. In the second stage, the intensification of the extraction was studied using ultrasounds, quantifying the energy used and the flavonoids extraction yield, using a response surface methodology approach. Finally, a complete identification and quantification of the flavonoids extracted in the different extraction conditions was done.

Table 1. Optimal extraction conditions of flavonoids from OSW found in the literature.

Authors	T (ºC)	Solvent	Time	Solvent-to-solid ratio	Extraction method	Quercetin extraction yield
(Lee et al., 2014)	60 ºC	EtOH, 70% v/v	3 h	10:1	Conventional stirred tank	2.78±0.05 mg QC/g onion peel
(Jin et al., 2011)	59.2 ºC	EtOH, 60% v/v	16.5 min	40:1	Conventional, stirred tank	3.70±0.21 mg QC/g onion skin
(Piechowiak et al., 2020)	44 ºC	MeOH	2.4 h	30:1	Conventional stirred tank	25.97 mg QC/g onion skin
(Khiari et al., 2008)	20-60 ºC	EtOH, 60% v/v	0.5-24 h	10:1	Conventional stirred tank	19-36 mg QE/g OSW
(Kiassos et al., 2009)	22 ºC	EtOH, 60% v/v	4.2 h	40:1	Conventional stirred tank	NR
(Price and Rhodes, 1997)	NR	MeOH, 70% v/v	1 min	25:1	High speed homogenizer	Up to 0.4 mg/g onion
(Jang et al., 2013)	49 ºC	EtOH, 59% v/v	36 min	60:1	Ultrasounds bath	11.08±0.30 mg QE/g OSW; 8 mg QC/g OSW
(Campone et al., 2018)	25 ºC	EtOH, 85% v/v	15 min	20:1	Ultrasounds bath	NR
(Kumar et al., 2014)	40 ºC	Water (pH 2.7-6.25)	NR	6:1	Ultrasounds bath	1.75 mg/g onion
(Kwak et al., 2017)	Room Temp.	MeOH:formic ac:water (50:5:45)	20 min	250:1	Ultrasounds bath	0.59 mg QC/g red onion 41.39 mg QC4'/g red onion
(Jin et al., 2011)	43.8 ºC	EtOH, 43.8% v/v	36 min	40:1	Ultrasounds tip	4.09±0.29 mg QC/g onion skin

NR: Not Reported

2. Materials and methods

2.1. Sample preparation

Onion (*Allium cepa* L. cv. Horcal) wastes were collected from a local factory. This factory produces blood sausages under the Protected Geographical Indication (IGP) "Morcilla de Burgos" (Official Journal of the European Union L224/3 of 05.09.2018 and C455/7 of 06.12.2016), being onions of the Horcal variety one of the main ingredients. Onion wastes were manually processed in order to separate the brown skins, which were dried at room temperature for 24 h. Subsequently they were milled using the cutting mill SM100 (Retsch GmbH, Germany), equipped with a 1 mm sieve. In order to quantify the residual moisture of the raw material, a sample was kept at 105 ºC for 24 h, according to the protocol NREL/TP-510-42621. Calculated moisture resulted to be 9.4±0.2%, and all the results obtained in this work were presented per gram of dry onion skin wastes (DOSW).

2.2. Extraction experiments

2.2.1. Conventional extraction (CE) experiments

The experimental work was divided in two stages: first to find the effect of the extraction temperature and the solvent used and second, to study the extraction kinetics. All the experiments were carried out in an incubator shaker (Model G25, New Brunswick Scientific Co., NJ, USA) and stirring was set at 275 rpm.

2.2.1.1. Effect of the solvent and the extraction temperature

In each experiment, 10 g of DOSW were transferred to an Erlenmeyer flask, where 100 mL of solvent were added. The solvents used were ethanol/water mixtures (%, v/v), ranging from 0% (pure water) to 100% (pure ethanol). The extraction experiments were carried out at 37 ºC and 50 ºC, for 60 min. After the extraction, the solid liquid mixture was separated under vacuum filtration. Solids were discarded and the liquid extract was kept at -20 ºC until analysis.

2.2.1.2. Kinetic experiments

In these experiments, 10 g of onion skin wastes were placed in the Erlenmeyer flask plus 100 mL of the solvent (ethanol 70%, v/v) at 37 ºC. Samples (0.75 mL) were taken periodically. Total extraction time was 60 min.

2.2.2. Ultrasound Assisted Extraction (UAE) experiments

The extraction of bioactive compounds from onion skin wastes was carried out using the ultrasonic processor Vibra-Cell 75043, 20 kHz, maximum power output 750 W (Bioblock Scientific, USA). It was equipped with a 13 mm titanium probe (maximum amplitude of oscillation was 79 µm, corresponding to the display setting value of 100%) that was submerged

into the sample at 2 cm from the bottom of the jacketed vessel. Water was circulating through the jacket in order to keep constant the extraction temperature (37 ºC). In each experiment, 60 mL of solvent were used and a variable amount of DOSW, as will be detailed. After the extraction, the total energy applied was registered and the solid-liquid mixture was centrifuged at 4500 rpm for 10 min at 15 ºC using the centrifuge Sorvall ST16R (Thermo Scientific, USA). The supernatant was kept at -20 ºC until analysis. In all the experiments, the sonication was applied in pulses in order to have a better control of the temperature and the energy delivered. The pulse mode selected was 5 s on, 5 s off, in order to complete the total sonication time of each experiment. The specific power (*P*, expressed as W/g DOSW) used in each experiment was calculated according to Eq. (1), from the total energy (*E*, expressed in J/g) measured in each extraction experiment, divided by the extraction time (*t*, expressed in s):

$$P = {}^{E}/_{t} \quad (1)$$

2.2.2.1. Kinetic experiments

In the experiments carried out to study the extraction kinetics, 3 g of onion skin wastes were placed in the extraction vessel plus 60 mL of the solvent (ethanol 70%, v/v). The amplitude of oscillation was changed from 20 to 100%. Samples were taken every 60 s, and the total sonication time for each experiment was 12 min.

2.2.2.2. Optimization experiments

For the experiments focused on the optimization of the UAE conditions, a central composite design (CCD) was used, selecting as variables the amplitude (40-80%), the sonication time (1-5 min) and the solvent-to-solid ratio (10-30 mL/g DOSW), using in all the cases 60 mL of ethanol 70% (v/v). The experimental plan consisted of 17 runs, including three repetitions of the central point of the experimental design. All the experiments were randomized. The total flavonoids content was selected as the response variable to be maximized.

Analysis of variance (ANOVA) with 95% confidence level was done for each response variable in order to test the model significance and suitability, calculating the second order polynomial equations, by means of the statistical software Statgraphics 18-X64.

2.3. Kinetic modelling: comparison between CE and UAE

In order to compare the extraction kinetics for both extraction techniques (CE and UAE), the experimental data were fitted to the Weibull model (Eq. 2), commonly used to model the extraction of bioactive compounds from natural matrices (Alonso-Riaño et al., 2020).

$$TFC\ (mg\ QE/g\ DOSW) = A \cdot [1 - exp(-k \cdot (t)^{n})] \quad (2)$$

Weibull model has three different parameters: *A* is a kinetic parameter that represents the maximum extraction yield at infinite extraction time and *k* is a kind of extraction rate constant; and *n* is the shape parameter of the extraction curve.

Since the extraction times for conventional process (60 min) and UAE process (12 min) were very different, for the comparison, the experimental data were represented versus the relative extraction time, obtained by dividing the extraction time by the maximum extraction time for each extraction process. In this way, it is possible to set a direct comparison between the trends obtained for the two extraction processes.

2.4. Freeze Drying process

The extract obtained in the optimal UAE conditions was subjected to freeze drying in order to get a dry powder. Prior to freeze drying, ethanol was removed in a rotary evaporator (temperature 37 ºC) and afterwards, it was equilibrated at -80 °C and freeze-dried in a Labconco Freeze Dry System (Labconco Inc., MO, USA) at 0.15 mbar for, at least, 48 h.

2.5. Characterization of the extracts

2.5.1. Total Phenolics Content (TPC) and Total Flavonoids Content (TFC)

The TPC in liquid extracts was measured in triplicate by the Folin–Ciocalteau method described by (Alonso-Riaño et al., 2020): a sample of 0.1 mL was mixed with 2.8 mL of water and 0.1 mL of Folin–Ciocalteau's reagent. After vortexing the solution, 2 mL of sodium carbonate in aqueous solution (7.5% w/w sodium carbonate) were added. After shaking, the mixture was incubated at room temperature in a dark place for 1 h. The absorbance was measured at 750 nm using a Jasco V-750 spectrophotometer (Jasco Corporation, Japan). Different concentrations of gallic acid were used to construct the standard curve and final results were expressed as milligrams of Gallic Acid Equivalents per gram of dry onion skin wastes *(mg GAE/g DOSW)* used in the extraction.

The TFC in liquid extracts was measured in triplicate according to the following procedure described by (Chang et al., 2002): 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. Samples were incubated for 30 min and, after being filtered (0.45 μm pore size), the absorbance was measured at 415 nm, using Jasco V-750 spectrophotometer. A quercetin standard curve in ethanol was used to calculate the TFC of the samples, which was expressed as milligrams of Quercetin Equivalent per gram of dry onion skin wastes *(mg QE/g DOSW)*. Due to the intrinsic color of the samples, a blank standard was also measured. In this case, instead of adding 0.1 mL of the reagent $AlCl_3$, 0.1 mL of water were

used, the rest of the reagents being equally added. After 30 min of incubation, the absorbance was measured and was subtracted from the sample absorbance readings.

To determine the TFC in the freeze-dried extract (FDE), a solution of 1 mg/mL of the FDE in 70% ethanol was used for the analytical procedures.

2.5.2. Antioxidant Activity (AA).

The total antioxidant activity was measured by the Ferric Reducing/Antioxidant Power (FRAP) assay developed by (Benzie and Strain, 1996). The working FRAP solution was prepared by mixing volumes of buffer acetate (pH 3.6) plus 10 mM of 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution plus 20 mM $FeCl_3$ solutions in the ratio 10:1:1. Then, 2.85 mL of the working FRAP reagent were added to 0.15 mL of the sample and incubated at 37 °C for 30 min. Absorbance was read at 593 nm in a Jasco V-750 spectrophotometer. As standard, a solution of $FeSO_4 \cdot 7H_2O$ was used. Different concentrations of this solution were used for the calibration curve. Results were expressed in *mg $FeSO_4$/g DOSW*.

To determine the antioxidant activity of the FDE, a solution of 1 mg/mL of the FDE in 70% ethanol (v/v) was used.

2.5.3. Identification and quantification of extract individual components

2.5.3.1. Individual flavonoids identification

Samples after extraction were characterized by High Performance Liquid Chromatography using a Diode Array Detector (HPLC-DAD, Agilent 1100, CA, USA) with a Kinetex® Biphenyl column (250 × 4.6 mm, particle size 5 μm, pore size 100 Å) supplied by Phenomenex (CA, USA), as described by (Benito-Román et al., 2020). The mobile phase consisted of ammonium acetate 5 mM with acetic acid (1%, v/v) in water (solvent A) and ammonium acetate 5 mM with acetic acid (1%, v/v) in acetonitrile (solvent B). The gradient profile was the following: from 0 to 7 min, 2% of solvent B (isocratic); from 7 to 20 min, from 2% to 8% solvent B; from 20 to 35 min, from 8% to 10% solvent B and from 35 to 55 min, 10% to 18% solvent B; from 55 to 65 min, increase from 18% to 38% of solvent B; from 65 to 75 min increase up to 65% of solvent B; from 75 to 80 min increase to 80% of solvent B. Post time was 10 min. The flow rate was set at 0.8 mL/min and temperature column was 25 ºC. Onion skin extracts were filtered (0.45 μm pore size; Filtros Anoia S.A., Spain) before injection. Samples were injected in duplicate, being 10 μL de injection volume. Three wavelengths were simultaneously used for sample characterization: 280 nm, 330 nm and 370 nm. ChemStation software (version A.06.03[509]) was employed to collect and analyze the chromatographic data delivered by the diode array detector and our own library was used to identify the different polyphenols by comparing retention times and UV spectra with those of standards.

Total Quercetin Equivalent *(Total QCE)* was calculated as the sum of QC, QC4', QC3 and QC3,4' expressed in mg quercetin equivalents (QCE)/g DOSW, according to Eq. (3):

$$\text{Total QCE}\left({}^{\text{mg}}/_{\text{g DOSW}}\right)=\text{QC}+\text{QC4}'\cdot\frac{M_W\text{QC}}{M_W\text{QC4}'}+\text{QC3}\cdot\frac{M_W\text{QC}}{M_W\text{QC3}}+\text{QC3,4}'\cdot\frac{M_W\text{QC}}{M_W\text{QC3,4}'} \quad (3)$$

where QC, QC4', QC3 and QC3,4' are the contents in OSW expressed in mg/g DOSW and M_w refers to the molecular weight of the respective flavonoids.

To determine the individual flavonoids of the FDE, a solution of 1 mg/mL of the FDE in 70% ethanol (v/v) was used, and results were expressed as mg flavonoid/g FDE.

2.5.3.2. Identification of soluble and structural carbohydrates in FDE

Identification and quantification of soluble carbohydrate in FDE was performed by HPLC-RID Agilent 1260 with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Laboratories, Inc., U.S.A.) using H_2SO_4 10 mM as mobile phase with a flow rate of 0.6 mL/min. The column and detector were maintained at 40 °C. Pure soluble carbohydrates were used for calibration (xylose, galactose, arabinose, glucose, sucrose and mannitol were purchased from Sigma-Aldrich). Results were expressed as g carbohydrate/100 g FDE.

The determination of structural carbohydrates and lignin was carried out according to the National Renewable Energy Laboratory (NREL) protocol NREL/TP-510-42618 for the determination of structural carbohydrates and lignin in biomass

2.6. Statistical analysis

All the statistical calculations were done using Statgraphics 18-X64. Optimization of the UAE process was based on the analysis of variance (ANOVA) with 95% confidence level, for each response variable in order to test the model significance and suitability.

The significance of the differences was determined based on an analysis of the variance with the Fisher's least significant difference (LSD) procedure at p-value ≤0.05. The correlation between different responses used in this work, was carried out using the Pearson product moment correlations at a 95% confidence level.

3. Results and discussion

3.1. Conventional Extraction Experiments

3.1.1. Effect of solvent and temperature

The amount of ethanol used in the mixture increased the TPC, TFC and AA results, up to a maximum (70% ethanol), decreasing when using higher concentration of ethanol; this trend was similar for both 37 and 50 ºC extractions (Table 2). Therefore, ethanol 70% (v/v) was chosen. When ethanol 70% (v/v) was used, the effect of the extraction temperature on the response variables was studied in detail. According to the results presented in Table 2, an increase in temperature resulted in lower values of TPC, TFC and AA (at a 95% confidence level, LSD test), therefore 37 ºC was chosen as the optimal temperature. The effect of the solvent on the flavonoids profile will be studied in detail (section 3.1.3.) in order to support these findings.

Table 2. Experimental results for the conventional extraction experiments

	TPC (mg GAE/g DOSW)		**TFC (mg QE/g DOSW)**		**AA (mg $FeSO_4$/g DOSW)**	
Solvent (% Ethanol)	**37 ºC**	**50 ºC**	**37 ºC**	**50 ºC**	**37 ºC**	**50 ºC**
0	9.8 ± 0.3^{A}	7.8 ± 0.1^{A}	1.7 ± 0.1^{A}	1.2 ± 0.1^{A}	12.3 ± 2.1^{A}	12.9 ± 0.1^{A}
25	15.9 ± 0.1^{B}	17.7 ± 0.3^{B}	5.9 ± 0.1^{B}	6.9 ± 0.2^{B}	26.7 ± 0.5^{B}	31.6 ± 0.6^{B}
50	41.8 ± 0.4^{E}	37.4 ± 0.9^{D}	19.4 ± 0.2^{E}	20.2 ± 0.3^{F}	63.0 ± 0.8^{E}	64.6 ± 1.1^{E}
70	$46.7\pm1.4^{G,b}$	$42.1\pm0.4^{E,a}$	$20.7\pm0.4^{F,a}$	$21.1\pm0.5^{F,a}$	$75.3\pm1.1^{F,b}$	$69.7\pm1.4^{F,a}$
80	$44.5\pm0.2^{F,b}$	$42.3\pm0.2^{E,a}$	$21.8\pm0.3^{G,b}$	$18.8\pm0.3^{E,a}$	$73.2\pm3.5^{F,a}$	$66.6\pm0.7^{F,a}$
90	33.6 ± 0.1^{C}	37.1 ± 0.5^{D}	16.0 ± 0.1^{D}	14.7 ± 1.1^{D}	58.8 ± 0.8^{D}	61.9 ± 0.7^{D}
100	21.6 ± 0.3^{D}	23.8 ± 0.2^{C}	11.5 ± 0.4^{C}	12.4 ± 0.2^{C}	36.3 ± 0.1^{C}	40.8 ± 0.1^{C}

**Different capital letters indicate there is statistically significant effect (95% confidence level, LSD test) of solvent used in the response studied for each temperature.*

***Different small letters indicate there is statistically significant effect (95% confidence level, LSD test) of the temperature on the studied response at a given concentration of ethanol (70 or 80%).*

The Pearson product moment correlations between each pair of variables presented in Table 2 (TPC-TFC; TPC-AA; TFC-AA) at both temperatures (37 and 50 ºC) was calculated using Statgraphics 18-X64. It showed strong linear relationship between the variables studied, at a 95% confidence level (p-values were zero in all cases; R^2 was in the range 0.94-0.99): increases in TPC or TFC mean increases in AA. These correlations were observed at both temperatures.

3.1.2. Extraction kinetics

In the conditions found as optimal (37 ºC and 70% ethanol, v/v) a longer extraction experiment was run, taking samples periodically in order to validate the flavonoids extraction kinetics. Results are presented in Figure 1 where it is possible to see that the extraction is fast, since after

12 min, around 80% of the total flavonoids have already been extracted. After 40 min, the extraction of flavonoids reaches a plateau at approximately 21 mg QE/g DOSW.

< Insert Fig. 1 >

Other studies on the extraction of bioactive compounds from onion skins reported different results for TPC and TFC, but in all cases, the best results were obtained when ethanol/water mixtures were used. (Kiassos et al., 2009) optimized the extraction of phenolic compounds from onion skin wastes (500 mg OSW in 20 mL of solvent), concluding that the optimal conditions were 60% aqueous ethanol, pH = 2 and 4.2 h of extraction time. Under these experimental conditions, the estimated TPC was 93.4±1.4 mg GAE/g onion. (Sharma et al., 2015) extracted phenolic compounds from 6 varieties of onion (1 red-skinned, 3 yellow-skinned and 2 white-skinned), using 1 g of sample and 20 mL of ethanol (70%, v/v) in a multi-step extraction, in which the whole bulb was used and not only the outermost layers. Important differences among the samples were observed. The best results were observed for a yellow-skinned variety, that provided a TPC of 55 mg GAE/g onion, TFC of 2.8 mg QE/g onion and antioxidant activity (determined using the FRAP assay) of 32.5 μmol Trolox equivalent/g onion. (Sagar et al., 2020) performed the extraction of flavonoids and other bioactive compounds from the skins of 15 different varieties of onion. The extraction was carried out using methanol in a ratio 1:25 (g onion:mL methanol). The TPC ranged from 14.55±0.41 to 288.74±1.27 mg GAE/g onion and the TFC was in the range 1.31±0.32 to 168.77±0.87 mg QC/g onion.

3.1.3. Individual flavonoid content

Figure 2 shows the composition of the conventional extraction experiments in terms of concentration quercetin and its derivatives, whereas in Table 3, the concentration of other phenolic compounds found in the extracts is presented.

< Insert Fig. 2 >

Regarding the quercetin profile of the extracts, similar trends can be observed for extractions done at 37 and 50 ºC: the amount of QC and derivatives increased up to a maximum when 70-80% ethanol was used, but the amount of compounds recovered at 50 ºC is slightly lower. It is possible to see that QC4' and QC are the main extracted compounds (at 37 ºC the concentration of these two compounds are 9.8±0.3 mg/g DOSW and 6.6±0.2 mg/g DOSW, respectively). Other quercetin glucosides, such as QC3,4' and QC3 had a concentration of 2.04±0.03 mg/g DOSW and 0.21±0.03 mg/g DOSW, respectively. In total, the sum of quercetin and its derivatives was 18.6±0.5 mg/g DOSW, yielding a total quercetin equivalent of 14.1±0.4 mg/g DOWS (Table 3). At 50 ºC the highest concentration for the sum of quercetin and derivatives was observed when ethanol 80%, v/v was used: 15.6±0.9 mg/g DOSW, almost

20% lower than the best result obtained at 37 ºC and total quercetin equivalent was 12.0±0.6 mg/g DOSW. The results presented in our work are in agreement with (Sharma et al., 2015) who identified QC, QC4', QC3,4' and isorhamnetin-3-O-glucoside, as the major flavonoids in onion. However, the QC and QC derivatives profile was different from that obtained in our work. (Jang et al., 2013) extracted 5.24±0.18 mg QE/g onion waste in an conventional extraction process with an ethanol/water mixture (59%, v/v) at 49 ºC for 35 min. (Jin et al., 2011) extracted 3.7±0.2 mg of quercetin/g OSW (16.5 min, 59.2 ºC and 40 mL of ethanol/water mixture 59.3%, v/v). (Khiari et al., 2008) showed that an ethanol/water mixture (60%, v/v) would lead to the highest recovery of flavonols from onion skin waste, when containing 0.1% of HCl (3 g OSW, 30 mL of solvent, stirring rate of 1250 rpm, 46 ºC for 24 h). Under those conditions total flavonols (sum of QC, QC4' and QC3,4') were 35.7±5.0 mg QE/g onion. It is difficult to compare the results among authors, because the content of quercetin and other bioactive compounds depends on several factors, such as cultivar, conditions during cultivation, the way the onion is stored and processed (Lu et al., 2011), or the layer of the onion used in the extraction process (Beesk et al., 2010).

Regarding other flavonoids extracted, kaempferol was only detected when the ethanol concentration was above 70%, with a maximum of 0.22±0.01 mg/g DOSW when 90% of ethanol was used at 37 ºC. Similar results were obtained for isorhamnetin (maximum, 0.26±0.02 mg/g DOSW; 80% ethanol at 37 ºC) which was detected when the concentration of ethanol was above 70%. The extraction of protocatechuic acid was favored at the lower ethanol concentration. In this sense, the highest concentration of this compound was detected when pure water was used (0.77±0.03 mg/g DOSW), in contrast to the result obtained when pure ethanol was used (0.07±0.01 mg/g DOSW). At 50 ºC, no significant effect of the amount of ethanol (20 to 90%) used on protocatechuic acid extraction was observed, being in the range 0.52-0.62 mg/g DOSW, but when absolute ethanol was used as solvent, a sharp decrease for protocatechuic acid was observed down to 0.18±0.02 mg/g DOSW. Other phenolics detected were myricetin (ethanol 70%, v/v: 0.52±0.09 mg/g DOSW, at 37 ºC), p-hydroxybenzoic acid (0.04-0.23 mg/g DOSW at 37 ºC), p-coumaric acid (up to 1.2 mg/g DOSW), isorhamnetin-3-glucoside and vanillic acid in lower amounts.

Table 3. Composition of the extracts as a function of the ethanol content at two different temperatures. Extraction time, 60 min.

		Concentration (mg/g DOSW)								
	Ethanol (%)	**Total QCs***	**Total QCE****	**Kaempferol**	**Isorhamnetin**	**Myricetin**	**Protocatechuic Acid**	**p-Hydroxybenzoic Acid**	**Coumaric Acid**	**Vanillic Acid**
	0	1.5 ± 0.1^{A}	0.88 ± 0.03^{A}	ND	ND	0.06 ± 0.01^{A}	0.77 ± 0.03^{E}	0.06 ± 0.01^{A}	0.52 ± 0.03^{B}	0.017 ± 0.001^{A}
	20	4.4 ± 0.2^{B}	3.1 ± 0.1^{B}	ND	ND	0.11 ± 0.01^{A}	0.39 ± 0.02^{B}	0.04 ± 0.01^{A}	0.76 ± 0.04^{D}	0.012 ± 0.002^{A}
	50	17.3 ± 0.4^{D}	13.1 ± 0.3^{E}	ND	ND	0.51 ± 0.10^{C}	0.56 ± 0.01^{D}	0.23 ± 0.02^{B}	1.2 ± 0.1^{E}	0.017 ± 0.001^{A}
37 ºC	**70**	18.6 ± 0.5^{E}	14.1 ± 0.4^{F}	0.09 ± 0.01^{A}	0.26 ± 0.02^{C}	0.52 ± 0.09^{C}	0.58 ± 0.02^{D}	0.22 ± 0.03^{B}	1.2 ± 0.1^{E}	0.016 ± 0.005^{A}
	80	17.4 ± 0.3^{D}	13.3 ± 0.2^{E}	0.10 ± 0.03^{A}	0.26 ± 0.02^{C}	0.50 ± 0.10^{C}	0.57 ± 0.02^{D}	0.23 ± 0.03^{B}	1.1 ± 0.1^{E}	ND
	90	12.8 ± 0.4^{C}	10.1 ± 0.2^{D}	0.22 ± 0.01^{B}	0.19 ± 0.01^{B}	0.29 ± 0.03^{B}	0.48 ± 0.06^{C}	0.06 ± 0.01^{A}	0.63 ± 0.09^{C}	ND
	100	4.9 ± 0.2^{B}	4.0 ± 0.1^{C}	0.14 ± 0.02^{B}	0.07 ± 0.01^{A}	0.11 ± 0.02^{A}	0.07 ± 0.01^{A}	ND	0.11 ± 0.01^{A}	ND
	0	0.94 ± 0.03^{A}	0.51 ± 0.02^{A}	ND	ND	0.04 ± 0.01^{A}	0.62 ± 0.03^{D}	0.04 ± 0.01^{A}	0.38 ± 0.02^{B}	0.013 ± 0.001^{A}
	20	4.8 ± 0.2^{B}	3.3 ± 0.1^{B}	0.03 ± 0.01^{A}	0.12 ± 0.02^{A}	0.15 ± 0.03^{B}	0.45 ± 0.02^{B}	ND	0.73 ± 0.01^{C}	0.006 ± 0.001^{B}
	50	12.8 ± 0.7^{D}	9.7 ± 0.6^{D}	0.03 ± 0.01^{A}	0.20 ± 0.01^{B}	$0.35\pm0.03^{C,D}$	$0.49\pm0.01^{B,C}$	0.09 ± 0.04^{A}	$1.01\pm0.03^{D,E}$	ND
50 ºC	**70**	14.8 ± 0.5^{E}	11.2 ± 0.4^{E}	0.03 ± 0.03^{A}	0.15 ± 0.01^{A}	$0.36\pm0.03^{C,D}$	$0.49\pm0.01^{B,C}$	ND	$1.00\pm0.01^{D,E}$	ND
	80	15.6 ± 0.9^{E}	12.0 ± 0.6^{E}	0.16 ± 0.06^{B}	0.23 ± 0.02^{C}	0.39 ± 0.03^{D}	0.53 ± 0.01^{C}	ND	1.10 ± 0.01^{E}	ND
	90	13.0 ± 0.7^{D}	10.1 ± 0.5^{D}	0.24 ± 0.02^{C}	0.20 ± 0.02^{B}	0.31 ± 0.02^{C}	$0.52\pm0.01^{B,C}$	ND	0.9 ± 0.1^{D}	ND
	100	10.0 ± 0.3^{C}	8.2 ± 0.2^{C}	$0.22\pm0.05^{B,C}$	0.14 ± 0.01^{A}	0.19 ± 0.03^{B}	0.18 ± 0.02^{A}	ND	0.19 ± 0.01^{A}	ND

The same capital letters indicate there is no statistically significant difference (95% confidence level, LSD test) of the solvent used in the response studied for each temperature

ND: Not detected

* Total QCs means the sum of QC, QC4', QC3 and QC3,4'

** Total QCE means total quercetin equivalents, calculated according to Eq. (3)

3.2. Ultrasound Assisted Extraction

3.2.1. Extraction kinetics

In total, five different amplitudes of oscillation were tested, being observed an important effect on the extraction yield: in general, an increase in the amplitude of oscillation involved an increase in extraction yield of TPC over time, as shown in Figure 3a. This Figure also reveals that the extraction is very fast since after only 1.5 min, 80% of the total flavonoids recovered are extracted.

< Insert Fig. 3 >

The amplitude of oscillation is a critical parameter affecting the sonication since it directly affects the ultrasonic intensity, which is defined as the energy transmitted per second and per area unit of the ultrasonic emitting surface (Chemat et al., 2017). In general, higher amplitudes of oscillation increase the cavitation intensity, increasing the contact area between solid matrix and solvent and favoring the penetration of the solvent into the solid matrix (Goula et al., 2017). However, the selection of the amplitude of oscillation has to be carefully done for two reasons. First, there is a threshold value of ultrasonic intensity to achieve cavitation. Second, too high amplitudes induce a rapid deterioration of the ultrasonic probe (which results in liquid agitation instead of cavitation and poor transmission of the ultrasound through the liquid media), or the degradation of the extracted compounds, reducing in both cases the overall extraction yield (Chemat et al., 2017; Tiwari, 2015). According to Figure 3a, 20% amplitude did not produce enough sonication intensity since the extraction of flavonoids was poor. In turn, higher amplitudes did increase the extraction yield, up to a maximum value close to 23 mg QE/g DOSW. In the specific case of the food industry, the amplitude of oscillation is usually optimized in order to maximize the extraction rate using the minimum power (Chemat et al., 2017). Therefore, if the results of the extraction kinetics of flavonoids are presented versus the specific energy used (expressed as kJ/g DOSW), as presented in Figure 3b, it is possible to see that specific energies above 10 kJ/g DOSW did not involve an increase of the extraction yield.

3.2.2. Optimization of the extraction conditions

Results of the central composite design used to optimize the extraction of flavonoids from onion skin are shown in Table 4. In this Table, the total phenolics content measured for each experiment is also presented.

Table 4. Results of the central composite design for the optimization of the ultrasound assisted extraction conditions

run	time (min)	Amplitude (%)	Solvent-to-solid ratio (mL/g OSW)	Total Energy (kJ)	Specific Energy (kJ/g OSW)	Specific Power (W/g OSW)	TPC (mg GAE/g DOSW)	TFC (mg QE/g DOSW)
1	1	40	10	1.49	0.25	4.14	7.8±0.8	15.5±0.3
2	5	40	10	3.07	0.51	1.70	10.1±0.3	17.0±0.2
3	1	80	10	3.23	0.54	8.98	6.5±0.3	18.3±0.3
4	5	80	10	16.27	2.71	9.03	6.7±0.2	18.8±0.2
5	1	40	30	0.66	0.33	5.52	58±4	21.0±0.2
6	5	40	30	3.24	1.62	5.36	73.3±1.8	23.8±0.1
7	1	80	30	3.18	1.59	26.29	61±2	22.7±0.1
8	5	80	30	15.95	7.98	26.47	102±5	23.3±0.3
9	1	60	20	3.60	1.20	19.80	34.8±1.7	16.9±0.2
10	5	60	20	14.08	4.69	15.61	59±2	21.2±0.3
11	3	26.4	20	1.48	0.50	2.72	51±3	20.6±0.4
12	3	93.6	20	16.66	5.55	30.61	56±1	21.1±0.3
13	3	60	3.18	1.53	0.08	0.45	4.6±0.9	7.7±0.1
14	3	60	36.82	7.96	4.88	27.60	65±2	22.5±0.1
15	3	60	20	7.98	2.66	14.76	54.2±1.2	20.3±0.1
16	3	60	20	7.88	2.63	14.59	53.7±1.8	20.4±0.2
17	3	60	20	8.35	2.78	15.36	55.4±1.7	20.7±0.2

The TFC ranged from 7.7±0.1 (experiment 13) >23.8 mg QE/g DOSW (experiments 6 and 8). Experiment 13 corresponded to the highest load of OSW, which was too high so the minimum conditions to reach cavitation were not obtained. It has to be considered that according to the extraction theory, high loads of solid reduce the driving force, reducing the extraction capacity of the solvent. The opposite phenomenon was observed in experiment 6, where a high solvent-to-solid ratio (30 mL/g DOSW) was used for 5 min and using low amplitude (40%). These experimental conditions allowed to extract significant amount of TPC (73.3±1.8 mg GAE/g DOSW), which were further increased to 102.1±5.1 mg GAE/g DOSW in experiment 8 (the only difference with experiment 6 was that amplitude was increased to 80%). Again, the lowest value of TPC was found in experiment 13 (4.6±0.9 mg GAE/g DOSW). The Pearson product moment correlations between TPC-TFC calculated using Statgraphics, showed a linear relationship between the variables studied, at a 95% confidence level (p-values in all the cases was 0), but compared to the conventional extraction process, in the UAE the correlation was not as strong (R^2 was 0.81). Despite the use of the same solvent, in most of the UAE experiments the TFC content was below the value (20.7±0.4 mg/g DOSW) obtained in the conventional extraction (same temperature and solvent). This can be due to the fact that probably the selection of another ethanol/water mixture would favor the extraction in those specific conditions of ultrasonic power (amplitude of oscillation), because physical properties of the solvent (viscosity, surface tension or vapor pressure) affect the acoustic cavitation and the cavitation threshold (Chemat et al., 2017; Tiwari, 2015).

The analysis of variance (Table 5, ANOVA) for the TFC, revealed that only the solvent-to-solid ratio had an effect on the extraction of flavonoids from onion skin wastes. Results revealed good fitting between the experimental results and the polynomial model (R^2=0.92). In Figure 4, a surface plot of the TFC extracted as a function of the extraction time and solvent-to-solid ratio is shown, when amplitude of oscillation equal to 80% is used. The importance of the solvent-to-solid ratio on the flavonoids extraction is shown.

Table 5. ANOVA results for the extraction of flavonoids (TFC) from onion skin wastes using ultrasounds

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:time	6.20944	1	6.20944	2.13	0.1874
B:amplitude	7.42173	1	7.42173	2.55	0.1542
C:solvent-to-solid-ratio	179.969	1	179.969	61.87	0.0001
AA	0.965442	1	0.965442	0.33	0.5826
AB	0.0968	1	0.0968	0.03	0.8604
AC	1.7298	1	1.7298	0.59	0.4658
BB	2.23716	1	2.23716	0.77	0.4096
BC	5.95125	1	5.95125	2.05	0.1957
CC	31.3829	1	31.3829	10.79	0.0134
Total error	20.3618	7	2.90883		
Total (corr.)	262.208	16			

< Insert Fig. 4 >

According to the results of the ANOVA study presented in Table 5, the solvent-to-solid ratio was critical for the final performance of the UAE of flavonoids from onion skin wastes. These observed results, the higher the solvent-to-solid ratio the higher the extraction yield, are in agreement with the mass transfer theory, which indicates that high solvent-to-solid ratio results in larger concentration gradient during the diffusion from the solid into the solvent. According to those results, small loads of solid are required to maximize the extraction yield, which leads to higher specific energies (kJ/ g raw material). Small loads of solid allow the ultrasonic wave to easily distribute through the solvent and there is lower resistance to the transference of the ultrasonic wave, so the mass transfer effect induced by the ultrasound is enhanced when small loads of raw materials are used. The optimization of the working conditions suggest that amplitudes in the low end of the studied range (40%), long times (around 5 min) and high solvent-to-solid ratios are needed to maximize the extraction of flavonoids from onion skin wastes.

Other studies investigating solvent-to-solid ratio when using UAE to recover phenolics from natural matrices are limited. For instance (Prasad et al., 2011) studied the effect of this parameter on the extraction of phenolic compounds from *Mangifera pajang* Kosterm peels in the range 20 to 50 mL/g raw material, finding the optimal in 32.7 mL/g. In the specific case of the use of ultrasounds to extract flavonoids from onion, (Jang et al., 2013) tried liquid to solid ratios (30-60 mL/g) in an ultrasonic bath. These authors found that solvent-to-solid ratio did not influence the extraction of quercetin form onion solid wastes; only temperature and solvent had effect on the extraction of quercetin (59% ethanol at 49 ºC, were the optimal conditions, setting the solvent-to-solid ratio at 60 mL/g). Other authors studied the recovery of flavonoids from onion using fixed solvent-to-solid ratios. For instance, (Jin et al., 2011) selected a ratio 40 mL/g

of onion skin to study the extraction of quercetin. They used an ultrasonic probe to study the effect of sonication time (9-25 min), ultrasound power (225-525 W) and ethanol concentration (25-81%) on the extractability of quercetin from onion skin. These authors found that ethanol concentration and, more especially, the ultrasonic power had statistically significant effect on the extraction of quercetin, but not the extraction time. The optimal conditions they proposed were 43.8% ethanol with ultrasound treatment using 606.4 W of power during 21.7 min. They also demonstrated that UAE led to higher extraction yield of quercetin (4.1 mg quercetin/g) compared to the conventional extraction process (10% increase, at lower temperature). (Kumar et al., 2014) performed the extraction in an ultrasonic bath (135 W) at a constant ratio of 6 mL solvent/g onion skin. Sonication times were in the range from 20 to 60 min, and did not have any effect on the extraction of quercetin, probably because, as shown in our work, the extraction of flavonoids is very fast and happens within the very first 20 min of extraction. These authors also demonstrated that pH did not affect the extraction of quercetin and that the extraction of quercetin was favoured when using microwaves (60-150 s) rather than ultrasounds. (Kwak et al., 2017) used a ratio equal to 250 mL solvent/g onion when studying the extraction of quercetin glycoside derivatives from three onion varieties. It seems that the effect of this parameter strongly depends on the matrix properties and the way it interacts with the solvent in the presence of the ultrasonic wave.

3.2.3. Individual flavonoids content

Table 6 presents the phenolics profile of the extracts obtained by UAE. It is possible to see that in all the conditions tried, QC4' and QC were the two major flavonoids extracted. Experiment 6 (5 min, 40% amplitude and 30 mL/g DOSW) provided the experimental conditions that led to the highest phenolics recovery; QC was 7.4±0.1 mg/g DOSW and QC4' was 10.4±0.1 mg/g DOSW; the total quercetin equivalent calculated according to Eq. (1) resulted to be 15.5±0.2 mg/g DOSW. The extraction of individual flavonoids compounds was specially affected by the solvent-to-solid ratio in agreement with the results obtained and already explained for the TFC (Table 4). The composition of the best extract obtained by UAE (experiment 6) was similar to that obtained by conventional extraction at 37 ºC using ethanol (70%, v/v). The advantage of the UAE is a reduction of the extraction time and an increase in the overall extraction yield of flavonoids.

Other authors that used UAE to extract quercetin and derivatives from onion skin wastes reported different results. (Jin et al., 2011) concluded that ultrasonic power increased the quercetin extraction yield by 10%, being 4.09±0.29 mg QC/g in the optimal conditions; (Jang et al., 2013) found that the yield of total quercetin equivalent was 11.08 mg/g DOSW in the optimal conditions; (Campone et al., 2018) reported an extraction yield of 93±7 mg/g of extract; (Kwak et al., 2017), tried different onion cultivars and analysed the effect of the part of the

union used for the extraction and found that the outermost layer of the onion showed the highest content of quercetin and quercetin derivatives: QC4' was the most abundant quercetin (41.39 mg/g onion) followed by QC7,4' (17.31 mg/g onion), QC3 (7.04 mg/g onion) and QC (0.59 mg/g onion) for red onion. Interestingly, these authors reported that quercetin glucosides are more soluble in water but tend to decompose during ultrasonic/microwave treatment.

According to the results presented in Table 6, it is possible to see that the profile of the phenolic compounds extracted does not vary with the extraction conditions, since in all the cases QC4' is the most abundant compound followed by QC. A small difference in the ratio QC4'/QC is detected as a function of the extraction method used: in the optimal conditions found for the conventional extraction process, this ratio was 1.48±0.05, whereas in the UAE process (experiment 6) was 1.41±0.02. The statistical analysis of the ratios revealed that QC4'/QC ratio for both extraction methods are homogenous groups, and there is not effect of the extraction method on the ratio QC4'/QC at al 95% confidence level according to the Fisher's least significant difference (LSD) test. In general, it has been demonstrated that UAE promoted the extraction of phenolics, and more specifically flavonoids.

Table 6. Composition of the extracts obtained in the CCD for the UAE

	Concentration (mg/g DOSW)											
Run	**QC**	**QC4'**	**QC3**	**QC3,4'**	**Kaempferol**	**Isorhamnetin**	**Myricetin**	**Protocatechuic acid**	**Hydroxybenzoic acid**	**p-Coumaric acid**	**Vanillic acid**	**Total QCE***
1	5.2±0.3	7.6±0.3	0.15±0.02	1.9±0.3	0.145±0.013	0.12±0.01	0.36±0.026	0.60±0.02	0.08±0.05	0.86±0.04	0.013±0.002	11.1±1.1
2	4.7±0.1	7.5±0.3	0.13±0.01	1.8±0.2	0.152±0.013	0.13±0.02	0.32±0.009	0.57±0.02	0.08±0.01	0.87±0.01	0.015±0.002	10.5±0.6
3	5.2±0.2	7.7±0.1	0.15±0.02	1.9±0.1	0.14±0.01	0.14±0.02	0.37±0.039	0.61±0.02	0.11±0.04	0.91±0.01	ND	11.2±0.5
4	5.7±0.1	8.0±0.2	0.15±0.03	1.9±0.1	0.127±0.004	0.14±0.02	0.37±0.042	0.62±0.01	0.15±0.02	0.93±0.04	ND	11.9±0.4
5	6.9±0.1	10.0±0.1	0.17±0.01	2.3±0.2	0.179±0.001	0.18±0.04	0.50±0.069	0.76±0.04	ND	1.08±0.13	ND	14.6±0.4
6	7.4±0.1	10.4±0.2	0.12±0.03	2.4±0.1	0.175±0.003	0.15±0.01	0.52±0.070	0.79±0.04	ND	1.13±0.14	ND	15.5±0.5
7	7.2±0.1	10.1±0.1	0.17±0.02	2.3±0.1	0.179±0.002	0.17±0.01	0.46±0.010	0.74±0.02	0.13±0.041	1.20±0.05	ND	14.9±0.3
8	7.2±0.1	10.1±0.1	0.17±0.03	2.3±0.2	0.201±0.005	0.16±0.01	0.51±0.067	0.76±0.03	ND	1.09±0.14	ND	15.0±0.4
9	6.1±0.2	9.5±0.2	0.16±0.01	1.6±0.2	0.11±0.01	0.07±0.00	0.14±0.009	0.16±0.01	ND	0.14±0.01	ND	13.0±0.6
10	6.9±0.2	9.1±0.1	0.19±0.01	2.4±0.1	ND	0.32±0.04	0.457±0.015	0.71±0.01	0.17±0.04	0.99±0.03	0.006±0.002	13.9±0.4
11	6.6±0.1	9.3±0.1	0.19±0.01	2.3±0.1	ND	0.25±0.06	0.462±0.012	0.71±0.01	0.17±0.01	1.05±0.01	ND	13.8±0.3
12	6.5±0.1	8.9±0.2	0.18±0.01	2.2±0.3	ND	0.24±0.05	0.44±0.01	0.68±0.01	0.17±0.02	0.97±0.02	ND	13.5±0.6
13	0.14±0.02	0.25±0.01	ND	0.1±0.0	ND	0.014±0.01	0.04±0.00	0.02±0.00	ND	ND	ND	0.32±0.1
14	6.4±0.4	10.1±0.1	0.31±0.07	2.4±0.1	0.204±0.004	0.36±0.02	0.74±0.13	0.83±0.03	ND	0.76±0.17	ND	14.5±0.5
15	6.8±0.1	9.5±0.1	0.18±0.02	2.3±0.1	0.165±0.004	0.15±0.02	0.47±0.02	0.71±0.01	0.16±0.02	0.97±0.06	ND	14.2±0.3
16	6.8±0.2	9.5±0.1	0.19±0.01	2.3±0.0	0.124±0.002	0.15±0.02	0.47±0.03	0.70±0.01	0.17±0.03	1.01±0.03	ND	14.1±0.4
17	6.8±0.1	9.6±0.2	0.19±0.01	2.3±0.1	0.126±0.008	0.15±0.02	0.47±0.01	0.70±0.01	0.17±0.01	1.03±0.02	ND	14.3±0.4

** Total QCE means total quercetin equivalents, calculated according to Eq. (3)*

3.3. Kinetic modelling. Comparison between CE and UAE processes

From the comparison between the conventional extraction process curve presented in Figure 1 and the UAE process presented in Figure 3a, it is possible to see an important reduction of the extraction time; however, the extraction curve for the conventional and UAE processes have similar trend: very fast initial extraction rate, decreasing to reach a plateau. According to (Chemat et al., 2017), the difference may be due to the ultrasonic capillary effect, phenomenon that refers to the increase of depth and velocity of penetration of liquid into channels and pores under some conditions of sonication. It is also known that sonication favors the solvent absorption by the solid matrix the diffusion of the target compound out of the matrix. In order to compare the extraction kinetics for both extraction techniques, the experimental data were fitted to the Weibull model (Eq. 1), commonly used to model the extraction of bioactive compounds from natural matrices (Alonso-Riaño et al., 2020; Kashaninejad et al., 2020).

The experimental kinetic curves for both extraction processes and the Weibull model calculated for each of them are presented in Figure 5.

< Insert Fig. 5 >

The kinetic parameters of the Weibull model are presented in Table 7. It is possible to see, from the values of the kinetic rate constant (*k*), how the UAE process provides faster extraction of flavonoids from onion skin wastes than conventional extraction process. It is possible to see that in the UAE process, the higher the amplitude the higher the kinetic rate.

Table 7. Kinetic model parameters obtained for the extraction of flavonoids from OSW

	Parameters -Weibull Model			
	A	**k**	**n**	**R^2**
Conventional process	20.330	0.196	0.926	0.996
UAE, 100% amplitude	22.475	1.328	0.485	0.993
UAE, 80% amplitude	20.624	1.193	0.599	0.994
UAE, 60% amplitude	20.201	1.114	0.804	0.989
UAE, 40% amplitude	17.196	0.399	1.153	0.995
UAE, 20% amplitude	1,243	0.001	0.592	0.825

In the UAE when 100% amplitude was used, the 80% of the total extraction was achieved in 1.5 min (relative time 0.125), whereas in the conventional extraction 12 min were required. If the productivity is calculated (to reach the 80% of the total extraction yield) it is possible to see that UAE increases it dramatically, shifting from 1.5 mg QE/(g DOSW · min) in the conventional process to 11.8 mg QE/(g DOSW · min), which leads to an important intensification of the extraction process.

3.4. Freeze dried extract (FDE)

The freeze dried extract (FDE) presented a brownish color and yielded around 9% (w/w) FDE of the dry onion skin waste. The FDE exhibited high antioxidant activity and very high content in phenolics and especially in flavonoids (18%), of which around 6.7% was quercetin and 9.4% QC4'. This extract also contained free sugars, such as glucose (12.8±0.1%), fructose (4.6±0.2%), arabinose (0.35±0.02%) and cellobiose (1.66±0.05%). The protein content of this extract is high (11.3±0.2%).

The FDE was stored for 180 days at three different temperatures. The antioxidant activity of the extract was affected by the storage conditions. The AA of the extract storage at 21 ºC was significantly decreased after 180 days (-20%), whereas at -20 ºC and 4 ºC the decrease was about 10% over the same period of time. These results correlate with the decrease in the TFC content, and the variation observed in the individual flavonoids profile presented in Table 8. Therefore, in order to preserve the excellent antioxidant properties and antioxidants content of this extract, it has to be preserved at low temperature.

Table 8. Stability study of the freeze-dried extract (FDE) obtained from onion skin wastes (cv. Horcal) stored under different temperatures

	Initial	**Storage Conditions (180 days)**		
		-20 ºC	**4 ºC**	**21 ºC**
TFC (g QE/100 g FDE)	21.3 ± 0.2^{A}	18.9 ± 0.4^{B}	18.1 ± 0.3^{B}	15.6 ± 0.3^{C}
FRAP (mg FeSO4/g FDE)	2005 ± 35^{A}	1813 ± 22^{B}	1809 ± 28^{B}	1669 ± 37^{C}
QC	6.7 ± 0.1^{A}	6.4 ± 0.3^{B}	6.3 ± 0.2^{B}	4.9 ± 0.1^{C}
QC4'	9.4 ± 0.2^{A}	9.1 ± 0.2^{B}	8.9 ± 0.4^{B}	7.6 ± 0.4^{C}
QC3,4'	2.2 ± 0.1^{A}	$2.1\pm0.2^{A,B}$	2.2 ± 0.2^{A}	1.7 ± 0.4^{B}
QC3	0.16 ± 0.02^{A}	0.16 ± 0.04^{A}	0.17 ± 0.02^{A}	0.15 ± 0.4^{A}
Myricetin	0.46 ± 0.03^{A}	0.46 ± 0.03^{A}	0.44 ± 0.01^{A}	0.39 ± 0.01^{B}
Kaempferol	0.20 ± 0.02^{A}	$0.16\pm0.03^{A,B}$	$0.18\pm0.03^{A,B}$	0.15 ± 0.01^{B}
Isorhamentin	0.13 ± 0.01^{A}	0.21 ± 0.03^{B}	0.24 ± 0.02^{B}	0.20 ± 0.06^{B}
Protocatechuic Ac.	0.72 ± 0.04^{A}	0.65 ± 0.05^{A}	0.64 ± 0.05^{A}	0.85 ± 0.03^{B}

**Different letters indicate statistically significant differences at confidence level of 95%*

4. Conclusions

In this work, the extraction of flavonoids from onion skin wastes has been studied using two different extraction techniques: conventional and ultrasound assisted. It has been observed that UAE reduces significantly the extraction time (from 1 h to around 5 min) and increases the overall extraction yield of flavonoids from 20.7±0.4 mg/g DOSW in the conventional extraction process to 23.9±0.2 mg/g DOSW. The UAE process has proved the importance of the solvent-to-solid ratio selection in the extraction yield, since small loads of raw material are required to increase the extraction yield. Small loads produce high specific powers, which enhance the mass transfer increasing the extraction yield of flavonoids. The combination of the extraction

parameters has to provide specific energies not higher than 10 kJ/g DOSW to maximize the extraction yield.

Regarding the profile of flavonoids extracted using both techniques, no significant differences were observed, since in both cases the same temperature (37 ºC) and solvent (ethanol 70%, v/v) were used, being QC and QC4' the main compounds extracted. The freeze dried extract resulted to be rich in QC and QC4', providing high antioxidant activity, which was kept for up to 6 months, when stored at temperatures below 4 ºC.

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6. Conflict of interest

The authors declare no Conflict of Interest.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE CAPTIONS

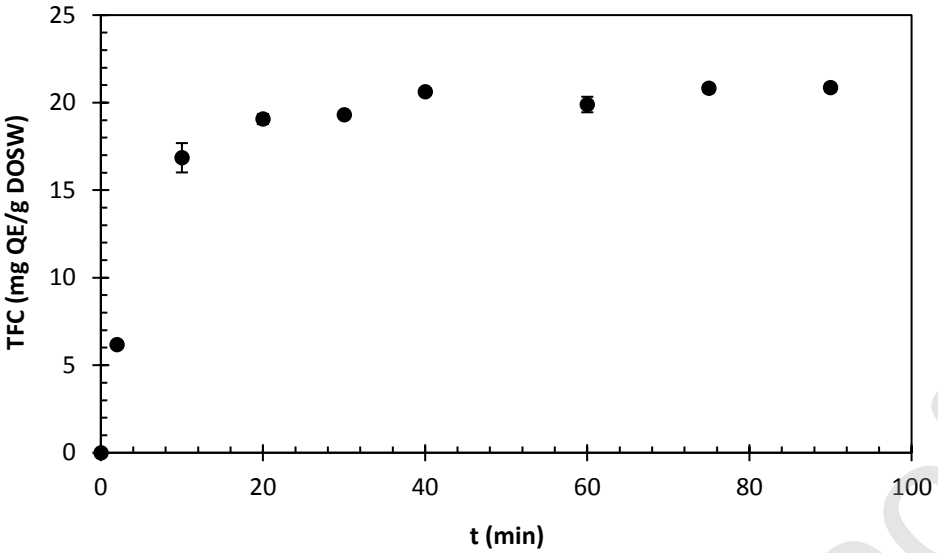
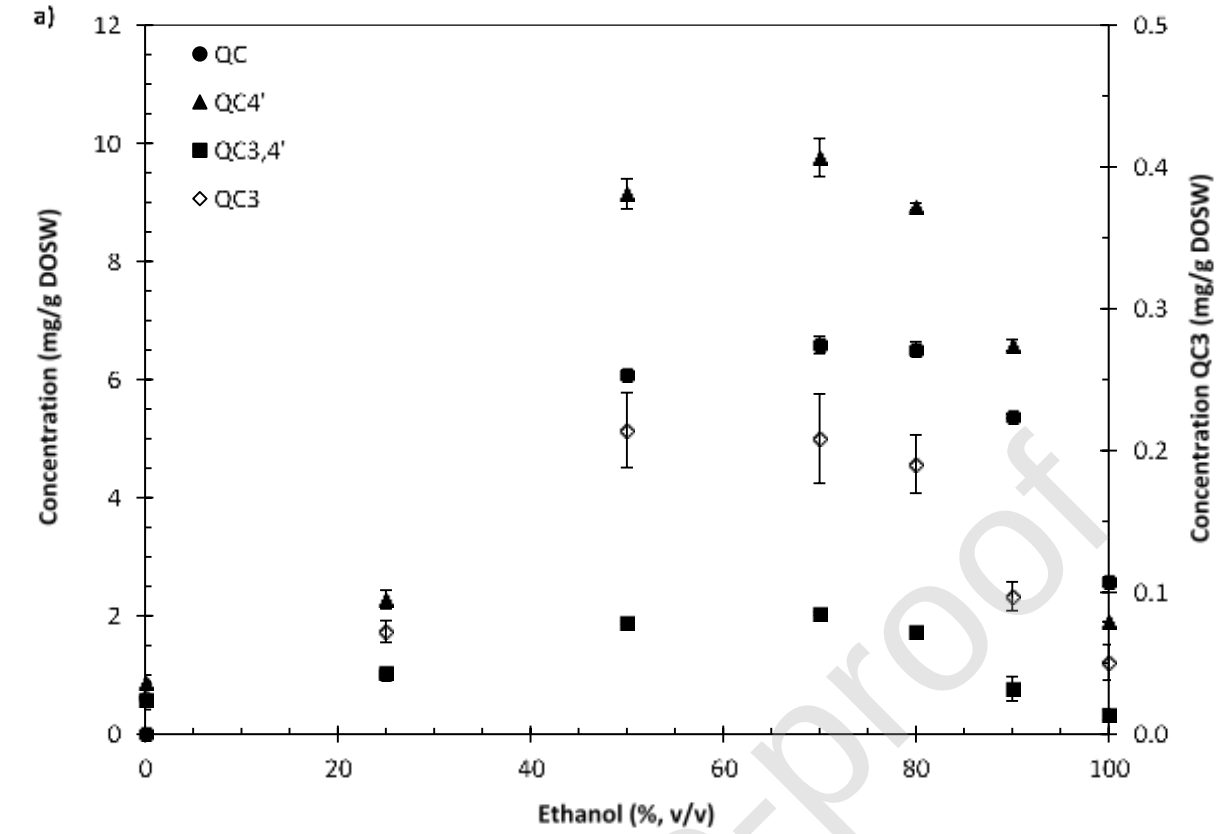


Figure 1. Extraction kinetics of flavonoids from OSW using the conventional extraction process (37 ºC and 70% ethanol, v/v)



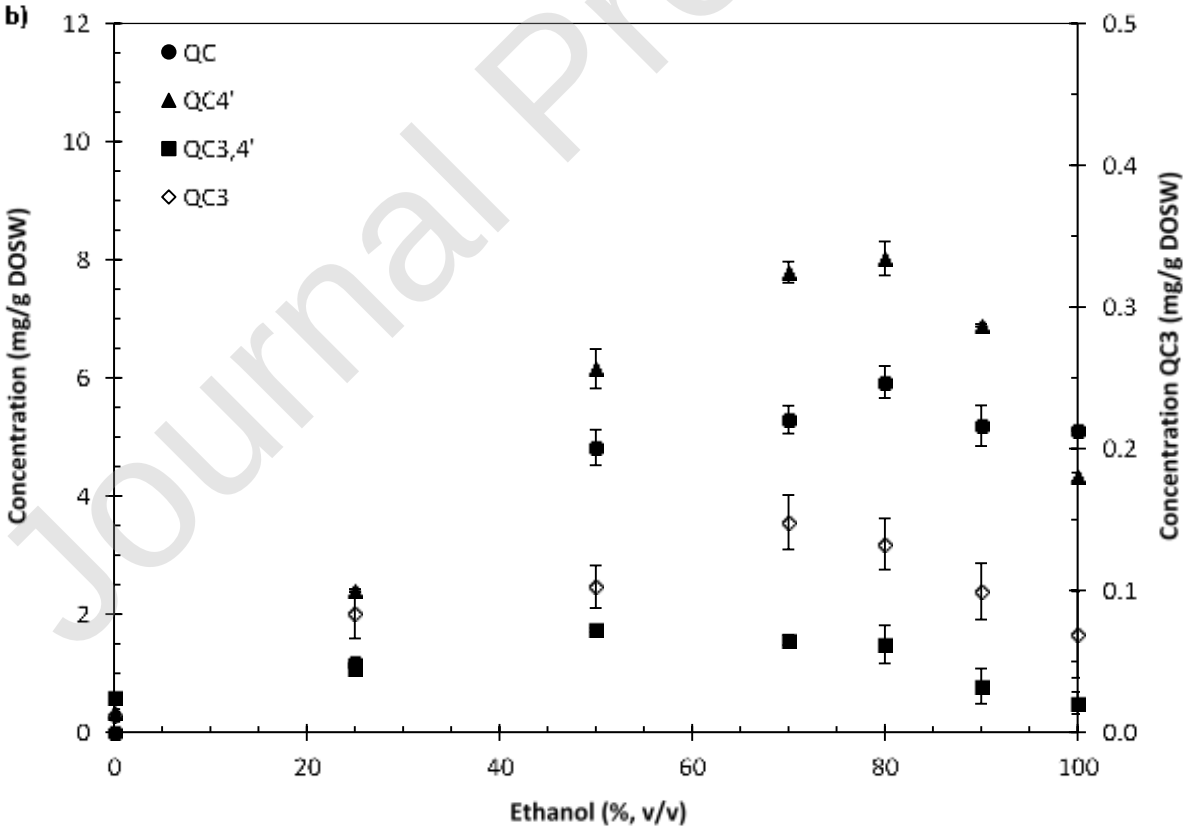


Figure 2. Concentration of quercetin and quercetin derivatives extracted at 37 ºC (a) and 50 ºC (b) using different water/ethanol mixtures. KEY: ●, QC; ▲, QC4'; ■, QC3,4';◇, QC3.

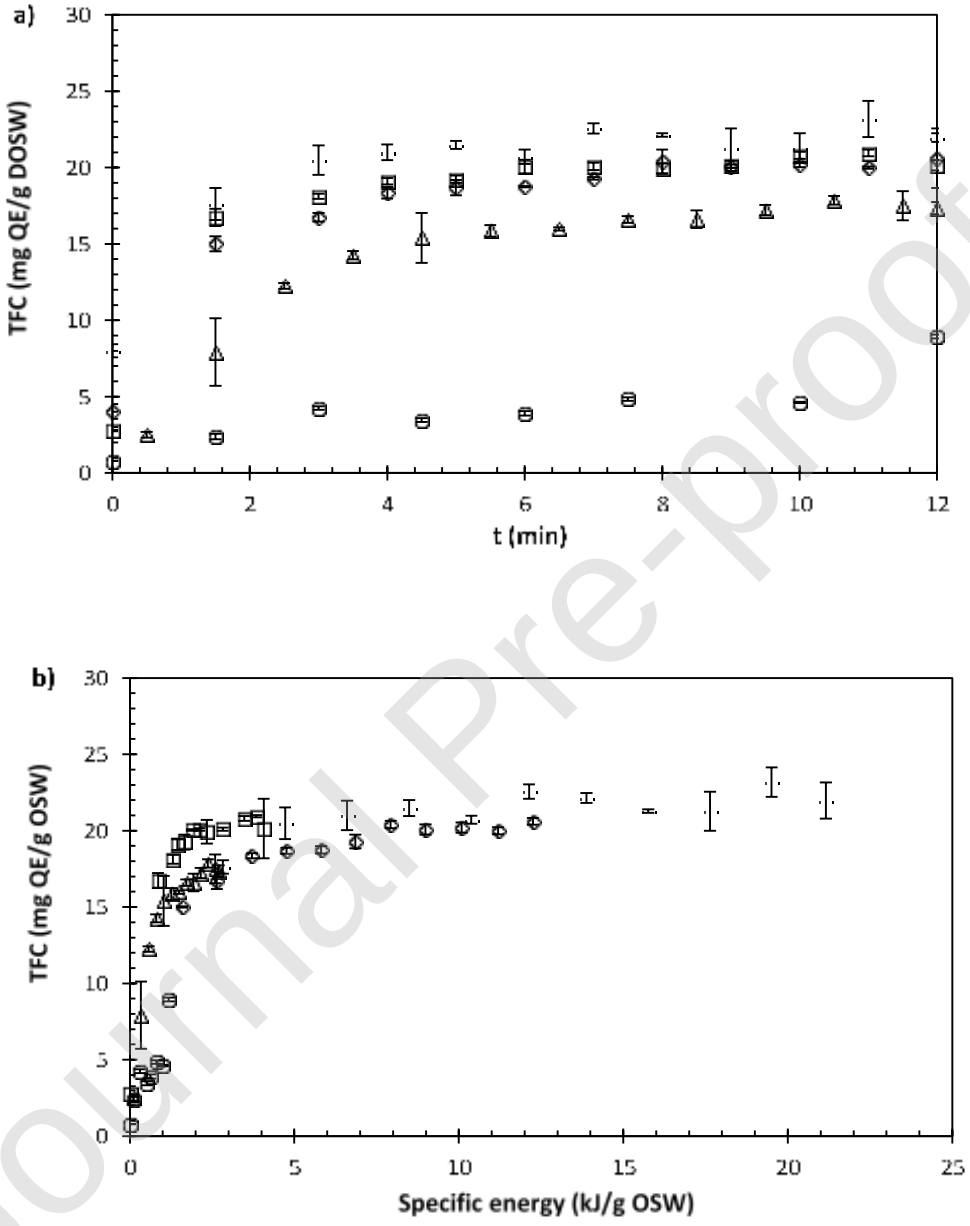


Figure 3. Extraction kinetics of flavonoids from DOSW for five different amplitudes of oscillation (○, 20%; △, 40%; □, 60%; ◇, 80%; –, 100%) expressed as a function of the extraction time (a) and the specific energy consumed (b). Temperature, 37 ºC; solvent, ethanol 70% (v/v).

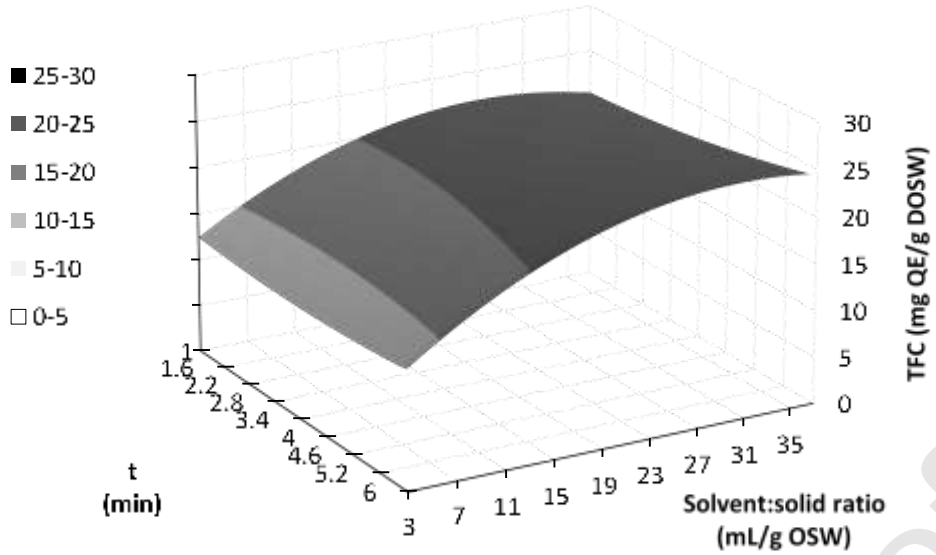


Figure 4. Surface plot for the TFC recovery as a function of the extraction time and the solvent-to-solid ratio, at 80% of amplitude

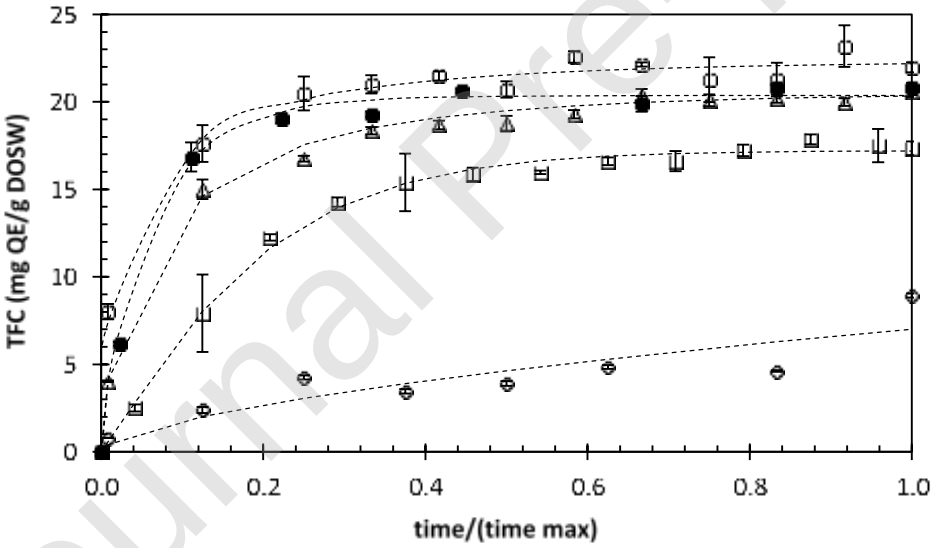


Figure 5. Experimental data for the extraction of flavonoids in a conventional process and ultrasound assisted process as a function of the relative extraction time. Dashed lines represent the kinetic curve obtained by Weibull model. KEY: ●, conventional extraction;○, UAE 100%; △, UAE 80%; □, UAE 40%;◇, UAE, 20%.