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3 1 **EFFECTS OF ACOUSTIC POWER AND pH ON PECTINS-**
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6 2 **ENRICHED EXTRACTS OBTAINED FROM CITRUS BY-**
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9 3 **PRODUCTS. MODELLING OF THE EXTRACTION PROCESS**

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11 4 Running Title: Extraction of pectin from citrus by-products with ultrasonic assistance

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ABSTRACT

BACKGROUND: The ultrasound-assisted extraction of pectins from orange by-products was investigated. Kinetics of mechanical agitation (0.2 xg) and acoustic (US1: 542 and US2: 794 W/L) extractions, were obtained and modelled at different pH values (1.5 and 2.0). All extractions were carried out at 25 °C, using citric acid as the extraction solvent.

RESULTS: Higher pectin extraction yields were obtained with ultrasonic assistance, in comparison with the results obtained using mechanical agitation. Moreover, yield increases were significantly higher using the more acidic pH. Thus, at pH 1.5, pectin yield increased from ~19%, obtained with agitation, to ~47%, applying ultrasounds; whereas, at pH 2.0, this increase was from ~10%, with agitation, to ~18%, applying ultrasounds. A considerable decrease of the galacturonic acid content was observed; when ultrasounds were applied for 60 min under pH 2.0. High methoxyl pectins were extracted at pH 1.5 whereas at pH 2.0, pectins exhibited a low methylation degree. Curves of acoustic and mechanical agitation extractions were properly represented by a second order rate model (average mean relative error $\leq 7.4\%$). The extraction rate constant, initial extraction rate and maximum yield were determined for all experimental conditions.

CONCLUSION: Overall, the results clearly indicated that the effect of ultrasounds was highly dependent on the pH. Therefore, adequate acidic conditions must be applied in order to improve the efficiency of ultrasounds on the pectin extraction process.

KEYWORDS

Pectin; Ultrasound-assisted extraction; Extraction kinetics; Degree of methylation; Carbohydrate composition

ABBREVIATIONS

AG	Mechanical agitation extraction
Ara	Arabinose
CI	Confidence intervals (95%)
Cp	Specific heat capacity of the solvent (J/kg K)
dm	Dry basis
DM	Degree of methylation
Gal	Galactose
GalA	Galacturonic Acid
Glc	Glucose
h	Initial extraction rate (% min ⁻¹)
HG	Homogalacturonans
HMP	High methoxyl pectins
k	Extraction rate constant (% ⁻¹ min ⁻¹).
LMP	Low methoxyl pectins
m	mass (kg)
Man	Mannose
MRE %	Mean relative error (%)
n	Number of samples
P	Ultrasounds power (W)
PI	Prediction limits (95%)
R	ratio of A ₁₇₄₀ over the sum of A ₁₇₄₀ and A ₁₆₃₀
RGI	Rhamnogalacturonan I
RGII	Rhamnogalacturonan II
Rha	Rhamnose
S _{calc}	Standard deviation of the calculated values
S _{exp}	Standard deviation of the experimental values
T	Temperature
t	Time
UA	Uronic acids
UAE	Ultrasound-assisted extraction
US	Power Ultrasounds
	Ultrasound-assisted extraction P= 542 ± 4
US1	W/L
	Ultrasound-assisted extraction P= 794 ± 4
US2	W/L
VAR %	Percentage of explained variance (%)
Xyl	Xylose
Y	Yield of extraction (%)
Y ₀	Initial yield of extraction (%)
Y _{calc}	Calculated yield of extraction (%)
Y _{exp}	Experimental yield of extraction (%)
Y _{max}	Maximum yield of extraction (%)

INTRODUCTION

41
42 Recently, there has been an increasing interest in re-valuing the waste generated by the food
43 industry, for both economical and environmental reasons.¹ As an example, around 70% of the
44 worldwide production of oranges is intended for the juice industry generating about 50% by
45 weight of residues, mainly composed of skins (flavedo and albedo), pulp, and seeds.^{2,3} These by-
46 products are rich in phenolic compounds, essential oils, pigments and, in particular, cell wall
47 polysaccharides such as pectins, hemicelluloses and cellulose.⁴⁻⁶

48 Polysaccharides have aroused considerable attention for their unique bioactivities and chemical
49 structures.^{7,8} Pectins are probably one of the most important types of cell wall polymers, being
50 widely used as gelling agents and stabilizers in a wide variety of food, pharmaceutical, and
51 cosmetic formulations.⁹ Pectins are a complex mixture of heteropolysaccharides, predominantly
52 formed by homogalacturonans (HG), a polymer largely composed of a backbone of linked D-
53 galacturonic residues (GalA).¹⁰ A second well-characterised component of pectins is the
54 rhamnogalacturonan I (RGI). In this type of polymer, the backbone is formed by GalA units
55 interspersed with L-rhamnose (Rha) residues which are linked to side-chains of neutral sugars
56 such as arabinose (Ara) and galactose (Gal).^{11,12} Pectin extracts can also contain
57 xylogalacturonan, and rhamnogalacturonan II (RGII), a highly complex branched structure
58 occurring with much less frequency than RGI.¹³

59 Most conventional methods applied for pectin extraction involve the use of mineral acids such as
60 HCl, HNO₃ or H₂SO₄, at pHs comprising between 1.5 and 3.0.¹⁴ Further, these processes are
61 regularly carried out under high temperature conditions (between 60 and 100 °C) and long
62 extraction times (from 0.5 to 7 h).^{9,15} However, only limited pectin yields are usually obtained
63 using these traditional procedures. Further, at industrial levels, the use of mineral acids and their
64 effluents may well cause serious environmental problems, representing an excessive cost for food
65 companies. Therefore, the substitution of these mineral acids by organic acids such as citric acid
66 has been investigated, obtaining similar or even higher yields.¹⁶ On the other hand, the use of

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3 67 elevated temperatures may promote the non-enzymatic degradation of pectins, which may affect
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5 68 their functional properties. Therefore, new technologies such as microwave assisted extraction,
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7 69 ^{17,18} moderate electric fields ⁹ and ultrasounds assisted extraction ¹⁹ have been proposed, to
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9 70 improve these conventional processes.

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12 71 Power ultrasounds (US) are characterized by their ability to improve mass transfer processes
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14 72 through a series of mechanisms activated by the non-linear effects of high amplitude ultrasonic
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16 73 waves. ²⁰ It is well accepted that the enhancement of extraction yields promoted by US could
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18 74 mainly be attributed to ultrasonic cavitation. This phenomenon consists in the formation, growth
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20 75 and implosion of gas nano/microbubbles into the liquid as a consequence of pressure fluctuations
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22 76 generated by the ultrasonic waves. ²¹ In recent years there has been an increasing interest in the
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24 77 use of US to intensify extraction procedures of pectins from different plant materials. ^{14,19,22} These
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26 78 studies agree that US could be a viable alternative for the extraction of pectins. However, it is
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28 79 important to determine the effect of US application not only on the yields but also on the
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30 80 characteristics of extracted pectins.

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34 82 In recent decades, from a food engineering point of view, mathematical modeling has been widely
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36 83 used since it may provide a quick and inexpensive assessment of the main effects of the different
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38 84 experimental conditions on the outcome of the extraction procedure. ²³ To the best of our
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40 85 knowledge, there are no studies based on the modelization of pectin extraction kinetics obtained
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42 86 by US application, using mild conditions; in particular the use of organic acids, and relatively low
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44 87 temperatures (below 30 °C).

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47 88 Within this context, the main aim of the present research was to investigate the effect of both pH
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49 89 and power ultrasound on the extraction of pectins from orange by-products. Thus, extraction
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51 90 yields and chemical characteristics such as the carbohydrate composition and the degree of
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53 91 methylation were determined. Furthermore, a mathematical model to simulate the extraction
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55 92 kinetics of both acoustic and agitation experiences was proposed.

MATERIALS AND METHODS

94 **Samples preparation**

95 Oranges of the *Navelina* variety, were purchased in a local market. Oranges whose juice had a
96 soluble solids content of 11.0 ± 0.5 °Brix were selected for the study. After extracting the juice,
97 the remaining material (by-product) was scalded to inactivate the endogenous enzymes,
98 lyophilized, ground and sieved (to a particle size between 0.355 and 0.710 mm). The moisture
99 content of the by-product was determined by AOAC method no. 934.06²⁴ in triplicate (647 ± 5
100 g/Kg). Finally, the lyophilized by-product was vacuum-packed and stored, protected from light
101 and kept under refrigeration (4 °C).

102 **Extraction procedure**

103 Extractions were performed in a vessel with a double glass layer (capacity of 250 mL). The
104 temperature was maintained at 25 ± 1 °C in a thermostatic bath by driving cooling water through
105 a jacketed extraction vessel (Selecta, Tectron Bio, Spain). In the case of UAE, it was necessary
106 to replace the water by a refrigerant liquid (ethylene glycol) and set the bath at 15 °C in order to
107 maintain the temperature at 25 °C.

108 Both, mechanical agitation extraction (AG) and ultrasound-assisted extraction (UAE) procedures
109 were carried out at pH 1.5 and 2.0, these values were chosen since previous studies have
110 demonstrated that high extraction yields of pectin can be obtained within this pH range.²⁵⁻²⁷ Thus,
111 citric acid solutions (0.5 mol/L and 0.05 mol/L for pH 1.50 ± 0.01 and 2.0 ± 0.01 respectively) were
112 prepared and adjusted to the desired pH using only citric acid. Approximately 3 g of by-product
113 were added to 100 mL of the citric acid solution. Individual experiments at different extraction
114 times (3, 6, 10, 20, 30, 40, 50 and 60 min) were carried out for each experimental condition. All
115 experiments were performed in triplicate.

116 **Mechanical agitation extractions (AG)**

117 AG were performed using a stirrer (RZR 2021, Heidolph, Germany) equipped with a 4-blade
118 propeller (50 mm diameter, 0.2 xg) placed at 2.8 cm from the liquid interface (Figure 1).

119 **Ultrasound-assisted extractions (UAE)**

120 UAE were carried out using an ultrasonic generator UP400S (Hielscher Ultrasonics GmbH,
121 Germany), with a power of 400 W and an ultrasonic frequency of 24 kHz. Two titanium probes
122 (Hielscher Ultrasonics GmbH, Germany) with tip diameters of 40 mm (US1) and 14 mm (US2)
123 were used in order to test different acoustic densities. The pulse and amplitude were adjusted to
124 50 and 100%, respectively. The probe was immersed in the soaking medium to a distance of 1.8
125 cm from the liquid interface. The setup of the ultrasonic treatment has been depicted in Figure 1.
126 Prior to UAE experiments, a calorimetric approach was applied to determine the effective
127 ultrasound power transferred into the medium by each probe.²⁸ Thus, the ultrasounds power was
128 measured in triplicate for the acoustic conditions applied using equation 1:

129 *Eq. 1* $P = \frac{dT}{dt} Cp m$

130 where P is the ultrasounds power (W), Cp is the heat capacity of the solvent (J/ kg K) and m is
131 the mass of the solvent (kg). Citric acid was the solvent used in all experiments, in which Cp was
132 calculated by differential scanning calorimetry (DSC 2920, TA Instruments, USA). Acoustic
133 power densities (W/L) were calculated as the ratio between the ultrasounds power applied (P) and
134 the total extraction volume: 542 ± 4 W/L (US1) and 794 ± 4 W/L (US2). On the other hand,
135 acoustic intensities (W/cm²) were determined as the ratio between the ultrasounds power applied
136 and the emitter surface of each probe system: 4.3 W/cm² (US1) and 51.6 W/cm² (US2).

137 **Purification of extracts**

138 Acid extracts were filtered through a screen of ~0.5 mm mesh. Next, the filtrates were precipitated
139 with ethanol 96 mL/100 mL (1:2, v/v) and stirred for 10 min. The mixtures were then centrifuged
140 at 1252 xg for 30 min and the precipitates separated by filtration (#40 glass fiber) and rinsed with

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3 141 acetone. Finally, extracts were dried at room temperature for 12 h and weighed. ²⁹ Extraction
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5 142 yields were calculated as g of extracted pectin by 100 g of by-product (%). The moisture of the
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7 143 by-product was considered in order to express all extraction yields on a dry matter (dm) basis.
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10 144 **Methods of analysis**

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12 145 In order to determine the main effects of US application, pH and extraction time on the
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14 146 physiochemical properties of the extracts, carbohydrate analysis and determination of the degree
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16 147 of methylation of pectins were carried out on extracts obtained after 30 and 60 min of extraction.
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19 148 **Alcohol insoluble residues (AIRS)**

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22 149 In order to determine the composition of the main types of cell wall polysaccharides present in
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24 150 the different extracts, AIRs were obtained by immersing the by-product samples in boiling
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26 151 ethanol.¹
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29 152 **Analysis of carbohydrate composition**

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31 153 Neutral sugars were released from cell wall polysaccharides by acid hydrolysis (Saeman
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33 154 hydrolysis). AIR samples (~5 mg) were dispersed in 12 mol/L H₂SO₄ for 3 h at room temperature.
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35 155 Then, dilution to 1 mol/L and incubation at 100 °C for 2.5 h was carried out in order to hydrolyze
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37 156 the samples. The released monosaccharides were transformed into their alditol acetates and
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39 157 separated by gas-liquid chromatography at 220 °C on 3% OV225 ChromosorbWHP100/120 mesh
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41 158 column (Hewlette Packard 5890A, Waldbronn, Germany) with Ar as the carrier gas flowing at
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43 159 20 mL/min. Temperatures of injector and FID detector were 230 °C and 240 °C, respectively. ¹
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45 160 Uronic acids (UA) were determined by colorimetry as total UA using a sample hydrolyzed for 1
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47 161 h at 100 °C, in this investigation UA were considered as equivalent to Galacturonic Acid (GalA).
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53 163 Moreover, in order to elucidate the main structural features of pectins, three molar ratios were
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55 164 calculated from the contents of GalA, Rhamnose (Rha), Galactose (Gal) and Arabinose (Ara). ¹⁵
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57 165 The ratio of GalA/(Rha+Gal+Ara), was calculated to represent the linearity of extracted pectins;
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3 166 the GalA/Rha ratio provided a global insight into the amount of RGI present in the sample, and
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5 167 the (Ara+Gal)/Rha ratio represented the approximate length of the RGI-type of pectins.
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8 168 **Determination of the degree of methylation (DM)**

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10 169 To determine the DM of pectins, extracts were pulverized and mixed with KBr (0.1 g of
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12 170 pulverized extract in 1 g of KBr) and pressed into a 2 mm pellet. FTIR spectra of these samples
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14 171 were recorded using a Bruker IFS66 instrument from 800 to 2000 cm⁻¹. The measuring resolution
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16 172 was 3 cm⁻¹. The resultant spectra were obtained using the OMNIC E.S.P. 5.1 software. DM was
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18 173 determined using the following equation: ³¹

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21 174 *Eq. 2*

$$22 \quad \quad \quad 23 \quad \quad \quad 24 \quad \quad \quad 25 \quad \quad \quad 26 \quad \quad \quad 27 \quad \quad \quad 28 \quad \quad \quad 29 \quad \quad \quad 30 \quad \quad \quad 31 \quad \quad \quad 32 \quad \quad \quad 33 \quad \quad \quad 34 \quad \quad \quad 35 \quad \quad \quad 36 \quad \quad \quad 37 \quad \quad \quad 38 \quad \quad \quad 39 \quad \quad \quad 40 \quad \quad \quad 41 \quad \quad \quad 42 \quad \quad \quad 43 \quad \quad \quad 44 \quad \quad \quad 45 \quad \quad \quad 46 \quad \quad \quad 47 \quad \quad \quad 48 \quad \quad \quad 49 \quad \quad \quad 50 \quad \quad \quad 51 \quad \quad \quad 52 \quad \quad \quad 53 \quad \quad \quad 54 \quad \quad \quad 55 \quad \quad \quad 56 \quad \quad \quad 57 \quad \quad \quad 58 \quad \quad \quad 59 \quad \quad \quad 60$$

$$DM = 124.7R + 2.2013$$

176 where R was calculated as the ratio of A₁₇₄₀ over the sum of A₁₇₄₀ and A₁₆₃₀, being A₁₇₄₀ and A₁₆₃₀
177 the absorbance intensities of bands for methyl-esterified and non methyl-esterified carboxyl
178 groups at 1740 cm⁻¹ and 1630 cm⁻¹, respectively. ³²

179 **Mathematical modeling**

180 A mathematical model was proposed with the aim of establishing a methodology to assess the
181 mass transfer process during extraction of pectins from orange by-products (either with AG or
182 UAE).

183 The extraction kinetics were mathematically described using a second order rate model. A second
184 order rate model has been previously used to describe solid-liquid extraction process. ³³ Moreover,
185 Patil *et al.* ³⁴ used different models to simulate kinetics of extraction, with the application of
186 ultrasounds, of camptothecin from *Nothapodytes nimmoniana*, reporting that with a second order
187 rate model obtained the best fit to the experimental values. The second order rate equation can be
188 described as follows:

189 *Eq. 3*

$$\frac{dY}{dt} = k(Y_{max} - Y)^2$$

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3 191 where, Y was the extraction yield (%) at a specific time t, Y_{max} was the maximum extraction
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5 192 yield (%), t the extraction time (min) and k the extraction rate constant (%⁻¹ min⁻¹). The initial
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7 193 extraction rate defined as h (% min⁻¹) when t approaches 0, can be expressed as,

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10 194 *Eq. 4*

$$h = kY_{max}t$$

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14 196 In order to obtain the kinetic parameters Eq. 3 was integrated under the initial and boundary
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16 197 conditions t= 0 to t and Y_t = 0 to Y_t, respectively:

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18 198 *Eq. 5*

$$Y = \frac{k Y_{max} t}{1 + k Y_{max} t}$$

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23 200 The linear form derived from equation (5) is,

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26 201 *Eq. 6*

$$\frac{t}{Y} = \frac{1}{kY_{max}} + \frac{t}{Y_{max}}$$

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30 203 Therefore, h and Y_{max} could be experimentally determined from the slope and intercept by
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32 204 plotting t/Y against t.

33 34 35 36 205 **Statistical analysis**

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38 206 All the experimental results represent the mean value and standard deviation from at least three
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40 207 replicates. The Parametric ANOVA test was used to evaluate the existence of significant
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42 208 differences (p < 0.05) between means of experimental data obtained on pectin characterization
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44 209 (carbohydrate composition and DM) and means were compared by Tukey's test. The statistical
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46 210 analyses were replaced by Kruskal- Wallis and pairwise-Wilcox (BH corrected) when data were
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48 211 not normally distributed and/or showed heterogeneity of variances after the application of Shapiro
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50 212 and Levene tests. This study was carried out using the statistical package R (version 2.14.2, R
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52 213 Foundation for Statistical Computing, Austria).

214 Regarding the mathematical model, the linear regression function of Microsoft Excel was used to
 215 calculate the confidence intervals (95%) of the parameters whereas the prediction limits (95%),
 216 were calculated by the function 'nlparci' from Matlab 7.5 (The MathWorks Inc., USA).

217 The average relative error (MRE %), given in Eq. 7, and the percentage of explained variance
 218 (VAR %), given in Eq. 8, estimated by the comparison of experimental and simulated data, were
 219 calculated to statistically evaluate the accuracy of the proposed mathematical models to simulate
 220 the extraction kinetics for both types of extraction procedures (AG and UAE).

221 Eq. 7

$$MRE \% = \frac{\sum_{i=1}^n \left(\frac{|Y_{exp} - Y_{calc}|}{Y_{exp}} \right)}{n} 100$$

223 Eq. 8

$$VAR \% = \left(1 - \frac{S_{exp}^2}{S_{calc}^2} \right) 100$$

225 RESULTS AND DISCUSSION

226 Extraction yields

227 The experimental results corresponding to the extraction yields obtained at different pH, either
 228 using mechanical agitation (AG = 0.2 xg) or acoustic assistance (US1 = 542 W/L and US2 = 794
 229 W/L), are depicted in Figure 2.

230 As can be observed, higher extraction yields were obtained with UAE in comparison to those
 231 obtained by AG. Further, an increase in US power also promoted a significant increase in the
 232 extraction yield. In particular, at pH 1.5 and after 30 min of extraction, the acoustic extraction
 233 rates were 2.3 (US1) and 2.8-fold (US2) higher than in the conventional extraction. When US
 234 density power increases, larger amplitude waves are generated throughout the solvent, resulting
 235 in the formation of a considerable number of bubbles which may collapse, accelerating the mass
 236 transfer rate.³⁵

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3 237 In general, these results are in agreement with several authors who have reported that US improve
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5 238 the extraction yield of pectins obtained from different plant tissues. For instance, Wang *et al.*¹⁴
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7 239 extracted pectins from grapefruit peel and reported that the yield obtained with US application
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9 240 (12.56 W/cm², 66 °C, for 28 min and pH 1.5) was ~1.2-fold higher than the yield obtained using
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11 241 a conventional procedure (80 °C, for 90 min pH 1.5). Also, Wang *et al.*²² observed a ~1.3-fold
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13 242 higher pectin extraction yield from mango peel with US application (20 kHz and 500 W, 20 °C
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15 243 for 15 min and pH 2.5) in comparison with a conventional method (mechanical stirring, 20 °C,
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17 244 for 2 h and pH 2.5). Further, Bagherian *et al.*,³⁶ who extracted pectin from grapefruit peel observed
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19 245 that the rate of ultrasonic extraction (power density was no specified) was more than 3 times faster
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21 246 than conventional extraction (for conventional extraction 90 °C for 90 min were used whereas
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23 247 for UAE a temperature range of 50-70 °C and different times of extraction from 4 to 30 min were
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25 248 applied, in both cases using pH of 1.5). These observations can be explained by the fact that the
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27 249 ultrasonic treatment enhances the swelling and softening processes of cell walls via the hydration
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29 250 of pectinous material from middle lamella, leading to the break-up of vegetal tissues³⁷.

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33 251 However, in this study, extraction yields were strongly influenced by the pH of the solvent. Thus,
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35 252 either for UAE or AG, significantly ($p < 0.05$) higher extraction yields were obtained at pH 1.5
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37 253 than at pH 2.0. This result is in agreement with the work of Levigne *et al.*³⁸ who obtained higher
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39 254 yields of pectins from sugar beet, by decreasing the pH from 3.0 to 1.0 in conventional extraction
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41 255 procedures (75 and 95 °C, for 30 and 90 min, extracting with HCl and HNO₃).³⁸ According to
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43 256 Maran *et al.*³⁹, a high acidic solvent has the potential to contact directly with the insoluble form
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45 257 of pectins hydrolyzing it into a soluble form and allowing a more efficient extraction.

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49 258 Furthermore, the results obtained in this study clearly indicate that the pH affects the capacity of
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51 259 US to enhance the extraction yield of pectins. Thus, at pH 1.5 and after 30 min of extraction, US
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53 260 improved the yield from ~19% with AG, to ~39 and ~47% with US1 and US2, respectively.
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55 261 However, at pH 2.0, the yields could only be improved from ~10% with AG, to ~14 and ~18%
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57 262 with US1 and US2, respectively. Therefore, although it has been reported that US may improve
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263 the pectin extraction yield, the extension of this increase seems to be clearly conditioned by the
264 pH of the medium.

265 Regarding the time of extraction, the highest yield increases were observed during the first 30
266 min of extraction. Similar results were obtained by Maran *et al.*³⁹ during the extraction of pectins
267 from *Musa balbisiana* using ultrasonic assistance.

268 It is important to note that with the US application at pH 1.5, very high extraction yields were
269 obtained after 60 min (~63%); this suggests that not only pectins but also other components were
270 extracted under these conditions.

271 **Carbohydrate composition**

272 In order to gain more insight into the pectin extraction process, extracts obtained after 30 and 60
273 min at different pH, either with mechanical agitation (AG = 0.2 xg) or with acoustic assistance
274 (US1 = 542±4 and US2 = 794±4 W/L) were submitted to carbohydrate analysis (Table 1).

275 The results suggested that pectic polysaccharides, as inferred from the presence of GalA, Rha,
276 Gal, Ara and Xyl were the main constituents of the extracts, ranging from 71 to 87 mol% of total
277 sugars. Moreover, other sugars such as Glucose (Glc) and Mannose (Man) were also detected, in
278 fact, the amount of Glc was one of the largest among all the determined monomers. In general,
279 the molar percentage of the different monomers was similar to the values reported by Wang *et al.*
280⁴⁰ and Wang *et al.*¹⁴, who extracted pectins from citrus products using both a conventional method
281 and also a UAE procedure (conventional method: 80 °C, for 1.5 h and pH 1.5; UAE: P= 410
282 W/L, at 67 °C for 28 min and pH 1.5).

283 The predominance of GalA, indicated that extracted pectins were mainly formed by
284 homogalacturonans (HG). Therefore, the solution of citric acid, either at pH 1.5 or 2.0, seems to
285 be selective for the extraction of this type of pectin chains.¹⁹ However, the GalA content detected
286 in this work ranged from 38.0 to 62.4 mol%, which, in general, is lower than the values reported
287 in other studies that have extracted pectins from citrus materials (41.5-95.3 mol%).⁴¹⁻⁴⁵ It has
288 been observed that different extraction conditions can affect the GalA content. For instance,

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3 289 Masmoudi *et al.*⁴¹ extracted pectins from lemon by-products and reported that the GalA content
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5 290 increase from 41.5 to 74.5 mol% when changing the temperature from 40 to 80 °C (after 1 h of
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7 291 extraction at pH 2.8). Hosseine *et al.*⁴³ extracted pectins from orange peel and also observed that
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9 292 as temperature increased, the GalA content also increased (from 57.0 to 73.0 mol% when
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11 293 temperature increased from 75 to 95 °C, after 30 min of extraction). In this study, the extraction
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13 294 was performed using a lower temperature (25 °C), and this could explain why pectins exhibited
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15 295 an overall lower GalA content than values reported by other authors. According to the FAO
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17 296 regulations⁴⁶ industrial pectins contain about 65% of GalA (w/w), therefore a purification process
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19 297 of the extracts should be carried out to suit this standard.

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23 298 The presence of minor amounts of Rha, Ara and Gal suggested that RGI-type pectin chains were
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25 299 also present in the different extracts.¹² In fact, Ara was the predominant of these three sugars,
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27 300 which is coherent since Ara exhibited one of the highest molar percentages among the monomers
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29 301 of the orange by-products used as raw material. Methacanon *et al.*⁴⁵ also observed relatively high
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31 302 amounts of this sugar on pectins extracted from pomelo (7.11-20.87 mol% extracting at 80 °C for 2
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33 303 h). Small amounts of Xyl has also been observed, thus, it can be deduced that the extracts also
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35 304 contain xylogalacturonan, the presence of these polysaccharides has already been observed by
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37 305 Wang *et al.*¹⁴ who extracted pectins from grapefruit peel.

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40 306 The results clearly indicated that the effect of US application on extracted pectins was dependent
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42 307 not only on the pH but also on the time of extraction. GalA was the sugar type mostly affected by
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44 308 the application of US. Thus, after 60 min of extraction at pH 2.0, significantly lower content ($p <$
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46 309 0.05) of GalA was observed with UAE than with AG; suggesting that the application of US under
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48 310 these conditions might have promoted an undesirable effect on the HG chain. Zhang *et al.*⁴⁷, also
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50 311 reported a decrease in the GalA content (from 83.8 to 75.7 mol%) on citrus pectins which were
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52 312 submitted to sonication on water (181 W/cm² for 1.5 h at 25 °C) and no significant decrease ($p >$
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54 313 0.05) was observed when sonication was performed using an acid solution (HCl 0.1 M). These
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56 314 observations agree with the results obtained in this investigation, since when extracting with the
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58 315 most acid solution (pH 1.5) no significant ($p > 0.05$) decrease of GalA content was observed with
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3 316 US application in both cases, i.e. after 30 and 60 min of extraction. Furthermore, no significant
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5 317 differences in GalA content was observed between AG and UAE for an extraction time of 30 min,
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7 318 regardless of the pH.
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10 319 Regarding non-pectic sugars, Glc was the most abundant sugar residue. Interestingly, an increase
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12 320 in this sugar was observed when comparing UAE with AG, in particular, after 60 min of
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14 321 extraction. This observation suggested that the application of acoustic energy might have caused
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16 322 the solubilization of non-pectic polysaccharides, such as hemicelluloses or even cellulose, since
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18 323 US lead to a better cell disruption via the formation of microjets, which improves the solvent
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20 324 penetration and increases the breakdown of the cell walls.^{45,48}
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23 325 Overall, significant differences ($p < 0.05$) were observed in the molar ratios of the different pectin
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25 326 sugars, when comparing pectins extracted by AG and those obtained by UAE (Table 1). On the
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27 327 contrary, no significant differences ($p > 0.05$) were observed between pectins extracted under
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29 328 US1 and US2 conditions, except for a significant ($p < 0.05$) increase of Man with US2 at 30 min
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31 329 and pH 2.0 and of Xyl at 60 min and pH 2.0.
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34 330 Further, at pH 2.0 and after 60 min of extraction, pectins obtained by UAE exhibited a significant
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36 331 ($p < 0.05$) decrease in linearity ($\text{GalA}/(\text{Ara}+\text{Gal}+\text{Rha})$) in comparison with those extracted by
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38 332 AG. Moreover, the ratio GalA/Rha calculated as a hypothetical representation of the ratio
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40 333 HG/RGI¹⁵, also decreased for pectins extracted with UAE compared to those obtained with AG.
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42 334 This phenomenon may indicate that UAE allows the extraction of pectins with a higher number
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44 335 of ramifications such as in RGI type of pectins. Nevertheless, no significant effect of US was
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46 336 observed when the extraction was carried out at pH 1.5 and after 30 min.
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50 337 Finally, the length of the RGI chains was calculated from the ratio $(\text{Gal}+\text{Ara})/\text{Rha}$. In general, US
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52 338 allowed the extraction of longer RGI chains compared with those extracted with AG. Although
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54 339 this increase was only significant ($p < 0.05$) after 60 min of extraction for both pH values.
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340 **Fourier transform infrared spectra (FTIR) and DM**

341 The infrared spectra of the extracts obtained with agitation (AG) and also with acoustic
342 assistance (US1 and US2) are shown in Figure 3. The characteristic absorption peak of
343 polysaccharides observed in these samples was the absorption peak between 3300 and 3500 cm^{-1}
344 due to OH stretching. The peak between 2850 and 3000 cm^{-1} was attributed to CH vibrational
345 modes, including CH, CH_2 , and CH_3 stretching and bending vibrations.¹⁰ An absorption at ~ 1740
346 cm^{-1} was caused by C=O stretching vibration of methylesterified carboxyl groups, while the
347 absorption at about 1630–1647 cm^{-1} was caused by C=O stretching vibration of free carboxyl
348 groups. The average of the ratio of the peak area at 1740 cm^{-1} (COO–R) over the sum of the peak
349 areas of 1740 cm^{-1} (COO–R) and 1630 cm^{-1} (COO–) was calculated as the DM.³² Some of
350 carboxyl group signals might also originate from phenolic compounds as indicated by the
351 presence of peaks at ~ 1523 cm^{-1} for the aromatic ring vibrations, which is lower in all cases on
352 the extracts obtained by UAE.¹⁴ A strong extensive absorption in the region of 1100–1000 cm^{-1}
353 was due to C–O–C stretching vibration of sugars (1091–1030 cm^{-1}) and stretching vibrations of C–
354 OH side groups.³⁹

355 According to the DM, pectins can be divided into two main groups: high methoxyl pectins (HMP)
356 exhibiting a DM higher than 50%, and low methoxyl pectins (LMP) with a DM lower than 50%.¹⁰
357 A very wide range of DM of pectins extracted from citrus materials has been reported in the
358 literature (17.0–91.58),^{42,43,51} which suggest that the extraction conditions might have a very
359 important effect on this parameter. The DM corresponding to extracted pectins are depicted in
360 Table 2. Overall and regardless of the method of extraction, the pH was the main parameter
361 affecting the DM. Thus, in general, pectins extracted at pH 1.5 exhibited a higher DM ($p < 0.05$)
362 than those extracted at pH 2.0, (on average, 55 ± 1 % for pectins extracted at pH 1.5 (HMP) and
363 46 ± 4 % for pectins extracted at pH 2.0 (LMP)). These results contrast with those reported by
364 Levigne *et al.*³⁸ who found a DM increase in pectins extracted from fresh sugar beet, when pH
365 increased from 1.0 to 2.0. However, the extraction conditions of that study were completely

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3 366 different from those presented in this work, since high temperature (between 70 and 90 °C) and
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5 367 mineral acids (HCl or HNO₃) were used.
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8 368 Moreover, US did not have a significant effect ($p > 0.05$) on the DM. With regard to the extraction
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10 369 time, significant differences ($p < 0.05$) were observed between pectins extracted at 30 min and
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12 370 those extracted after 60 min at pH 2.0. The highest DM was obtained after 60 min of extraction.
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14 371 These results are in general agreement with those reported by Minjares-Fuentes *et al.*¹⁹, who
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16 372 performed UAE (P= 50 W/L, at 55 °C) of pectins from grape pomace and observed that relatively
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18 373 high DM (> 50%) could be obtained using citric acid as a solvent (pH 2.0) and an extraction time
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20 374 of 60 min.
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23 24 375 **Mathematical modeling**

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26 376 In order to evaluate the entire extraction process, a second order rate model was used to
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28 377 mathematically describe the UAE and AG experiments carried out under different pH conditions.
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31 378 The parameters of the kinetic models: Y_{max}, h and k were identified for all extractions by plotting
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33 379 t/Y_t against t and considering the initial extraction yield equal to zero ($Y_0 = 0$). The parameters
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35 380 and their corresponding confidence intervals ($p < 0.05$) (CI) are depicted in Table 3.
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38 381 As can be observed, the maximum yield (Y_{max}) increased with the application of US at both pH
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40 382 values (1.5 and 2.0), obtaining the highest Y_{max} at pH 1.5. The same trend was observed for the
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42 383 h parameter, which is related to the initial extraction rate. This behavior could be due to the fact
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44 384 that US accelerate the extraction rate, especially during the first stages of the process.⁵² The
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46 385 increases of the Y_{max} and h parameters when applying US, are coherent with the experimental
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48 386 results, and are also in accordance with many studies which have mathematically described
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50 387 extraction procedures assisted by US, using a second order rate model.^{34,53,54} Thus, a similar
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52 388 trend was observed by Xu *et al.*⁵⁵ who model the pectin extraction from grapefruit peel with US
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54 389 application (400 W/L) considering that it takes place into two main stages: transformation of
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56 390 insoluble pectins followed by degradation of partially dissolved pectic polymers.
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3 391 On the other hand, the extraction rate constant (k) decreased with the application of US, for both
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5 392 pH values, 1.5 and 2.0. Yao *et al.*³⁴ and Goula,⁵³ who extracted camptothecin from *Nothapodytes*
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7 393 *nimmoniana* and oil from pomegranate seeds respectively, with US application (76.4-191 W/cm²
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9 394 and 130 W respectively) using different temperatures (30-60 °C and 20-80 °C respectively)
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11 395 observed a decrease of k when the temperature of extraction increased, even when higher yields
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13 396 were obtained under those conditions. Since the k parameter is inversely proportional to Y_{max} ;
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15 397 higher Y_{max} values corresponded to lower k values.

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18 398 The experimental extraction curves were simulated by using the identified parameters from the
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20 399 second order model (Table 3). Simulated versus experimental values are presented in Figure 4.
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22 400 Mathematical adjustment of the second order rate model, the corresponding confidence intervals
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24 401 ($p < 0.05$) (CI), and the prediction limits ($p < 0.05$) (PI) have also been included in Figure 4.

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27 402 In spite of the experimental variability, mainly attributed to the heterogeneity of the raw material,
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29 403 a high correlation coefficient between the proposed model and experimental data was observed
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31 404 not only for UAE ($R^2 \geq 0.9759$) but also for AG extractions ($R^2 \geq 0.9622$).

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34 405 The mean relative error (% MRE) and the explained variance (% VAR) obtained through the
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36 406 comparison of experimental and simulated extraction curves for UAE experiments were $\leq 7.4\%$
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38 407 and $\geq 97.2\%$ respectively (Table 3). These results would confirm that the second order rate model
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40 408 might provide an adequate description of the UAE kinetics. The agreement of the second order
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42 409 model with the experimental results may suggest that there are primarily two phenomena taking
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44 410 place during the extraction procedure: Initially, there is an intense dissolution of pectins in which
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46 411 maximum leaching takes place, followed by a second, much-slower stage, which corresponds to
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48 412 external diffusion and may be related to the remaining soluble material.³³

413 CONCLUSIONS

414 Overall, the results presented in this study prove that the application of US is able to improve the
415 efficiency of the pectin extraction process in comparison with a conventional solid-liquid

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3 416 extraction carried out with mechanical agitation; even when the conditions used could be
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5 417 considered as mild (low temperature: 25 °C and using an environmentally friendly solvent such
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7 418 as citric acid). However, the significance of this improvement was clearly dependent on the pH
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9 419 of the solution. Thus, considerably higher yields were observed when the citric acid solution was
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11 420 adjusted to a lower pH (1.5).

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14 421 Further, the US effect on pectin characteristics was influenced not only by the pH but also by the
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16 422 extraction time. For instance, the application of US at pH 2.0, for 60 min promoted a considerable
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18 423 decrease of GalA. Interestingly, this effect was not observed when the extraction procedure was
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20 424 carried out using a more acidic solvent (pH 1.5) or when shorter extraction times were applied
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22 425 (30 min).

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25 426 Moreover, the pH had also a significant effect on the DM. In this sense, LMP pectins could be
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27 427 obtained using a pH of 2.0, whereas HMP pectins were obtained adjusting the pH at 1.5.

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30 428 On the other hand, a second order rate model provided an adequate description of the kinetics of
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32 429 both pectin extraction procedures ($\% \text{ MRE} \leq 7.4\%$ and $\% \text{ VAR} \geq 95.5\%$). Thus, the maximum
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34 430 yield, the initial extraction rate and the extraction rate constant were obtained by modeling the
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36 431 extraction process. Compared with AG, UAE increased maximum yield and the initial extraction
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38 432 rate, which indicated that US mainly enhanced the first stage of the extraction process.

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41 433 In conclusion, taking into consideration environmental and waste valorization aspects, it could be
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43 434 interesting for the food industry to introduce US as a technology able to improve the extraction
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45 435 of pectins from food by-products; avoiding or reducing the use of mineral acids and high
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47 436 temperatures. However, US should be applied under adequate conditions, since the present study
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49 437 clearly shows that US efficiency might depend on key variables such as the pH of the medium.
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51 438 Therefore, further studies are required to obtain more information about the system behavior over
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53 439 higher ranges of other important parameters as the extraction temperature.
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3 440 **ACKNOWLEDGEMENTS**
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6 441 The authors would like to acknowledge the financial support of the National Institute of Research
7
8 442 and Agro-Food Technology (INIA) and co-financed with ERDF funds (RTA2015-00060-C04-
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10 443 03), the Ministerio de Educación, Cultura y Deporte of Spain for the research fellowship
11
12 444 (FPU15/03040) and the Balearic Government for the research fellowship (FPI/1814/2015).
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FIGURE LEGENDS

605
606 **Figure 1.** Experimental setup for pectin extraction from orange by-products corresponding to
607 experiments carried out with agitation (A) and with ultrasonic assistance (B)

608 **Figure 2.** Plot of extraction yield (Y) (%) versus time at different pH (A: pH 1.5 and B: pH 2.0)
609 for experiments carried out with agitation (● AG = 0.2 xg) and with UAE (■ US1 = 542±4 W/L
610 and ▲ US2 = 794±4 W/L)

611 **Figure 3.** The Fourier Transform Infrared Spectra (FTIR) of pectin extracted with agitation (—
612 AG = 0.2 xg) and with UAE (— — US1 = 542 W/L and . . . US2 = 794 W/L)

613 **Figure 4.** Simulated (Y_{calc}) vs. experimental yield (Y_{exp}) at different pH (A: pH 1.5 and B: pH
614 2.0). For experiments carried out with agitation (● AG = 0.2 xg) and with UAE (■ US1 = 542±4
615 W/L and ▲ US2 = 794 W/L). The mathematical model (—), the confidence intervals ($p < 0.05$)
616 (— —), and the prediction limits ($p < 0.05$) (.....) are also represented in the figure

TABLES LEGENDS

620
621 **Table 1.** Carbohydrate composition (mol%) of extracts obtained with mechanical agitation (AG=
622 0.2 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

623 **Table 2.** Degree of methylation (DM) of extracts obtained with mechanical agitation (AG= 0.2
624 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

625 **Table 3.** Identified parameters of the second order rate model, and the corresponding confidence
626 intervals (CI) associated to each parameter for the kinetics obtained with mechanical agitation
627 (AG= 0.2 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

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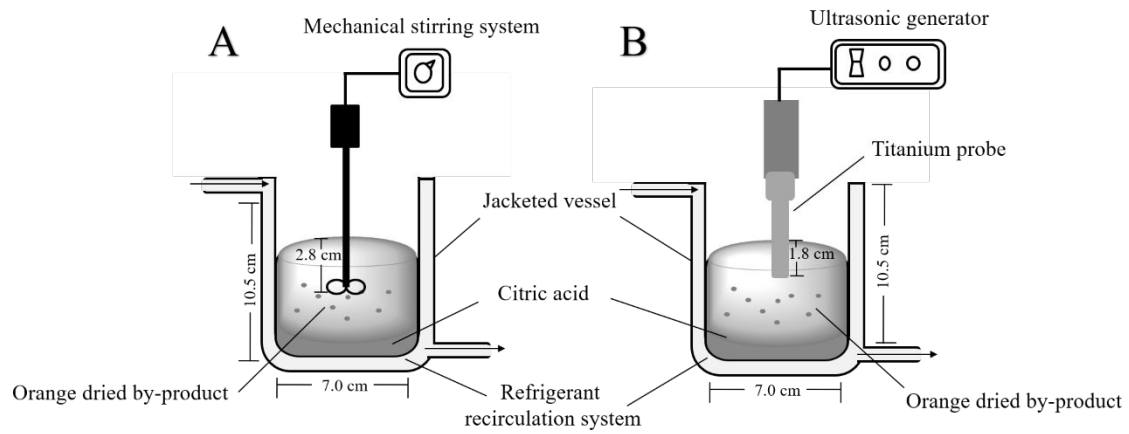


Figure 1. Experimental setup for pectin extraction from orange by-products corresponding to experiments carried out with agitation (A) and with ultrasonic assistance (B)

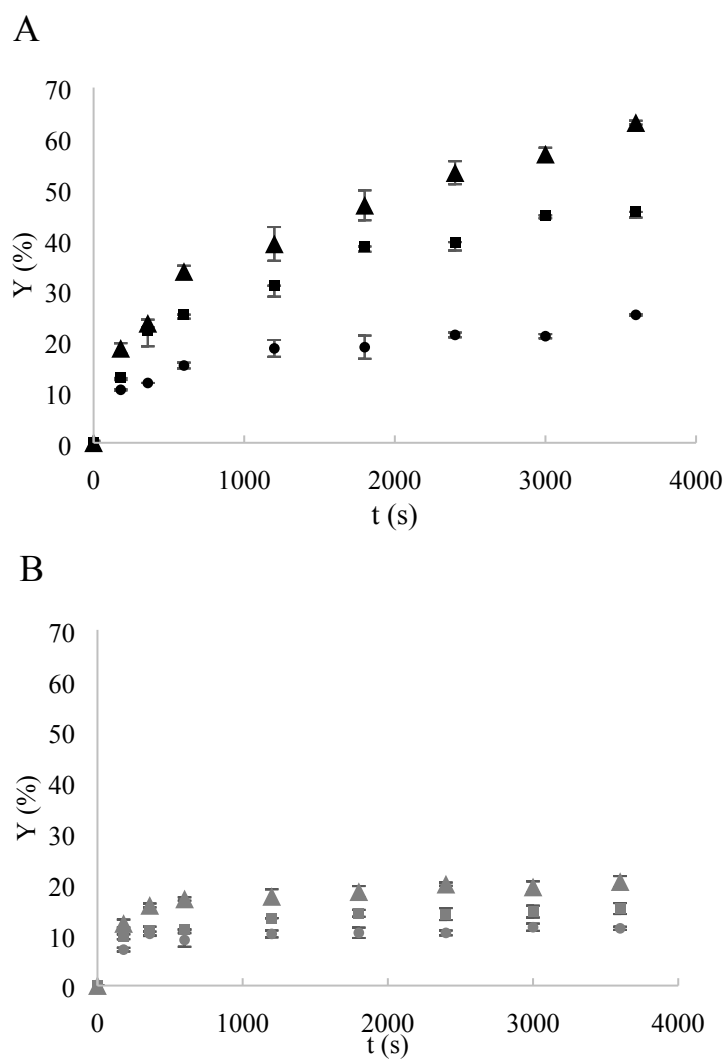


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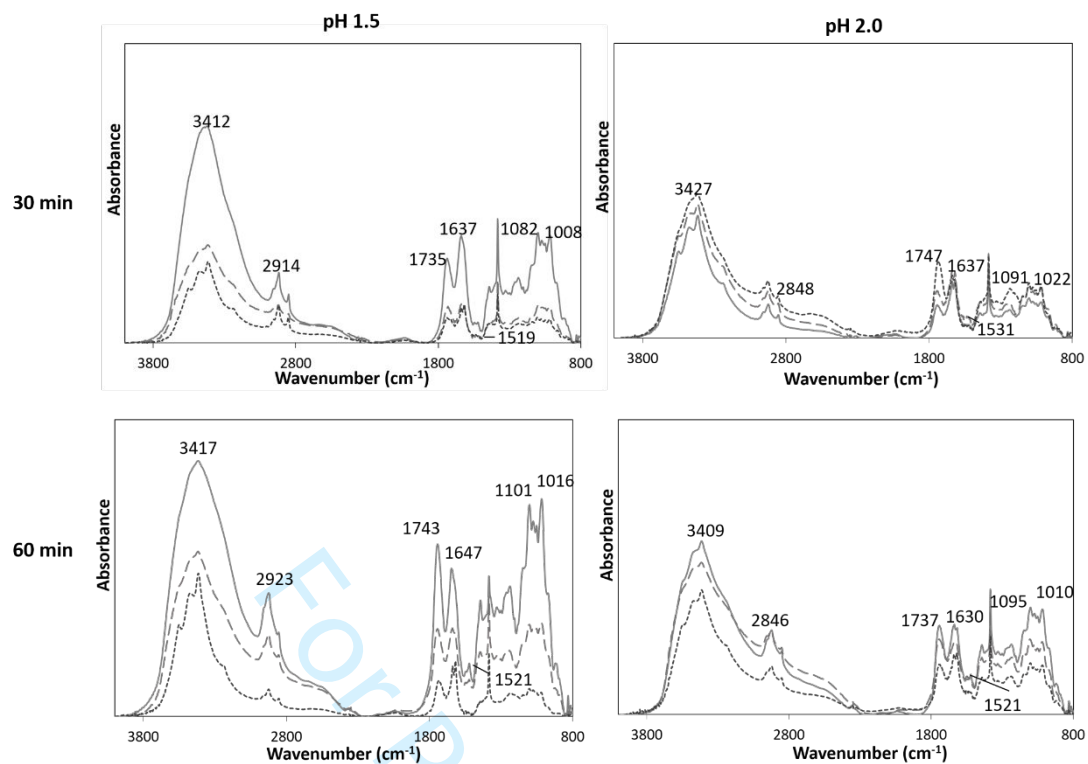


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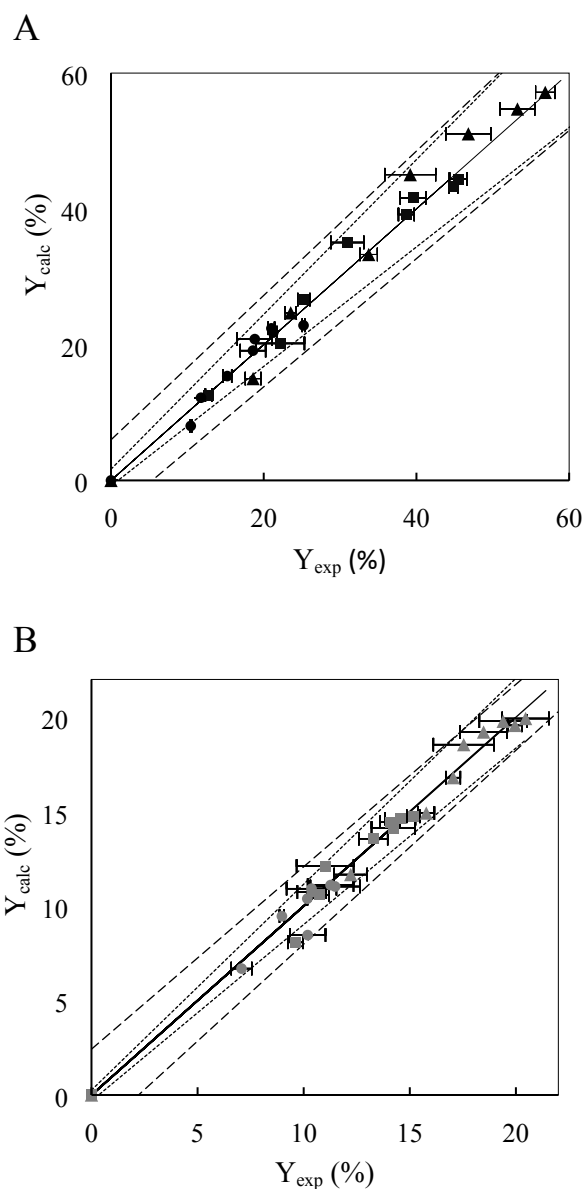


Figure 4. Simulated (Y_{calc}) vs. experimental yield (Y_{exp}) at different pH (A: pH 1.5 and B: pH 2.0). For experiments carried out with agitation (● AG = 0.2 xg) and with UAE (■ US1 = 542±4 W/L and ▲ US2 = 794 W/L). The mathematical model (—), the confidence intervals (p < 0.05) (---), and the prediction limits (p < 0.05) (.....) are also represented in the figure

Table 1. Carbohydrate composition (mol%) of extracts obtained with mechanical agitation (AG= 0.2 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

Time (min)	pH	Experiment	Rha	Ara	Xyl	Man	Gal	Glc	GalA	GalA/Rha+Gal+Ara	GalA/Rha	Gal+Ara/Rha	
30	1.5	AG	3.1 ± 0.1 ^{bcd}	17.5 ± 1.5 ^{ab}	3.8 ± 0.8 ^a	2.3 ± 0.2 ^{de}	4.2 ± 0.2 ^{de}	10.3 ± 1.3 ^g	57.4 ± 2.1 ^a	2.2 ± 0.1 ^{bcd}	18.4 ± 0.6 ^{bc}	5.7 ± 0.4 ^{abcd}	
		US1	3.0 ± 0.4 ^{bcd}	13.1 ± 3.4 ^{de}	2.6 ± 0.2 ^b	3.7 ± 0.2 ^a	5.0 ± 0.8 ^{bcde}	16.9 ± 1.3 ^{cdef}	56.7 ± 3.2 ^{ab}	2.9 ± 1.0 ^{abc}	21.6 ± 4.7 ^{ab}	5.2 ± 0.2 ^{bcd}	
		US2	3.1 ± 0.1 ^{bcd}	14.6 ± 1.1 ^{bcde}	2.7 ± 0.2 ^b	2.9 ± 0.2 ^{abcd}	5.3 ± 0.3 ^{bcd}	16.3 ± 0.7 ^{def}	54.9 ± 2.0 ^{abc}	2.4 ± 0.2 ^{abcd}	21.1 ± 1.6 ^{ab}	6.3 ± 0.1 ^{ab}	
	2.0	AG	3.5 ± 0.2 ^b	13.3 ± 1.0 ^{cde}	2.5 ± 0.0 ^b	2.5 ± 0.1 ^{cde}	4.5 ± 0.4 ^{cde}	14.4 ± 0.8 ^{efg}	59.4 ± 1.2 ^a	2.8 ± 0.2 ^{abc}	20.0 ± 1.8 ^{abc}	4.9 ± 0.5 ^{cd}	
		US1	2.7 ± 0.1 ^{cd}	11.3 ± 0.6 ^e	1.2 ± 0.1 ^c	2.2 ± 0.2 ^{de}	4.7 ± 0.1 ^{cde}	14.9 ± 1.5 ^{ef}	62.4 ± 2.2 ^a	3.3 ± 0.2 ^a	27.3 ± 1.7 ^a	5.7 ± 0.3 ^{abcd}	
		US2	2.5 ± 0.1 ^{cd}	12.0 ± 1.2 ^e	1.3 ± 0.1 ^c	3.3 ± 0.6 ^{abc}	4.0 ± 0.4 ^e	17.2 ± 0.0 ^{cde}	57.4 ± 5.0 ^a	3.1 ± 0.5 ^{ab}	27.4 ± 3.4 ^a	6.2 ± 0.4 ^{abcd}	
	60	1.5	AG	4.4 ± 0.3 ^a	17.0 ± 1.0 ^{abc}	3.7 ± 0.2 ^a	2.2 ± 0.1 ^{de}	5.5 ± 0.3 ^{abc}	12.8 ± 0.8 ^{fg}	54.3 ± 0.9 ^{abc}	2.0 ± 0.1 ^{cde}	14.5 ± 1.0 ^{bc}	4.9 ± 0.4 ^{cd}
			US1	3.0 ± 0.0 ^{bcd}	16.6 ± 1.1 ^{abcd}	3.3 ± 0.5 ^{ab}	2.6 ± 0.3 ^{bcde}	5.0 ± 0.1 ^{bcde}	21.0 ± 1.9 ^{bc}	48.6 ± 1.7 ^{bcd}	2.0 ± 0.1 ^{cde}	19.0 ± 0.7 ^{bc}	6.8 ± 0.3 ^a
			US2	3.3 ± 0.2 ^{bc}	16.6 ± 0.3 ^{abcd}	4.1 ± 0.2 ^a	2.7 ± 0.3 ^{bcde}	6.1 ± 0.5 ^{ab}	20.3 ± 1.2 ^{cd}	47.0 ± 2.2 ^{cd}	1.8 ± 0.1 ^{de}	16.7 ± 1.5 ^{bc}	6.6 ± 0.5 ^{ab}
2.0		AG	3.5 ± 0.4 ^b	13.2 ± 0.5 ^{cde}	2.6 ± 0.3 ^b	2.0 ± 0.2 ^e	5.3 ± 0.1 ^{bcd}	13.3 ± 1.9 ^{efg}	61.4 ± 3.5 ^a	3.1 ± 0.6 ^{ab}	21.2 ± 3.4 ^{ab}	4.7 ± 1.1 ^d	
		US1	3.4 ± 0.1 ^b	17.7 ± 1.1 ^{ab}	1.5 ± 0.2 ^c	3.4 ± 0.3 ^{ab}	7.1 ± 0.7 ^a	25.6 ± 2.5 ^a	41.3 ± 2.1 ^{de}	1.5 ± 0.1 ^e	14.6 ± 1.4 ^{bc}	7.2 ± 0.6 ^a	
		US2	3.6 ± 0.2 ^b	19.8 ± 0.9 ^a	3.0 ± 0.3 ^{ab}	3.3 ± 0.3 ^{abc}	6.9 ± 0.3 ^a	24.9 ± 1.7 ^{ab}	38.0 ± 2.0 ^e	1.3 ± 0.1 ^e	12.7 ± 1.5 ^c	7.2 ± 0.3 ^a	
Orange by-product			1.8 ± 0.1	26.2 ± 1.6	6.2 ± 0.2	3.0 ± 0.4	10.5 ± 0.5	22.2 ± 3.0	29.5 ± 1.6				

* Rha: Rhamnose, Ara: Arabinose, Xyl: Xylose, Man: Mannose, Gal: Galactose, Glc: Glucose, GalA: Galacturonic acid.

**Values are means ± standard deviation of three replicate experiments. Values with different letters in the same column indicate significant differences (p < 0.05)

Table 2. Degree of methylation (DM) of extracts obtained with mechanical agitation (AG= 0.2 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

Time (min)	pH	Experiment	DM (%)
30	1.5	AG	55.7 ± 0.9 ^{abc}
		US1	54.9 ± 1.3 ^{abc}
		US2	55.5 ± 0.8 ^{ab}
	2.0	AG	40.0 ± 1.4 ^d
		US1	44.4 ± 2.2 ^d
		US2	43.2 ± 1.0 ^d
60	1.5	AG	56.6 ± 1.1 ^a
		US1	53.5 ± 3.2 ^{abc}
		US2	55.3 ± 3.1 ^{ab}
	2.0	AG	49.7 ± 0.3 ^c
		US1	49.6 ± 0.1 ^c
		US2	51.2 ± 2.5 ^{bc}

* Values are means ± standard deviation of three replicate experiments. Values with different letters indicate significant differences ($p < 0.05$)

Table 3. Identified parameters of the second order rate model, and the corresponding confidence intervals (CI) associated to each parameter for the kinetics obtained with mechanical agitation (AG= 0.2 xg) and those extracted with UAE (US1=542 W/L and US2=794 W/L)

pH	Experiment	Ymax (%)		h (% min ⁻¹)		K (% ⁻¹ min ⁻¹)		R ² *	MRE** (%)	VAR** (%)
		Value	CI	Value	CI	Value	CI			
1.5	AG	25.28	[23.36, 27.47]	3.90	[2.78, 6.61]	6.1x10 ⁻³	[5.1x10 ⁻³ , 8.7x10 ⁻³]	0.9632	7.4	96.7
	US1	51.22	[46.15, 54.70]	5.55	[4.52, 7.19]	2.1x10 ⁻³	[1.9x10 ⁻³ , 2.4x10 ⁻³]	0.9861	5.2	98.2
	US2	69.66	[64.31, 75.99]	6.37	[5.09, 8.53]	1.3x10 ⁻³	[1.2x10 ⁻³ , 1.4x10 ⁻³]	0.9730	7.4	97.2
2.0	AG	11.51	[11.00, 12.07]	5.33	[3.11, 14.56]	4.0x10 ⁻²	[2.1x10 ⁻¹ , 1.2x10 ⁻¹]	0.9881	5.5	95.5
	US1	15.42	[14.70, 16.21]	5.66	[3.55, 13.94]	2.4x10 ⁻²	[1.6x10 ⁻² , 5.3x10 ⁻²]	0.9870	4.4	97.5
	US2	20.70	[19.90, 21.57]	8.92	[5.67, 20.85]	2.1x10 ⁻²	[1.4x10 ⁻² , 4.5x10 ⁻²]	0.9913	3.4	98.9

* R² Correlation coefficient of the linear form of the model

** % MRE: Mean relative error, % VAR: percentage of explained variance, were estimated by comparison of experimental and simulated extraction kinetics.