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Studies of degradation of pectin derived compounds from onion skins in subcritical water

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HIGHLIGHTS

- Galacturonic acid degraded in subcritical water yielding formic acid and furfural.
- Heating rate affected the degradation of GalA.
- The degradation of GalA followed a first order kinetics.
- GalA hydrolysis was promoted by temperature.
- The activation energy of the GalA hydrolysis was determined to be 96.7 ± 3.4 kJ/mol.

GRAPHICAL ABSTRACT

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ABSTRACT

The degradation kinetics of Galacturonic Acid (GalA) in subcritical water (SubW) was studied in a batch reactor. GalA degradation was faster than other monosaccharides and could be described by the first order kinetic model. The initial concentration of GalA did not affect the degradation kinetics, but the heating rate and the reaction temperature played an important role in the degradation of GalA. The energy of activation was calculated according to the Arrhenius equation and resulted to be 97 ± 3 kJ/mol. Degradation products formation, mainly formic acid and furfural, was faster with temperature until a maximum concentration was reached (0.2 g C in formic acid/g C in initial GalA at 155°C and 45 min, severity factor 3.2), followed by a fast degradation whereas a plateau concentration was reached in the case of furfural (around 0.16 g C /g C in initial GalA at 185 °C after 45 min).

1. Introduction

Pectins are a set of complex heteropolysaccharides present in the middle lamella of the plant cell walls [1], which are widely used in the industry to improve some technological aspects in their formulations [2]. Pectins are composed mainly of galacturonic acid (GalA), an acid sugar which is the oxidized form of D-galactose [2], which forms different structures: a linear and homogenous one named homogalacturonan (HG) pectin formed only by GalA, and a branched one named rhamnogalacturonan (RG) pectin. RG structure has rhamnose inserted into the linear GalA chain, with some other neutral sugars (mainly galactose or arabinose) attached to it [3].

Pectin has a growing worldwide demand: it is estimated to be as high as 40,000 t per year, with an annual growth of about 5% [4]. In order to

Abbreviations: SubW, Subcritical water; GalA, Galacturonic acid; GluA, Glucuronic acid; FA, Formic acid; Fur, Furfural.

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meet the increasing demand of pectin, new sources and extraction strategies are necessary. In this sense, subcritical water (SubW) offers attractive properties to separate and fractionate different components of lignocellulosic biomass [5]. SubW refers to water at temperatures ranging from 100 °C (boiling point) to 374 °C (critical point), which remains in a liquid state due to the application of pressure. SubW possesses two different features from room temperature water: one is the lower dielectric constant and the other is a higher ionic product (K_w, higher the higher the temperature), which implies that the pH changes from 7.0 at room temperature to about 5.7 at 180 °C, resulting in higher ionic strength of hydronium and hydroxide ions than at ambient temperature [6]. Properties of SubW can be tuned by changing the working conditions, enhancing mass transfer and the extractability of barely water-soluble bioactive compounds, since subcritical water favors the hydrolysis reactions [7]. For instance, the hydrolysis of the hemicelluloses releases xylose and arabinose that are transformed into furfural; hexoses from cellulose yield levulinic acid [8], pectin can be hydrolyzed into smaller molecules using SubW (120 °C, 40 min) [9], or polygalacturonic acid that can be degraded to GalA oligomers (degree of polymerization <14) in a batch reactor at temperatures of 125 and 135 °C (pressure 10 MPa) and reaction times from 10 to 120 min following very complex reaction mechanisms, involving parallel and consecutive reactions [10]. Therefore, it is necessary to study the hydrolysis kinetics of polysaccharides promoted by SubW [11], in order to identify and quantify the byproducts formed.

Given the potential of SubW for the extraction of pectin from biomass, basic research on the degradation (kinetics and byproducts formed) of pectin and its monomer (GalA) is necessary, since temperature promotes pectin degradation [3] and a careful selection of the extraction conditions is needed. According to the literature, the conversion of sugars and uronic acids into organic acids and other products in subcritical/supercritical water results to be very complex [12–14], and fast [15]. More specifically, it has been demonstrated that hexuronic acids are more susceptible to degradation than hexoses although they are structurally related [16]. GalA has an aldehyde group at C1 and a carboxylic acid group at C6 [17], and it is precisely the presence of the carboxylic groups at carbon C6 which seems to be responsible for the higher reactivity of the uronic acids [18]. Among the uronic acids, glucuronic acid (GluA) suffers a faster degradation than GalA in SubW at temperatures in the range from 140 to 160 °C [19]. Wang et al. [11] studied the degradation of GalA in SubW in the temperature range from 160 to 200 °C, in a continuous reactor, with residence times up to 300 s, reporting that GalA degradation follows a first order kinetics and pH of the media increases as the GalA is degraded and the degradation of GalA is much faster than the degradation of galactose, which barely degrades at temperatures close to 180 °C. Pińkowska et al. [20] studied the hydrolysis of high methyl ester citrus-apple pectin as a model substance for plant biomass waste in a batch reactor using SubW (120–200 °C, residence times up to 60 min), reporting that formic acid was one of the main degradation products from GalA obtained.

Most of the works that can be found in the literature are focused on describing the degradation kinetics of the uronic acids, but more emphasis should be paid on the degradation products formed from the uronic acids as a function of the working conditions. The purpose of this work is to study the degradation of GalA under SubW conditions, in order to find out the degradation kinetics followed by this compound and the main degradation products that can be expected under different SubW conditions. This work will help to obtain useful insights regarding the degradation products from pectin that can be expected during the SubW extraction, considering as experimental factors affecting the degradation of GalA the following: temperature, GalA concentration and reactor heating rate. In addition to GalA, the degradation of other saccharides, including galactose, arabinose, and glucuronic acid, will be investigated under specific conditions (145 °C for 120 min—the optimal temperature for pectin extraction in SubW, as determined by Benito-Román et al. [36]). This exploration is essential as these compounds may also be present in the pectin structure obtained during SubW extraction.

2. Materials and methods

2.1. Chemicals

D-galacturonic acid monohydrate (GA, purity >97%) was obtained from Alfa Aesar (ThermoFischer GmbH, Kandel, Germany). Glucose (glu, 99.5%), galactose (gal, 99%), arabinose (ara, 99%), glucuronic acid (GluA, ≥98%) and norfuraneol (97%) were supplied by Sigma-Aldrich Inc. (St. Louis, MO, USA). Furfural (F, 99%), 2-furancarboxylic acid (>98%) and 5-formyl-2-furanoid acid (>98%) were purchased from TCI – Tokyo Chemical Industry CO Ltd (Tokyo, Japan). Formic acid (FA, 98%) and acetic acid glacial (AcA, 99.7%) were purchased from Panreac Química S.L.U. (Barcelona, Spain) and lactic acid was supplied by Fluka Chemic GmbH (Buchs, Switzerland). Potassium hydrogen phthalate and sodium hydrogen carbonate were supplied by Nacalai Tesque, Inc. (Kyoto, Japan). Sulfuric acid used for the sample hydrolysis was 96% purity and was provided by Labbox Labware (Barcelona, Spain), whereas the one used for the HPLC mobile phase preparation was 96% Suprapur provided by Merck KGaA (Darmstadt, Germany). All chemicals were used without further purification.

2.2. Acid hydrolysis experiments

The degradation of GalA (0.5 g/L; 2.28 mM) in acid medium (1 h, 121 °C, 4% H_2SO_4) was carried out in an autoclave reactor (AUTESTER-G, P-Selecta), according to the NREL laboratory analytical procedure 510–42623, issued for the determination of sugars, byproducts and degradation products in liquid fraction process samples.

2.3. Subcritical water hydrolysis experiments

Experiments were carried out in the 500 mL batch reactor (maximum pressure 7 MPa and maximum temperature, 250 °C, designed in our laboratory) shown in Fig. 1. In each experiment, 350 mL of the GalA solution were placed in the reactor. Then, the system was pressurized by means of N_2 to achieve the desired working pressure (5 MPa). The solution in the reactor was stirred using a magnetic stirrer placed under the reactor. A ceramic electric band heater (2000 W) around the extractor was used to heat the reactor to the desired working temperature. Then, the effect of the GalA initial concentration, the reactor heating rate and the working temperature on the GalA concentration was studied.

The effect of the GalA initial concentration (from 0.1 to 0.5%, v/v, a range that can be commonly found in the literature) and the reactor heating rate (from 4.4 to 8.5 °C/min) were initially studied at a temperature of 145 °C. Samples were collected periodically, with the initial sample (t = 0) obtained upon reaching the reaction temperature to

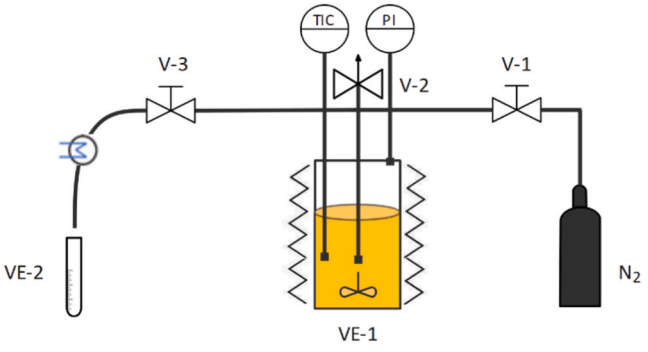

Fig. 1. Subcritical water batch reactor used in this work. Key: VE-1, SubW batch reactor; VE-2, samples collecting tube; V-1, N_2 line valve; V-2, purge valve; V-3, sampling valve.

assess potential degradation of GalA during the heating-up period, up to a maximum reaction time of 120 min

Once the effect of the GalA initial concentration and the heating rate were studied, the effect of the reaction temperature was studied in the range from 125 to 185 °C for a maximum reaction time of 180 min. Samples were taken periodically from the reactor to complete the total reaction time. The simultaneous effect of both temperature and time is estimated using the so-called severity factor in which the temperature profile in heating and the isothermal period were considered, according to (Eq. [1]) [21], where t_H is the time needed to achieve the desired working temperature, t is the isothermal treatment time (min), T is the working temperature (°C), T_{ref} is equal to 100 °C and ω is an empirical value related with activation energy and temperature, and typically takes a value of 14.75 for glycosidic bond cleavage of carbohydrates [21].

$$\log R_0 = \log\left[R_{0Heating} + R_{0Isothermal}\right] = \log \int_0^{t_H} \exp\left(\frac{T(t) - T_{ref}}{\omega}\right)\cdot dt + \log\left(t\cdot e^{\left(\frac{T-T_{ref}}{\omega}\right)}\right) \quad (1)$$

The degradation studies for additional compounds (GluA, Ara, Gal) were conducted at 145 °C for a duration of up to 120 min, with an initial concentration of each compound set at 0.1%. The heating rate employed for these experiments was 4.4 °C/min.

2.4. Samples characterization

2.4.1. GalA and degradation products identification

Liquid samples were centrifuged (5 min at 4500 rpm) and then filtered through a 0.22 μm filter. Then, they were analyzed by HPLC: the chromatographic system used was the model 1260 Infinity II (Agilent), with the following specific modules: injector (G7129A), isocratic pump (G7110B), refractive index detector (G7162A) and variable wavelength detector (G7114A). The column used for the analysis was Aminex HPX-87 H (BioRad) coupled with the guard column Micro-Guards Cation H^+ (BioRad). Both the column and RI detector were kept at 40 °C and, as mobile phase, 5 mM H_2SO_4 solution was used at 0.6 mL/min, with a total running time of 67 min per injection. The identification of the compounds was done by comparing the retention time with pure standards, which are detailed in Section 2.1. A representative chromatogram is presented as supplementary material (Fig. S1).

Regarding the concentration of GalA, the ratio C/C_0 was subsequently calculated with C_0 representing the concentration of GalA measured at t = 0 and C the concentration at a given time. The yield of the degradation products (formic acid and furfural) was expressed using as a reference the carbon content of those species compared to the initial carbon content present in the galacturonic acid solution (g of C/g of C in the initial GalA), as reported by [22].

2.4.2. Total organic carbon (TOC) determination

TOC was determined using the Shimadzu TOC-V CSN Analyzer (Shimadzu Co., Japan). TOC was calculated as the difference between total carbon (TC) and inorganic carbon (IC). The standard for the TC determination was potassium hydrogen phthalate ($C_8H_5KO_4$), in a concentration range from 0 ppm to 1000 ppm. For inorganic carbon (IC) determination, a solution of sodium hydrogen carbonate ($NaHCO_3$) up to 200 ppm was used as standard. This determination allowed to calculate the TOC balance, as the ratio between the mass of organic carbon in the solution after the SubW treatment and in the feed solution, expressed in %.

2.5. Kinetic modeling

The first order kinetic model was used to describe the degradation of GalA under the different experimental conditions studied, since it is commonly used to describe the degradation of compounds under SubW conditions. This model assumes that the natural logarithm of the residual concentration of the substrate decreases linearly with the time. The Eq. (2) describes mathematically this model:

$$\ln(C/C_0) = -kt \quad (2)$$

C is the remaining concentration of GalA in the reactor effluent, C_0 the initial concentration of GalA in the feed, k is the degradation rate constant at given pressure and temperature conditions and t is residence time in the reactor.

2.6. Statistical analysis

All the statistical calculations were done using Statgraphics 18-X64. 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.

To estimate the kinetic parameters, non-linear regression was performed by using the Marquardt algorithm (Statgraphics 18-X64). Experimental results were then compared to those of the model prediction through the values of the Root Mean Square Deviation (RMSD), calculated according to Eq. (3):

$$RMSD = \sqrt{\frac{\sum_{i=1}^{n}\left(Response_{\exp} - Response_{calc}\right)^2}{n}} \quad (3)$$

3. Results and discussion

3.1. Degradation of GalA in acid medium

The degradation of GalA in acid medium revealed that nearly 40% of the initial GalA (final concentration 1.38 mM) was degraded, yielding mainly furfural (0.06 g C/g C in initial GalA) and formic acid (0.10 g C/g C in initial GalA). These results were used as a reference in order to study the degradation of GalA in SubW, and also to determine the main degradation products formed. The TOC analysis revealed that almost 62% of the initial carbon present in GalA is identified in formic acid, furfural and unreacted GalA, whereas the remaining 38% is present in other unidentified species. This results are in agreement with these reported by Danfeng et al. [23], who studied the determination of GalA in pectin and pectin products by acid hydrolysis, using either H_2SO_4 or HCl in different concentrations. These authors reported a degradation of the monomers released from the pectin chain by the action of the acid and heat, yielding furfural and other compounds. They also reported that acid-hydrolysis methods can induce a partial or incomplete hydrolysis of pectin, result also reported by Liu et al. [24]. The latter authors reported that the hydrolysis procedure is a relatively easy operation, while the reaction mechanism of acid hydrolysis is complicated. Although uronic acids can be easily hydrolyzed, they yield unstable compounds that suffer further degradation, in contrast to pentoses or hexoses that degrade to 2-furfural and to 5-hydroxymethyl-2-furfural that further degrades to levulinic acid and formic acid, respectively [23].

3.2. Degradation of GalA in SubW

3.2.1. Effect of the GalA concentration

Three different concentrations of GalA were tried: 0.1%, 0.25% and 0.5% at 145 °C, at a heating rate of 4.4°C/min. It was possible to observe that the initial concentration of GalA did not affect the degradation rate of the uronic acid, according to the results presented in Table 1. There was no statistically significant difference (95% confidence level) between the kinetic constants calculated for the three different experimental conditions studied, although a trend can be observed, as the lower the concentration the higher the kinetic constant. GalA degradation was linear and was modeled using the first order kinetics.

Table 1
First order model kinetic constants determined for the GalA degradation experiments in SubW and TOC balance calculated.

		k (min^{-1})	R^2	RMSD	TOC balance (%)
Effect of the concentration	0.1%	0.0172 ± 0.0008^{A}	0.991	0.12	83.0 ± 0.7^{A}
	0.25%	0.0166 ± 0.0006^{A}	0.983	0.09	82.2 ± 1.4^{A}
	0.5%	0.0155 ± 0.0007^{A}	0.975	0.11	83.4 ± 1.2^{A}
Effect of the heating rate	4.4 °C/min	0.0172 ± 0.0008^{C}	0.991	0.12	83.0 ± 1.7^{A}
	5.5°C/min	0.0196 ± 0.0009^{B}	0.972	0.14	78.1 ± 1.1^{B}
	8.5°C/min	0.031 ± 0.001^{A}	0.986	0.15	69.7 ± 0.3^{C}
Effect of the temperature	125°C	0.0045 ± 0.0002^{A}	0.992	0.03	90.7 ± 0.8^{A}
	145°C	0.0172 ± 0.0008^{B}	0.991	0.12	83.0 ± 0.7^{B}
	155°C	0.0376 ± 0.0008^{C}	0.993	0.13	69.2 ± 1.1^{C}
	165 °C	0.0703 ± 0.0062^{D}	0.989	0.32	62.5 ± 0.5^{D}
	185 °C	0.1974 ± 0.0096^{E}	0.985	0.31	56.1 ± 0.5^{E}

Different letters indicate statistically significant differences at confidence level of 95%, among the results obtained at each experimental factor studied, according to the LSD test.

The results obtained in our work agree with those reported by Wang et al. [11], who studied the GalA degradation in SubW in a continuous tubular reactor. They studied the effect of the GalA concentration (range 0.5–2.5%), and concluded that the degradation followed a first order kinetics and there was no effect of the concentration on the degradation kinetics.

The TOC balance (Table 1), indicated that around 83% of the initial carbon remained in the solution, the remaining 17% moved to the gas phase, and no differences were observed among the three different concentrations tried. Regarding the degradation products, formic acid and furfural accounted for the 95% of the identified degradation products formed in all cases. In Fig. 2 it is presented the evolution of the concentration of these two degradation products versus time. After 60 min, the concentration of formic acid reached a plateau around 0.23 g of carbon per g of carbon in the initial GalA (g C/g C in GalA), whereas the concentration of furfural kept steadily increasing with time, up to 0.06 g C/g GalA at 120 min, but a plateau was not reached. The results presented in this work are in agreement with the results presented by Pińkowska et al. [20], who showed that high methyl ester citrus-apple pectin, at 150°C after 40 min, yielded 63.7 mg/g raw material of formic acid and 18.6 mg/g raw material of furfurals, whereas almost 40% of the formed products were unidentified, and by Miyazawa and Funazukuri [22], who found that furfural was a secondary product from the degradation of monomers and oligomers formed from the degradation of polygalacturonic acid in pressurized hot water (180–280 °C) in a semicontinuous reactor, where a maximum yield of 0.47% (calculated as g of C/g of C of the initial sample) was measured (220 °C). Furfural was produced in lower extent than formic acid, which agrees with the results presented by Usuki et al. [19], who reported that furfural is scarcely produced from hexuronic acids.

All in all, the reaction pathway of GalA under SubW is complex and yet not clear. Zhao et al. [25] proposed that furfural can be degraded to form formic acid. Therefore, according to these results, GalA can degrade to furfural and this compound can suffer a further degradation to formic acid. Other authors have attributed a key role to formic acid in the degradation of pentoses to yield furfural [26]. According to those authors, in order to obtain furfural yields above 50%, the increase in the formic acid concentration allows to reduce the reaction temperature: at 170 °C low formic acid concentrations are needed whereas the temperature can be reduced down to 150 °C if more concentrated formic acid solutions (above 64.4%, w/w) are used. In any case, as the GalA degradation reaction goes on, the pH of the solution tends to increase from 2.9–3 to 3.4–3.5 after the complete degradation of the GalA solution (0.1%, w/w) at 145°C. Wang et al. [11] also reported the same behavior, pointing out that the pH increase might be related to the decarboxylation of GalA or to the ketone formation under the specific reaction conditions. Fatouros et al. [18] reported that an important degradation step of uronic acids is the heat-induced loss of carbon dioxide; according to these authors the ring opening velocity of GalA was enhanced due to the presence of the carboxylic function, but this cannot

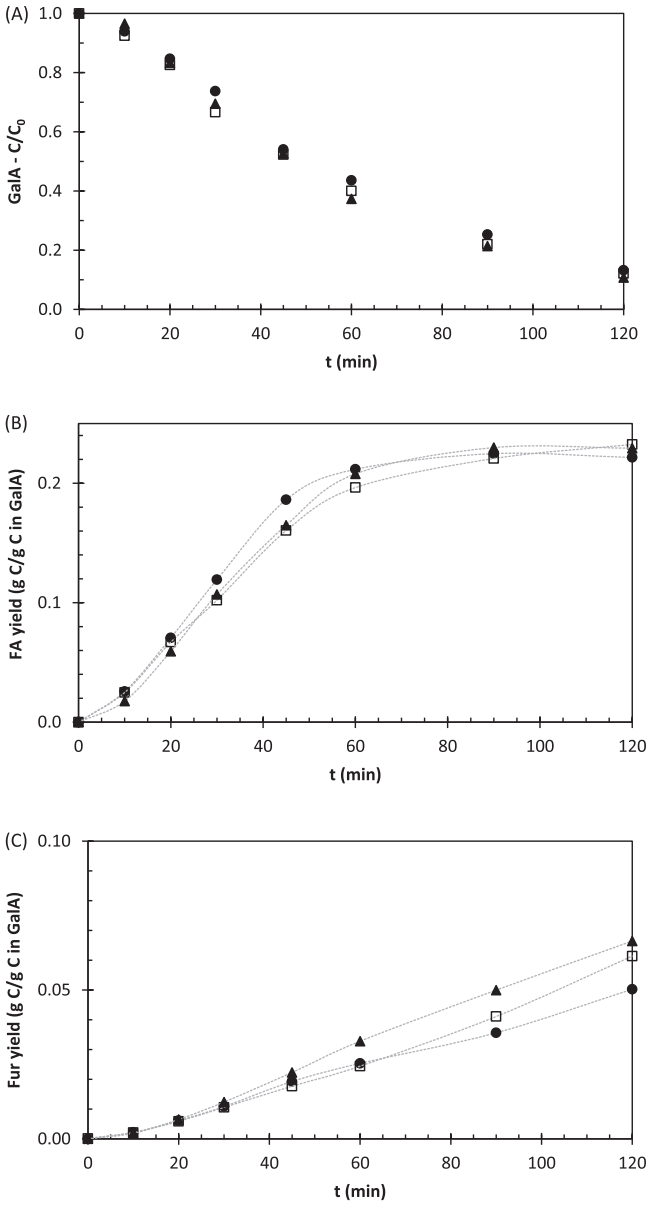

Fig. 2. Evolution of the concentration of the main degradation products (A, GalA; B, formic acid; C, furfural), formed from GalA at 145 °C and a heating rate of 4.4 °C along time, when the effect of the initial GalA concentration is studied. KEY: ●, 0.5%; □, 0.25%; ▲, 0.1%. Dashed lines are included to guide the eye.

be the only explanation of the increased reactivity. These authors pointed out that the high reactivity of the uronic acids is related to their ability to release CO_2 that leads to the formation of very reactive intermediate products. Mohamad et al. [27] proposed that the fact that may help to explain the high reactivity of uronic acids is related to the fact that the oxygen atoms in the carboxylic group tend to attract electrons, which weakens the bond next to the carboxyl group. However, not all the uronic acids behave in the same way: GluA has a significantly lower reactivity tan GalA, and according to Fatouros et al. [18] the reason must be found in the position of the OH group in carbon 4, which is different in both compounds. Also Usuki et al. [19], reported different degradation patterns for GalA and GluA: GalA shows a different degradation pattern compared to other uronic acids. These authors observed that initially GalA degraded rapidly to a certain extent, after which, the degradation process advanced much more slowly than GluA degradation.

The work developed by Bornik and Kroh [28] tried to shed light on the degradation pathway of GalA under different pH, at 100 °C for 2 h. These authors revealed that, under weak acidic conditions, GalA undergoes a reaction pathway that leads to the formation of browning compounds such as reductic acid (2,3-dihydroxy-2-cyclopenten-1-one) which has more browning power than furfural, DHCP (4,5-dihydroxy-2-cyclopenten-1-one), and furfural. In sum, the browning power of the GalA solutions is ten times higher than that of pentoses and hexoses. All these three compounds have a common intermediate: 2-ketoglutaraldehyde, formed by eliminative decarboxylation at high temperatures in slightly acid to neutral conditions. Other compounds such as norfuraneol is typically formed under alkaline conditions. The decarboxylation process of GalA to arabinose only happened in the presence of concentrated mineral acids. According to this fact, under weak acidic conditions, the decarboxylation of GalA to yield arabinose is not the preferred degradation pathway. Other authors have been able to identify other degradation products formed as a consequence of the GalA exposure to acidic conditions at high temperatures, such as Madson and Feather [29]. These authors reported the hexuronic acids suffer decarboxylation reaction, leading to the formation of furfural, reductic acid (2,3-dihydroxycyclopenten-I-one), and traces of 5-formyl-2-furoic acid. Urbisch et al. [16] used GalA solutions to study its degradation process at temperatures of 130 °C and heating times of 240 min. In the first place, these authors probed that GalA solutions had a 10 times higher browning power than Gal or Ara solutions, which supports the idea of uronic acids having higher reactivity than the equivalent hexose. In the second place, these authors were able to identify the following degradation products: furfural and norfuraneol (the authors demonstrated that were also formed during the degradation of D-galactose and D-arabinose) 2-furoic acid, 5-formyl-2- furoic acid, which together with reductic acid are specific degradation products for the uronic acids.

In this work, only 5-formyl-2- furoic acid was detected in the experiments that had an initial concentration of 0.5% of GalA. It was observed that the concentration of this compound increased with time, reaching the highest concentration (0.026 mg C/g C in GalA) after 120 min. On the other hand, 2-furoic acid was not detected in any of the experiments carried out, according to our analytical method. Norfuraneol (4-Hydroxy-5-methyl-3-furanone), a highly reactive compound, did not appear as a degradation product when GalA was used as raw material, probably due to slightly acid reaction conditions, which according to Bornik and Kroh [28], demands pH in the range to 5–8, to be formed, compared to the lower pH obtained in the reaction conditions (see Fig. S2 in supplementary material), which was below 4 in most of the cases.

3.2.2. Effect of temperature

All the experiments were carried out using a 0.1% GalA solution in a temperature range from 125 to 185 °C at a heating rate of approximately 4.4 °C/min. It was observed a clear effect of the temperature, with a GalA degradation that resulted to be faster the higher the temperature, as demonstrate the kinetic constants presented in Table 1. Moreover, the TOC balance (Table 1) also revealed a higher gasification rate of the organic matter the higher the temperature, reaching a gasification rate of almost 50% at 185 °C.

The temperature dependence of the kinetic constant was expressed by the Arrhenius equation:

$$k = k_0 e^{(-E_a/RT)} \tag{4}$$

where E_a is the activation energy (J/mol), k_0 is the frequency factor (min^{-1}), R is the gas constant (8.3145 J/mol·K), and T is the absolute temperature (K). The E_a and k_0 values for GalA were evaluated from the plots shown in Fig. 3, after linearization of the Eq. (4). From this figure, the activation energy resulted to be 97 ± 3 kJ/mol and the frequency factor $3.82{\cdot}10^{10}$ min^{-1}.

Considering that the ω parameter in Eq. (1) is a function of the activation energy according to Eq. (5) [21],

$$\omega = \frac{T_f^2 \cdot R}{E_a} \tag{5}$$

with T_f denoting a temperature selected to be midway among the biomass fractions under subcritical conditions (which is 155 °C in this study), R representing the universal gas constant (8.314 J·mol/K) and E_a indicating the apparent activation energy (97 kJ/mol in the present study), it is possible to calculate it for the specific reaction of GalA degradation in SubW. The ω parameter resulted to be 15.7, rather than the conventionally used 14.75. The ω calculated value was used to calculate the severity factor for the GalA degradation reaction in Section 2.2.2 according to Eq. (1).

The effect of temperature on the degradation kinetics of GalA using SubW has also been studied by other authors in the literature. Usuki et al. [19] reported a value of 427 kJ/mol for the activation energy (temperature range 140–160 °C), calculated after adjusting the experimental data to the Weibull model. Wang et al. [30] reported the activation energy for GluA and glucuronate in SubW (160–200 °C), which resulted to be 88.5 and 63.2 kJ/mol, respectively. These authors used the first order model. In a previous work, the same authors [11] studied the degradation of GalA in SubW (160–220 °C). They demonstrated that the degradation process obeyed a first-order kinetics, demonstrated also that the dependence of the degradation kinetics constant could be expressed by the Arrhenius equation, being the activation energy and the frequency factor 131 kJ/mol and $4.81{\cdot}10^{12}$ s^{-1}, respectively. After having studied the degradation of GalA and GluA in SubW, these authors were able to confirm the higher degradability of the uronic acids compared to the corresponding hexose. In this sense, Khajavi et al. [31] reported activation energies in the range 90–170 kJ/mol for some hexoses (galactose, glucose, sorbose, fructose, and mannose: 170, 155,

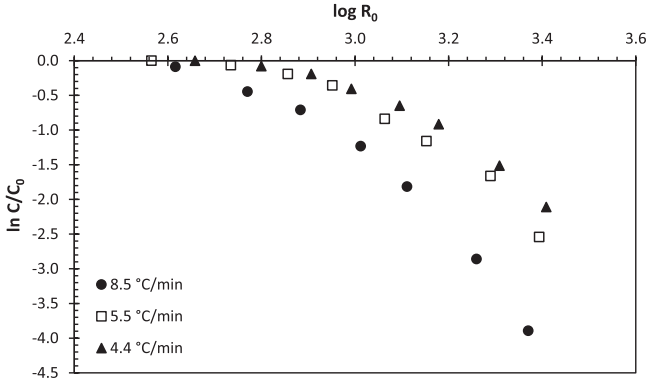

Fig. 5. Evolution of the concentration of GalA with time when different heating rates were tried at 145 °C versus the severity factor. Initial concentration of GalA was 0.25%. Key: ●, 8.5 °C/min; □, 5.5 °C/min; ▲, 4.4 °C/min.

132, 120, and 90 kJ/mol, respectively) whose degradation in SubW was studied in the temperature range 180–260 °C and fitted to the Weibull model.

Mohamad et al. [27] studied the degradation of other uncommon uronic acids mannuronic acid (MA) and guluronic acid (GA), obtained from alginic acid primarily obtained from kelp. They used a continuous flow reactor in a temperature range from 170 to 250 °C, residence time up to 100 s and pressure equal to 25 MPa. The activation energies for the decomposition of MA and GA were 28.3 and 20.6 kJ/mol, using the first-order model, and it was found that these two uronic acids had a decomposition rate significantly higher than those of GalA and GluA.

The formation of the degradation products was clearly affected by temperature, following different patterns (see Fig. 4). Formic acid and furfural were formed in low extent at 125 °C, which agrees with the low degradation rate detected for GalA (C/C_0 after 120 min was 57%).

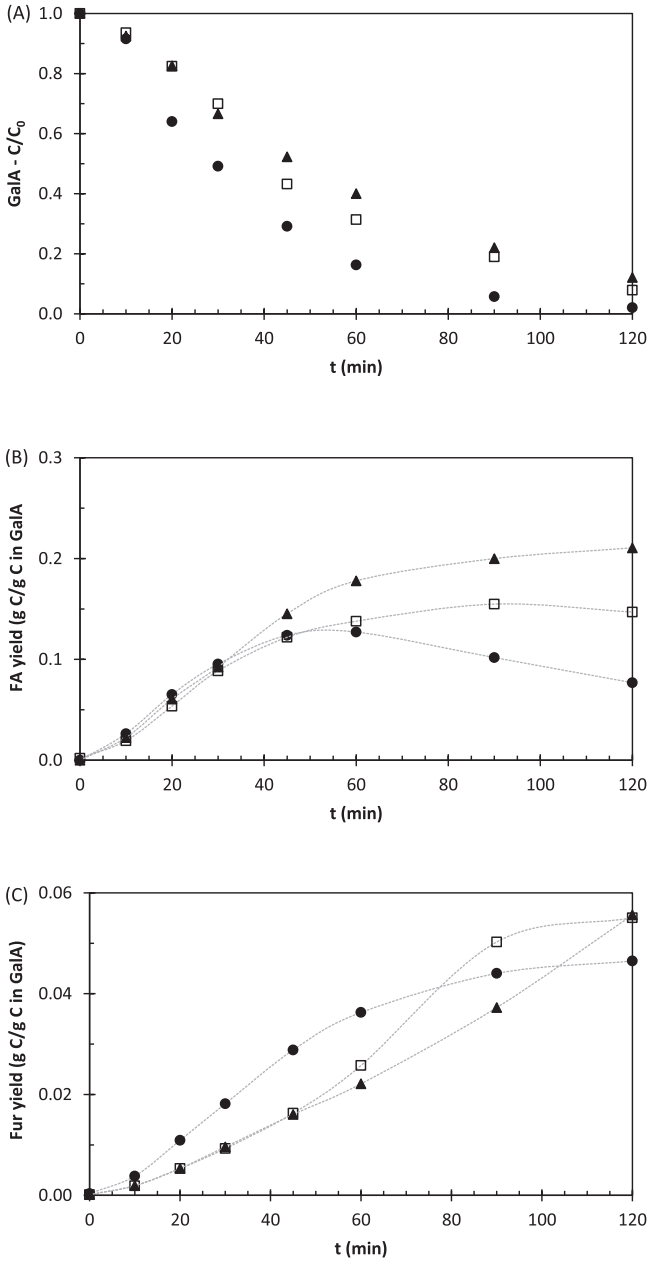

Fig. 6. Effect of the heating rate on the concentration of the main degradation products (A, GalA; B, formic acid; C, furfural) formed from GalA (0.25%, v/v) at 145 °C. Key: •, 8.5 °C/min; □, 5.5 °C/min; ▲, 4.4 °C/min. Dashed lines are included to guide the eye.

However, when reaction temperature was increased, the formation of formic acid and furfural was increased as well. Regarding formic acid, the highest amount was formed at 145 °C after 90 min, when a plateau was reached (approximately 0.2 g C/g C in GalA). When temperature was increased, the highest concentration of formic acid was detected after shorter experimental times: 45 min at 155 °C, 30 min at 165 °C and only 10 min at 185 °C. After this maximum, the concentration of formic acid began to decrease, the faster the higher the temperature. In general, formic acid has been considered as an effective catalyst for biomass processing [32], which is usually formed when processing biomass at high temperatures forms other compounds such as furfural, together with glycolic acid, 2-furoic acid, lactic acid, pyruvic acid, and various C5 and C4 hydroxylic acids among others [33] at temperatures in the range from 125 to 200 °C. These authors have demonstrated that formic acid is quite unstable under acidic conditions at high temperatures, as has been demonstrated in our work when working temperatures are above 155 °C (Fig. 4). In parallel, the concentration of furfural increased with temperature, but in all the temperatures above 155 °C, a plateau was reached after 60 min of experiment. Besides the formation of formic acid and furfural, changes in the concentration of arabinose were detected (Fig. 4 (D)). This is indicating that the GalA degradation via decarboxylation is also taking place, as indicated Pińkowska et al. [20]. This reaction is strongly affected by the temperature and the reaction time. At 125 °C a steady increase in arabinose concentration is observed, reaching the highest values at 135 and 145 °C after 45 min, decreasing from that moment. Then, at the highest temperatures, the maximum concentration of arabinose is found at very short times, undergoing a fast degradation.

Furfural is a highly reactive compound due to its furan ring and aldehyde group, which can be easily oxidized at high temperatures [34]. Furfural tends to be unstable at high temperatures under acidic conditions [33], being involved in different side reactions, which comprise oxidation, degradation, condensation, and polymerization [34]. More specifically, furfural degradation includes self-polymerization (furfural resinification, which produces a black, insoluble resin so called humin), ring opening and decomposition reactions, whereas furfural condensation denotes reactions with sugar intermediates [32] such as d-xylose or d-xylose intermediates (when furfural is produced from biomass hydrolysis) [35]. As has been presented in our samples, the GalA degradation reaction media resulted to have a concentration of formic acid several times higher than that of furfural at low temperature. Some authors have demonstrated that the presence of formic acid in the media reduces the furfural degradation extent, so the undesired humin by-products are prevented [33], due to a shift of the reaction equilibrium. The conversion of furfural to formic acid is affected by temperature, furfural concentration and the presence of a catalyst (usually an acid). High acidities promote the degradation of furfural to formic acid and other aliphatic compounds, which can continue reacting to form highly branched compounds; on the other hand, high concentrations of furfural promote condensation reactions. Almhofer et al. [33] observed that formic acid formation follows an acid-catalyzed mechanism and that this reaction is reversible. Formic acid may thus slow down the degradation of furfural to some extent. To prevent these side-reactions, some additives were added into reactions for their feature of inhibiting polymerizations [34], such as thiourea. This fact can explain why in our samples, despite of the high temperature, the concentration of furfural remains constant and the concentration of formic acid decreases. For instance, Lamminpää et al. [32], in their experiments had furfural concentrations in the range (4.7–15 g/L) and formic acid in the range 20–300 g/L, up to 64 times more formic acid than furfural in the reaction media at temperatures from 160 to 200 °C.These authors demonstrated that the formic acid concentration affects the degradation mechanism of furfural.

3.2.3. *Effect of the heating rate*

The heating rate is a key parameter in the SubW batch experiments.

In general, all systems are designed in order to minimize the time necessary to reach the experimental temperature. In this study all the experiments were carried out at 145 °C using a GalA initial solution of 0.25%. Then the heating rate was varied by controlling the power used in the heating band, so three different heating rates were obtained: 4.4, 5.5 and 8.5 °C/min.

In this case it was possible to see that the heating rate did have an effect of the degradation kinetics of GalA, being faster the faster the heating rate, according to the kinetic constants reported in Table 1. In this case, the TOC balance (Table 1) revealed a significant effect of the heating rate on the organic carbon dissolved after the treatment: the faster the heating rate the lower TOC in the final solution, indicating a higher share of the gaseous products formed (higher gasification of the organic matter). Moreover, the pH determination (Fig S1, B) also revealed and increase of the pH when the faster heating rate was tried, which might be indicating the presence of different species formed during the GalA degradation process, following a different degradation route. Fig. 5 shows the evolution of the GalA concentration versus the severity factor calculated using Eq. (1) and the ω parameter obtained according to Eq. (5), and can be seen that faster heating rates induce a faster degradation of GalA despite leading to smaller severity factors (since heating times are shorter). Severity factor considers the time a sample is exposed at temperatures above 100 °C which is supposed to be the temperature that induces degradation.

Regarding the degradation products, the formation of formic acid was affected by the heating rate. The formic acid forming rate was similar for the three heating rates observed in the first 45 min of experimental work. However, at the fastest heating rate, the highest concentration of formic acid was observed at 45 min, decreasing from this moment. The medium heating rate (5.5 °C/min) revealed an increase of the formic concentration up to 90 min, beginning to decrease afterwards. The slowest heating rate (4.4 °C) provided a slow concentration increase after the initial 45 min and a plateau in the formic acid concentration was observed afterwards, as observed in Fig. 6.

The concentration of the furfural formed seems also to be affected by the heating rate: at the slowest heating rate the concentration of furfural increased during all the experimental time, however at the fastest heating rate the concentration of furfural reached a maximum after 90 min of experiment.

3.3. Degradation of other compounds at 145 °C

Besides the degradation of GalA in SubW, the degradation of glucuronic acid (GluA), galactose (Gal) and arabinose (Ara) was studied at 145 °C for 120 min. This temperature was the optimal proposed by Benito-Román et al. [36] for the extraction of pectin from onion using SubW. Galactose and arabinose are two key compounds that can be found in the branched RG-I pectin domain, therefore it might be useful to study their degradation kinetics under the extraction conditions.

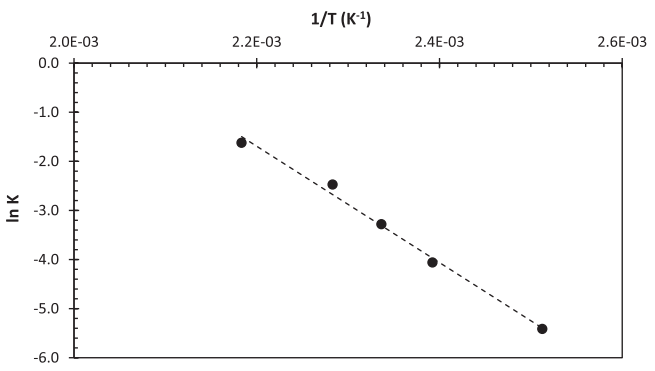

Fig. 3. Temperature dependence of the kinetic constant, according to the Arrhenius equation.

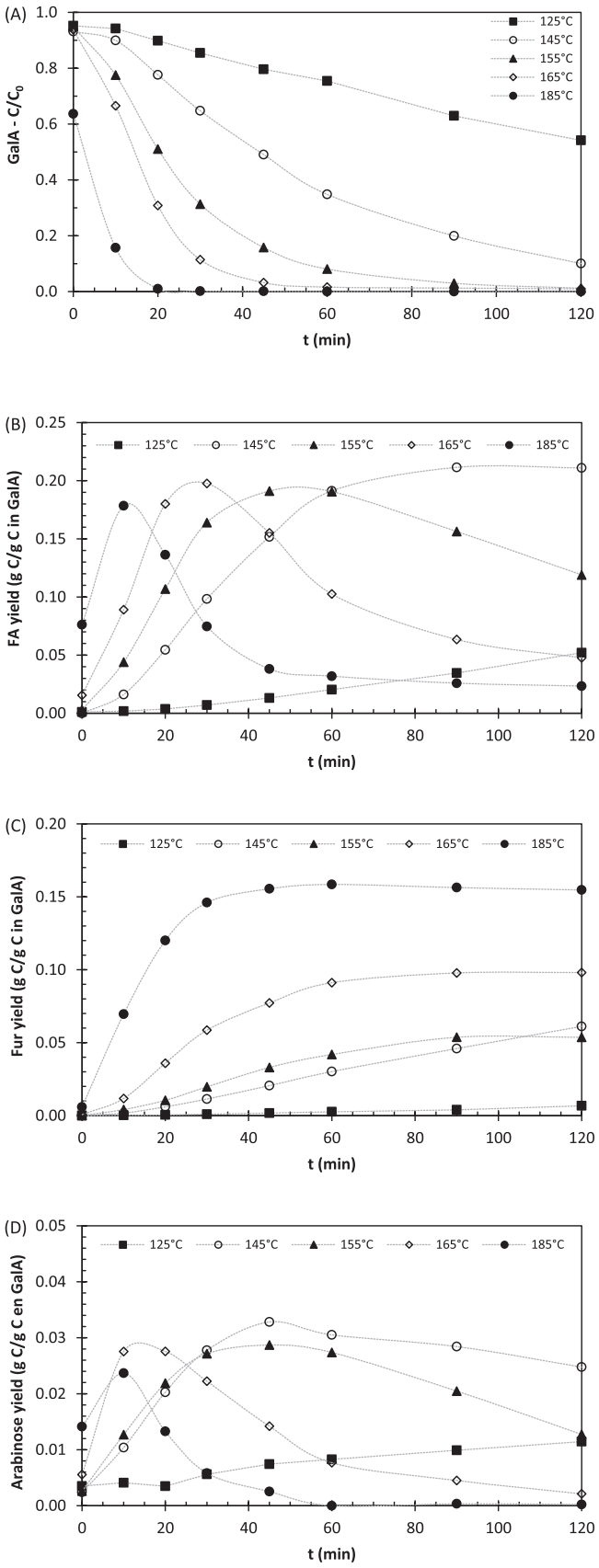

Fig. 4. Effect of temperature on the concentration of the main degradation products ((A), GalA; (B) formic acid; (C) furfural; (D) arabinose) formed from GalA (0.1%, v/v) with a heating rate of 4.4 °C/min. Dashed lines are included to guide the eye.

Table 2
First order model kinetic constant determined for GalA, GluA, Gal y Ara degradation experiments at 145 °C.

	k (min^{-1})	R^2	RMSD
GalA	0.0172 ± 0.0008^{B}	0.991	0.12
GluA	0.0337 ± 0.0002^{A}	0.992	0.26
Gal	0.0010 ± 0.0005^{C}	0.925	0.26
Ara	0.0002 ± 0.0000^{D}	0.967	0.03

The degradation kinetic constant for each of the aforementioned compounds is presented in Table 2. It is possible to see that the highest reactivity corresponds to GluA, followed by GalA, and finally the degradation kinetics of the sugars resulted to be very slow compared to the uronic acids, which agrees with the results presented in the literature. Galactose and specially arabinose were relatively stable at high temperature: only 20% of the initial arabinose was degraded, yielding formic acid and furfural, whereas galactose concentration was decreased by 50%, yielding formic acid and 5-hydroxymethylfurfural.

4. Conclusions

The degradation of GalA in SubW has been studied in this work. It has been demonstrated that the initial concentration of GalA did not have any effect on the degradation kinetics. However, the heating rate and the temperature used did affect the degradation kinetics, promoting it the higher the temperature and the faster the heating rate. It was also demonstrated that the degradation of GalA could be expressed by the first order model; which allowed to calculate the activation energy (97 kJ/mol) and which served to calculate the specific ω parameter used in the severity factor calculations for the GalA degradation in SubW. As the main degradation products, formic acid and in lower extent furfural were identified, which in general accounted for a maximum of 50% of the initial carbon present in GalA, not being possible to identify the compounds in which the remaining initial carbon has been transformed into. The TOC balance revealed an important extent of gasification reactions, which were promoted the higher the temperature and the faster the heating rate. No other reaction intermediates, but 5-formyl-2- furoic acid, reported in the literature have been identified in this work. All in all, useful insights regarding the degradation kinetics of GalA in SubW were obtained, which will serve for future experiments regarding the extraction of pectin from natural matrices using SubW.

CRediT authorship contribution statement

Ó. Benito-Román: Conceptualization, Formal analysis, Investigation, Project administration, Writing – original draft, **M.T. Sanz:** Data curation, Funding acquisition, Supervision, Writing – review & editing. **S. Beltrán:** Funding acquisition, Supervision, Writing – review & editing.

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Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.supflu.2023.106155.

References

[1] R. Ciriminna, A. Fidalgo, R. Delisi, L.M. Ilharco, M. Pagliaro, Pectin production and global market, Agro Food Ind. Hi. Tech. 27 (2016) 17–20.
[2] L.R. Adetunji, A. Adekunle, V. Orsat, V. Raghavan, Advances in the pectin production process using novel extraction techniques: a review, Food Hydrocoll. 62 (2017) 239–250, https://doi.org/10.1016/j.foodhyd.2016.08.015.
[3] K. Klinchongkon, P. Khuwijitjaru, S. Adachi, Degradation kinetics of passion fruit pectin in subcritical water, Biosci. Biotechnol. Biochem. 81 (2017) 712–717, https://doi.org/10.1080/09168451.2016.1277941.
[4] Z. Lu, J. Wang, R. Gao, F. Ye, G. Zhao, Sustainable valorisation of tomato pomace: a comprehensive review, Trends Food Sci. Technol. 86 (2019) 172–187, https://doi.org/10.1016/j.tifs.2019.02.020.
[5] M.J. Cocero, Á. Cabeza, N. Abad, T. Adamovic, L. Vaquerizo, C.M. Martínez, M. V. Pazo-Cepeda, Understanding biomass fractionation in subcritical & supercritical water, J. Supercrit. Fluids 133 (2018) 550–565, https://doi.org/10.1016/j.supflu.2017.08.012.
[6] M. Plaza, C. Turner, Pressurized hot water extraction of bioactives, TrAC Trends Anal. Chem. 71 (2015) 39–54, https://doi.org/10.1016/j.trac.2015.02.022.
[7] Ó. Benito-Román, B. Blanco, M.T. Sanz, S. Beltrán, Subcritical water extraction of phenolic compounds from onion skin wastes (Allium cepa cv. horcal): effect of temperature and solvent properties, Antioxidants 9 (2020) 1–20, https://doi.org/10.3390/antiox9121233.
[8] C. Antonetti, D. Licursi, S. Fulignati, G. Valentini, A.M.R. Galletti, New frontiers in the catalytic synthesis of levulinic acid: From sugars to raw and waste biomass as starting feedstock, Catalysts 6 (2016) 1–29, https://doi.org/10.3390/catal6120196.
[9] J. Chen, C. Zhang, Q. Xia, D. Liu, X. Tan, Y. Li, Immunosuppression and modulated gut microbiota composition in ICR mice cyclophosphamide-induced, pectin ameliorated, Molecules 25 (2020) 1–16.
[10] C.S. Valdivieso Ramirez, J.E. Sanchez Gallego, M. Gänzle, F. Temelli, M.D. A. Saldaña, Carboxylic acid-catalysed hydrolysis of polygalacturonic acid in subcritical water media, J. Supercrit. Fluids 169 (2021), https://doi.org/10.1016/j.supflu.2020.105103.
[11] R. Wang, T. Kobayashi, S. Adachi, Degradation kinetics of D-galacturonic acid and sodium D-galacturonate in subcritical water, J. Appl. Glycosci. 56 (2009) 181–184, https://doi.org/10.5458/jag.56.181.
[12] T.M. Aida, Y. Sato, M. Watanabe, K. Tajima, T. Nonaka, H. Hattori, K. Arai, Dehydration of d-glucose in high temperature water at pressures up to 80 MPa, J. Supercrit. Fluids 40 (2007) 381–388, https://doi.org/10.1016/j.supflu.2006.07.027.
[13] F.S. Asghari, H. Yoshida, Acid-catalyzed production of 5-hydroxymethyl furfural from D-fructose in subcritical water, Ind. Eng. Chem. Res. 45 (2006) 2163–2173, https://doi.org/10.1021/ie051088y.
[14] C.M. Martínez, T. Adamovic, D.A. Cantero, M.J. Cocero, Ultrafast hydrolysis of inulin in supercritical water: fructooligosaccharides reaction pathway and Jerusalem artichoke valorization, Ind. Crops Prod. 133 (2019) 72–78, https://doi.org/10.1016/j.indcrop.2019.03.016.
[15] K. Klinchongkon, P. Khuwijitjaru, J. Wiboonsirikul, S. Adachi, Extraction of oligosaccharides from passion fruit peel by subcritical water treatment, J. Food Process Eng. 40 (2017), https://doi.org/10.1111/jfpe.12269.
[16] A. Urbisch, U. Einhorn-Stoll, H. Kastner, S. Drusch, L.W. Kroh, Formation of phenolic compounds from d -galacturonic acid, J. Agric. Food Chem. (2018) 2–8, https://doi.org/10.1021/acs.jafc.8b04158.
[17] L.N. Gerschenson, The production of galacturonic acid enriched fractions and their functionality, Food Hydrocoll. 68 (2017) 23–30, https://doi.org/10.1016/j.foodhyd.2016.11.030.
[18] A. Fatouros, U. Einhorn-Stoll, H. Kastner, S. Drusch, L.W. Kroh, Influence of the carboxylic function on the degradation of d -galacturonic acid and its polymers, J. Agric. Food Chem. 69 (2021) 9376–9382, https://doi.org/10.1021/acs.jafc.1c02388.
[19] C. Usuki, Y. Kimura, S. Adachi, Degradation of pentoses and hexouronic acids in subcritical water, Chem. Eng. Technol. 31 (2008) 133–137, https://doi.org/10.1002/ceat.200700391.
[20] H. Pińkowska, M. Krzywonos, P. Wolak, A. Złocińska, Production of uronic acids by hydrothermolysis of pectin as a model substance for plant biomass waste, Green. Process. Synth. 8 (2019) 683–690, https://doi.org/10.1515/gps-2019-0039.
[21] H.A. Ruiz, M. Galbe, G. Garrote, D.M. Ramirez-Gutierrez, E. Ximenes, S.N. Sun, D. Lachos-Perez, R.M. Rodríguez-Jasso, R.C. Sun, B. Yang, M.R. Ladisch, Severity factor kinetic model as a strategic parameter of hydrothermal processing (steam explosion and liquid hot water) for biomass fractionation under biorefinery

concept, Bioresour. Technol. 342 (2021), https://doi.org/10.1016/j.biortech.2021.125961.
[22] T. Miyazawa, T. Funazukuri, Hydrothermal production of mono(galacturonic acid) and the oligomers from poly(galacturonic acid) with water under pressures, Ind. Eng. Chem. Res. 43 (2004) 2310–2314, https://doi.org/10.1021/ie0202672.
[23] D. Li, X. Hua, J. Luo, Y. Xu, Quantitative determination of galacturonic acid in pectin and pectin products by combined pectinase hydrolysis and HPLC determination, Food Addit. Contam. - Part A. 40 (2023) 319–327, https://doi.org/10.1080/19440049.2023.2165171.
[24] D. Liu, W. Tang, J.Y. Yin, S.P. Nie, M.Y. Xie, Monosaccharide composition analysis of polysaccharides from natural sources: hydrolysis condition and detection method development, Food Hydrocoll. 116 (2021) 106641, https://doi.org/10.1016/j.foodhyd.2021.106641.
[25] Y. Zhao, K. Lu, H. Xu, L. Zhu, S. Wang, A critical review of recent advances in the production of furfural and 5-hydroxymethylfurfural from lignocellulosic biomass through homogeneous catalytic hydrothermal conversion, Renew. Sustain. Energy Rev. 139 (2021) 110706, https://doi.org/10.1016/j.rser.2021.110706.
[26] K. Dussan, B. Girisuta, M. Lopes, J.J. Leahy, Conversion of hemicellulose sugars catalyzed by formic acid: kinetics of the dehydration of d -Xylose, l Arab. D. Glucose (2015) 1411–1428, https://doi.org/10.1002/cssc.201403328.
[27] R. Mohamad, T. Aki, Y. Nakashimada, Y. Okamura, T. Tajima, Y. Matsumura, Decomposition kinetics of uronic acids obtained from kelp under hydrothermal condition, J. Energy Inst. 90 (2017) 185–190, https://doi.org/10.1016/j.joei.2016.02.005.
[28] M.A. Bornik, L.W. Kroh, D-galacturonic acid as a highly reactive compound in nonenzymatic browning. 1. Formation of browning active degradation products, J. Agric. Food Chem. 61 (2013) 3494–3500, https://doi.org/10.1021/jf303855s.
[29] M.A. Madson, M.S. Feather, The acid-catalyzed decarboxylation of D-xyluronic, D-galacturonic, and D-gly-cerp-D-gulo-hepturic acid, Carbohydr. Res. 70 (1979) 307–311.
[30] R. Wang, T.L. Neoh, T. Kobayashi, Y. Miyake, A. Hosoda, H. Taniguchi, S. Adachi, Degradation kinetics of glucuronic acid in subcritical water, Biosci. Biotechnol. Biochem. 74 (2010) 601–605, https://doi.org/10.1271/bbb.90818.
[31] S.H. Khajavi, Y. Kimura, T. Oomori, R. Matsuno, S. Adachi, Degradation kinetics of monosaccharides in subcritical water, J. Food Eng. 68 (2005) 309–313, https://doi.org/10.1016/j.jfoodeng.2004.06.004.
[32] K. Lamminpää, J. Ahola, J. Tanskanen, Kinetics of furfural destruction in a formic acid medium, RSC Adv. 4 (2014) 60243–60248, https://doi.org/10.1039/c4ra09276g.
[33] L. Almhofer, R.H. Bischof, M. Madera, C. Paulik, Kinetic and mechanistic aspects of furfural degradation in biorefineries, Can. J. Chem. Eng. (2022) 1–17, https://doi.org/10.1002/cjce.24593.
[34] W. Xu, S. Zhang, J. Lu, Q. Cai, Furfural production from corncobs using thiourea as additive, Environ. Prog. Sustain. Energy 36 (2017) 676–680, https://doi.org/10.1002/ep.
[35] J. Köchermann, J. Mühlenberg, M. Klemm, Kinetics of hydrothermal furfural production from organosolv hemicellulose and d-xylose, Ind. Eng. Chem. Res. 57 (2018) 14417–14427, https://doi.org/10.1021/acs.iecr.8b03402.
[36] O. Benito-Román, P. Alonso-Riaño, E. Díaz De Cerio, M.T. Sanz, S. Beltrán, Semi-continuous hydrolysis of onion skin wastes with subcritical water: pectin recovery and oligomers identification, J. Environ. Chem. Eng. 10 (2022) 107439, https://doi.org/10.1016/j.jece.2022.107439.