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Degradation kinetics of sugars (glucose and xylose), amino acids (proline and aspartic acid) and their binary mixtures in subcritical water: Effect of Maillard reaction

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ABSTRACT

A systematic kinetic study was conducted in subcritical water medium in the temperature range from 150 to 200 °C for pure glucose, xylose, proline and aspartic acid as well as binary mixtures of sugars + amino acids to understand the reaction kinetics and interactions among biomass components and to discern the influence of Maillard reaction (MR) on the overall reaction kinetics.

The main degradation products identified for glucose and xylose were the respective dehydration products, hydroxymethyl furfural and furfural, yielding an increasing solid residue with temperature (15.9 wt% at 200 °C) with an augmented heating value. The degradation of sugars and amino acids in binary systems was faster compared to pure compounds due to MR and the production of dehydration products was delayed when considering total sugar conversion. Higher relative reactivity in MR was observed for xylose over glucose showing also higher antioxidant activity.

1. Introduction

Subcritical water (subW) is defined as water at a temperature in the range from 100 to 374 °C and at a pressure high enough to keep it in its liquid state. subW exhibits unique temperature-dependent properties, such as a decrease in its dielectric constant, an increase of diffusivity as well as an increase of the ionic product of water (Barea et al., 2023). Therefore, subW has been demonstrated to be a good solvent, not only in extraction processes, but also in chemical reactions due to its increasing self-ionization at high temperatures. Nevertheless, there is still much work to be done to comprehensively understand the degradation reactions of the hydrolysis products obtained from complex raw materials in this highly reactive medium, specially considering the variability of the substrates in the hydrolysis of lignocellulosic biomass, leading in multiple reactions. Different compounds are realeased into the medium that can interact with each other and undergoing different reaction processes making difficult the overall understanding of the process.

A variety of feedstocks have been treated with subW yielding diverse results based on composition and processing conditions. Proteins and carbohydrates are two of the major components of biomass. In previous studies, subW was considered as a hydrolytic medium to recover and fractionate different components of the brewer's spent grain (BSG) (Alonso-Riaño et al., 2021, 2023). BSG is a lignocellulosic solid by-product generated in the brewing industry after mashing and wort filtration processes. In a discontinuous configuration, 56 % of the total carbohydrates present in the BSG were released at 170 °C and 22 min of subW treatment. This included 80 % of the hemicelluloses (18 % as monomer and 82 % as oligomer) and 20.8 % of the glucans (as oligomers from the residual starch fraction of the BSG). Addtionally, BSG contains also a considerable fraction of protein that was also released into the reaction medium. An extraction/hydrolysis yield of 64 % of the total protein fraction of the BSG was also obtained in the subW reaction medium. Under these conditions, the amount of free amino acids present in the subW extracts reached values up to 24 mg free amino acid/g protein. Intereactions between proteins and carbohydrates released into the medium can led to interactions similar to Maillar reaction (MR).

MR involves the condensation between a carbonyl group of reducing sugars (aldehydes or ketones) and an amine group (such as amino acids,

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peptides and proteins) (Kim & Lee, 2009a). Therefore, in the subW reactive medium, MR can be initiated due to the presence of a reducing-end carbonyl group from the hydrolysis of the polysaccharide fraction, and a free primary amine group from free amino acids and small peptides in the subW reaction medium, leading to a further reduction of their content (R. Fan & Gao, 2022) and the formation of new MR products, including dark-brown polymeric compounds. Consequently, the presence of different compounds might influence the mechanism and pathway of the reactions of the components released, being worth the investigation with model compounds to better understand the behaviour of sugars and amino acids in the degradation reactions under subW conditions. Susbstrates, like simple sugars and amino acids, can help to understand the complex reaction pathways in a subW medium.

In this work, some basic kinetic studies were conducted in subW focusing on main sugars derived from the polysaccharide fraction of the BSG (glucose and xylose) and some of the amino acids present in the BSG (proline and aspartic acid) as well as their binary mixtures. These studies could contribute to enhance our understanding of the effect of the MR on the content of both sugars and amino acids released in the subW medium. Monitoring the content of sugars and amino acids could help us to assess the extent and significance of MR in the subW treatment of lignocellulosic biomass with a high protein content. Additionally, it helps to evaluate how the decomposition rates of sugars might be affected by the presence of some amino acids released from the BSG, serving as an example of lignocellulosic biomass with a relative high content of protein.

While there are some studies exploring the degradation kinetics of sugars and individual amino acids in subW, there is a gap regarding the degradation of mixtures of these compounds considering the interactions between both components.

In this study, it is hypothesized that degradation kinetics of compounds released during subW fractionation/hydrolysis of lignocellulosic biomass are influenced by the concurrent release of compounds into this reactive medium. Application of subW treatment to natural and food product is mainly focused on extraction of bioactive compounds, but limited attention has been paid about intereactions of released compounds. The MR is one of the most important in the food industry, typically occurring at temperature below 150 °C or in dry heat; however, it has rarely been considered under the conditions of interest in the hydrothermal process of biomass, along with its impact on current kinetic models (Peterson et al., 2010). Therefore, the goal of this study was to understand the degradation kinetics of the main monomers, glucose and xylose, present in the polysaccharide fraction of various lignocellulosic biomass, such as BSG. Additionally, it is aimed to compare the kinetics, taking into account the presence of free amino acids resulting from the hydrolysis of the protein fraction. Glycine, as the simplest proteinogenic amino acid, has been extensively studied in literature for its degradation in different model mixtures under subcritical water conditions (Körner, 2021). In this work, proline, the major amino acid present in the BSG, and aspartic acid, the amino acid preferentially released during the subW treatment from BSG, were chosen as model amino acids. According to previous studies, aspartic acid exhibited the highest hydrolysis yield in subW reactive medium in a batch configuration with yield values reaching 20 %, considering the amino pofile of the BSG biomass (Alonso-Riaño et al., 2021). Reactivity of sugars and amino acids was assessed based on the kinetics profile of the different components, including browning development and reducing capacity of the medium.

2. Experimental section

2.1. Chemicals

Glucose (99 %), xylose (99 %), xylulose (≥99 %), furfural (99 %), glyceraldehyde (98 %) and glycoaldehyde (98 %) were purchased from Sigma-Aldrich (St Louis, MO, USA). Proline (>99 %), aspartic acid (>99 %) and 1,6 anhydroglucose (>99 %) were purchased from TCI chemicals (Tokyo Chemical Industry,Co.). Standards of 5-HMF (97 %) from Alfa Aesar (ThermoFischer, Kandel, GmbH, Erlenbachweg), formic acid (98 %) and fructose (>99 %) from Fluka (Honeywell, Charlotte, NC, USA), acetic acid (99.8 %) from VWR, 1,3-dihydroxyacetone (97 %) from Fluorochem (Hadfield, UK), and levulinic acid (99 %) from Merck (Merck KGaA, Darmstadt, Germany).

2.2. Equipment for subcritical water hydrolysis

The subcritical water treatment was carried out in a lab-assambled batch system featuring a 0.5 L capacity reactor. The reactor was covered by a ceramic heating jacket (230 V, 4000 W, ø 95 mm, 160 mm height) to reach the selected working temperature. A Pt100 sensor placed inside the reactor and connected to a PID system allowed to control and register the temperature during the extraction. To enhance mass transfer, a magnetic stirring bar was placed in the bottom of the reactor. In a typical run, 350 mL of different water solutions were charged into the reactor according to the carbohydrate and amino acid profile of BSG for 5 % of biomass loading in a discontinuous reactor as it was carried out in previous studies (Alonso-Riaño et al., 2023). Two types of solutions were utilized: (1) pure component water solutions consisting of 5 wt% of glucose, 1.1 wt% of xylose, 0.15 wt% of proline, 0.06 wt% of aspartic acid and (2) binary mixtures, composed of glucose + proline and xylose + proline and xylose + aspartic acid. The aim was to compare the degradation kinetics and analyze the potential effect of MR. All the experiments were performed without the addition of any external catalyst.

Three different temperatures were assayed, 150, 180 and 200 °C and the working pressure was set at 50 bar by using N_2 as the pressurization agent. The effect of pressure on the hydrolysis performance has been observed to be non-significant in different studies, compared to the effects of temperature and time, as long as water remains in its liquid state (Rivas-Vela et al., 2021). A needle valve (Autoclave Engineers) followed by a cooling system was connected to collect samples along the hydrolysis in order to determine the kinetics of the process. After a certain reaction time, the vessel was cooled and it was depressurized when the temperature was lower than 90 °C. After subW treatment, the solid residue retained after filtering was dried and weighed and analyzed for its elemental composition.

The autocatalytic performance of subW and the effect of the presence of free amino acids in the reaction medium was evaluated through the conversion of sugar monomers and the C-yield of the different products obtained in the reaction medium:

$$Conversion = \frac{moles\ \ of\ \ sugar_o - moles\ \ of\ \ sugar_t}{moles\ \ of\ \ sugar_o}\cdot 100 \tag{1}$$

$$\mathrm{C} - Yield = \frac{C\ \ moles\ \ of\ \ reaction\ \ product_t}{C\ \ moles\ \ of\ \ sugar_o}\cdot 100 \tag{2}$$

where the subscripts *“o”* and *“t”* correspond to the beginning of the reaction and the reaction time, t, respectively. The yield in Eq. 2 is reported as the percentage of the identified degradation products to initial raw material, on a carbon basis.

2.3. Analytical methods

2.3.1. Sugars and its degradation products

Glucose, xylose and their degradation products were determined by High-Performance Liquid Chromatography (HPLC), Agilent 1260 Infinity II (Agilent Technologies, Sta. Clara, CA, USA) following the method described by Alonso-Riaño et al. (2021). The HPLC apparatus is equipped with a Biorad Aminex-HPX-87H column with its corresponding pre-column and two detectors, a variable wavelength detector (VWD), and a refractive index detector (RID). Sulfuric acid (0.005 M) was the mobile phase. The column and the refractive index detector were maintained at 40 °C. For analysis, samples (10 μL) of sugars and their degradation products were directly injected after filtering through a 0.22 μm pore size syringe filter (Scharlab, Barcelona, España). Although not all the standards were determined in the subW medium according to the described HPLC method, calibration was performed with all the pure standards detailed in section 2.1 concerning sugars and their degradation products in subW medium. Peak identification was done by comparison of the sample peak retention times with the standard pure compounds.

2.3.2. *Amino acids*

The amino acids selected in this work, proline and aspartic acid, were analyzed by gas chromatography after derivatization by using the EZ: faast^{TM} kit (Phenomenex, Torrance, CA, USA) and a GC-FID instrument (Hewlett Packard, HP 5890 Series II). Details can be found elsewhere (Alonso-Riaño et al., 2021; Trigueros et al., 2021). Briefly, aliquots (4 μL) of the derivatized amino acids were injected at 1:15 split ratio at 250 °C into a Zebron column (ZB –AAA, 10 m and 0.25 mm in diameter) programmed from 110 to 320 °C at 32 °C/min. Helium was used as a carrier gas at 60 kPa and nitrogen was used as a make-up gas. The detector temperature was 320 °C. Amino acids were identified uisng standards provided in the mentioned kit, with norleucine as internal standard.

2.3.3. *Elemental analysis*

Elemental composition (C, H, N, S) of the solid residue generated after subW treatment was determined by an organic elemental microanalyzer equipment (Thermo Scientific^{TM} Model Flash 2000, Waltham, MA, USA). The combustion temperature was 900 °C and sulphanilamide was used as standard. The higher heating value (HHV) of the solid residue and raw materials was evaluated by the following equation (Friedl, Padouvas, Rotter & Varmuza, 2005) for a better comparision between these two values.

$$\mathrm{HHV(kJ/kg)} = 3.55 \cdot C^2 - 232 \cdot \mathrm{C} - 2230 \cdot \mathrm{H} + 51.2 \cdot \mathrm{C} \cdot \mathrm{H} + 131 \cdot \mathrm{N} + 20600. \quad (3)$$

2.3.4. *Browning intensity*

The browning intensity was determined according to Laroque et al. (2008). The absorbance of the solutions at 420 nm was measured using a Jasco V-750 spectrophotometer (Jasco Analítica Spain, S.L.) as an index of the brown polymers formed in the degradation of pure sugars and in the different stages of MR.

2.3.5. *FRAP "Ferric reducing power"*

The reducing capacity of the liquid products of subW treatment was determined by the ferric reducing ability (FRAP) method according to Benzie and Strain (1996). A standard curve was prepared using different concentrations of $FeSO_4$ and the results are expressed as mM of Fe^{2+}/mL of sample.

2.3.6. *Kinetic analysis of subcritical water treatment for sugars, amino acids and their mixtures*

For the kinetic analysis, the changes in concentration for the sugars and the amino acids were determined as a function of the reaction time. A simple first-order reaction model was assumed to fit the total decrease in concentration for pure sugar and amino acid systems, as well as for their binary mixtures, in order to compare the decrease in concentration in subW at differente temperatures:

$$\frac{dC_i}{dt} = -k_i C_i \quad (4)$$

The relationship between the rate constants of Equation 4, k_i, and temperature was established by the Arrhenius law:

$$k = k_o \exp\left(\frac{-E_a}{RT}\right) \quad (5)$$

where k_o is the preexponential factor (min^{-1}), E_a the activation energy ($kJ \cdot mol^{-1}$) and T the absolute temperature (K).

A more in-depth kinetic study was carried out for the individual sugars degradation in subW considering different literatures studies for glucose (Aida et al., 2007; Fang et al., 2011; Peterson et al., 2008; Yu et al., 2008) and xylose degradation (Kammoun et al., 2019; Kang et al., 2021; Paksung & Matsumura, 2015) (Fig. 1s and 2S) assuming first-order reaction steps.

As described in the literature, the main reactions of sugars in subW have been found to be tautomerization, dehydration and *retro*-aldol reactions. Glucose isomerizes to fructose via the Lobryede Bruyneva Ekenstein (LBE) transformation or produces 1,6 anhydroglucose (also known as levoglucosan). Both isomers, glucose and fructose, can further decompose to 5-hydroxymethylfurfrual (HMF) via dehydration reaction or produce retroaldol products. Additionally, water can rehydrate HMF to form levulinic and formic acid (Aellig & Hermans, 2012). All these products are highly reactive and, as reaction time increases, they can repolymerize to form water insoluble humic acids (humins) or other degradation products (Fang et al., 2011). According to the proposed routes and the results described later in this work, the following equations were considered for glucose degradation:

$$\frac{dC_G}{dt} = -\left(k_{G-F} + k_{G-Lg} + k_{G-HMF} + k_{G-U}\right)C_G + k_{F-G}C_F \quad (6)$$

$$\frac{dC_F}{dt} = k_{G-F}C_G - \left(k_{F-G} + k_{F-HMF} + k_{F-Gal} + k_{F-U}\right)C_F \quad (7)$$

$$\frac{dC_{Lg}}{dt} = k_{G-Lg}C_G - k_{Lg-U}C_{Lg} \quad (8)$$

$$\frac{dC_{HMF}}{dt} = k_{G-HMF}C_G + k_{F-HMF}C_F - \left(k_{HMF-Fu} + k_{HMF-LH} + k_{HMF-FH} + k_{HMF-U}\right)C_{HMF} \quad (9)$$

$$\frac{dC_{Fu}}{dt} = k_{HMF-Fu}C_{HMF} - k_{Fu-U}C_{Fu} \quad (10)$$

$$\frac{dC_{Gal}}{dt} = k_{F-Gal}C_F - k_{Gal-U}C_{Gal} \quad (11)$$

$$\frac{dC_{LH}}{dt} = k_{HMF-LH}C_{HMF} - k_{LH-U}C_{LH} \quad (12)$$

$$\frac{dC_{FH}}{dt} = k_{HMF-FH}C_{HMF} - k_{FH-U}C_{FH} \quad (13)$$

where the subscript G refers to glucose, F to fructose, Lg to levoglucosan, HMF to hydroxymethyl furfural, Fu to furfural, Gal to glyceraldehyde, LH to levulinic acid, FH to formic acid, U to unknown compounds and k_{i-j} and C_i the corresponding kinetic constants and concentrations, respectively (see Fig. 1S.

Regarding xylose degration in subW, similar to glucose, isomerization of xylose to xylulose is also proposed in the literature (Paksung & Matsumura, 2015), as well as the dehydration to furfural and decomposition into *retro*-aldol products of both isomers. Other products, such as formic acid, could also be formed from xylose, xylulose and furfural (Fig. 2S) (Lau et al., 2014). Based on the proposed literature routes and the data obtained in this work, as they will be later described, the following equations are proposed to fit the xylose degradation in subW:

$$\frac{dC_X}{dt} = -\left(k_{X-Xu} + k_{X-Fu} + k_{X-Gly} + k_{X-FH} + k_{X-U}\right)C_X + k_{Xu-X}C_{Xu} \quad (14)$$

$$\frac{dC_{Xu}}{dt} = k_{X-Xu}C_X - \left(k_{Xu-X} + k_{Xu-Fu} + k_{Xu-Gly} + k_{Xu-FH} + k_{Xu-U}\right)C_{Xu} \quad (15)$$

$$\frac{dC_{Fu}}{dt} = k_{X-Fu}C_X + k_{Xu-Fu}C_{Xu} - (k_{Fu-FH} + k_{Fu-U})C_{Fu} \tag{16}$$

$$\frac{dC_{FH}}{dt} = k_{Fu-FH}C_{Fu} + k_{X-FH}C_X + k_{Xu-FH}C_{Xu} - k_{FH-u}C_{FH} \tag{17}$$

$$\frac{dC_{Gly}}{dt} = k_{X-Gly}C_X + k_{Xu-Gly}C_{Xu} - k_{Gly-U}C_{Gly} \tag{18}$$

where the subscript X refers to xylose, Xu to xylulose, Gly to glycoaldehyde, Fu to furfural, FH to formic acid, U to unknown compounds and $k_{i\text{-}j}$ and C_i the corresponding kinetic constants and concentrations, respectively.

The rate constants for the different kinetic equations proposed were obtained by simultaneously solving the set of differential equations for each sugar. The differential equations were solved using a fourth order Runge-Kutta method and the parameters were obtained by minimizing the following objective function (O.F.) using the simplex Nelder-Mead method:

$$O.F. = \sqrt{\frac{\sum_{allcompounds}\sum_{i=1}^{n} abs\left(C_{i,exp} - C_{i,calc}\right)}{n}} \tag{19}$$

The quality of the fitting was evaluated by the root mean square error (RMSE):

$$RMSE = \sqrt{\frac{1}{n}\sum_{allcompounds}\sum_{i=1}^{n}\left(C_{i,exp} - C_{i,calc}\right)^2} \tag{20}$$

where the subscript "i" refers to the different experimental kinetic data points for each individual compounds, the subscripts "exp" and "*calc*" refer to the experimental and calculated mole concentration (mM) of the different components, respectively, and n is the total experimental data (the sum of all kinetic data for all the compounds).

3. Results and discussion

3.1. Glucose degradation in subcritical water

The drecrease of glucose within the temperature range from 150 to 200 °C has been plotted in Fig. 1 (a-c). A temperature increase led to a faster degradation of glucose in subW and an increasing substrate conversion. The total decomposition rate of glucose was fitted to a first order reaction model to fit the decrease in concentration at the three different temperatures. Results are presented in Table 1S. The rate constant continuously increased with temperature with the highest value at 200 °C ($k = 0.0213\ \text{mim}^{-1}$). This observed temperature-dependent trend in the rate constant values aligns with findings from Möller et al. (2012), who reported a rate constant of $5\cdot10^{-4}\ \text{s}^{-1}$ ($0.03\ \text{min}^{-1}$) at 220 °C for the decomposition of glucose for microwave-assisted in subW processes. The activitacion energy for glucose degradation in subW (150–200 °C) was evaluated as 90.8 kJ/mol. Other values reported in the literature for degradation of glucose in subW are higher than the value reported in this work. For instance, Khajavi et al. (2005) reported a value of 155 kJ/mol for glucose degradation in the temperature range from 180 to 260 °C (10 MPa, residence time up to 300 s in a continuous reactor) by fitting their kinetic data to a Weibull model.

A common factor used when dealing with subW is the severity factor that combines the effect of treatment time and temperature according to the following equation (Alonso-Riaño et al., 2023):

$$\log R_o = \log\left(t\cdot exp\left(\frac{T - T_{ref}}{14.75}\right)\right) \tag{21}$$

where t is the subW treatment time (min), T is the operating temperature (°C) and T_{ref} is equal to 100 °C. Fig. 3Sa shows the percentage of remaining glucose as a function of the severity factor. A common trend at the three different temperatures can be observed, showing that severity factors higher than 5.0 led to total glucose degradation.

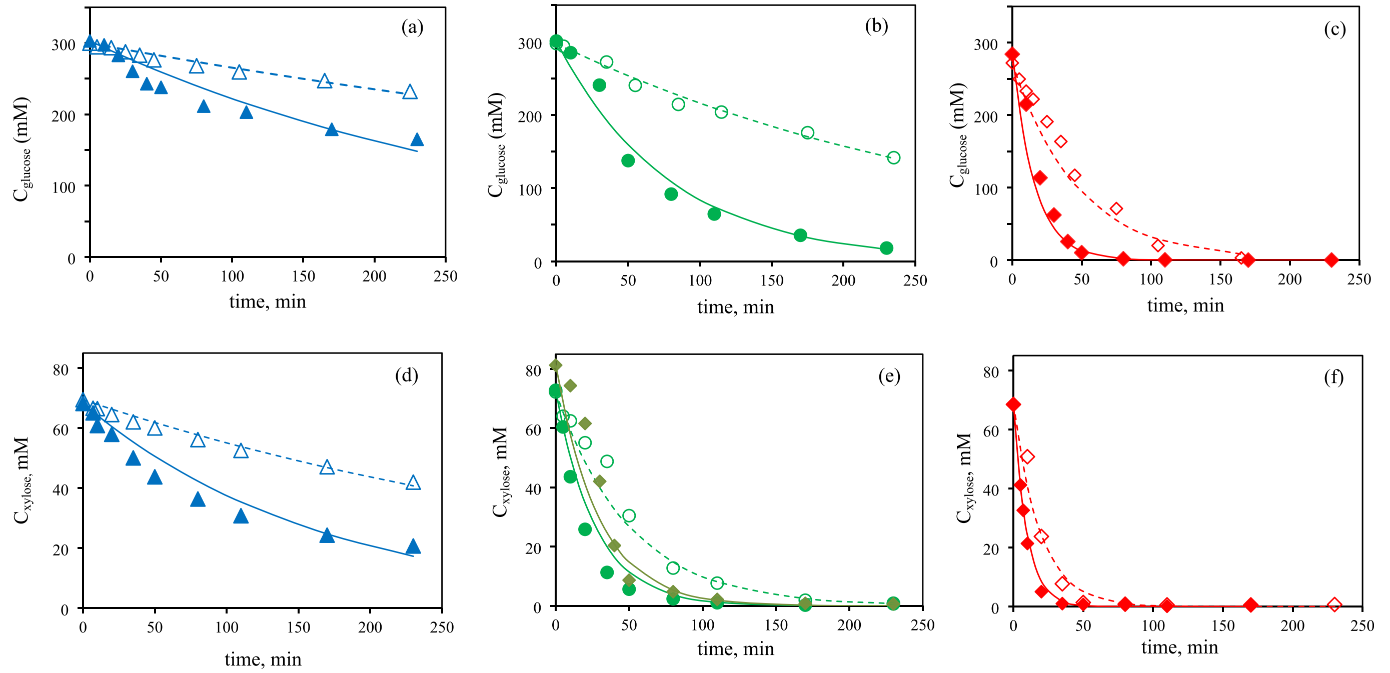

Fig. 1. Total decomposition rate for glucose (a,b,c) and xylose (d,e, f) in pure sugars subW treated systems and sugar + amino acid systems. (a,d) 150 °C (△ sugar, ▲ sugar + proline); (b,e) 180 °C (○ sugar, ● sugar + proline, ◆ xylose + aspartic acid); (c,f) 200 °C (◇ sugar, ◆ sugar + proline). Lines represent the first order model for total decomposition rate (Table 1S): dashed lines for pure sugars and continuous lines for mixtures sugar + amino acid.

The kinetic profiles of some of the degradation compounds from glucose in subW reaction medium have been plotted in Fig. 2 together with the glucose concentration profile at three working temperatures studied in this work, for a better comparison. At subW conditions, the main reaction pathway involved the dehydration of glucose (or fructose) with HMF as the main degradation product. The maximum conversion yield at 150 and 180 °C for HMF, evaluated according to Eq 2 in a C basis, was obtained at the final reaction time performed reaching values of 8.7 and 12.41 %, respectively (26.1 and 36.9 mM). However, at 200 °C, there was a maximum in the HMF yield (13.8 %, 37.5 mM) followed by the formation of larger amounts of levulinic and formic acids from HMF. This behaviour of HMF aligns with findings in literature where from a fixed reaction time a bell-shape for reaction intermediates yields, such as HMF was described in the fructose and glucose degradation reactions, with subsequent observation of levulinic acid formation and polymerization products (Möller et al., 2012). Furfural was aldo determined in the glucose decomposition route, but at much lower yields compare to HMF. In this study it has been proposed as a product of hydrothermal HMF degradation (see Fig. 1S) Retroaldol condensation products were also determined in the liquid product, but at much lower concentrations than HMF, being glyceraldehyde the main retro aldol product reaching maximum C-yield values around 2.3 – 3 % evaluated according to Equation 2 (14–18 mM, see Fig. 2b-d). The main compounds indentified in this work agree with the literature (Yu et al., 2008) where dehydration (C—O bond splitting) and *retro*-aldol condensation (C—C bond breaking) were found the key reactions, being *retro*-aldol condensation predominant at higher temperatures than the temperatures used in this work.

Among the different compounds reported in the literature as degradation products from glucose, 1,3 dihydroxiacetone (the isomer of glyceraldehyde, see Fig. 1S) was also detected in this work, but at very much low concentrations with yield increasing slowly with temperature; however the maximum yield in a C basis was less than 0.5 % at the highest temperetarure essayed in this work. Both, glyceradehyde and dihydroxyacetone can be dehydrated to yield pyruvaldehdye, but it was not determined in our HPLC as compared with the corresponding standard. Moller et al. (2012) proposed also the formation of trihydroxybenzene from HMF; however, this compound was also not observed in this work, considering the retention time for the standard peak. Therefore, these two compounds were not included in the proposed kinetic model. The kinetic data of glucose degradation in subW were fitted to the equations proposed (Eq 6–13) and the adjustable kinetic parameters obtained in the fitting procedure are presented in Table 2S along with the value of the RMSE. Fig. 2 shows good agreement between experimental data and the results obtained with the proposed kinetic route and model.

By increasing temperature, an increasing formation of insoluble compounds was taking place, as indicated in Table 3S, expressed as weight percentage relative to the initial mass of sugar charged into the reactor. An increase in temperature favors the ionic reactions that promote the formation of aromatic compounds that facilitate the char formation in subcritical regions (Promdej & Matsumura, 2011). In the

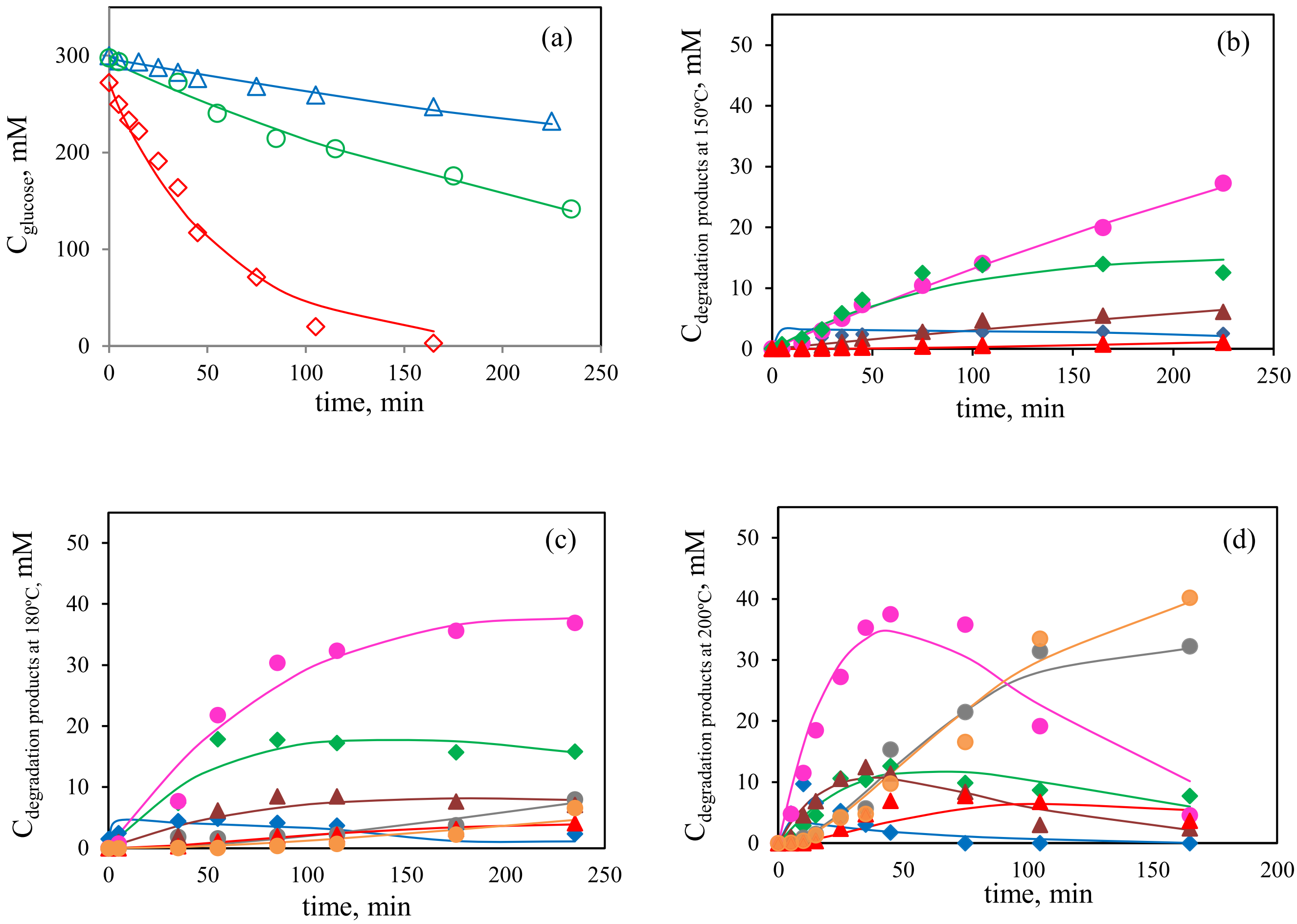

Fig. 2. (a) Kinetic profile of glucose (△ 150 °C, ○ 180 °C, ◇ 200 °C) (b-d) Kinetic profile of degradation products of glucose (b-150 °C, c-180 °C, d-200 °C): fructose (◆), levoglucosan (▲), HMF (●), glyceraldehyde (◆), furfural (▲), levulinic acid (●), formic acid (●). Continuous lines represent the kinetic model according to Eq. 6–13 (parameters are listed in Table 2S(a)).

literature, this solid has been recently referred as "secondary char" representing the fraction derived from the condensation and polymerization of the dissolved organics in the liquid phase (Ischia et al., 2022). At 200 °C, the solid residue accounted for up to 15.9 % of the initial glucose charged into the reactor. The amount of solid collected at the lowest temperature was too small to determine its elemental composition. Nevertheless, the trend in the solid residue composition is evident in Table 3S. There was an increase in the carbon content and a decrease in the hydrogen and oxygen content that led to lower molar ratios H/C and O/C in the solid residue compared to glucose due to more carbonized hydrochars (Ischia et al., 2022). The higher carbon content of the solid residue resulted in higher values of higher heating values (HHV) for the solid residue generated after subW treatment. The evolution of the elemental composition of the series of reactions in subW from glucose to hydrochar is depicted in the van Krevelen diagram (Fig. 4Sa). It can be observed that the final residues composition line up in a line with a slope of 1.90 (close to 2 as corresponding to dehydration reactions from glucose to HMF). This alignment suggests that HMF is involved in self-condensation reactions, forming dimers or oligomers that contribute to hydrochar formation; however slight deviations from the 2 slope are due to other hydrochar reactions such as aromatization and gasification, impacting the molar composition (Ischia et al., 2022). This hydrochar, as observed, proves to be challenging to liquefy.

3.2. *Xylose degradation in subcritical water*

The decrease of xylose in subW treatment has been plotted in Fig. 1 (d-f). Similar to glucose, an increase in temperature led to faster xylose degradation. Total decomposition rate of xylose was fitted to a first order reaction model showing that the rate constant continuously increased with temperature yielding an activation energy value of 95.4 kJ/mol. When comparing decomposition rates of glucose and xylose, xylose suffered faster degradation than glucose. This fact can be also observed in Fig. 3S(b) where xylose decomposed completely at severity factors higher than 4.5, compared to 5.0 for glucose.

The kinetic profiles of the degradation products from xylose, as determined in this study, have been plotted in Fig. 3 (b-d) for the three different temperatures studied in this work together with the xylose concentration profile (Fig. 3a) at the three working temperatures. Similar to glucose degradation, xylose dehydration is favoured under the ionic reaction conditions of subW, being furfural the primary degradation product within the temperature range covered in this work. A smooth maximum was observed at the highest temperatures assayed in this work (180 and 200 °C) corresponding to the maximum conversion yield in a C basis (Eq. 1) of 31 % and 50 %, respectively (24 and 40 mM, respectively). An increase in temperature and reaction time led to furfural degradation into other soluble compounds such as formic acid or any other unidentified compounds, but also to the formation of insoluble solids. In any case, the degradation of xylose and the production of furfural were faster than glucose degradation and HMF formation.

Considering the *retro*-aldol condensation, the reaction intermediate products are obtained by the cleavage at the alpha (α) carbon and the beta (β) carbon. Therefore, the *retro*-aldol condensation of xylose yields glycolaldehyde and glyceraldehyde; the latter of which may further produce glycolaldehyde and formaldehyde. In the case of xylulose, it is converted into glycolaldehyde and dihydroxyacetone. Among the retroaldol condensation products, glycoaldehyde was found in the largest

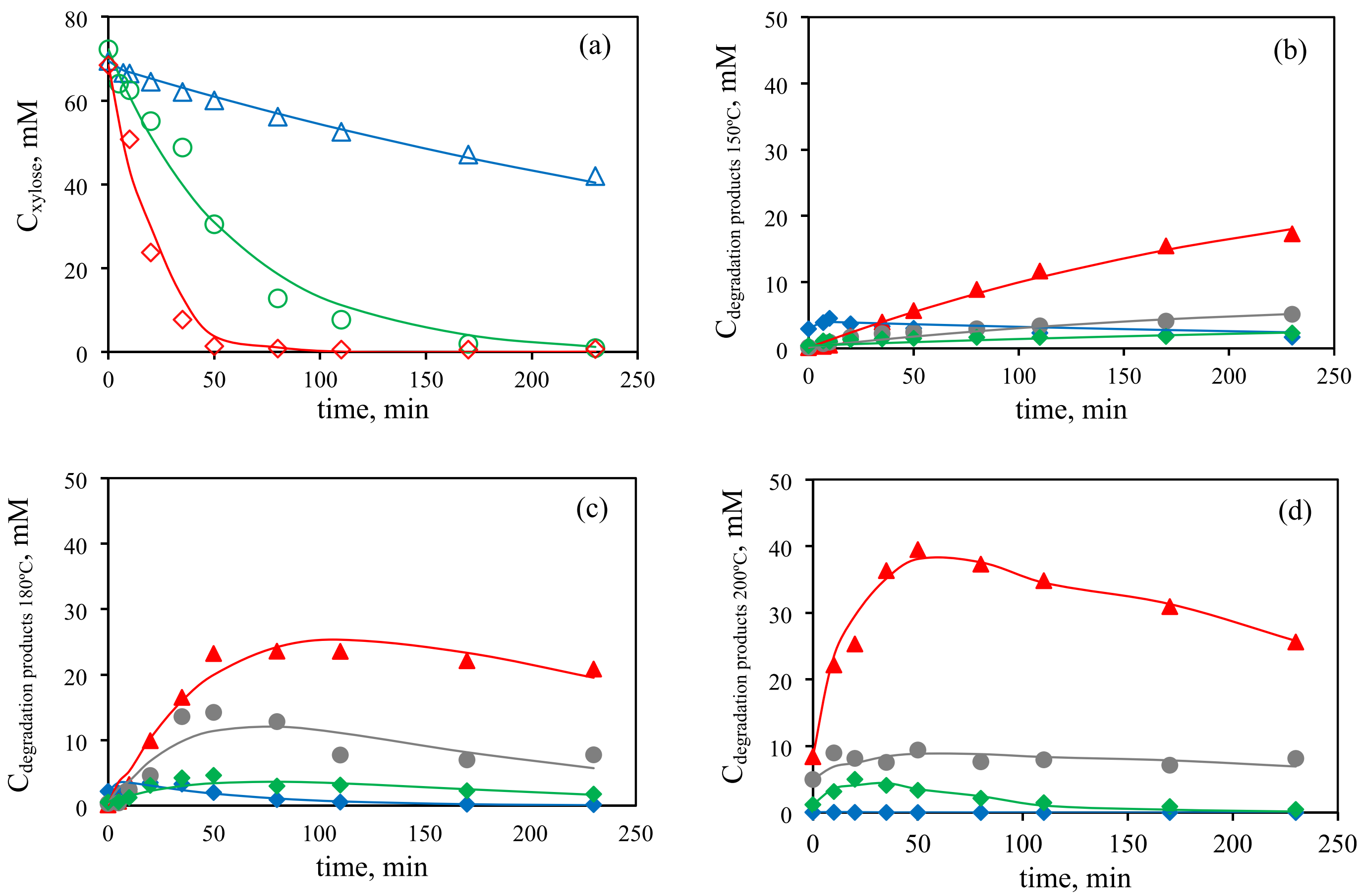

Fig. 3. (a) Kinetic profile of xylose (△ 150 °C, ○ 180 °C, ◇ 200 °C) (b-d) Kinetic profile of degradation products of xylose (b-150 °C, c-180 °C, d-200 °C): xylulose (◆), furfural (▲), glycoaldehyde (◆), formic acid (●). Continuous lines represent the kinetic model according to Eq. 14–18 (parameters are listed in Table 2S(b)).

amount at all the temperatures covered in this work. Glyceraldehyde and dihydroxyacetone were also found, but their concentrations were very small. At all conditions tested, concentration for these two compounds was lower than 1 mM, resulting in C-yields lower than 1 %. Therefore, these two compounds were not included in the equations proposed for xylose degradation (Eq. 14–18). The adjustable kinetic parameters obtained in the fitting procedure for these equations are presented in Table 2S(b) together with the RMSE value. A good fit between the experimental data and the kinetic equations proposed was achieved as shown in Fig. 3. The main degradation compounds reported in literature for pentoses (xylose and arabinose) were also mainly furfural and glycoaldehyde being consistent with *retro*-aldol and dehydration products (Yu et al., 2008). In support of the results obtained in this work, Paksung & Matsumura (2015) also reported glycoaldehyde as the main retroaldol condensation products, while glyceraldehyde was found in very small amounts under subW conditions.; however, these authors reported a relatively large amount of dihydroxyacetone, whereas in this study, a very low amount was determined according to our analytic HPLC method.

Similar to glucose degradation, by increasing temperature, an increase in the amount of the insoluble solids, referred to as “secondary char” was also observed for xylose (Table 3S) reaching values up to 12.9 % of the initial xylose charged into the reactor at 200 °C. CHN elemental analysis showed an increase in the carbon weight percentage content and a drecrease of the corresponding values of H and O compared to xylose. Due to the low amounts of “secondary char” collected at 150 °C, its elemental analysis could not be determined. The C content of the “secondary char” at 180 and 200 °C was higher than the corresponding values for glucose degradation (at 180 °C/200 °C 62.8/66.4 % for glucose residue and 67.9/70.3 % for xylose residue). This CHO composition resulted in lower molar ratios of H/C and O/C for the “secondary char” obtained from xylose than those obtained from glucose, also leading to higher HHV due to more carbonized hydrochars.

The evolution of the elemental composition of the series of reactions in subW from xylose to “secondary hydrochar” is depicted in the van Krevelen diagram (Fig. 4S). The final residues compositions line up in a line with a slope of 1.829 from xylose-furfural, showing deviation from the slope of 2 for dehydration reactions. The chemical structure of secondary char mainly consists of condensed and linked furan rings deriving from HMF and furfural (Ischia et al., 2022). However, molar composition is affected due to other reactions such as gas production, aromatization, dissolution of chemical species into the liquid phase, or other phenomena like functionalization of the outer layers of the hdryochar (Ischi 2022).

3.3. *Pure amino acid degradation*

Fig. 4 (a-c) shows the degradation kinetics of proline within the temperature range from 150 to 200 °C. An increase in temperature led to faster degradation rate of the amino acid proline. However, degradation rate of proline was much slower than that of glucose and xylose. A first order reaction model provided a good fit of the experimental data and resulted in an activation energy of 85.37 kJ/mol. Abdelmoez et al. (2010) reported a value of the activation energy for proline of 49.3 kJ/mol in the temperature range from 503 to 563 K at a pressure value corresponding to the saturated vapor pressure of water mixture at the

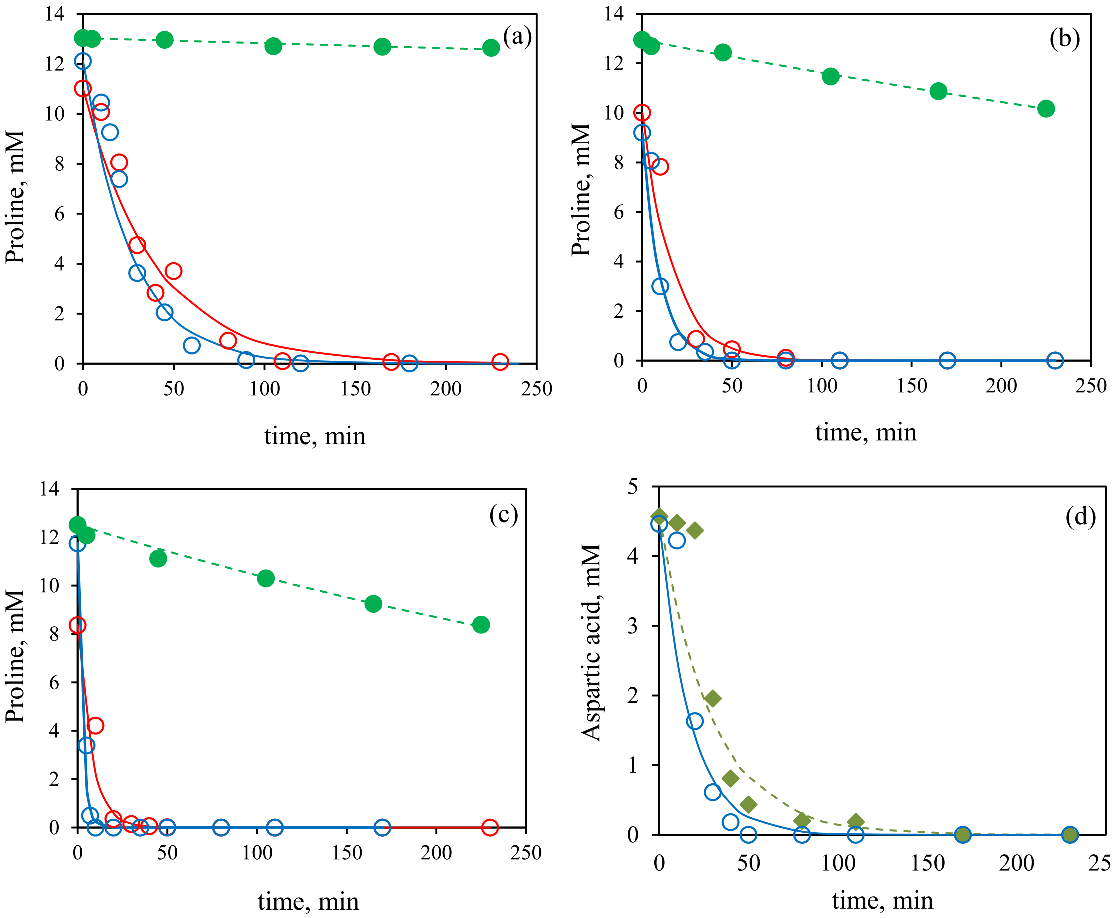

Fig. 4. Kinetic profile of proline (a,b,c) and aspartic acid (d). in pure amino acid subW treated systems and sugar + amino acid systems at different temperatures (a) 150 °C (b,d) 180 °C and (c) 200 °C: pure proline (●), pure aspartic acid (♦), glucose + proline system (○), xylose + amino acid system (○). Lines represent the first order model for decomposition rate (parameters listed in Table 1S). Dashed lines for pure amino acids and continuous lines for sugar + amino acid systems.

working reaction temperature. Proline was considered in this study, since it is the major amino acid present in the protein fraction of the selected biomass, BSG. However, as previously explained, aspartic acid was the most abundant free amino acid under typical subW condtions, 170–180 °C, usually employed for biomass processing when delaing with hemicellulose removal. Therefore, it was considered appropriate to focus on analyzing the degradation kinetics of aspartic acid at 180 °C. Fig. 4 d shows the degradation kinetic of aspartic acid at 180 °C. A significantly faster degradation rate was observed for aspartic acid compared to proline, as it can also be observed in Table1S where the degradation reaction rate constants for both amino acids at 180 °C can be compared, being 30 times higher for aspartic acid. Aspartic acid was also found to be one of the most labile amino acids by Aldelmoez et al. (2010) when studying its degradation kinetics in the temperature range from 503 to 563 K. In that temperature range, decomposition rates for aspartic acid were too fast that it was not possible the calculation of the activation energy. At 230 °C (503 K) these authors reported a reaction rate constant of 0.73 min^{-1}. The different stability of amino acid under subW conditions can be also observed in Fig. 3S(c) where the percentage of proline and aspartic remaining has been plotted as function of the severity factor showing that more than two thirds of the initial proline remained in the subW medium at severity factors higher than 5, whereas for aspartic acid, severity factors higher than 4.0 led to the total degradation of this compound. Unlike sugars, there is limited literature regarding the reaction pathways of amino acids, peptides as well as protein during subW treatment (Y. Fan et al., 2018). Studies on proline degradation in subcritical water medium are scarce. Tressl et al. (1985) reported different pyrrolidines and piperidines in an equimolar model system of L-proline with different monosaccharides heated for 1.5 h at 150 °C (pH 5–6); however, no kinetic data were reported in that work. On the other hand, in the literature, it has been reported that glycine and alanine can be formed as degradation products from other amino acids, especially aspartic acid (Körner, 2021); however, in this work, these two small amino acids were not determined according to CG experimental method described in section 2.3.2. In fact, for aspartic acid, deamination has been reported as the main degradation pathway yielding maleic and fumaric acids. Rehydratation of these acids yields malic acid, which may further be converted into pyruvic acid and this further into acetic or lactic acid (Körner, 2021).

3.4. Study of the systems reducing sugar and amino acid in subcritical water

The time course for the decrease of glucose, xylose and proline content in the binary mixtures, sugar + proline, can be observed in Figs. 1 and 4 At all the temperatures studied in this work, a faster depletion rate was observed for all the compounds in the binary mixture compared to the individual degradation rate in subW. Kinetic data were fitted to a first order reaction model (Table 1S). The faster reaction rates can be also observed in the values of the reaction rate constants for the binary systems compared to the individual compounds. Due to the higher relative stability of proline in subcritical water, the increase in the reaction rate constant was much higher for proline (up to an increase factor of 100) than for the sugars (increase factor between 2 and 4).

The kinetic behaviour of the binary system aspartic acid + xylose was also study at 180 °C, considering that aspartic acid was identified as the most abundant free amino acid after subW treatment of BSG. Additionally, xylose was the most prevalent monomer released from the polysaccharide fraction of the hemicellulose at working temperatures around 170–180 °C, being these two components significant in the subW medium. Figures 1e and 4d provide the kinetic profile of the binary mixture aspartic acid + xylose at 180 °C, for xylose and aspartic acid, respectively. A faster depletion rate of aspartic acid was also observed in the binary system compared to the individual system (Fig. 4d); however, considering the rapid degradation of aspartic acid in subW, the increase in the reaction rate constant for aspartic in the binary system was not as pronounced as compared to proline, increasing by a 2-fold factor.

The xylose consumption profile followed a similar trend for both binary systems (xylose + proline and xylose + aspartic acid). This fact was reflected in the similar reaction rate constant values for the disappearance of xylose with proline and aspartic acid, 0.0369 and 0.0340 min^{-1} for xylose in the presence of proline and aspartic acid, respectively. These results indicated no significant different effect of these two types of amino acids on the degradation kinetics of xylose.

Activation energy in the binary mixtures sugar + amino acid was determined by the Arrhenius equation (see Table 1S). An ANOVA test was applied to test if there were any statistically significant differences among the slopes of Arrhenius plot (related to the activation energy) for individual and binary systems. For glucose and xylose, there were not statistically significant differences at the 90 % or higher confidence level in both sugar-systems and sugar + amino acid systems. However, in the case of proline, lower values of the activation energy were obtained in the sugar + proline systems compared to proline showing that the presence of a reducing sugar decreased the sensitivity of the amino acid to temperature in subW medium.

The faster decrease in reducing sugars (glucose and xylose) and individual amino acids in sugar + amino acid systems indicate that both compounds were used as reactants in the MR, being faster this reaction by increasing temperature. It seems clear that MR has a significant effect on the disappearance reaction rate of the compounds released in subW medium, such as carbohydrates and amino acids. Previous studies on interactions between glucose and glycine in subW medium at 250 °C and 10 MPa (7.5 s residence time in a plug flow reactor) also provide supporting evidence of Maillard-type reaction between glucose and glycine (Peterson et al., 2010).

MR is one the most complex reactions, producing hundreds of reaction products and low and high molecular weight compounds, melanoidins, responsible for colour development (Davies et al., 1997). Fan et al. (Y. Fan et al., 2018) reported two different pathways for lysine + sugars systems treated by subW. In the first pathway, sugars react with amino groups forming glycosylamine which rearranges to Amadori compounds. These compounds then undergo cyclization and cleavage to yielf pyrazines. In the second pathway, fragments of the *retro*-aldol reaction products from sugars can condense with nitrogen from amino acids to form alkyl-pyrazines, with the second pathway becoming more significant at higher temperatures.

Therefore, browning colour development is the easiest measurable parameter of the MR due to its visual determination. Colour development at 420 nm was followed and determined for all the experiments carried out in this work. No browning was observed when proline or aspartic acid were treated alone in subW medium, with values of less than 0.1 of absorbance along treatment time. For sugars (refer to Fig. 5a and 5b), browning gradually increased over treatment time, attributed to caramelization reactions of sugars when heating. Notably, browning development was higher for glucose than for xylose. In any case, it must be highlighted that before browning determination, samples were centrifuged to remove finer black hydrochar suspended particles in the reaction medium that could potentially disturb browning determination.

In the case of binary sugar + proline mixtures, there was an increase in the browning degree due to MR compared to the treatment of individual sugars in subW (Fig. 5a and 5b), since, as it is well-stablished, melanoidins constitute and end-product of the MR (Körner, 2021). For the binary systems, there was a sharp increase in browning development in the early stages of the MR followed by a decrease and then a plateau (see Fig. 5a and 5b). Xylose presented a higher propensity to produce the precursors of MR compared to glucose. This behaviour has been also reported in the literature when heating sugars and amino acids at lower temperatures than the working temperatures covered in this work. Murata et al. (Murata et al., 2007) observed that solutions containing xylose (or arabinose) turned more brown than those with glucose after heating with a lysine solution in acetate buffer at 95 °C for 1 h. Similar

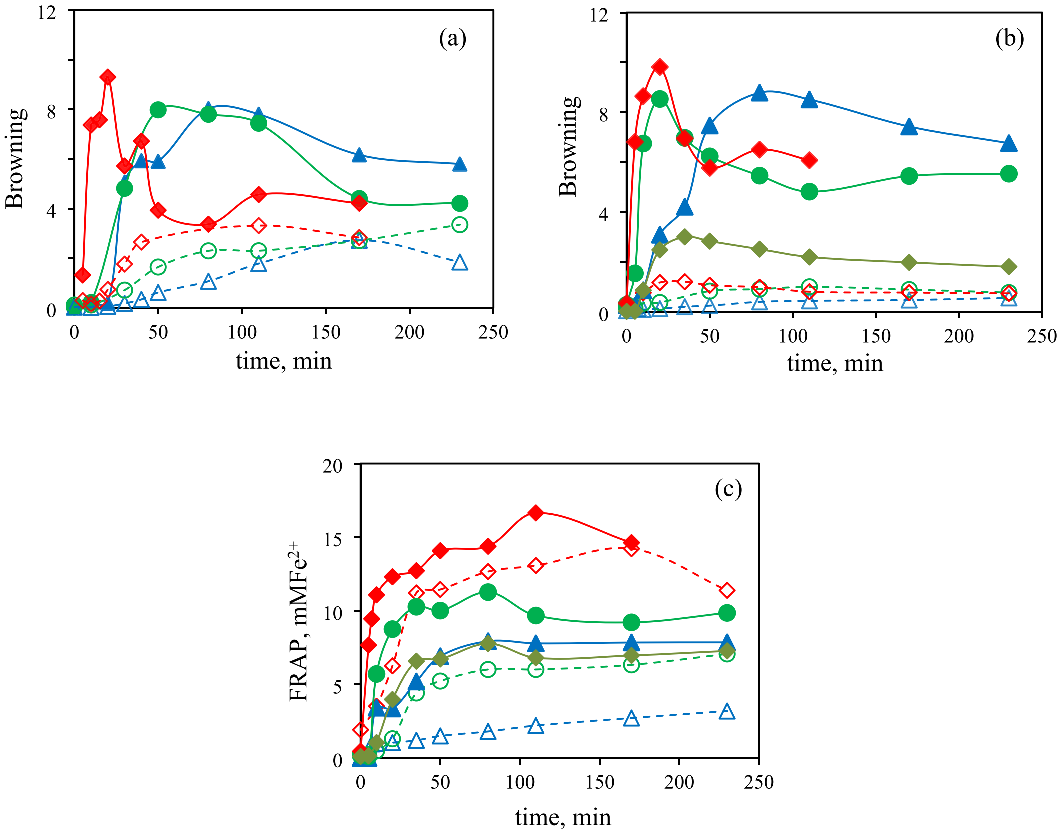

Fig. 5. Browning degree for pure sugar systems and sugar + amino acid systems during subW treatment at different temperatures: (a) glucose (b) xylose. (c) Reducing capacity for xylose and xylose + amino acid systems. 150 °C (△ sugar, ▲ sugar + proline); 180 °C (○ sugar ● sugar + proline, ◆ xylose + aspartic acid); 200 °C (◇ sugar, ♦ sugar + proline). Lines are to guide the eye: dashed lines for pure sugars and continuous lines for sugar + amino acid systems.

conclusion was reached by Laroque et al. (Laroque et al., 2008), at 55 °C, for 24 h of heating.

Browning development of the system xylose + aspartic acid at 180 °C has been also plotted in Fig. 5b. Compared to the xylose + proline system, lower degrees of browning were observed for xylose + aspartic acid, probably due to the faster degradation of aspartic acid at subW conditions, making it less available for MR with reducing sugars. Nevertheless, as pointed out by Ajandouz et al. (2008), it was rather difficult to determine any direct relationships between the type of amino acid and its ability to induce browning for systems like glucose and different amino acids, what can be extrapolated to xylose and amino acid systems.

Products of browning reactions have been shown to possess varying degreees of antioxidant capacities. Reducing capacity was determined for the xylose, xylose + proline and xylose + aspartic acid systems (see Fig. 5c) showing an increase of reducing power with temperature for all the systems with higher reducing capacity for the xylose + amino acids systems than xylose supporting the formation of MR products with pronounced antioxidant capacity as it has been reported in the literature (Lertittikul et al., 2007). It was also observed a correlation between browning degree and reducing power for the two amino acids studied in this work, with the xylose + proline system exhibiting higher reducing power. In the literature, it has been reported that reducing activity for glucose/glycine, diglycine, and triglycine model systems heated at 100 °C can be attributed to compounds formed during the thermolysis of Amadori products in the primary phase of MR or as heterocyclic products of MR or caramelization of sugars (Kim & Lee, 2009b). Solids formed at 180 °C in the binary system xylose + proline were collected and their elemental composition was determined exhibited higher C percentage and lower percentages of H, O and N, resulting in lower H/C, O/C and N/C ratios and higher HHV of the solid residue compared to the initial raw material mixture (xylose + proline mixture). The evolution route of the elemental composition ratios from the mixture xylose + proline to the the solid residue is presented in the van Krevelen diagram (Fig. 4S (b)) showing higher deviations from the dehydration pathways than the ones observed in pure sugar systems. This supports the formation of solid formation from other products such as MR reaction products. Indeed, as described in the literature, the most important pathway of proline consumption is not degradation but condensation with other compounds, acting as a precursor to nitrogen-containing humins (Chacon-Parra et al., 2022; Körner, 2021). This is further supported by the data in Table 3S, where the percentage of the solid residue in the binary xystem xylose-proline at 180 °C was more than 20 % higher compared to the xylose system at the same temperature.

Sugar degradation reactions may occur simultaneously with the MR, and the degradation sugar reaction products could subsequently take also part in the MR network becoming even more intricated (Kim & Lee, 2009b). To check the impact of MR on sugar degradation reaction products profile, the main degradation products from glucose and xylose under subW conditions, HMF and furfural, respectively, have been plotted as a function of sugar conversion evaluated by Equation 1 (Fig. 6). For subW treatment of sugar systems, production of HMF and furfural continuously increased from the beginning of the process as the sugar conversion. However, for sugar + amino acid systems, there was a clear retard in HMF and furfural production supporting the fact of the presence of proline and aspartic acid is altering the early stages of decomposition path of glucose and xylose. This alteration led to a sharp increase in the browning development at the beginning of the process (see Fig. 5a and 5b). Peterson et al. (2010) similarly observed that the production of HMF from the degradation of glucose in a glucose +

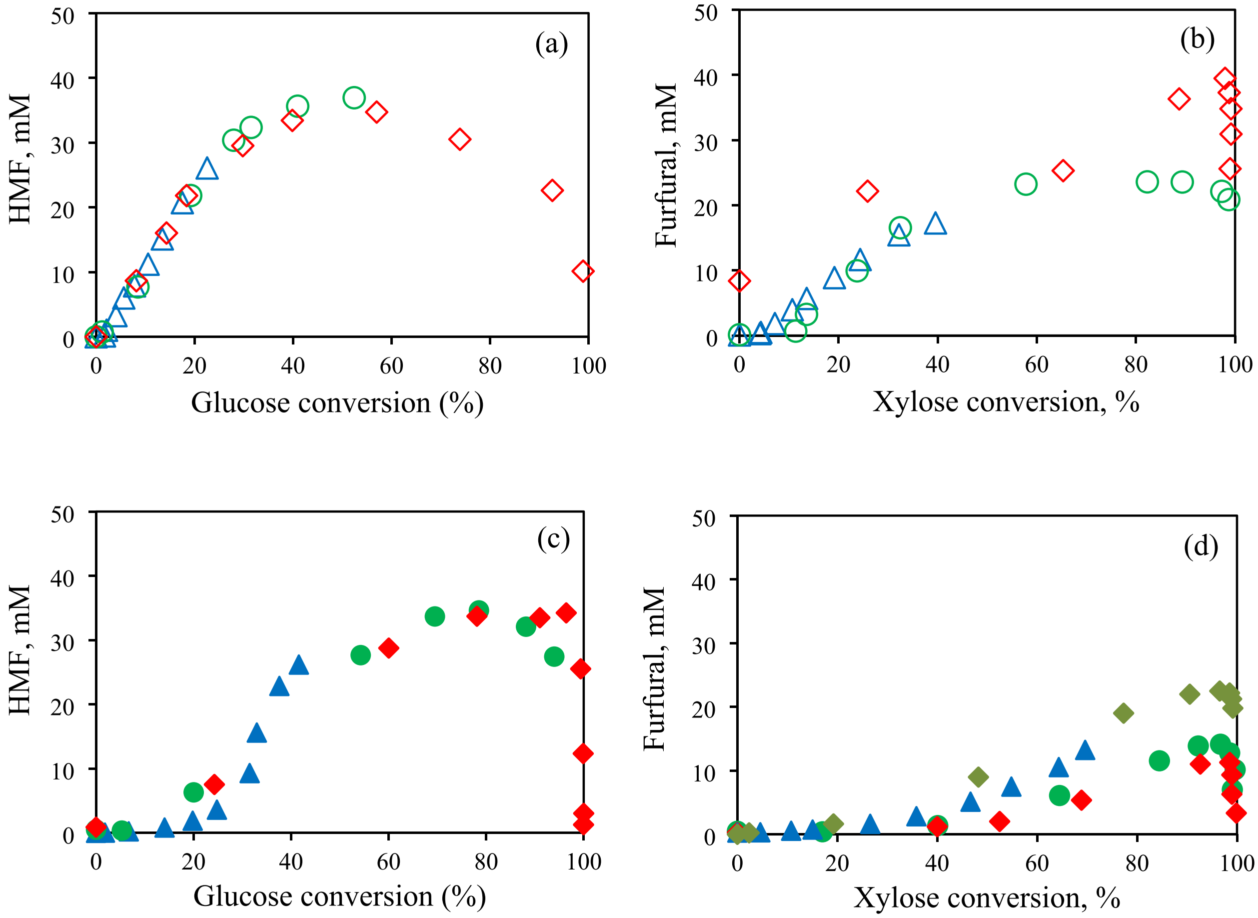

Fig. 6. Profile of main dehydration products from sugars as a function of sugar conversion (glucose → HMF, xylose → Furfural) for sugar and sugar + amino acid systems (a) pure glucose system (b) pure xylose system (c) glucose + proline system (d) xylose + proline and xylose + aspartic acid systems during subW treatment at different temperatures: 150 °C (△ sugar, ▲ sugar + proline); 180 °C (○ sugar ● sugar + proline, ◆ xylose + aspartic acid); 200 °C (◇ sugar, ◆ sugar + proline).

glycine system under subW conditions at 250 °C, 10 MPa and 7.5 s residence time in a plug flow reaction was hindered by the presence of glycine concluding that the degradation pathway was altered by the presence of glycine. Maximum HMF concentration was similar in both systems; however, for furfural, lower maximum concentrations were obtained for sugar + amino acid systems supporting previous results of higher browning degree for pentoses due to higher tendency to be involved in MR.

The connection between the degradation pathways of sugars and amino acids under subW conditions introduces a unique aspect to the MR dynamics. Peterson et al. (2010) have highlighted that in the degradation route of sugars under subW conditions, different compounds are generated that also have carbonyl groups (refer to Fig. 1S and 2S). The presence of carbonyl groups makes these compounds reactive in the MR. Therefore it could be also assume that these degradation compounds could take also part in the degradation of the amino acids under subW conditions. The impact of amino acids degradation may be even more pronounced than that of glucose itself, as it was demonstrated in the system pyruvaldehyde and glycine (Peterson et al., 2010), contributing to the complexity of the reaction occurring in subW medium.

4. Conclusions

This work provides a framework for a better understanding the interaction kinetics among the released biomass components in subW reactive medium. These intereactions have been demonstrated by changes in the degradation kinetics of individual sugars and amino acids, coupled with the development of browning and antioxidant capacity of the reaction mixture.

A detailed kinetic study has been carried out for aqueous glucose and xylose solutions under subW conditions by determining the concentration profile of sugars and their degradation products included dehydration and retro aldol condensation. This study could also provides valuable insights for optimizing the production of specific chemical compounds formed in the degradation routes. The time-evolution of liquid intermediates allowed the proposal of reaction pathways based on first order kinetic steps.

The results obtained in this work showed that the presence of sugars and amino acids in subW medium greatly increased the reaction loss of both type of compounds in subW due to the MR. Formation of dehydration products from sugars (HMF and furfural) was retarded considering the monosaccharide consumption in sugar + amino acid systems supporting that MR were taking place. Furthermore, xylose showed higher reactivity in MR compared to glucose showing the hydrolysates also higher antioxidant activity. Therefore, this work provides new insights in the basics of complex process taking place in this highly reactive medium. Nevertheless, MR is a highly complex reaction, and the effect of the presence amino acids and peptides of different chain length on degradation kinetics of sugar derived biomass requires further investigation, since different reaction pathways can be expected.

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CRediT authorship contribution statement

P. Alonso-Riaño: Data curation, Investigation. **A.E. Illera:** Data curation, Methodology. **O. Benito-Román:** Data curation, Methodology. **R. Melgosa:** Data curation, Methodology. **A. Bermejo-López:** Methodology. **S. Beltrán:** Funding acquisition, Writing – review & editing. **M.T. Sanz:** Data curation, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: M.T. Sanz reports financial support was provided by Agencia Estatal de Investigación. M.T. Sanz reports financial support was provided by Junta de Castilla y León (JCyL) and the European Regional Development Fund (ERDF).

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138421.

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