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Treatment of supermarket vegetable wastes to be used as alternative substrates in bioprocesses

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ABSTRACT

Fruits and vegetables have the highest wastage rates at retail and consumer levels. These wastes have promising potential for being used as substrates in bioprocesses. However, an effective hydrolysis of carbohydrates that form these residues has to be developed before the biotransformation.

In this work, vegetable wastes from supermarket (tomatoes, green peppers and potatoes) have been separately treated by acid, thermal and enzymatic hydrolysis processes in order to maximise the concentration of fermentable sugars in the final broth.

For all substrates, thermal and enzymatic processes have shown to be the most effective. A new combined hydrolysis procedure including these both treatments was also assayed and the enzymatic step was successfully modelled. With this combined hydrolysis, the percentage of reducing sugars extracted was increased, in comparison with the amount extracted from non-hydrolysed samples, approximately by 30% in the case of tomato and green peeper wastes. For potato wastes this percentage increased from values lower than 1% to 77%. In addition, very low values of fermentation inhibitors were found in the final broth.

1. Introduction

One-third of all food produced globally for human consumption, about 1.3 billion tons, is lost each year according to the Food and Agriculture Organization (Gustavsson et al., 2011). Food is wasted throughout the food supply chain, from initial agricultural production down to final household consumption. Actually, in industrialized countries more than 40% of the food losses occur at retail and consumer levels. In the European Union this loss of produced food is attributed as: 5.5% by improper post-harvest handling, 20% by supermarkets and food industries, 7.5% due to expiring best-before-date, and 13% as consumer household garbage for not being consumed (Nanda et al., 2015). In fact, nowadays, food waste represents worldwide the single largest component of municipal solid waste reaching landfills (Kosseva, 2013). In this context, fruits and vegetables have the highest wastage rates and, specifically, in the European Union, about 50% of all fruits and vegetables go to waste throughout the entire food chain (Gustavsson et al., 2011; Nanda et al., 2015).

Food wastes are a significant global problem for economic, environmental and food security reasons. Therefore, government efforts have focused on diverting waste away from landfill through regulation, taxation, and public awareness. According to the European Landfill Directive (1999/31/EC), the amount of biodegradable waste sent to landfills in member countries by 2016 must be 35% of the levels reached in 1995 (Kosseva, 2013).

The solid extract of these wastes is mainly constituted by carbohydrates, proteins, lipids and minor amounts of vitamins and minerals. Indeed, carbohydrates are the main component of fruit and vegetables and represent 70–90% of their dry weight. So, due to their high polysaccharide content, waste fruits and vegetable have promising potential for being converted into value-added products, such as fuels or chemicals, through thermochemical and biological pathways (Nanda et al., 2015). Several works have been carried out to transform these wastes to value added products like enzymes, organic acids, flavouring compounds, food colorants, bioethanol, bio-methane, etc. (Panda et al., 2016; Ravindran and Jaiswal, 2016).

In the past decade, important issues about the world climate change, along with the rising demand for renewable energy, have led to the development of alternative technologies for the production of biofuels like ethanol or butanol (Aguiar et al., 2013). Current production of bioethanol relies on ethanol from starch and sugars but there has been considerable debate about its sustainability (Alvira et al., 2010). For this reason, biofuels produced from food vegetable wastes is an interesting alternative (Kennes et al., 2016).

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Many of vegetal carbohydrates are in polymeric form, so production of fuels and/or chemicals from these waste materials need to be accomplished by hydrolysing the starch, cellulose and/or hemicellulose to soluble sugars, which can be fermented to the desired products (Thulluri et al., 2013), such as ethanol and hydrogen (Han et al., 2015, 2017). Thereof, the pretreatment of the wastes to facilitate the polysaccharides hydrolysis is a key step in the biotechnological procedure of revalorization. Nowadays various pretreatment methods have been developed which include physical, chemical, biological, thermal approaches and even combined processes (Thulluri et al., 2013; Vavouraki et al., 2014; Wu et al., 2016). In addition, it is well known that hydrolysis that require severe conditions (high temperatures, low pH . . .), may be limited to be used as pretreatment for bioprocesses because of the formation of fermentation inhibitory by-products like furfural or 5-hydroxymethylfurfural (HMF) (Khawla et al., 2014; Wu et al., 2016).

Hence, it can be concluded that a pretreatment of vegetable wastes to facilitate the hydrolysis of polysaccharides into monomeric sugars results to be fundamental for revalorization processes via microbial ways. In this work, vegetable supermarket wastes (tomatoes, green peppers and potatoes) have been treated by different hydrolysis procedures with the purpose to maximise the concentration of fermentable sugars in the final broth. The assayed treatments were evaluated by means of monitoring the evolution of sugar concentrations and analysing the possible formation of fermentation inhibitors (furfural, HMF and acetic acid). The last aim was to optimise the hydrolysis process in order to use vegetable wastes as raw material for fermentation processes.

2. Materials and methods

2.1. Raw materials

Vegetable wastes (tomatoes, green peppers and potatoes) was selected as model vegetables taking into account three factors:

(i) These wastes represent a considerable percentage of total vegetable wastes generated at retail (the three usually sum up approximately 40% of total wastes in the local supermarket that supplied the raw materials).
(ii) The carbohydrates of these wastes are mainly complex polymers that need to be hydrolysed.
(iii) These vegetables are not seasonal products and their wastes are available along all the year.

Wastes were supplied by a local market and once in the laboratory they were washed with distilled water and stored at 4 °C during a maximum of three days until being treated. Additionally, nutritional composition and moisture content of these vegetables are shown in Table 1.

2.2. Determination of sugars in raw materials

2.2.1. Potential reducing sugars

For the determination of potential reducing sugars, samples were treated according to Lenihan et al. (2011). Samples were first ground in pieces under 2 mm. An amount of 0.3 g of minced sample was introduced into a test tube and 3 mL of 85% H_2SO_4 that has been cooled to 15 °C was added. Samples were stirred thoroughly before being placed in a water bath at 30 °C. This temperature was maintained for 2 h, stirring the samples every 10 min. After this time, the mixture was washed from the tube into an Erlenmeyer flask and distilled water was added to 89.11 g. The dilute solution was then autoclaved at 121 °C and 1 atm for 1 h. Finally, sample was cooled to room temperature, then bigger solids were removed passing the sample through a 1 mm mesh sieve and the liquid was centrifuged at 20 °C and 5000 rpm during 5 min (Kubota 6500 High Speed Refrigerated Centrifuge) to remove the remaining particles. Supernatant was frozen and potential reducing sugars were determined in these samples according to the DNS method described in Section 2.4.1.

2.2.2. Soluble sugars

In order to determine the amount of total sugars and reducing sugars that can be extracted from the considered wastes just by using water, they were treated as follows. Distilled water was added to waste in a relation 1:2 (100 g of waste and 200 mL of water) and the mixture was homogenized in a kitchen blender during 3 min. Solids were removed with a sieve and the liquid phase was centrifuged at 20 °C and 5000 rpm during 5 min (Kubota 6500 High Speed Refrigerated Centrifuge). The pellet was discarded and the supernatant were frozen until being analysed, as described in Section 2.4.

2.3. Hydrolysis treatments

Each treatment was assayed with each kind of waste separately carried out at least in triplicate using different batches of each residue. All reagents employed in the different hydrolysis were supplied by Sigma-Aldrich.

2.3.1. Thermal hydrolysis

(A) This treatment was modified from Del Campo et al. (2006) as follows, 100 g of each waste were minced in pieces smaller than 2 cm, and then this material was dried at 55 °C during 24 h in an incubator (Heidolph Unimax 2010). The dried material was grinded in a kitchen robot (Moulinex Minirobot D81) obtaining a particle size below 2 mm. Distilled water was added to the grinded samples in a relation of 5% (w/v) in 250 mL Pyrex bottles, which were treated in an autoclave at 110 °C and 1.5 atm during 5 min. Solids were removed with a sieve and the liquid phase was adjusted to pH 6.5–7 with 6 M NaOH or 1 M HCl. Finally, samples were centrifuged at 20 °C and 5000 rpm during 5 min (Kubota

Table 1
Nutritional composition of the vegetables employed as substrate (given per 100 g of fresh product).

	Carbohydrates (g)[a]	Lipids (g)[a]	Proteins (g)[a]	Water content (g)[b]	Vitamins (mg)[a]	Minerals (mg)[a]
Tomato	3.9	0.2	0.9	93.0	15.7[A]	272[A]
Pepper	4.6	0.2	0.9	93.5	133[B]	218[B]
Potato	17	0.1	2.0	63.5	21.5[C]	521[C]

[a] Average values adapted from USDA (United States Department of Agriculture).
[b] Average values of own data.
[A] Vitamins (A, B_1, B_3, B_6, C, E, K)/Minerals (Mg, Mn, P, K).
[B] Vitamins (A, B_1, B_2, B_3, B_6, C, E, K)/minerals (Ca, Fe, Mg, P, K, Na, Zn).
[C] Vitamins (B_1, B_2, B_3, B_6, C)/minerals (Ca, Fe, Mg, P, K, Na).

6500 High Speed Refrigerated Centrifuge) and supernatants were frozen until being analysed.

(B) According to Correa et al. (2012) procedure, distilled water was added to 10 g of each waste in a relation 1:1 (w/v) and the mixture was homogenized in a kitchen blender. The samples were then treated in an autoclave at 135 °C and 3 atm during 5 min. After that, samples were treated in the same way as described in (A) section.

2.3.2. Acid hydrolysis

Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation (Taherzadeh and Karimi, 2008). Indeed, lignin acts as a physical barrier that protects polysaccharides from enzyme action, so lignin removal increases enzyme effectiveness by eliminating non-productive adsorption sites and by increasing access to cellulose and hemicellulose (Kumar et al., 2009; Sun et al., 2011). Hence, with the aim of removing the lignin from the samples before the acid hydrolysis, 10 g of each sample was minced in the kitchen robot (Moulinex Minirobot D81) until a particle size below 2 mm was obtained. Then, samples were immersed in 20 mL of NaOH 0.1 N and, after 15 min 0.8 g of $CaSO_4$ were added and the mixtures were let stand for 3 h at room temperature. After this time, liquid was removed employing a sieve and solid residue was washed twice with distilled water.

Solid material was mixed with 5% H_2SO_4 in a relation 2:1 (w/v) and then it was treated in an autoclave at 125 °C and 2 atm during 15 min. Finally, solids were removed with a sieve and the liquid phase was neutralized to pH 6.5–7 with NaOH 6 M, centrifuged and supernatants were frozen until being analysed (Monsalve et al., 2006).

2.3.3. Enzymatic hydrolysis

The first step in the enzymatic hydrolysis consisted on the delignification of samples as it was described in Section 2.3.2. After that, distilled water was added to solid residue in a 6% relation (w/v), pH was adjusted to 4.5 and the mixture was incubated in a bath at 75 °C during 5 min. Once samples were cooled to room temperature, the combination of enzymes was added as follows: 83 μL of cellulase from *Trichoderma reesei* (C2730) (enzymatic activity ≥700 Beta-Glucanase units/g, density 1.10–1.30 g/mL), 50 μL of α-amylase from *Aspergillus oryzae* (A8220) (enzymatic activity ≥800 Fungal Alpha Amylase units/g, density 1.10–1.30 g/mL) and 8 μL of amyloglucosidase from *Aspergillus niger* (A7095) (enzymatic activity ≥260 units/mL, density 1.2 g/mL). Then, 250 mL Erlenmeyer flasks containing the samples were shaken to homogenize and incubated at 60 °C in static during 60 min. Afterwards, bottles were subjected to 95 °C during 5 min in a water bath in order to stop the enzymatic reaction. Finally, bottles were shaken and samples were taken and sieved, centrifuged and frozen as it was explained above for thermal hydrolyses.

The mixture of enzymes was selected according to the nature of the complex polymers contained in the vegetable wastes (mainly cellulose in tomatoes and red peppers and starch in potatoes) (Van Dyk et al., 2013). Cellulase catalyses the breakdown of cellulose into glucose, cellobiose, and higher glucose polymers. α-Amylase and amyloglucosidase hydrolyze the α-(1,4) glucosidic bonds in starch into glucose. The pH and incubation conditions were set with the aim to be accurately for all the employed enzymes.

2.3.4. Thermal-enzymatic hydrolysis

In order to optimise the pretreatment, a procedure that combined thermal and enzymatic hydrolysis was assayed. Lignin was removed from the samples as it was described in Section 2.3.2. Once solid material was obtained, water was added to solid residue in a relation 1:1 (w/v) and samples were then treated in an autoclave at 135 °C and 3 atm during 5 min. Afterwards, the mixture was cooled to room temperature and pH was adjusted to 4.7, then enzymes were added in the same way described above and the rest of the procedure was the same as explained in the enzymatic hydrolysis section. Additionally, samples taken during the enzymatic step were subjected to 95 °C during 5 min in a water bath in order to stop the reaction and then were centrifuged and supernatants were frozen until being analysed. Before taken each sample, Erlenmeyer flasks were shaken to homogenize. Along this process some weight of solids is lost, remaining a solid residue after extracting the reducing sugars. So, in order to establish the losses registered, samples were taken and dry extract were analysed in triplicate. Solid weighed throughout the successive steps are shown in Table 2.

2.4. Analytical methods

All reagents were supplied by Sigma-Aldrich and analyses were carried out in triplicate.

2.4.1. Dinitrosalicylic acid (DNS) method

The percentage of total reducing sugars was determined with 1% dinitrosalicylic acid reagent (DNS) according to the Miller method (Zhang et al., 2011). This method has been selected because it is widely used to estimate the reducing sugars content of different samples. In this procedure, 0.5 mL of DNS reagent was added to 0.5 mL of sample to be analysed and the mixture was vortexed and incubated in a boiling water bath for 5 min. Afterwards, 5 mL of distilled water was added to the tubes and samples were cooled down in ice bath to quench the oxidation reaction. The absorbance of samples was recorded at 540 nm against a reagent blank. Analyses were performed with an UV-Spectrophotometer (ThermoScientific Heλios γ). The concentration of reducing sugars was determined according to the standardisation performed on glucose.

2.4.2. Phenol-sulphuric acid method

To determine the amount of total sugars, the DuBois phenol-sulphuric acid assay modified as follows was employed (Hall, 2013). In this method, 0.5 mL of 5% phenol and 2.5 mL of 96% H_2SO_4 were added to 1 mL of sample and the mixture was incubated at room temperature for 1 h. Finally, the absorbance was recorded at 492 nm against a reagent blank. Measurements were performed with an UV-Spectrophotometer (ThermoScientific Heλios γ). The concentration of total sugars was determined according to the standardisation performed on glucose.

2.4.3. Determination of fermentation inhibitors

Concentrations of fermentation inhibitors, i.e., furfural, HMF and acetic acid, were analysed by high performance liquid chromatography (HPLC). To determine acetic acid concentration, the Agilent 1200 chromatograph (Agilent Technologies) was equipped with an ICSep ICE-ION-300 column (Transgenomic) coupled to a

Table 2
Solids weights throughout the thermal-enzymatic hydrolysis expressed on dry weight basis (%).

	Initial solid weight	Remaining solid weight after delignification process	Remaining solids weight after thermal-enzymatic hydrolysis
Tomato	100	94.9	48.7
Pepper	100	82.1	30.5
Potato	100	83.5	47.5

refractive index detector (RID). The mobile phase employed was sulphuric acid (0.450 mM, pH 3.1) at a flow rate of 0.3 mL/min with the column temperature set at 75 °C (Alonso et al., 2014). To determine furfural and HMF, the method was modified from Abu-Bakar et al. (2014) and De Andrade et al. (2016), the instrument was equipped with a Gemini-NX 5 μm C18 110A column (Phenomenex) coupled to a diode-array detection (DAD) system and the flow was fixed at 1 mL/min. The mobile phase was acetonitrile/water (20:90) and final UV detection was carried out at 260 nm for furfural, whereas methanol/water (10:90) and 285 nm were employed for HMF determination. Data acquisition and analysis were performed with ChemStation software (Agilent Technologies). All compounds were determined employing as reference external analytical standards (Sigma-Aldrich).

3. Results and discussion

3.1. Soluble and potential reducing sugars in raw wastes

Firstly, the amount of sugars that could be extracted from raw materials just by using distilled water, i.e. soluble sugars, was determined. Fig. 1 shows a comparison between reducing and total sugars extracted from several batches of vegetable wastes supplied by a local market at different dates. It is remarkable the small differences observed between batches. In fact, values are within the following ranges: 21–25, 20–27 and 0.05–2 for soluble reducing sugars and 31–40, 24–31 and 1–3 for soluble total sugars in tomato, pepper and potato, respectively (given as g per 100 g of waste dry weight). It can be observed that values of total and reducing sugars in case of tomato and pepper are in the same order of magnitude. On the contrary, the amount of sugars extracted from potato is in both cases much lower. Choi et al. (2016) described that the amount of total sugars (including, fructose, glucose and sucrose and excepting starch) found in potato was 0.9–1.5 g/100 g dry weight, whereas the amount of reducing sugars (fructose and glucose) was within the range 0.3–0.9 g/100 g dry weight. These values were in accordance with those extracted with water from potato waste in the present work as total and reducing sugars, as expected since potato native starch, the main carbohydrate in these vegetable, is not easily solubilized in water at room temperature (Hong et al., 2016). Likewise, values of available sugars of approximately 28 g/100 g dry weight were reported by Guil-Guerrero et al. (2006) for pepper, almost the same than that obtained in our work for total sugars. Smoleń et al. (2015) found values of 22.8 g/100 g dry weight of fructose and glucose in tomato, values that are very similar to those we found for soluble reducing sugars (23.3 g/100 g dry weight), as fructose and glucose are the reducing sugars present in tomato. Regarding pepper, a value of 56 g/100 g dry weight of total sugars was found by Guil-Guerrero et al. (2006), value slightly higher than that reported in this work for potential reducing sugars (around 48 g/100 g dry weight).

Additionally, it was observed that more than a half of soluble total sugars were reducing sugars in case of tomato and pepper (around 64% and 86%, respectively), whereas only slightly more than a third (approximately 36%) of soluble total sugars were reducing sugars in case of potato. With the aim to determine the maximum amount of reducing sugars that could be recovered from these vegetable wastes by means of hydrolysis treatments, potential reducing sugars were quantified. Values obtained from several batches of tomato, pepper and potato, were within the ranges: 50–52, 46–51 and 63–65 (g/100 g dry weight), respectively. Again, small differences were found between batches. In this case, the values obtained were in the same order of magnitude for all the tested wastes. Del campo et al. (2006) described a total content of carbohydrates in tomato of around 56% (w/w) (4% sucrose and 35% lignin), value similar to that here measured for potential reducing sugars. It also should be noticed that the potential reducing sugars for tomato and pepper were approximately twofold the soluble reducing sugar values. With regards to potato, value of potential sugars was more than 60 times the value obtained in potato for soluble reducing sugars. Potential sugar values determined here for potato wastes is within the range (60–80 g/100 g dry weight) reported by Raatz et al. (2016) for starch content in fresh potatoes. This means that employing only water as extraction agent nearly half of potential reducing sugars are extracted in case of tomato

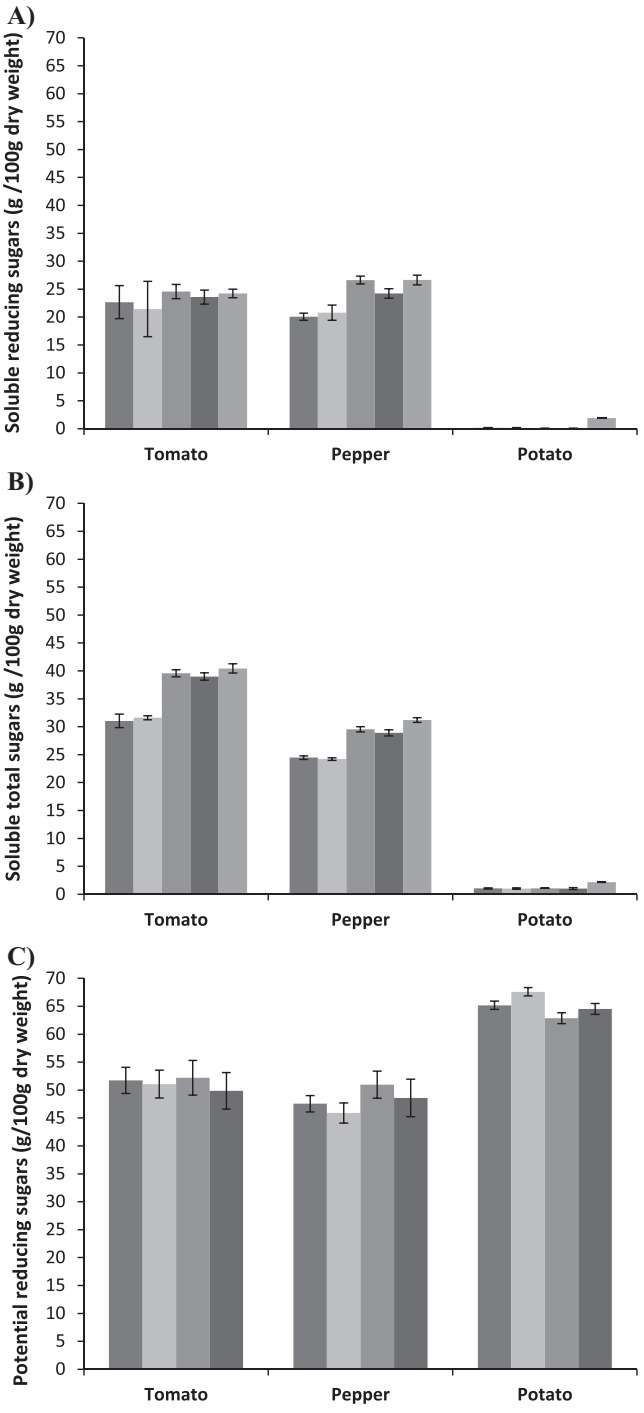

Fig. 1. Soluble reducing sugars (A), soluble total sugars (B) and potential reducing sugars (C) extracted from different batches of vegetable wastes.

and pepper, however less than 1% of potential reducing sugars are recovered in case of potato. Hence, these results showed the importance of pretreat these wastes as a key step in their revalorisation by fermentation processes, mainly in the case of potato wastes.

3.2. Performance of hydrolysis methods

Thermal, acid and enzymatic hydrolysis have been tested in order to maximise the amount of reducing sugars that can be obtained from wastes. In Fig. 2 it is shown the comparison between reducing and total sugar extracted from the vegetable wastes after the different hydrolyses treatments. For each substrate, similar values of total and reducing sugars were obtained employing 110 °C thermal and acid hydrolyses (Fig. 2A and C). Del Campo et al. (2006), employing a 110 °C hydrothermal hydrolysis with similar procedure as that developed in this work, recovered an amount of single sugars (glucose and fructose) of 35 g/100 g for tomato and 50 g/100 g for red pepper, values much higher than those obtained in this work for reducing sugars of tomato and green pepper (around 18 and 14 g/100 g, respectively). Summoogum-Utchanah and Swami (2015) employed fruit and vegetable wastes treated by dilute acid hydrolysis achieving a maximum extraction of 23 g/100 g (dry weight) of reducing sugars. This value is slightly higher than those obtained in this work with acid treatment for tomato and pepper (19 and 15 g/100 g,

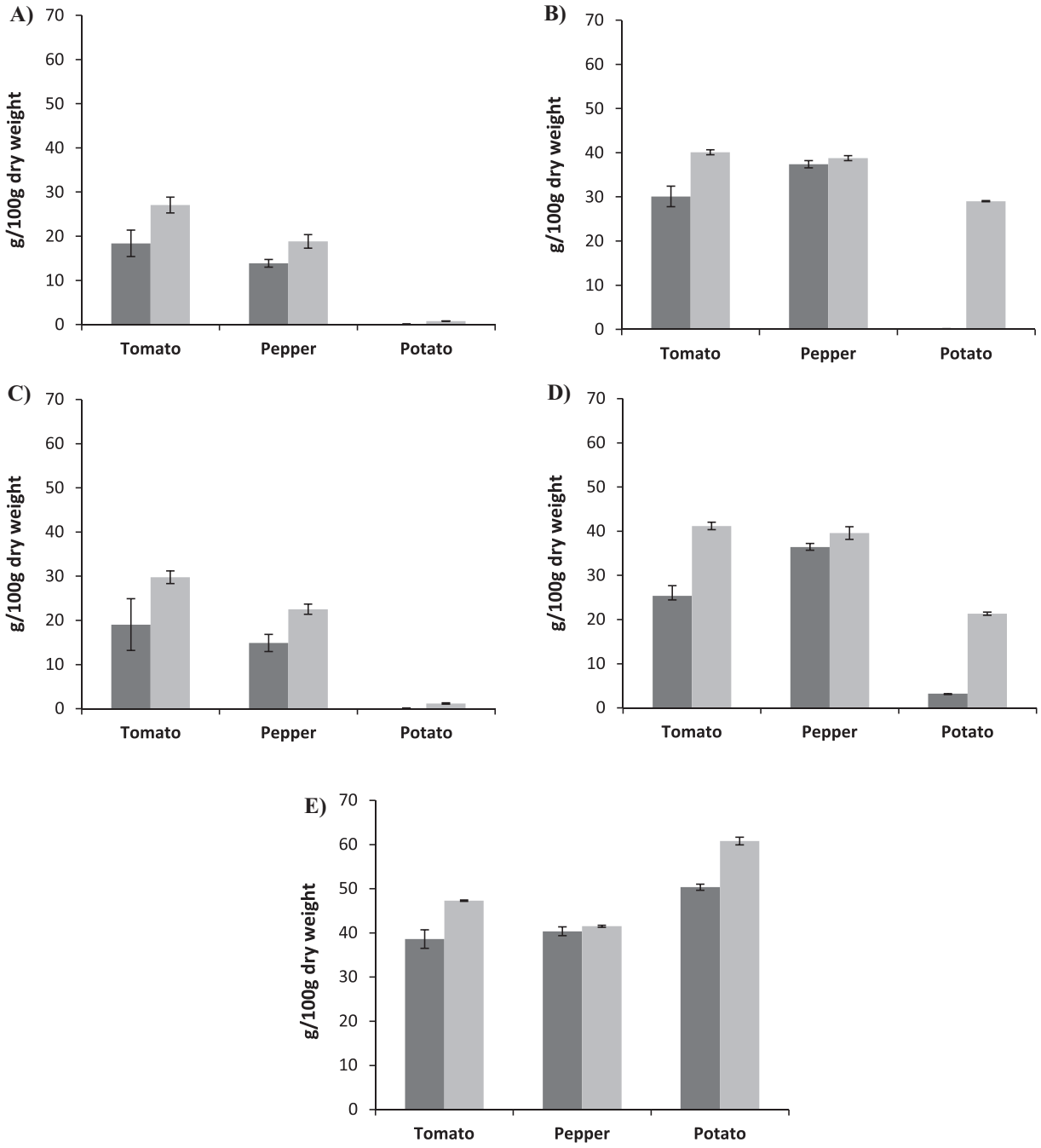

Fig. 2. Reducing (■) and total (■) sugars extracted from vegetable wastes after the hydrolyses: (A) thermal 110 °C, (B) thermal 135 °C, (C) acid, (D) enzymatic and (E) thermal-enzymatic.

respectively). Better results were obtained with 135 °C thermal and enzymatic treatments (Fig. 2B and D). with these hydrolysis methods, the amount of reducing sugars recovered from tomato wastes were 25–30 g per 100 g of initial waste (dry weight) and around 37 g/100 g for pepper. Table 3 shows the percentage of reducing sugars extracted with respect to the potential reducing sugars contained in each substrate. The higher percentages were obtained with 135 °C thermal and enzymatic treatments for tomato and pepper. In case of pepper the amounts extracted were above 75% for both treatments.

Concerning potato, it should be remarked that 110 °C thermal and acid hydrolysis (Fig. 2A and B) does not seem to be efficient enough to break down the starch contained in this substrate. In addition, 135 °C thermal hydrolysis increased the total sugars extracted from potato but not the amount of reducing sugars. Higher temperatures favour the solubilisation of starch, as reported (Fuentes-Zaragoza et al., 2010). However, this treatment is not efficient enough to convert this polymer into reducing sugars. Finally, with enzymatic treatment an increase in total and reducing sugars in potato hydrolysate were achieved. Nevertheless, the amount of reducing sugars extracted was still far from the potential reducing sugars of potato wastes, and the percentage extracted hardly achieved 5% (see Table 3).

3.3. Thermic-enzymatic hydrolysis

Considering data shown in Table 3 (excepting for potato thermally treated), the best results were achieved for all wastes with thermal (135 °C) and enzymatic hydrolysis. For this reason, both treatments were combined in a two-step hydrolyses. Indeed, in all cases, the highest amount of reducing and also total sugars were achieved employing the thermal-enzymatic hydrolyses (Fig. 2). Furthermore, this increment was notably higher in case of potato wastes (Fig. 2E). Moreover, as shown in Table 3, the highest percentages of extracted reducing sugars with respect to potential reducing sugars were obtained with the thermic-enzymatic hydrolyses for tomato, pepper and potato (in all cases higher than 75%).

Çöpür et al. (2012) employed a mixture of tomato, pepper, and eggplant stalks as substrates for reducing sugars production. Stalks were chemically pretreated with sodium borohydrate and then enzymatically hydrolysed. Best results achieved sugar yields of 30% (w/w). This value is similar to those obtained here for tomato and pepper wastes in enzymatic treatment, whereas it is lower than the values obtained in the combined thermal-enzymatic treatment for these vegetables residues (around 40 g/100 g dry weight).

Potato residues can be compared with vegetable residues with high content in starch such as cassava. Indeed, tapioca starch factories generate a large amount of solid waste that contains a high level of starchy-lignocellulosic biomass, especially cassava pulp. This waste can be hydrolysed enzymatically achieving values of approximately 50 g/100 g dry extract (Virunanon et al., 2013), values very similar to those obtained in our work for thermal-enzymatic treatment of potato residues.

In the hydrolysis treatments by chemical and/or physical technologies the temperature and time of reaction are determinant factors. Furthermore, the total content of hydrolysable biopolymers and the nature of these polysaccharides are also key factors. However, it is important to highlight that, in general, for vegetable wastes, specially, those that contain high levels of starch, best results are obtained with enzymatic processes with a previous pretreatment (Patle and Lal, 2007; Preeti et al., 2012; Virunanon et al., 2013).

The differences observed in this work between enzymatic and thermal-enzymatic results are due to the fact that a thermal treatment at 135 °C previous to enzymatic treatment contributes to the solubilisation of carbohydrates making easier to the enzymes to break the complex polysaccharides. These differences found between these treatments were notably greater in case of potato waste. As it has been commented above, potato carbohydrates are mainly constituted by starch and starch in potatoes is generally 70–80% amylopectin with the remainder being amylose (Raatz et al., 2016). As in enzymatic hydrolyses a combination of cellulose, α-amylase and amyloglucosidase were employed, amylopectin and amylose can be easily degraded. It is difficult to solubilise potato native starch (Hong et al., 2016), nevertheless, the structure and properties of starch are altered upon hydrothermal treatment induced in excess water. This phenomenon is well recognised as gelatinisation which is an irreversible order-disorder transition (Somboonchan et al., 2016). Gelatinised starch is easily accessible to enzymes that can break the starch's structural units, amylose and amylopectin, into glucose.

In order to acquire a deeper knowledge of the enzymatic step, samples were taken during the combined treatment and the evolution with time of extracted reducing and total sugars were measured. It is noticeable that the initial amount of reducing sugars after the thermic step was much lower in potato compared to tomato and pepper. As can be seen in Fig. 3, along the enzymatic hydrolysis, the concentration of reducing sugars in tomato and pepper wastes increased slowly, whereas in case of potato residue this increment was notably sharper. During the enzymatic step the concentration of reducing sugars in the broth increased in all cases, just 21% and 15% for tomato and pepper (respectively), whereas for the potato the reducing sugars concentration increases 11 times.

After the 60 min that the enzymatic step lasted, a percentage around 25, 16 and 23 for tomato, pepper and potato, respectively, of the potential reducing sugars initially measured for the wastes remained without being hydrolysed into reducing sugars. It was observed that, in case of tomato and potato wastes a maximum content in reducing sugars was achieved at 45 min. On the contrary, in case of potato waste, the trend of the curve indicated that a higher amount of reducing sugars could be achieve if the enzymatic reaction goes on for some more time.

The possible formation of fermentation inhibitors, i.e. furfural, HMF and acetic, was investigated by analyzing the final products and in all cases the amount of these sugar degradation products was below the detection limit (<1 mg/L). For furfural or HMF, a concentration of 4 g/L has been reported to inhibit sugar fermenta-

Table 3
Comparison of reducing sugars yield extraction of the different hydrolysis assayed. Average values are shown.

		Reducing sugars extracted with respect to reducing potential sugars (%)[a]		
		Tomato	Pepper	Potato
Non-hydrolysed samples		45.5 ± 0.0	49.0 ± 0.1	0.72 ± 0.01
	Thermic (110 °C)	37.2 ± 5.9	30.8 ± 2.0	0.12 ± 0.05
	Thermic (135 °C)	58.8 ± 2.3	77.5 ± 0.8	0.18 ± 0.02
Hydrolysed samples	Acid	35.9 ± 3.0	28.7 ± 0.9	0.09 ± 0.02
	Enzymatic	49.5 ± 1.0	75.5 ± 0.7	4.93 ± 0.02
	Thermic-enzymatic	75.4 ± 2.1	83.7 ± 1.0	77.4 ± 0.7

[a] Average values were used for reducing potential sugars.

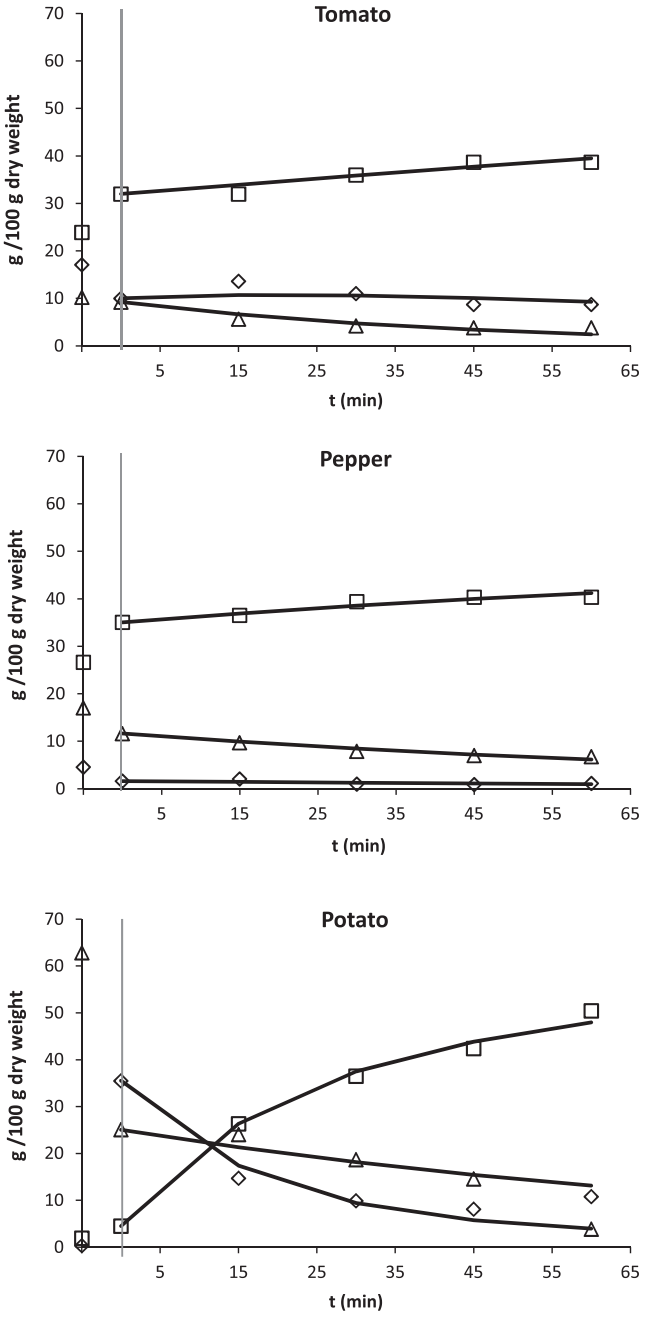

Fig. 3. Evolution of solubilised reducing sugars (M: □), solubilised non-reducing-sugar intermediates (D: ◇) and potential reducing sugars in solid phase (S: Δ) with time. Symbols correspond to experimental data (or calculated from experimental data) and lines correspond to model results. All concentrations are expressed as equivalent glucose. Vertical grey line divides the thermic and the enzymatic steps. In all cases, SD ≤ 2.0 g/100 g.

tion by yeast. In addition, for acetic acid the reported inhibition level for *S. cerevisiae* was 6 g/L (Zheng et al., 2013). According to the results obtained in this work it can be assured that furfural, HMF and acetic acid concentrations are quite below those concentrations described as necessary to cause fermentation inhibition problems.

3.4. Modelling

A model based on irreversible first-order reactions was developed for the enzymatic step. It considers that solid carbohydrates are hydrolysed into soluble intermediates that are subsequently degraded to monomers of glucose. So, S is the polymers in solid phase (estimated as potential reducing sugars minus dissolved total sugars), D is the dissolved non-reducing-sugar intermediates (estimated as dissolved total sugars minus dissolved reducing sugars) and M is the dissolved reducing sugars, all of them expressed as equivalent glucose. k_1 is the solubilisation rate of polymers in solid phase and k_2 is the hydrolysis rate of soluble intermediates into glucose. The inhibitors formation was not included in the model because, as it was commented above, the amount formed during the enzymatic hydrolysis is very low and can be considered negligible for modelling purpose. Parameters were determined by fitting the model to experimental data using Microsoft Excel software. Model reactions are represented below.

$$S \xrightarrow{k1} D \xrightarrow{k2} M$$

Table 4
Kinetic constants calculated from experimental data.

	k_1 (min^{-1})	k_2 (min^{-1})	r^2
Tomato	0.0221	0.0121	0.993
Pepper	0.0106	0.0804	0.999
Potato	0.0108	0.0577	0.987

r^2 values were obtained from fitting by a linear regression experimental and model data.

The comparison between experimental and model data is shown in Fig. 3. It can be observed that the fitting is very good in all cases. Furthermore, in Table 4 are shown the values of kinetic constants k_1 and k_2 and also the r^2 obtained from fitting by a linear regression experimental data and model data. It should be noticed that the correlations represented by r^2 values were in all cases above 0.98 which indicated the high level of accuracy achieved by the model. Although all the constants values are of the same order, the solid polymers are solubilized to non-reducing-sugar intermediates in half time for tomato with respect to pepper and potato. However, the transformation of the solubilised non-reducing-sugar intermediates to solubilised reducing sugars takes place faster in pepper and potato residues than in case of tomato wastes. Lenihan et al. (2011), employed a similar model to analyze the kinetics of acid hydrolysis of lignocellulosic biomass and reported that the reaction rate of hydrolysis depends on a number of variables, i.e.: temperature, time, substrate concentration and substrate composition.

The particular behavior of potato wastes is determined by starch content which is the main carbohydrate found in potato. On the contrary, the principal polysaccharides contained in tomato and pepper are pectin and cellulose, hemicellulose is also present but in lower concentrations, around 2% (w/w) (Egüés et al., 2013; Deinychenko and Yudicheva; 2016). As the composition of these vegetables is very similar, the different behavior during the combined hydrolysis process may be defined by the specific structure and interactions between their compounds. In recent works, it was reported that the organization and interactions of vegetable wall components is not known with certainty, so the effect of treatments are determined by chemical components, physical properties and supermolecular structures (Çöpür et al., 2012; Sun et al., 2016).

4. Conclusions

The high content of carbohydrates in vegetable wastes indicates that these materials may be valuable recourses to be used as substrates for fermentation purpose. A pretreatment to facilitate the polysaccharides solubilisation and hydrolysis is recommended for cellulose-containing materials, i.e. tomato and peppers, and

essential for starchy wastes, i.e. potato. The combined thermic-enzymatic hydrolysis turned out as the most efficient method of the assayed procedures to obtain monomeric sugars from tomato, pepper and potato wastes. Specifically, with this combined pretreatment the percentage of reducing sugars extracted (with respect to the reducing potential sugars) was increased in approximately 1.7 times for tomato and green peeper wastes and in 107 times for potato wastes. It is remarkable that the obtained hydrolysates are suitable to be directly used as fermentation media due to the low concentrations of growth inhibitors, which allow avoiding the purification step. Additionally, it should be highlighted that, according to results reported here, and for the assayed enzymes, 45 min was time enough to achieved the maximum concentration of reducing sugars for tomato and pepper wastes, whereas, in the case of potato waste, 1 h did not allow to obtain the maximum yield of reducing sugars. So, the duration of the enzymatic process resulted to be an important parameter that should be adjusted depending on the specific composition of vegetable wastes mixture. The kinetic model developed for the enzymatic step showed a good fitting, so it could be employed in future technical studies.

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