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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.

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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, bio-ethanol, 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 (Aguir 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.

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, B₁, B₃, B₆, C, E, K)/Minerals (Mg, Mn, P, K).

^B Vitamins (A, B₁, B₂, B₃, B₆, C, E, K)/minerals (Ca, Fe, Mg, P, K, Na, Zn).

^C Vitamins (B₁, B₂, B₃, B₆, C)/minerals (Ca, Fe, Mg, P, K, Na).

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% H₂SO₄ 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

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