Carbohydrate Polymers 92 (2013) 2065–2074

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Conversion of a non-water soluble potato starch waste into reducing sugars under non-conventional technologies

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ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form
15 November 2012
Accepted 19 November 2012
Available online 5 December 2012

Keywords:
Biomass
Starch-based waste
Microwave irradiation
Ultrasound irradiation

ABSTRACT

In this exploratory work, the comparison of the utilisation of different non-conventional technologies (ultrasound and microwave irradiations) for the depolymerisation of a complex industrial starch-based waste into reducing sugars was investigated. Reducing sugars could then be converted into higher value-added compounds such as higher alcohols. The experiments were performed on three different starting materials named as 'Potato flour', 'Wet potato sludge' and 'Dry potato sludge'. The conversion of 'Potato flour' into reducing sugars reached in acidic conditions 61% within an hour under microwave irradiation, 70% and 84% within 120 min under low and high frequency ultrasonic irradiation, respectively.

1. Introduction

The world energy consumption has soared by 30% during the last two decades and the oil consumption has increased of about 4% in 2010. Almost 80% of the fossil fuels are employed for primary energy consumption and 58% are consumed by the transport sector (Nigam & Singh, 2011). Biomass can be considered as one of the alternative resources to reduce the consumption of fossil fuel, as it is an abundant and emerging source of energy that may be used as raw materials. In 2007, the global production of biofuels reached 62 billion litres, which is corresponding only to 1.8% of the global transport fuel consumption in energy terms. Almost 80% was ethanol fuel and the remaining was biodiesel (Scheffran, 2010, chap. 2).

However, the chosen biomass should not compete with food and be inedible to solve ethics problems. Waste-to-energy processes can be a renewable energy without depending on fossil fuels. We would like hence to present our exploratory work dedicated to the transformation of inedible waste into high added value compounds, which can then undergo fermentation to provide biofuels. Thus, the raw material used in our process is a starch-based waste, potato peel, provided by a Finnish company called Jepuan Peruna Oy, with an average of 20 tonnes per day generated in 2010. The challenge of converting potato waste into biofuels can be divided into two main stages; the conversion (depolymerisation) of starch-based waste into reducing sugars and then its transformation into higher alcohols like butanol or pentanol. The work presented is focused on the first part of the catalytic conversion.

The production of biomass must be also sustainable, emphasising the necessity to design an as clean as possible process to enable a progressive giving up of fossils energies leading subsequently to a more eco-friendly society. This can be achieved through the help of the twelve principles of green chemistry (Anastas & Warner, 1998). At first, water as the cheapest and greenest alternative to molecular organic solvents was chosen. However, starch is a non-water soluble biopolymer leading to heterogeneous systems potentially less reactive media than homogeneous ones. To overcome this limitation, we turned ourselves on the use of non-conventional activation methods. The use of ultrasound and microwave technologies appeared to us as rapid, clean, safe and adapted means to afford a rapid depolymerisation of starch-based materials in a heterogeneous medium.

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http://dx.doi.org/10.1016/j.carbpol.2012.11.048

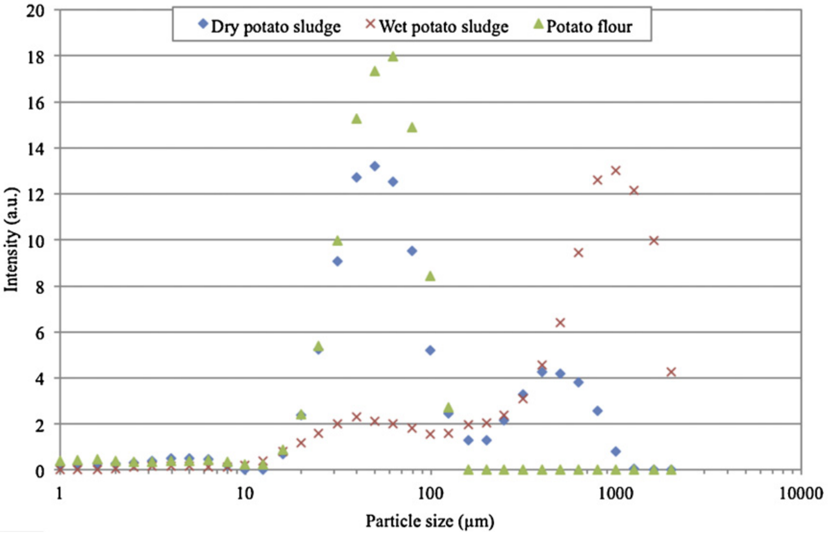

Fig. 1. Particle size distribution of 'Potato flour', 'Wet potato sludge' and 'Dry potato sludge' potato raw materials.

Ultrasound is defined as a sound wave within a frequency range of 20 kHz to 3 MHz. Sonochemistry is the application of ultrasound to chemical reaction. Piezoelectric transducers are used to generate the ultrasound. The reactivity of a liquid medium can be improved thanks to ultrasound irradiation, which can be done through the generation, growth and collapse of bubbles caused by the unique phenomenon of cavitation leading to several physical or chemical effects according to the incident frequency in liquids. At low frequency, shock waves allow an efficient stirring of the medium together with erosion effects, whereas, at high frequency, the collapsing time is shorter and free radicals are formed (Mason & Lorimer, 2002). The degradation of polysaccharides under ultrasound irradiation has been investigated since the 30s (Flosdorf & Chambers, 1933). The effect of ultrasound over carbohydrates has been also explored in sulphuric acid at low frequency (Choi & Kim, 1994; Koda, Taguchi, & Futamura, 2011), in neutral conditions at low frequencies (Portenlänger & Heusinger, 1997) and at high frequencies (Czechowska-Biskup, Rokita, Lotfy, Ulanski, & Rosiak, 2005; Koda et al., 2011). However, most of the works have been performed on water-soluble carbohydrates, cellulose-based or pullulan but rarely on starch.

The effects of microwave heating have been studied since the 80s (Gedye et al., 1986; Giguere, Bray, Duncan, & Majetich, 1986). Microwaves are electromagnetic radiation within wavelengths of 1 m to 1 mm, which corresponds to the frequencies of 300 MHz to 300 GHz, respectively. Microwave heating is a rapid heating device for polar media. Polar molecules undergo intermolecular frictions while the alternative electric field is applied. These frictions provoke the heating of the polar molecules (Loupy, 2006). The starch depolymerisation under microwave irradiation was already investigated in 1979 under neutral pH in water (Khan, Johnson, & Robinson, 1979), in diluted hydrochloric acid (Khan, Robinson, & Johnson, 1980; Yu, Chen, Suree, Nuansri, & Wang, 1996) and also chloride-based catalyst to enhance the hydrolysis (Kunlan et al., 2001).

To the best of our knowledge, all these studies explored the degradation process of biopolymer under ultrasound or microwave irradiations with pure authentic materials but never with a complex matrix. The aim of this work is to valorise an inedible industrial starch-based waste by using non-conventional methods of irradiation into reducing sugars, which can be further transformed into higher alcohols, what will be treated in another article.

2. Experimental details

2.1. Materials

The ultrasonic bath is a 5L "Kerry Pulsatron" with a working frequency at 24 kHz. A 100 mL homemade ultrasonic reactor was designed to perform experiments at 500 kHz. The acoustic power of 30 W was measured by calorimetry according to the standardisation performed by Kimura et al. (1996). A Pyrex® and metallic ultrasonic 20 kHz probes delivered an acoustic power of 17 and 86 W, respectively, with 75% amplitude with the metallic probe. All ultrasonic reactors were fitted with cooling circulating systems to ensure an efficient temperature control. The experiments under microwave irradiation were performed in a mono-mode synthesis microwave Prolabo Synthewave S402 (electric power 600 W). The reactors were glassware tubes suitable for the microwave with a 20 mL capacity. Experiments under and without mechanical stirring ("Zero" experiments) were carried out in an oil bath with a Stuart hot plate stirrer SC162 for comparison with the non-conventional activation methods. The heater power of the device was 700 W.

2.2. Experimental method

The experiments were performed in distilled neutral, basic (1 mol L^{-1} NaOH) or acidic (3 mol L^{-1} H_2SO_4) water with three kinds of raw material – Potato flour, Wet potato sludge and Dry potato sludge. 'Potato flour' is pure potato, purchased at the supermarket, composed solely of starch and considered as a reference material. 'Wet potato sludge' is the sludge waste product of the industrial production of potato peels, provided by the Finnish company Jepuan Peruna Oy. This sludge is composed of two third of water (67%) and one third of dry matter (33%). This latter is issued from the removal of roughly a centimetre of all around the potato with a potato rotating peeler machine. The weight percentage of sugars was done by a total hydrolysis of the sludge dry matter, which is mainly composed of glucose (80.2%), mannose (4.9%) and galactose (3.2%) units. More than 88% of the dry matter of the 'Wet potato sludge' can be subsequently considered as the total sugar potential. The third raw material used called 'Dry potato sludge', is the wet sludge dried under vacuum line and ground with a mortar and pestle prior to use.

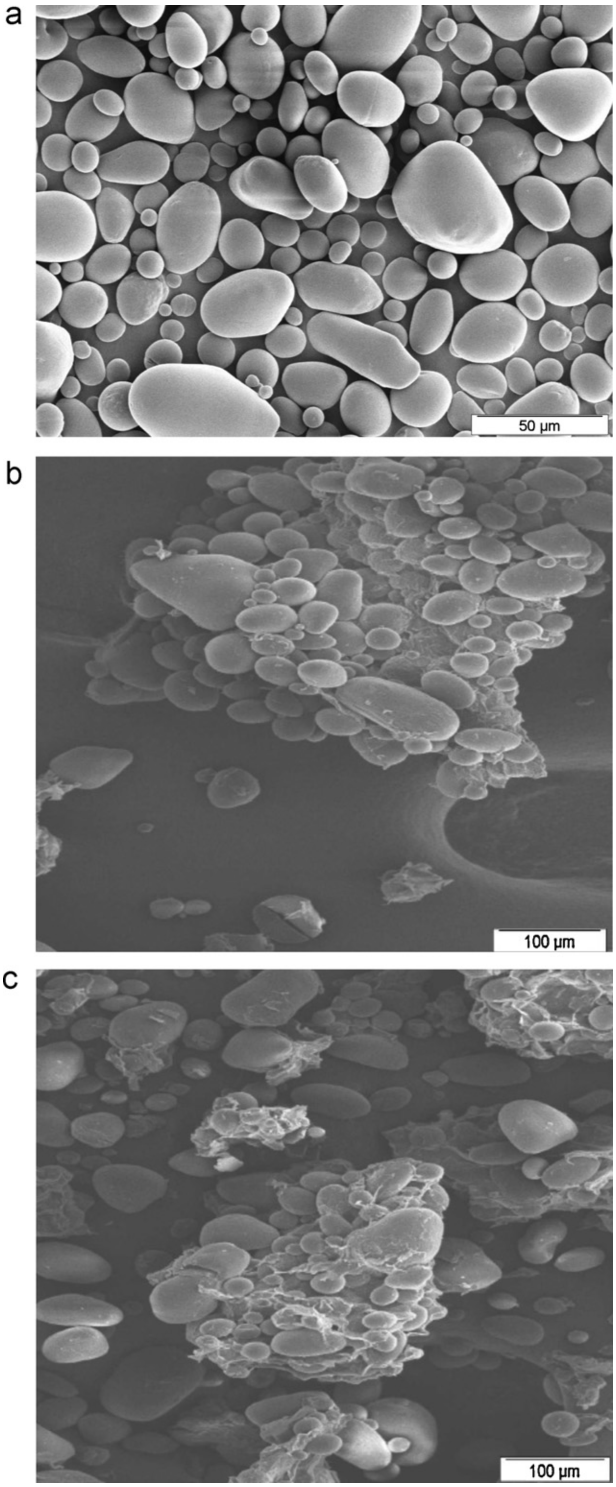

Fig. 2. SEM pictures of (a) 'Potato flour'; (b) 'Wet potato sludge' and (c) 'Dry potato sludge' raw materials.

Each run was performed as follow. Each starting material was added to H_2SO_4 (sulphuric acid 3 mol L^{-1}), distilled water or NaOH (sodium hydroxide 1 mol L^{-1}) to obtain a 3 wt% solution. Due to the weak results obtained and also that saccharification can be hardly done under basic conditions, only few experiments were performed with NaOH. Sodium hydroxide is more utilised for the pre-treatment of cellulose before hydrolysis (Silverstein, Chen, Sharma-Shivappa, Boyette, & Osborne, 2007). Mechanical stirring and ultrasound irradiation processes were performed for 120 min whereas microwave processes were irradiated 60 min. The reactions were carried out at room temperature and 60 °C. Afterwards, the mixture obtained was separated by centrifugation at 7000 tr min^{-1} for 15 min. The liquid phase was filtered through a 47 mm nylon filter membrane of 0.45 µm to obtain clear solution and the amount of sugar was analysed. The acidic solutions were separated in two, a part was neutralised with few pellets of sodium hydroxide, in order to reach a basic pH for total reducing sugar analyses, and the other part was neutralised with barium carbonate and filtered on Celite®. On the other hand, the solid phase was dry with the vacuum line or low temperature oven. The raw material depolymerised under acidic conditions was first washed 3 times with distilled water to quench the hydrolysis and centrifuged at 7000 tr min^{-1} for 30 min, before drying with the vacuum line. The results were the average of two measurements.

2.3. *Analyses*

The amount of reducing sugars was determined according to the total reducing sugars (TRS) method with 1% dinitrosalicylic acid (DNS) reagent according to the Miller technique as follows (Miller, 1959). A sample of 2 mL of the depolymerized solution of starch-based materials was added to 2 mL of 1% DNS reagent solution. The resulting solution was boiled for 10 min. Then, 1 mL of a 40% solution of potassium sodium tartrate was poured in order to keep the colouration of the reaction and cooled down to room temperature to quench the oxidation reaction. The analysis of the solution was performed with a UV–visible spectrophotometer, Varian Cary50 Scan, at the wavelength of 575 nm. The TRS (total reducing sugar) concentration in each solution was calculated according to a standard curve performed with glucose. The highest rates of reducing sugars were analysed with a Bruker Autoflex speed (Bruker Daltonics) matrix assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectroscopy to reveal the composition and the nature of the obtained reducing sugars. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix with a concentration of 50 mg mL^{-1} in water. The size distribution of the raw particles was measured by means of a laser diffraction granulometer Malvern Mastersizer 2000. The raw materials were coated with Au/Pd and observed in secondary electron mode with a JEOL JSM-6100 scanning electron microscope operated at 8 kV.

3. Results and discussion

3.1. *Characterisation of raw materials*

The particle size distributions of the three raw materials are shown in Fig. 1.

As shown in Fig. 1, the three starting materials display different particle size distributions. The 'Wet potato sludge' material displays the highest particles size distribution with a median diameter of 656 µm with a dual population at 40 and 1000 µm. Both 'Potato flour' and 'Dry potato sludge' materials display much smaller particle size distributions. Whereas the former has a median diameter of 42 µm with a single population, the 'Dry potato sludge' one has a median population of 53 µm with a main dual population: one around 50 µm, which represents the individual grains, and another one above 450 µm, which represents the aggregates grains. From these results, one could foresee that the 'Wet potato sludge' material, displaying a 656 µm median size distribution, might be the less adapted material for the heterogeneous depolymerisation reaction compared to both 'Dry potato sludge' and 'Potato flour' materials, displaying both much smaller median size distributions.

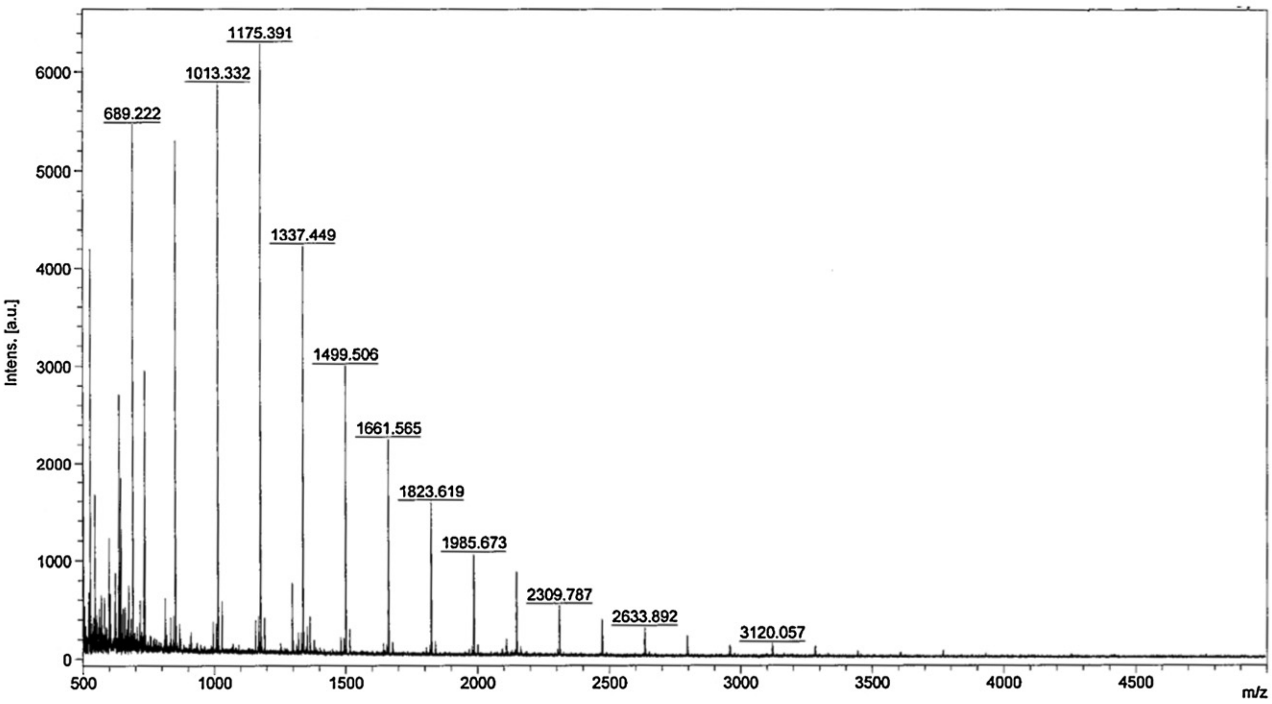

Fig. 3. MALDI-TOF analyses of resulting reducing sugars under mechanical stirring (Potato flour – H_2SO_4 3 mol L^{-1} – 60 °C – 2 h – 3 wt%).

The SEM analyses matched the previous analyses, whereas the ‘Potato flour’ material showed only single starch grains (Fig. 2a), both ‘Wet potato sludge’ and ‘Dry potato sludge’ materials showed single but also aggregated grains (respectively Fig. 2b and c). In addition, the rate of aggregation was much higher for the ‘Wet potato sludge’ material than for the ‘Dry potato sludge’ one, which also displayed single grains. This aggregation made plausible the first drawn hypothesis on the expected lower reactivity of the ‘Wet potato sludge’ material compared to the others.

3.2. *No treatment samples*

“Zero” samples were performed in order to determine the amount of reducing sugars in acidic solution without any stirring with the same experimental conditions – 3 wt%, 3 M sulphuric acid solution, 60 °C, 2 h. The starting materials deposed at the bottom of the reactor throughout the reaction. The results of reducing sugars were 2.0%, 0.5% and 2.5% (±0.1%) for ‘Potato flour’, ‘Wet potato sludge’ and ‘Dry potato sludge’, respectively. The experiments in neutral and basic conditions or room temperature were discarded according to the results obtained (Section 3.3).

3.3. *Mechanical stirring*

Experiments on mechanical stirring were done at first not only to optimise the experimental conditions but also for comparison then with the results obtained with the use of ultrasound and microwave activations. A 3 wt% load of starting material was chosen to avoid any supplementary problem of viscosity that could hide the effect of the nature of the starting materials at 2 different temperatures, room temperature and 60 °C (below the starch gelatinisation temperature), for 120 min in a 50 mL vessel at basic, neutral and acid pHs. The results obtained for all the three substrates are displayed in Table 1.

Whatever the pH, the depolymerisation reaction did not occur at room temperature (entries 1–9). When rising the temperature to 60 °C, the reaction did not take place or a little at both neutral (entries 13–15) and basic (entries 10–12) pHs. Under acidic conditions, the three starting materials afforded reducing sugars in different amounts with the highest recovered yield obtained with the ‘Potato flour’ material. As expected the ‘Wet potato sludge’ material, displaying a very complex matrix and the biggest particles size distribution, afforded the lowest depolymerisation rate.

According to these preliminary results, the basic conditions were discarded. Even if the acidic conditions afforded the best conversion yields, all the three starting materials displayed also a weak but not negligible reactivity under neutral conditions. Even if very small amounts of reducing sugars were obtained, the effects

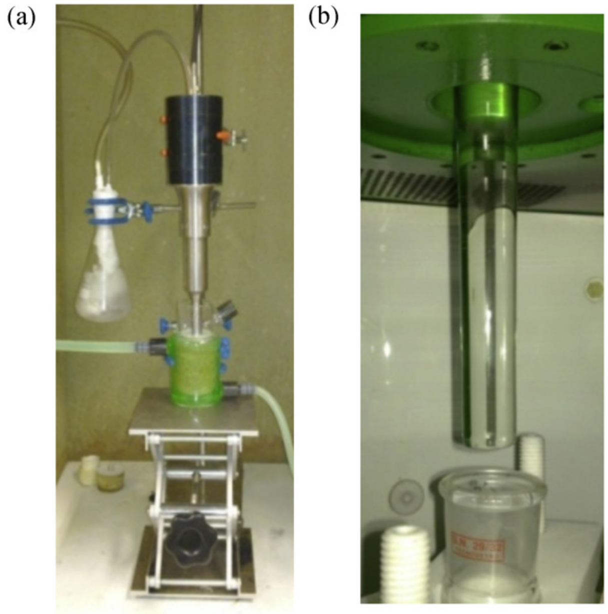

Fig. 4. (a) Ultrasonic horn with a 20 kHz titanium probe and (b) 20 kHz Pyrex probe (Prof. Giancarlo Cravotto, University of Torino).

Table 1
Effect of mechanical stirring with the three starting materials (Potato flour/Wet potato sludge/Dry potato sludge – NaOH 1 mol L^{-1}/neutral/H_2SO_4 3 mol L^{-1} – room temperature/60 °C – 2 h – 3 wt%).

Entry	Starting material	pH	Temperature (°C)	% Reducing sugars
1	Potato flour	Basic	RT	–
2	Wet potato sludge	Basic		–
3	Dry potato sludge	Basic		0.1 ± 0.1
4	Potato flour	Neutral		0.2 ± 0.1
5	Wet potato sludge	Neutral		3.0 ± 0.2
6	Dry potato sludge	Neutral		3.0 ± 0.2
7	Potato flour	Acidic		0.2 ± 0.1
8	Wet potato sludge	Acidic		0.4 ± 0.1
9	Dry potato sludge	Acidic		0.4 ± 0.1
10	Potato flour	Basic	60	–
11	Wet potato sludge	Basic		–
12	Dry potato sludge	Basic		–
13	Potato flour	Neutral		0.2 ± 0.1
14	Wet potato sludge	Neutral		3.0 ± 0.2
15	Dry potato sludge	Neutral		2.6 ± 0.1
16	Potato flour	Acidic		36.0 ± 1.8
17	Wet potato sludge	Acidic		8.5 ± 0.4
18	Dry potato sludge	Acidic		29.3 ± 1.5

brought up by ultrasound or microwave irradiations might enhance the process and neutral conditions could not thus be discarded. A MALDI-TOF analysis was performed on the resulting reducing sugars of Potato flour in sulphuric acid, at 60 °C for 120 min (36% of reducing sugars). The spectrum acquired showed an abundance of various oligosaccharides (4, 5 and 6 glycosidic units) and many polysaccharides in smaller amount. The two next sections present the results obtained with at first the use of ultrasonic irradiation and the second one with the use of microwave irradiation.

3.4. Ultrasonic irradiation

Two incident ultrasonic frequencies were used in this section, 20 and 500 kHz. The former is a low frequency that favours the occurrence of strong physical effects. With such a frequency, it could be expected that aggregations of the 'Wet potato sludge' material might suffer disintegration, subsequently allowing the release of trapped starch grains into the solution to enhance the overall reactivity. However, the strong acidic conditions avoid normally the direct immersion of a metallic ultrasonic probe into the solution to prevent damages by corrosion. Several substitution solutions have been therefore tried to overcome this limitation. At first, the reacting solution was immersed in an ultrasonic bath filled with water. This system remains probably the less powerful one, as the reacting solution is not directly in contact with the ultrasonic source; it is therefore an indirect irradiation mode. At second, we used either a sacrificial metallic (Fig. 4a) or a Pyrex probe (Fig. 4b) one directly dipped into the reacting solution. It is obvious that the metallic probe suffered strong corrosion phenomena; however, the ultrasonic power delivered to the reacting solution is much higher than with Pyrex® probe. This difference is due to the mechanical properties of both materials to transmit sound waves. That is the reason why we sacrificed a metallic horn for comparison. The results obtained at 20 kHz are summarised and displayed below, Table 2 and Fig. 5:

As already noticed previously, no reaction took place at room temperature whatever the ultrasonic device, the nature of the starting material and the pH value (Table 2, entries 1–6). The increase of the working temperature up to 60 °C generated a depolymerisation of all three starting starch-based materials. In the ultrasonic bath, the 'Wet potato sludge' material afforded less reducing sugars (Table 2, entry 11) than the 'Potato flour' one (Table 2, entry 10) or the 'Dry potato sludge' one (Table 2, entry 12). Even if the ultrasonic bath is an indirect irradiation method, once again the particles size distributions found for the three starch materials followed the trend of the depolymerisation rate. The total reducing sugars concentration is almost twice higher under indirect ultrasonic irradiation (Table 2, entry 10)

Table 2
Effect of different low frequency system on the depolymerisation rate of starch materials (Potato flour/Wet potato sludge/Dry potato sludge – neutral/H_2SO_4 3 mol L^{-1} – room temperature/60 °C – 2 h – 3 wt%).

Entry	Starting material	Apparatus and frequency (kHz)	pH	Temperature (°C)	% Reducing sugars
1	Potato flour	Bath; 24	Neutral	RT	0.2 ± 0.1
2	Wet potato sludge	Bath; 24	Neutral	RT	–
3	Dry potato sludge	Bath; 24	Neutral	RT	0.2 ± 0.1
4	Potato flour	Bath; 24	Acidic	RT	0.2 ± 0.1
5	Wet potato sludge	Bath; 24	Acidic	RT	0.3 ± 0.1
6	Dry potato sludge	Bath; 24	Acidic	RT	0.3 ± 0.1
7	Potato flour	Bath; 24	Neutral	60	0.3 ± 0.1
8	Wet potato sludge	Bath; 24	Neutral	60	0.3 ± 0.1
9	Dry potato sludge	Bath; 24	Neutral	60	0.2 ± 0.1
10	Potato flour	Bath; 24	Acidic	60	69.8 ± 3.5
11	Wet potato sludge	Bath; 24	Acidic	60	6.1 ± 0.3
12	Dry potato sludge	Bath; 24	Acidic	60	47.9 ± 2.4
13	Potato flour	Pyrex probe; 20	Acidic	60	10.5 ± 0.5
14	Wet potato sludge	Pyrex probe; 20	Acidic	60	0.4 ± 0.1
15	Dry potato sludge	Pyrex probe; 20	Acidic	60	12.7 ± 0.6
16	Potato flour	Pyrex probe; 20	Neutral	60	0.0 ± 0.0
17	Wet potato sludge	Pyrex probe; 20	Neutral	60	0.0 ± 0.0
18	Dry potato sludge	Pyrex probe; 20	Neutral	60	0.0 ± 0.0
19	Potato flour	Sacrifial probe; 20	Acidic	60	100.0 ± 5.0

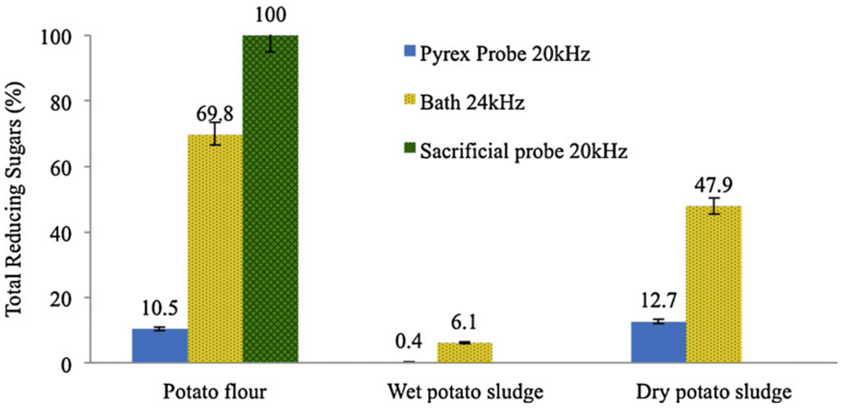

Fig. 5. Comparison of different low frequency ultrasound apparatus for the depolymerisation of 'Potato flour', 'Wet potato sludge' and 'Dry potato sludge' (H_2SO_4 3 mol L^{-1} – 60 °C – 2 h – 3 wt%). Percentage of reducing sugars obtained.

compare to a simple mechanical stirring. Similar results were obtained with direct irradiation by Choi and co-workers (Choi & Kim, 1994). An 81% glucose yield from a 3 wt% pure maize starch was reached with a 25 kHz horn irradiation for 120 min at 100 °C in 0.5 mol L^{-1} sulphuric acid solution. However, our experiments were performed at lower temperature (60 °C) in order to avoid the gelatinisation that occurs beyond 60/65 °C and also to reduce the energy consumption. Temperature is a parameter that has to be considered. While high temperature can decrease the efficiency of the cavitation bubbles – the vapour pressure bubbles penetrate the cavitation bubbles – it was shown that the hydrolysis of sucrose was faster at 75 °C than 50 °C (Kardos & Luche, 2001). The experiments were subsequently performed at higher sulphuric acid concentration. Thus, about 69% of reducing sugars were obtained for the depolymerisation of Potato flour (starch) in the ultrasonic bath for 120 min at 60 °C in 3.0 mol L^{-1} sulphuric acid and up to 100% with the sacrificial 20 kHz probe (identical experimental conditions). Comparable results were obtained with the hydrolysis of standard soluble starch into glucose in an ultrasonic 35 kHz bath (Mecozzi, Acquistucci, Amici, & Cardarilli, 2002). 99.2% and 88.1% of glucose were reached with 120 min of irradiation in CH_3COOH and HCOOH 1 M, respectively. The authors employed non-oxidant acids because of the colorimetric interferences in Dubois analyses. The lowest depolymerisation rate was obtained with the Pyrex ultrasonic probe. A calorimetry was performed on the probe with obtaining an acoustic power of 17 W. A weak acoustic power and a too low temperature may be the explanation of the low depolymerisation rate of reducing sugars. The best result obtained under ultrasonic irradiation as low frequency was reached with the sacrificial metallic probe (Table 2, entry 19). Ultrasound can disrupt and damage the granule of starch at low frequency irradiation, probably helped by the high acoustic power generated (86 W). Jambrak and co-workers revealed that high acoustic power of an ultrasonic 24 kHz probe disrupted the crystalline region of starch granules and reduced the turbidity of a corn starch 10 wt% suspension after irradiation (Jambrak et al., 2010). The decreasing of the turbidity is directly related to the degradation of the starch granule. Reducing sugars are water-soluble whereas starch is non-soluble. Unfortunately, we could not perform the other experiments with the 'Wet potato sludge' and 'Dry potato sludge' materials as the probe was severely damaged after 120 min strong acidic medium. This indicates the potential of low frequency ultrasound to afford reducing sugars but also an obstacle to be overcome by designing effective acid resistant ultrasonic probes.

Afterwards, we explored the effect of the high frequency ultrasonic irradiation on the reaction rate. On the contrary, at such a range of frequency, notably between 200 and 600 kHz, physical effects are suppressed but the sonolysis of water affords $OH^{\bullet}$ which are the main responsible of the degradation of polymers. However the disappearance of physical effects at such a high frequency, avoids the use of strongly heterogeneous solutions, preventing us to use more than 3% solutions. The results obtained are shown in Table 3:

Once again, the best results were obtained under acidic conditions at 60 °C whatever the starting starch-based material. The hydrolysis of the dried matters 'Potato flour' and 'Dry potato sludge' brought the highest conversion into sugars compared to the 'Wet potato sludge' one. In this case, during the experiment, the agglomeration of the potato peels could be easily observed and can be explained by its high particle size distribution (Fig. 1). At such

Table 3
Effect of high frequency system on the depolymerisation rate of starch materials (Potato flour/Wet potato sludge/Dry potato sludge – neutral/H_2SO_4 3 mol L^{-1} – room temperature/60 °C – 2 h – 3 wt%).

Entry	Starting material	Apparatus and frequency (kHz)	pH	Temperature (°C)	% Reducing sugars
1	Potato flour	500	Neutral	RT	0.2 ± 0.1
2	Wet potato sludge	500	Neutral	RT	1.5 ± 0.1
3	Dry potato sludge	500	Neutral	RT	1.7 ± 0.1
4	Potato flour	500	Acidic	RT	0.2 ± 0.1
5	Wet potato sludge	500	Acidic	RT	0.3 ± 0.1
6	Dry potato sludge	500	Acidic	RT	0.3 ± 0.1
7	Potato flour	500	Neutral	60	0.4 ± 0.1
8	Wet potato sludge	500	Neutral	60	1.6 ± 0.1
9	Dry potato sludge	500	Neutral	60	1.3 ± 0.1
10	Potato flour	500	Acidic	60	87.4 ± 4.4
11	Wet potato sludge	500	Acidic	60	12.6 ± 0.6
12	Dry potato sludge	500	Acidic	60	25.3 ± 1.3

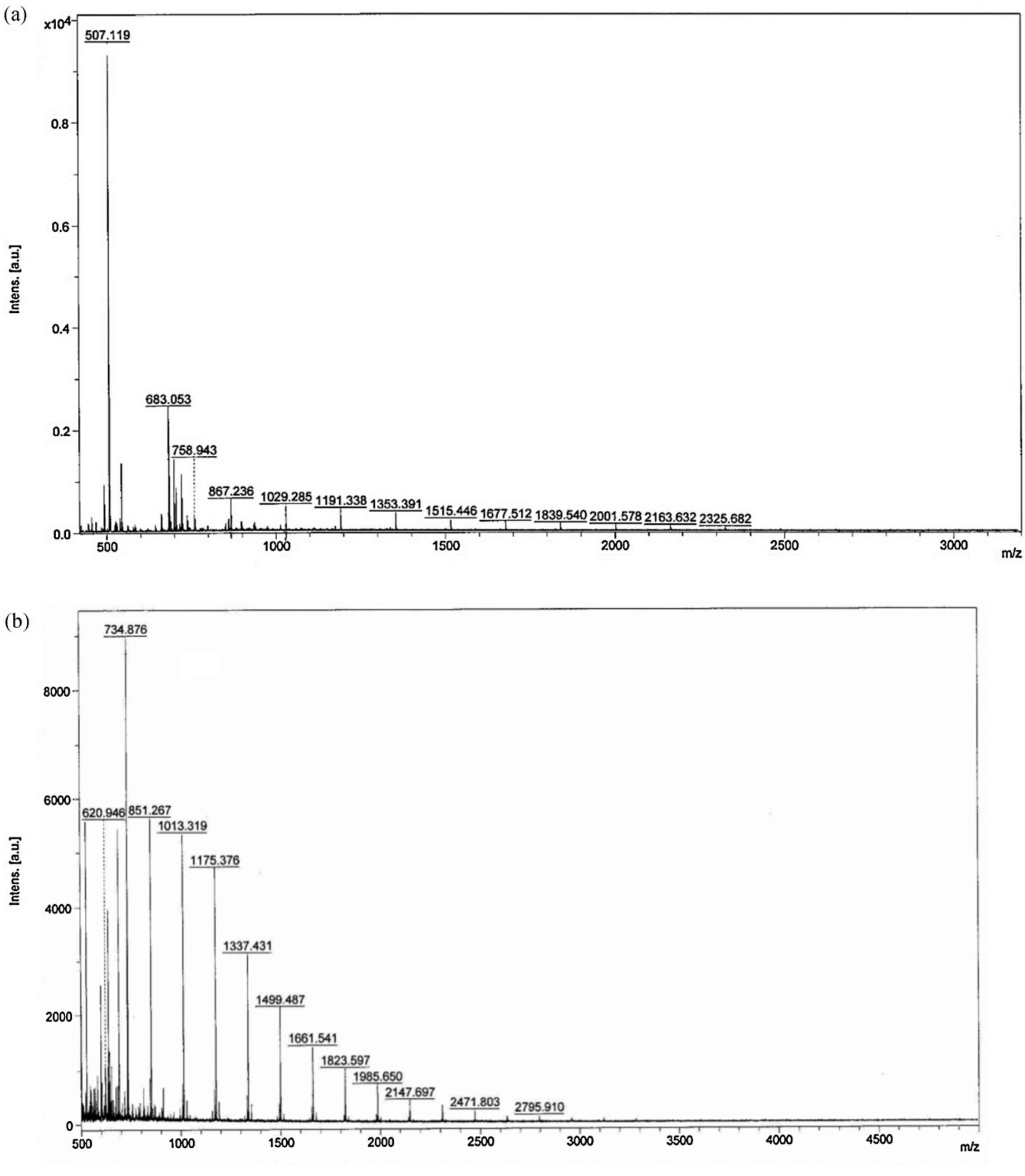

Fig. 6. MALDI-TOF analyses of resulting reducing sugars under (a) 24 kHz and (b) 500 kHz ultrasonic irradiation (Potato flour – H_2SO_4 3 mol L^{-1} – 60 °C – 2 h – 3 wt%).

a high frequency, the cavitation bubbles imploded too rapidly to provoke efficient mixing effects in the medium, preventing a good depolymerisation rate. The highest depolymerisation yield of 87% was reached with the 'Potato flour' material (Table 3, entry 10), which is solely composed of a simple matrix with a single particle size distribution.

The three series of experiments (mechanical stirring, low frequency and high frequency) follow a similar trend with always the lowest conversion yield for the 'Wet potato sludge' material and the highest for the 'Dry potato sludge' and the 'Potato flour' ones. The grinding pre-treatment of the potato peel ('Dry potato sludge') allowed the liberation of the granule of the potato starch and thus permitted accessibility of the α-1,4-glycosidic bonds for the hydrolysis, whereas the high aggregation of particles in the 'Wet potato sludge' material reduced the accessibility of these same bonds for the hydrolysis. However, it cannot be admitted that low and high ultrasonic frequencies can work out through the same effects. Different effects subsequently afforded very similar conversion yields. To clarify this point, MALDI-TOF analyses were performed on the 'Potato flour' material treated under acidic conditions at 60 °C under

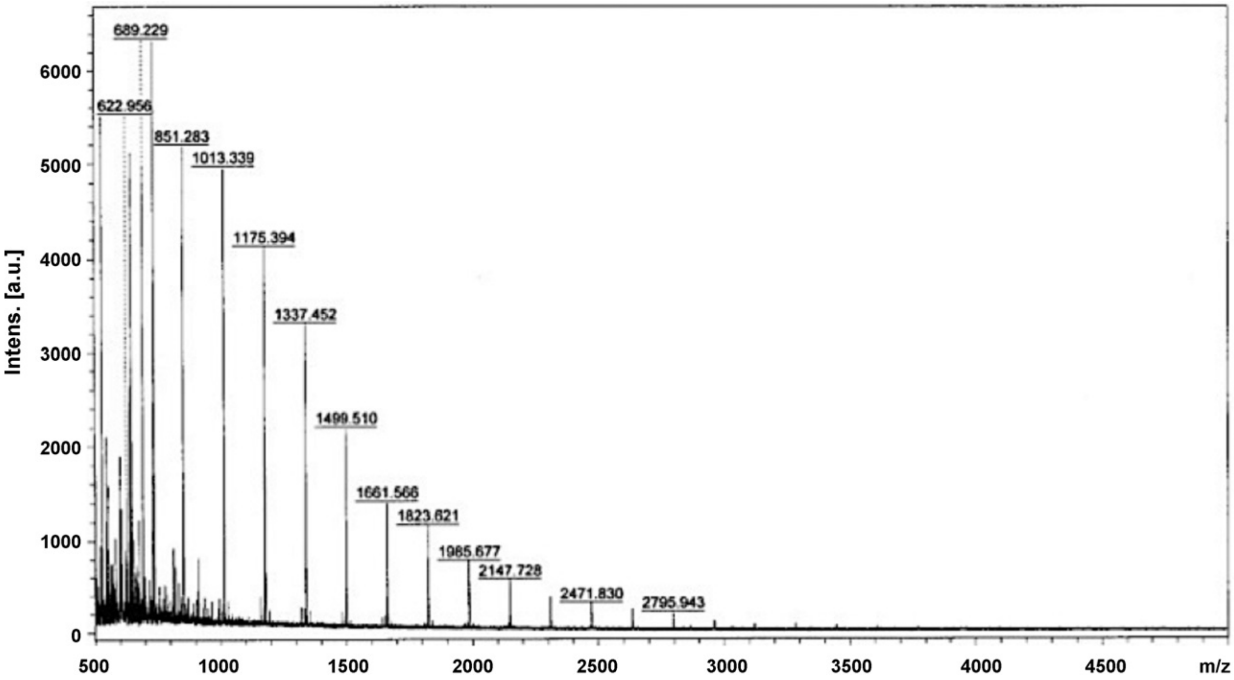

Fig. 7. MALDI-TOF analyses of resulting reducing sugars under microwave irradiation (Potato flour – H_2SO_4 3 mol L^{-1} – 60 °C – 1 h – 3 wt%).

low and under high ultrasonic frequencies to match eventual differences of the reducing sugars composition. The analyses are given in Fig. 6.

The MALDI-TOF analyses revealed some differences in the composition of the resulting reducing sugars issued from the depolymerisation under different processes, mechanical stirring (Fig. 3), low frequency ultrasound and high frequency ultrasound (Fig. 6a and b). Ultrasound has a direct effect on the degradation of starch with different intensities and selectivities between oligosaccharides according to the incident frequency. At 24 kHz (Fig. 6a), a main intensive peak can be observed at 507 g.mol^{-1}, which can represent oligosaccharides with three glycosidic units, whereas, at 500 kHz (Fig. 6b), the main peak at 734 represents oligosaccharides with four glycosidic units. A strong ultrasonic selectivity subsequently occurred under high frequency irradiation probably due to the radical degradation done by OH$^{\bullet}$ radicals produced by the sonolysis of water at 500 kHz. Koda and co-workers noticed already this effect on the depolymerisation of four water-soluble biopolymers (dextran, methyl cellulose, pullulan, and poly ethylene oxide) under 20 and 500 kHz (Koda et al., 2011). The utilisation of a radical scavenger *t*-BuOH at both frequencies showed that high frequency irradiation generates radicals responsible of the degradation of the carbohydrates. At 20 kHz, the degradation rate was slightly reduced, whereas at 500 kHz the radical scavenger completely inhibited the degradation. Finally, under mechanical stirring (Fig. 3), the amount of oligosaccharides with 4–6 glycosidic units is about the same, contrary to ultrasonic irradiation.

3.5. *Microwave irradiation*

Even if some researchers specialised in this field believe in the existence of specific effects, we discarded experiments at room temperature. In addition, previous displayed results have shown that the depolymerisation reaction did not occur or very little in basic or neutral conditions. These experimental conditions were thus discarded. This is confirmed by Khan and co-workers who

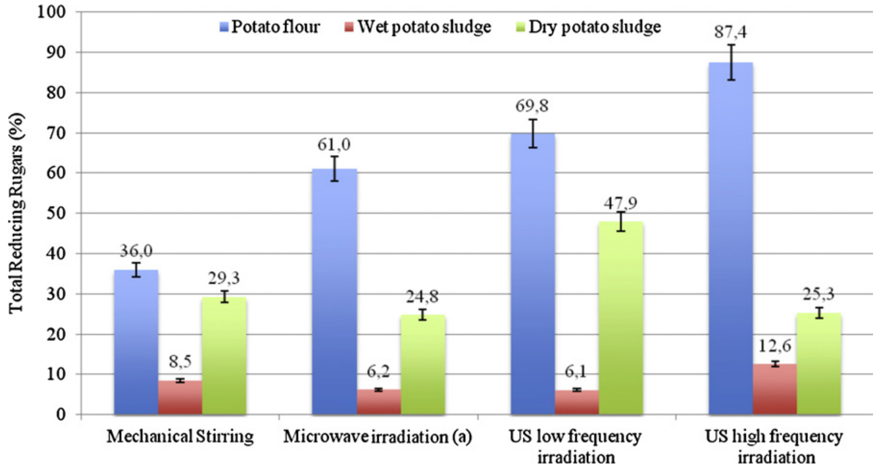

Fig. 8. Total reducing sugars yields of the experiments performed under mechanical stirring, microwave and ultrasound irradiation on 'Potato flour', 'Wet potato sludge' and 'Dry potato sludge' (60 °C, H_2SO_4 3 mol L^{-1}, 1 h (a)/2 h, 3 wt%).

Table 4
Effect of microwave irradiation on the depolymerisation rate of starch materials (Potato flour/Wet potato sludge/Dry potato sludge – H_2SO_4 3 mol L^{-1} – 60 °C – 1 h – 3 wt%).

Entry	Starting material	Time min.	% Reducing sugars
1	Potato flour	60	61.0 ± 3.1
2	Wet potato sludge	60	6.2 ± 0.3
3	Dry potato sludge	60	24.8 ± 1.2

performed irradiations on a 10% starch suspension solution in neutral solution under strong temperature and pressure (191–198 °C and 12.8–14.9 bar) within 20 min and obtained barely 10% of reducing sugars (Khan et al., 1979), whereas 88% was reached in hydrochloric acid (0.014 mol L^{-1}) solution within 5 min in similar conditions (Khan et al., 1980). Microwave was employed as a pre-heating treatment before acid hydrolysis (HCl – 0.45 mol L^{-1}) for the depolymerisation of 20 g L^{-1} of pure amylose (Warrand & Janssen, 2007). Within 10 min of pre-treatment at 20% power of irradiation and 10 min of acid hydrolysis, more than 97% of monosaccharides were achieved, whereas 1 h of pre-treatment at 90 °C and 6 h of acid hydrolysis was necessary to reach the same yield. Microwave heating is known as a powerful tool for the synthesis of mono-di- and oligosaccharides (Corsaro, Chiacchio, Pistara, & Romeo, 2004; Richel, Laurent, Wathelet, Wathelet, & Paquot, 2011).

The results obtained under acidic conditions are shown in Table 4. The highest rate of sugars was again obtained with the ‘Potato flour’ material by reaching 61% of reducing sugars in 60 min. This yield drops to a poor 6% with the ‘Wet potato sludge’ material. It is likely that the intermolecular frictions of the aqueous medium do not allow an efficient mixing, provoking the natural decantation of the heterogeneous substrate even when the microwave oven was fitted with a mechanical stirrer. The lowest conceivable reaction time of 60 min was chosen due to the high-energy consumption generated by the microwave device. In order to compare the composition of the resulting sugars obtained under microwave irradiation, MALDI-TOF analysis of the ‘Potato flour’ material after 60 min of reaction (Table 4, entry 1) was performed and is shown in Fig. 7.

The MALDI-TOF analysis of microwave irradiation and mechanical stirring reveal similar results with no detectable oligosaccharides selectivity, contrary to ultrasound condition. The depolymerisation of the starch-based materials under stirring and microwave conditions is rather due to a thermal effect than any specific one.

4. Conclusions

Fig. 8 summarises all depolymerisation yields obtained under acidic conditions under all used different activation methods. The highest rates were obtained with the ‘Potato flour’, used as the reference, and the lowest ones with the ‘Wet potato sludge’, whatever the activation method. These results confirm our first hypothesis where the complexity of the matrix drove out the depolymerisation rate. The highest depolymerisation rate has been found at high frequency ultrasound, with an 87% in 120 min. Not only the high conversion yield but also the MALDI-TOF analysis confirmed that such a frequency displays promising potential for the depolymerisation of biopolymer. Under low frequency ultrasound, the most powerful the system is, the highest the conversion yield into reducing sugars is. As a typical example, the acoustic power of an ultrasonic bath is not high enough as it is on an indirect mode and the energy released from the collapse of the cavitation bubbles cannot break down the cells of the potato sludge. A direct irradiation brings a higher acoustic power and mixing. However, the strong acidic conditions avoided us to explore further the use of a metallic-type probe, apart from the single experiment ran with a sacrificial metallic horn, while a total depolymerisation of the ‘Potato flour’ was obtained in 120 min thanks to its intensive acoustic power provided. Work is under progress to design a corrosion-protected ultrasonic probe. The high conversion yield of 70% of ‘Potato flour’ under microwave irradiation within 60 min was promising. However, as microwave technology does not bring out any particular or specific mechanical effect, the conversion yields of the ‘Wet potato sludge’ and of the ‘Dry potato sludge’ materials dramatically falls under such a technique.

This exploratory study was performed with a low weight percentage, where an industrial process cannot be considered. Experiments have been performed under low frequency ultrasonic bath on more concentrated starting material. An optimal depolymerisation rate seems to be reached around 13 wt%, however, some complementary works are still under progress.

Acknowledgements

This project is a part of research programme Sustainable Energy (2008–2011) funded by the Academy of Finland. The authors gratefully acknowledge the Région Rhône-Aples with the Programme CIBLE-2011 for the financial support. The authors also would like to thank Jepua Peruna Oy for providing the raw materials and A. Durand-Terrasson (CERMAV-CNRS) for his participation to microscopic analysis. The authors thank Prof. Giancarlo Cravotto, University of Torino (Dipartimento di Scienza e Tecnologia del Farmaco), for the utilisation of the ultrasonic 20 kHz Pyrex® probe. Fortum Foundation is acknowledged for personal grant (A.H.).

References

Anastas, P. T., & Warner, J. C. (1998). *Green chemistry: Theory and practice*. Oxford: Oxford University Press.

Choi, J. H., & Kim, S. B. (1994). Effect of ultrasound on sulfuric acid-catalysed hydrolysis of starch. *Korean Journal of Chemical Engineering*, *11*, 178–184. http://dx.doi.org/10.1007/BF02697463

Corsaro, A., Chiacchio, U., Pistara, V., & Romeo, G. (2004). Microwave-assisted chemistry of carbohydrates. *Current Organic Chemistry*, *8*(6), 511–538. http://dx.doi.org/10.2174/1385272043485828

Czechowska-Biskup, R., Rokita, B., Lotfy, S., Ulanski, P., & Rosiak, J. M. (2005). Degradation of chitosan and starch by 360-kHz ultrasound. *Carbohydrate Polymers*, *60*(2), 175–184. http://dx.doi.org/10.1016/j.carbpol.2004.12.001

Flosdorf, E. W., & Chambers, L. A. (1933). The chemical action of audible sound. *Journal of the American Chemical Society*, *55*(7), 3051–3052. http://dx.doi.org/10.1021/ja01334a509

Gedye, R., Smith, F., Westaway, K., Ali, H., Baldisera, L., Laberge, L., et al. (1986). The use of microwave ovens for rapid organic synthesis. *Tetrahedron Letters*, *27*(3), 279–282. http://dx.doi.org/10.1016/S0040-4039(00)83996-9

Giguere, R. J., Bray, T. L., Duncan, S. M., & Majetich, G. (1986). Application of commercial microwave ovens to organic synthesis. *Tetrahedron Letters*, *27*(41), 4945–4948. http://dx.doi.org/10.1016/S0040-4039(00)85103-5

Jambrak, A. R., Herceg, Z., Subaric, D., Babic, J., Brncic, M., Brncic, S. R., et al. (2010). Ultrasound effect on physical properties of corn starch. *Carbohydrate Polymers*, *79*(1), 91–100. http://dx.doi.org/10.1016/j.carbpol.2009.07.051

Kardos, N., & Luche, J.-L. (2001). Sonochemistry of carbohydrate compounds. *Carbohydrate Research*, *332*(2), 115–131. http://dx.doi.org/10.1016/S0008-6215(01)00081-7

Khan, A. R., Johnson, J. A., & Robinson, R. J. (1979). Degradation of starch polymers by microwave energy. *Cereal Chemistry*, *56*(4), 303–304.

Khan, A. R., Robinson, R. J., & Johnson, J. A. (1980). Starch hydrolysis by acid and microwave energy. *Journal of Food Science*, *45*(5), 1449. http://dx.doi.org/10.1111/j. 1365-2621.1980.tb06581.x

Kimura, T., Sakamoto, T., Leveque, J.-M., Sohmiya, H., Fujita, M., Ikeda, S., et al. (1996). Standardization of ultrasonic power for sonochemical reaction. *Ultrasonics Sonochemistry*, *3*(3), S157–S161. http://dx.doi.org/10.1016/S1350-4177(96)00021-1

Koda, S., Taguchi, K., & Futamura, K. (2011). Effects of frequency and a radical scavenger on ultrasonic degradation of water-soluble polymers. *Ultrasonics Sonochemistry*, *18*(1), 276–281. http://dx.doi.org/10.1016/j.ultsonch.2010.06.007

Kunlan, L., Lixin, X., Jun, L., Jun, P., Guoying, C., & Zuwei, X. (2001). Salt-assisted acid hydrolysis of starch to -glucose under microwave irradiation. *Carbohydrate Research*, *331*(1), 9–12. http://dx.doi.org/10.1016/S0008-6215(00)00311-6

Loupy, A. (2006). *Microwaves in organic synthesis – Second, completely revised and enlarged edition* Weinheim: Wiley-VCH.

Mason, T. J., & Lorimer, J. P. (2002). *Applied sonochemistry: The uses of power ultrasound in chemistry and processing*. Weinheim: Wiley-VCH.

Mecozzi, M., Acquistucci, R., Amici, M., & Cardarilli, D. (2002). Improvement of an ultrasound assisted method for the analysis of total carbohydrate in environmental and food samples. *Ultrasonics Sonochemistry*, *9*(4), 219–223. http://dx.doi.org/10.1016/S1350-4177(01)00125-0

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. http://dx.doi.org/10.1021/ac60147a030

Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science*, *37*(1), 52–68. http://dx.doi.org/10.1016/j.pecs.2010.01.003

Portenlänger, G., & Heusinger, H. (1997). The influence of frequency on the mechanical and radical effects for the ultrasonic degradation of dextranes. *Ultrasonics Sonochemistry*, *4*(2), 127–130. http://dx.doi.org/10.1016/S1350-4177(97)00018-7

Richel, A., Laurent, P., Wathelet, B., Wathelet, J.-P., & Paquot, M. (2011). Microwave-assisted conversion of carbohydrates. State of the art and outlook. *Comptes Rendus Chimie*, *14*(2–3), 224–234. http://dx.doi.org/10.1016/j.crci.2010.04.004

Scheffran, J. (2010). The global demand for biofuels: technologies, markets and policies. In A. A. Vertès, N. Qureshi, H. P. Blaschek, & H. Yukawa (Eds.), *Biomass to Biofuels: Strategies for Global Industries*. Oxford, UK: Blackwell Publishing Ltd. http://dx.doi.org/10.1002/9780470750025.ch2

Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D., & Osborne, J. (2007). A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresource Technology*, *98*(16), 3000–3011. http://dx.doi.org/10.1016/j.biortech.2006.10.022

Warrand, J., & Janssen, H.-G. (2007). Controlled production of oligosaccharides from amylose by acid-hydrolysis under microwave treatment: Comparison with conventional heating. *Carbohydrate Polymers*, *69*(2), 353–362. http://dx.doi.org/10.1016/j.carbpol.2006.10.021

Yu, H.-M., Chen, S.-T., Suree, P., Nuansri, R., & Wang, K.-T. (1996). Effect of microwave irradiation on acid-catalyzed hydrolysis of starch. *The Journal of Organic Chemistry*, *61*(26), 9608–9609. http://dx.doi.org/10.1021/jo961160p