

## Bioethanol production from Jerusalem artichoke by acid hydrolysis

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### Abstract

The hydrolysis of inulin from Jerusalem artichoke (JA) slices by HCl under different regimes of temperature and hold (contact time) time was investigated. Final reducing sugars concentration in the hydrolyzates depended on temperature, pH, hydromodule (JA:water) and hold time. Acid hydrolysis at higher temperature and longer hold time increased the degradation of fructan to fructose and increased the concentrations of inhibitory compounds such as 5-hydroxymethylfurfural (HMF). Acid hydrolysis at 126 °C, hydromodule 1:1 and pH 2, resulted in less than 0.2 g/L HMF at both hold times (30 min and 60 min.). Fermentation of the hydrolyzates obtained by hydrolysis at 126 °C, hydromodule 1:1, pH 2 and hold time 60 min, resulted in the highest ethanol yield of 7.6 % w/w, which corresponds to volumetric productivity of ethanol 1.52 g /Lh and 94.12 % (w/w) of theoretical yield of ethanol.

**Key words:** Bioethanol, Jerusalem artichoke, acid hydrolysis, *S. cerevisiae*

### Introduction

Nowadays, bioethanol produced by alcoholic fermentation is widely used as a renewable biofuel. Main feedstock for ethanol production are sugar cane and corn grain, while many other agricultural raw materials rich in fermentable carbohydrates, or those locally available that could be converted to yield the fermentable sugars, are used worldwide [1]. One major problem with ethanol production is the availability and the price of the raw materials, because feedstock costs account for more than one-third of the final production costs [2]. Feedstock based on corn, sorghum, Jerusalem artichoke, potato and lignocellulosic biomass are of the greatest interest for ethanol production. Jerusalem artichoke (JA) (*Helianthus tuberosus* L.) tubers represent an alternative feedstock for bioethanol production, because ethanol yield is equivalent to that obtained from sugar beets and twofold that of corn [3]. The tubers accumulate high levels of polysaccharides (fructans) during their growth. On a dry weight basis, the tubers contain 68-83% fructans, 15-16% proteins, 13% insoluble fiber and 5% ash [4]. Inulin is a linear polymer of D-fructose joined by  $\beta$  (2→1) linkages and terminated with a D-glucose molecule linked to fructose by an  $\alpha$  (1→2) bond, as in sucrose. When JA is to be used for ethanol fermentation by *Saccharomyces cerevisiae* it is first converted to fermentable sugars by enzymes or acid [5]. Kim and Hamdy [6] concluded that JA slurry should be hydrolyzed in 0.1 M HCl at 97°C and then subjected to alcohol fermentation. It was reported that between pH 1 and 2 the extent of dehydration of fructose varied linearly with time and pH but was minimal at pH 2 [7]. Fructan in *Helianthus tuberosus* can be easily extracted then be hydrolyzed to fructose by acid which is also the first step in dimethylfuran production [8]. Fleming and GrootWassink [4] compared various acids (hydrochloric, sulfuric, citric and phosphoric) for their effectiveness on inulin hydrolysis. The available literature provides little information about the kinetics and efficiency of acid hydrolysis of JA tubers into sugar or about the by-product of hydrolysis such as 5-hydroxymethylfurfural (HMF) which can inhibit the yeast growth and fermentation.

In this paper we established the optimal conditions for complete acid hydrolysis of JA with minimum side reactions and maximum sugar- ethanol production. The main goal of present study was to examine effect of the process conditions such as the temperature, hold time and hydromodule on the JA hydrolysis e.g. the maximum fructose equivalent (FE) and the synthesis of 5-hydroxymethylfurfural (HMF). In addition, the JA hydrolyzates obtained under various experimental conditions of hydrolysis were tested as a substrate for bioethanol production using pure culture of *Saccharomyces cerevisiae*.

## Materials and Methods

*Microorganism:* Pure culture of *Saccharomyces cerevisiae* strains DTN from the collection of the Faculty of Technology, Novi Sad, was used for the ethanol fermentation of Jerusalem artichoke (JA) hydrolyzates. The culture was maintained on a malt agar slant. The agar slant consisted of malt extract (3 g/L), yeast extract (3 g/L), peptone (5 g/L), agar (20 g/L) and distilled water (up to 1 L). Before use as an inoculum for the fermentation, the culture was aerobically propagated in 500 mL flasks in a shaking bath at 30°C for 48 h and then separated by centrifugation. The liquid media consisted of yeast extract (3 g/L), peptone (3.5 g/L),  $\text{KH}_2\text{PO}_4$  (2.0 g/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (1.0 g/L),  $(\text{NH}_4)_2\text{SO}_4$  (1.0 g/L), glucose (10 g/L) and distilled water. The concentration of 2.5 % (w/w) inoculum was used for the fermentation of JA hydrolyzates.

*Raw material, preparation and chemicals.* Jerusalem artichoke used as raw material was provided by the Institute for Crops and Vegetables, Novi Sad (Vojvodina, Serbia). The entire JA tubers were washed and sliced (3 mm thick) before use. The 5-HMF was obtained from Merck (Darmstadt, Germany). HCl was obtained from Pliva-Lachema (Czech Republic) and NaOH from Zorka (Šabac, Serbia).

*Acid hydrolysis and factors involved.* The hydrolysis was performed in 0.5 L flasks in an autoclave. The slices were mixed with water in various weight ratios and acid solution of HCl was poured into the flask, so that the total volume of mixture was 200 mL and pH 2. Then the mixture was heated under various regimes of temperature and hold time in the autoclave. The hydrolyzates were cooled rapidly in ice slush for 5 min, neutralized with 1 M NaOH, and then quantitatively assayed for reducing sugars (as fructose equivalent-FE), HMF and non-hydrolyzed residue (NHR). The level of reducing sugar in the fresh JA tuber prior to acid hydrolysis was also determined. Various temperature levels, 124°C, 126°C, 128°C, 130°C, 132°C, and 134°C at hold time (30 min and 60 min) and hydromodule (JA:water) 1:1 were set and the contents of reducing sugar, HMF, and non-hydrolyzed residue were determined. Also, the hydrolysis was performed at 126 °C and various hydromodules as 1:0.3, 1:0.5 and 1:1 (hold time 30 min and 60 min) and the content of reducing sugar was determined.

*Ethanol fermentation of JA hydrolyzates.* The JA hydrolyzates obtained using various regimes of acid hydrolysis were subjected to ethanol fermentation by *S. cerevisiae* under anaerobic conditions for 72 h. The hydrolyzates were rapidly cooled and adjusted to pH 5.0 by adding 1 M NaOH. Then, hydrolyzates were poured into an Erlenmayer flask (0.5 L), and the yeast inoculum (2.5 % w/w) was added. The flask was then fixed on a rotary shaker (100 rpm) and placed in a thermo stated cabinet at 30°C. During the fermentation, the consumption of the substrate was recorded as well as the formation of ethanol.

*Analytical methods.* Reducing sugars in the fresh JA slices as well as those liberated during the acid hydrolysis were determined by 3,5-dinitrosalicylic acid [9] and the data herein are reported as fructose equivalent (FE). FE (%) was calculated as ratio of reducing sugar of sample measured as fructose mass (g) per sample dry mass weight (g), and multiplied by 100. The fructose and glucose contents were determined by HPLC (Jasco, pump PU-980, detector RI-930, sampler AS-950, 20  $\mu\text{L}$  injector loop, column Sugar KS-801, eluent: water at a flow

rate of 0.6 mL/min and elution time 30 min). The dry mass was determined by the standard drying method in an oven at 105°C to constant mass. Protein content was estimated as the total nitrogen by the Kjeldahl method multiplied by 6.25, and the ash content was determined by slow combustion of the sample at 650°C [10]. The 5-HMF concentration was measured at 285 nm using the HPLC method of Del Campo et al. [11]. The ethanol concentration was determined on the basis of the density of alcohol distillate at 20°C and expressed in weight % (w/w). Non-hydrolyzed residue was determined by filtration of 10 g of hydrolyzate through drying filter paper. Filter cake obtained after the filtration was rinsed with water three times and the content of NHR was determined by standard drying method in an oven at 105°C to constant mass.

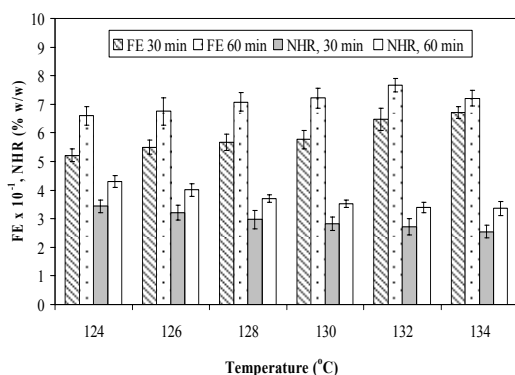
*Statistical analysis.* The data were obtained from experiments conducted in triplicate, and were analyzed for statistical significance by a one-way analysis of variance (ANOVA). A 5% probability level ( $p=0.05$ ) was used to accept or reject the null hypothesis. All statistical analyses were performed with the software Microsoft Office Excel 2003 for Windows.

## Results and discussion

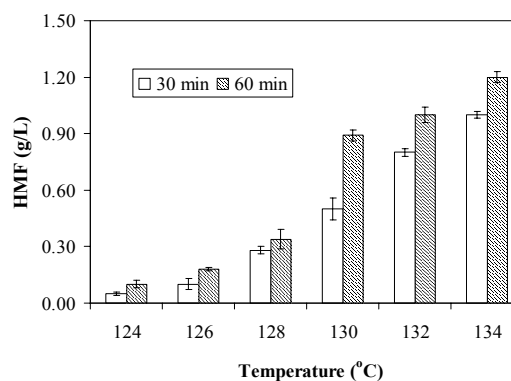
The content of the main components of Jerusalem artichoke was the following % (w/w): 15.6 reducing sugars, 11.8 fructose, 3.8 glucose, 20.75 dry mass, 10.01 protein, 1.8 ash. JA contains high amount of sugars, mainly fructose. The sugars are easy for hydrolysis under acidic conditions and conversion into fuel ethanol. These results are in accordance with the typical composition of JA reported by Kisgeci et al. [12].

### *Effect of temperature and hydromodule on the acid hydrolysis*

The effect of acid on the hydrolysis of JA slices at various reaction temperatures and hold time on the FE values of hydrolyzates and non-hydrolyzed residues is summarized in Figure 1.



**Figure 1.** Effect of temperature and hold times on FE values and non-hydrolyzed residue of hydrolyzates obtained at pH 2; hold time 30 min and 60 min; hydromodule 1:1.



**Figure 2.** Concentration of HMF in hydrolyzates obtained at various temperatures and hold time of acid hydrolysis at pH 2; hold time 30 and 60 min; hydromodule 1:1.

A maximum FE value of 76.67 % (w/w) was reached upon heating (at 132°C) the slices of JA (hydromodule 1:1) for 60 min at pH 2 with the addition of HCl. Minimal NHR value of 3.35 % (w/w) was produced at 134°C. The increase in temperature resulted in marked effect on the hydrolysis of JA slices as evidenced by the reducing sugar content of samples heated for two hold times. Increasing the temperature from 124°C to 132°C increased significantly ( $p<0.05$ ) the FE values. However, when the temperature was further increased to 134°C, a considerable ( $p<0.05$ ) decline in the FE values was observed. This is in agreement

with the conclusions reported by Kim and Hamdy [6], who postulated that the reducing sugar, reported as fructose equivalent, is very heat sensitive in acidic conditions and easily destroyed at 97°C. After 40 min, 59.5 FE and 10.2 % HMF (based on dry weight) were detected in the 8 % JA slurry. Further heating after the maximum FE value had been reached, at each reaction temperature, resulted in a decline in the reducing sugar content which was indicative of faster d-fructose decomposition through recombination and dehydration than its formation as the result of acid catalysis. In this work, similar and rather high conversions could be achieved with lower temperatures of acid hydrolysis in a longer period of hold time (FE was 65.98% w/w, NHR was 4.3 % w/w at 124°C and FE was 66.5% w/w, NHR was 4.01 w/w at 126°C, hold time of 60 min). However, in our opinion, acid hydrolysis should be performed at low temperature and our recommendation is to apply temperature of 126°C. This is justified from economical point of view since temperature of hydrolysis is important to improve the energy balance. Based on the results, all further experiments were conducted at 126°C. In all experiments within 30 min more than 80% of the total conversion performed within 60 min was attained.

Dilute acid treatment, in particular, may also cause formation of furfural and 5-hydroxymethylfurfural (HMF) from the dehydration of released sugars. These side-products are a concern because they act as microbial inhibitors and negatively affect fermentation of sugars [13].

The effect of various temperatures on the HMF content of samples was also determined and the results are shown in Figure 2. HMF concentration increased with increasing temperature levels. The highest ( $p < 0.05$ ) concentration of HMF was detected at 134°C, after 60 min hold time (1.05 g/L). Acid hydrolysis at 124°C and 126°C, respectively, resulted in less than 0.2 g/L HMF at both hold times. The concentrations of HMF ranged from 0.05 g/L to 1.2 g/L which is lower than the concentration considered to inhibit fermentation [14]. Kim and Hamdy [6] observed that the concentration of HMF increased significantly at temperatures above 100°C, reaching the critical value that inhibited the alcoholic fermentation.

*Ethanol fermentation of the JA hydrolyzates obtained at various temperatures of acid hydrolysis.*

Taking into the consideration the aforementioned results and our opinion on the importance of conducting the hydrolysis process at low temperature because considerable energy savings, fermentation of the hydrolyzates was conducted at various temperatures and two hold times (30 min and 60 min). The results are presented in Table 1.

The results showed that the content of reducing sugars in the hydrolyzates increased with increasing process temperature and hold time until 132°C was reached, after which a decrease in this parameter was observed. This is in agreement with the results presented in Figure 1. In contrast to that, the highest ethanol yield (7.6% w/w) was achieved at 126°C during 60 min period. This has proved that the choice of optimal parameters for acid hydrolysis (126°C, 60 min, pH 2) was right. The fermentation of hydrolyzates at temperatures higher than 126°C showed a significant ( $p < 0.05$ ) reduction in maximum ethanol concentrations, maximum values of theoretical yield of ethanol, product yield on substrate ( $Y_{P/S}$ ) and the volumetric productivities of ethanol (P). This can be further confirmed by the changes of caramel intermediate (HMF and furfural). Furfural and HMF can be degraded further to formic and levulinic acid [15]. The inhibitory effects of furfural and HMF are synergistic in combination with other compounds [16]. Inhibitor compounds present in the hydrolyzate showed a synergistic effect on toxicity with respect ethanol and biomass yield [17]. Degradation compounds present in the JA hydrolyzates can inhibit the yeast or even enzymes [18]. This higher inhibitory effect observed when using thermo-tolerant yeast could also be due to the effect caused by high temperatures on cell membrane [19].

**Table 1.** Results of the batch fermentation of hydrolyzates obtained at various temperatures and hold time of acid hydrolysis

Temperature (°C)	Reducing sugar <sup>a</sup>		Maximum of ethanol <sup>b</sup>		% (w/w) of theoretical yield of ethanol		Y <sub>PS</sub>		P	
	(‰ w/w)		(‰ w/w)				(g/g)		(g/L·h)	
	Hold time (min)									
	30	60	30	60	30	60	30	60	30	60
124.00	11.82 ± 0.20	14.78 ± 0.12	5.76 ± 0.06	7.30 ± 0.15	72.55 ± 2.35	88.24 ± 2.16	0.37 ± 0.012	0.45 ± 0.011	0.80 ± 0.02	1.01 ± 0.03
126.00	12.58 ± 0.12	15.48 ± 0.11	6.01 ± 0.26	7.60 ± 0.12	77.17 ± 1.57	94.12 ± 2.55	0.39 ± 0.008	0.48 ± 0.013	0.87 ± 0.01	1.52 ± 0.02
128.00	12.81 ± 0.18	15.94 ± 0.13	5.60 ± 0.08	7.00 ± 0.09	68.63 ± 2.94	86.27 ± 1.57	0.35 ± 0.015	0.44 ± 0.008	0.78 ± 0.06	0.97 ± 0.03
130.00	13.01 ± 0.16	16.24 ± 0.15	4.88 ± 0.14	6.10 ± 0.11	58.82 ± 4.51	72.55 ± 1.76	0.30 ± 0.023	0.37 ± 0.009	0.67 ± 0.03	0.85 ± 0.05
132.00	13.89 ± 0.19	17.39 ± 0.20	4.58 ± 0.08	5.80 ± 0.08	50.98 ± 3.14	64.71 ± 3.14	0.26 ± 0.016	0.33 ± 0.016	0.64 ± 0.02	0.80 ± 0.04
134.00	13.00 ± 0.20	16.80 ± 0.20	4.43 ± 0.20	5.60 ± 0.11	47.06 ± 2.55	60.78 ± 1.57	0.24 ± 0.013	0.31 ± 0.008	0.61 ± 0.01	0.78 ± 0.02

<sup>a</sup> Acid treatment: pH 2, hydromodule 1 : 1

<sup>b</sup> Ethanol fermentation: 30°C, 72 h, 2.5 % (w/w) of *S.cerevisiae*

**Table 2.** Results of the batch fermentation of JA hydrolyzates obtained at various hydromodule and hold time of acid hydrolysis

Hydromodule (JA : water)	Reducing sugar <sup>a</sup>		Nonhydrolyzed residue		Maximum of ethanol <sup>b</sup>		% (w/w) of theoretical yield of ethanol		Y <sub>PS</sub>		P	
	(‰ w/w)		(‰ w/w)		(‰ w/w)				(g/g)		(g/L·h)	
	Hold time (min)											
	30	60	30	60	30	60	30	60	30	60	30	60
1 : 0.3	10.67 ± 0.09	13.33 ± 0.22	9.62 ± 0.11	13.22 ± 0.22	1.84 ± 0.17	2.30 ± 0.14	27.45 ± 2.35	33.33 ± 1.76	0.14 ± 0.012	0.17 ± 0.009	0.26 ± 0.02	0.32 ± 0.03
1 : 0.5	11.11 ± 0.15	13.89 ± 0.18	6.61 ± 0.20	8.26 ± 0.18	4.12 ± 0.12	5.20 ± 0.09	58.82 ± 3.92	72.55 ± 3.14	0.30 ± 0.020	0.37 ± 0.016	0.58 ± 0.04	0.72 ± 0.04
1 : 1.0	12.58 ± 0.12	15.48 ± 0.23	3.82 ± 0.15	4.07 ± 0.13	6.10 ± 0.26	7.60 ± 0.12	77.17 ± 1.57	94.12 ± 2.55	0.39 ± 0.008	0.48 ± 0.013	0.87 ± 0.01	1.52 ± 0.02

<sup>a</sup> Acid treatment: pH 2, temperature 126°C

<sup>b</sup> Ethanol fermentation: 30°C, 72 h, 2.5 % (w/w) of *S.cerevisia*

*Ethanol fermentation of JA hydrolyzates obtained at various hydromodules*

Further objective was to determine the parameters of fermentation of the JA hydrolyzates obtained at various hydromodules. Pretreatment at high solid content can reduce the energy input for heating the water which can increase the economic feasibility of the biorefinery process [20]. For this purpose, mixtures of various amounts of JA slices and water which corresponded to hydromodule were prepared and hydrolyzed by the treatment with acid (pH 2; hold time 30 and 60 min; temperature 126°C). After that the temperature was decreased to 32°C and the hydrolyzate was inoculated with 2.5% (w/w) of *S. cerevisiae* and subjected to ethanol fermentation. The results are presented in Table 2.

The hydromodule had a pronounced effect on both the acid hydrolysis and the ethanol fermentation. Regarding the initial yield, higher hydromodule (which corresponded to lower substrate concentrations) is more suitable since substrate inhibition could be thus avoided. Similar substrate inhibition was also noticed for ethanol fermentation [21]. Thus, low performance from fermentation with hydrolyzates obtained at lower hydromodule probably is due to accumulation of inhibitory by-products from the acid treatment. According to the results presented in Table 3, the maximum ethanol concentration and maximum value of product yield on substrate ( $Y_{P/S}$ ) were achieved for the hydromodule 1:1. For an economically feasible process, ethanol yield need to be as high as possible which can decrease the energy input of the following distillation, which is considerable in the economical evaluation of the overall process [22]. In addition, the use of higher initial substrate concentrations is economically more favorable, since it could decrease the reactor volumes [23]. Taking into account the ethanol concentrations achieved, the yield of products per substrate ( $Y_{P/S}$ ) and the volumetric productivities of ethanol (P) presented in Table 2, and related economic points, we can suggest utilization of hydromodule 1:1.

## Conclusions

The hydrolysis of JA slices by mineral acid was studied. Acid hydrolysis at higher temperature and longer hold time increased the degradation of fructan from JA to fructose and increased the concentrations of inhibitory compounds such as HMF. Acid hydrolysis at 126°C and hold time 30 min and 60 min, resulted in less than 0.2 g/L HMF. Fructose equivalent of 67.55 % w/w was achieved at 126°C, pH 2 and hold time of 60 min. From the industrial point of view, it is very important to underline that, under the optimal hydrolysis conditions; the final 5-HMF level should be much lower than the limit for yeast growth inhibition. The fermentation of the obtained hydrolyzates resulted in the highest ethanol yield (7.6 % w/w) produced in the case of hydrolyzates prepared at 126°C, hydromodule 1:1 and pH 2, when compared to the hydrolyzates obtained under the other conditions. In view of present hydrolysis and fermentation results, considerable energy savings could be attained since the overall process was effectively performed with hydrolyzates obtained at 126°C, hydromodule 1:1 and pH 2 of hydrolysis. These results confirmed that this agriculture product can be successfully processed by acid hydrolysis under relatively slight reaction conditions for the use in bioethanol production. The techno-economic analysis is required to get an objective comparison of two catalytic processes, e.g. acid and enzymatic hydrolysis into degradation of JA for ethanol production. Mineral acid can be considered as a respectable catalyst having some advantages over enzymes. Therefore, acid hydrolysis should not be neglected as an alternative saccharification process.



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