Academia Journal of Environmetal Science 6(12): 310-316, December 2018
DOI: 10.15413/ajes.2018.0147
ISSN: ISSN 2315-778X

Academia Publishing

Research Paper

Melon husks and seeds as potential energy source

Accepted 29th October, 2018

M. L. Montoro*, A. Mamaní, M. L. Herrero, M. F. Sardella and A. C. Deiana

Instituto de Ingeniería Química, Facultad de Ingeniería,
Universidad Nacional de San Juan, Avenida Libertador 1109 oeste-C.P.5400 San Juan – Argentina.

*Corresponding author. E-mail: mmontoro@unsj.edu.ar. Tel: +54 2644211700 int.458 subint.38.

ABSTRACT

In the challenge of fossil fuel substitution, lignocellulosic biomass is considered the feedstock of the future, due to its economic and environmental advantages in comparison with bioethanol from starch or sugar. However, physical and chemical barriers caused by the close association of the main components of lignocellulosic biomass hinder the hydrolysis of cellulose and hemicellulose to fermentable sugars. For this reason, biomass must be pretreated to expose the cellulose of the vegetable fibers. These processes intend to eliminate lignin and hemicellulose, decrease cellulose crystallinity and increase the porosity of lignocellulosic materials. Melon is one of the main horticultural crops of San Juan Province, Argentina. From its industrialization, hundreds of tons of melon husk are discarded, generating environmental problems. Since this material has potential as second generation biofuel feedstock, a study for the enhancement of sugars bioavailability for ethanol production is proposed. An acid pretreatment with sulfuric acid, followed by enzymatic hydrolysis and fermentation was applied. The most influential variables were determined and the optimal route to use this residue as energy source is proposed.

Key words: Melon, husk, bioethanol, pretreatment, acid.

INTRODUCTION

The worldwide interest in the use of different biomass as a renewable source of clean biofuels, green chemicals and power generation is gaining momentum (Nyakuma, 2015). The need to expand the energy matrix has led to the diversification of raw materials for the production of biofuels. Countries such as Brazil and the United States are pioneers in the research and implementation of bioethanol, the latter being the largest producer of this type of biofuel (Mussatto et al., 2010; Staff, 2008; Ajanovic, 2011).

Fifty percent of the fruit industrially processed is discarded, generating important environmental problems (Orozco et al., 2014). Fruit residues, composed by husks, seeds and pulp, constitute a useful source of sugars, being potential raw materials for second-generation ethanol (Sánchez Orozco et al., 2012), This application represents an alternative to add value to these wastes, in addition to contributing to the environment balance (Sarkar et al., 2012).

The production of ethanol from lignocellulosic feedstocks involves three basic steps (Bañuelos et al., 2018): pretreatment (physical, chemical, thermal, combination of some of them), enzymatic hydrolysis and fermentation. Pretreatment step aims to reduce the crystallinity of cellulose and improve the access of enzymes by reducing the loss carbohydrates. The choice of this first stage is a key step in these processes and mainly depends on the characteristics of the raw material (Zhang et al., 2016). During the enzymatic hydrolysis, complex carbohydrates are degraded to monomeric sugars (Gupta and Verma, 2015), which are later converted to ethanol during fermentation with yeast *Sacharomyces cerevisiae* or bacteria *Zymomonas mobilis*. Currently melon consumption presents a growing demand especially in countries such as United States and Europe, where in addition to its marketing as a whole fruit, another alternatives such as fresh processed product (Aguayo et al., 2001), jams and juice concentrates are commercialized. These alternatives of industrialization of melon generate significant quantities

of waste, such as shells and seeds, which can be used for the generation of valuable by-products, such as bioethanol.

This study presents the results of studies carried out to evaluate the behavior of melon husk and seeds pretreated with acid as bioethanol feedstock. Acid pretreatment of these lignocellulosic fractions of melon was implemented to increase fermentable sugars content. These studies are complementary to those made previously with the whole fruit (pulp, peel and seeds) in order to assess the impact of these fractions on the performance of reducing sugars and ethanol. The methodology involves treatment of husks and seeds with sulphuric acid, including validation of the optimal conditions predicted by an experimental design, followed by enzymatic hydrolysis and subsequent fermentation for the production of bioethanol. These studies have the purpose of finding alternatives for the valorization of discarded production of melon, which has a markedly negative impact on regional economies.

MATERIALS AND METHODS

Husks and seeds used for this study were from melons (*Cucumis melo*) belonging to Honey Dew variety, from San Juan province. The fresh fruit was washed with 10% v/v sodium hypochlorite. Pulp, seeds and peel were separated using a knife. After separation, husks and seeds were grinded in a blender model LAR2220CC2 Metvisa^{MR} of 2 L capacity. This soft paste, with a high humidity content, was stored at a temperature below - 15°C for later use in experimental trials. In all cases, the reagents used during pretreatment, hydrolysis and analytical analysis were of analytical grade.

Ground melon husks and seeds were subjected to a treatment with dilute sulfuric acid, applying an experimental design. Optimal conditions were experimentally validated, and the samples were submitted to hydrolysis tests and subsequent fermentation to obtain ethanol.

Pretreatment with dilute sulfuric acid

Melon husks and seeds were treated in a thermostated reactor with mechanical stirring with sulfuric acid solutions in concentrations ranging from 0.5 to 2% w/w and solid to liquid ratios between 8:1 and 11:1 (w/v). Temperature was kept constant at 55°C (± 3°C).

Experimental design

For this study, a single block 2^3 factorial design with 2 central points was chosen. Software Stagraphics Centurion 16.1.15, was used for data handling. The factors studied were acid concentration, time and liquid / solid ratio, while the response variable selected to evaluate the effect of acid treatment was total sugars content.

Table 1: Factors and levels adopted of acid pretreatment study.

Variable	Levels		
	-	+	Central point
H_2SO_4 conc. (% w/w)	0.5	2.00	1.25
Time (min)	30	90	60
Liquid/Solid ratio (ml/g)	8:1	11:1	9.5:1

Table 1 shows the minimum and maximum level adopted for the factors involved in the pretreatment, as well as the central points adopted. This ranges were adopted from previous screening assays.

Enzymatic hydrolysis

The material treated at the optimal operating conditions was hydrolyzed with cellulase from *Trichoderma reesei* ATCC26921 (SigmaAldrich) and hemicellulase from *Aspergillus niger* (SigmaAldrich), both added with a load of 20 units per gram of substrate (dry basis), maintaining a solid: liquid ratio of 0.11 g/l. This enzymatic load was adopted from previous assays.

In all samples, pH was adjusted to 4.8 after adding NaOH 1 N. The experiments were conducted in 500 ml Erlenmeyer flasks at 45°C in an orbital shaker with temperature control (Lab.CompanionSI600) for 24 h. Reducing sugars content was determined at the beginning and at the end of the hydrolysis step. To evaluate the effectiveness of this stage, the content of reducing sugars was determined at the beginning and at the end of hydrolysis.

Fermentation

Preparation of inoculum

The yeast used was *S. cerevisiae* PM-16, obtained from the collection of autochthonous microorganisms, Biotechnology Institute (IBT), Engineering Faculty – National University of San Juan. Yeast strain was reactivated in liquid YEPD (10 g/L Yeast Extract; 10 g/L Peptone; 10 g/L Dextrose) and the pH adjusted to 4.5 using HCl 1N.

Inoculum for fermentations was prepared in 125 ml Erlenmeyer flasks, with liquid sterile YPD and incubated in a temperature-controlled orbital shaker (Lab.Companion SI-600) at 25°C for 24 h. Yeast adaptation was carried out in 25 ml of sterile melon juice at 25°C, 100 rpm for 24 h.

Fermentation

For fermentation studies, 50 ml of pretreated and enzymatic hydrolyzed melon husks and seeds were placed

in 50 ml Erlenmeyer flasks and autoclaved at 121ºC and 15psi for 20 min. Once cold, they were inoculated with a biomass concentration of $1.64.10^8$ cells/ml and incubated at 27°C for 48 h. The samples were taken at 0, 6, 12, 24 and 48 h to determine yeast cell growth, ethanol productivity and residual sugar content.

To reduce the possibility of contamination, the process was reproduced in a number of Erlenmeyer flasks equal to the number of samples to be taken. Each flask was used as a sample, and residual sugar content, pH and ethanol were determined. Assays were done by triplicate. Yeast cell growth was calculated from cell count using Neubauer Chamber. The ethanol concentration was determined by FTIR Analyzer Alpha Bruker. The fermentation efficiency was calculated using the following formula (Arumugam and Manikandan, 2011):

$$Efficiency(\%) = \frac{Ethanol\ yield\ obtained}{Theorical\ maximun\ ethanol\ yield\ from\ sugar} \times 100$$

Analytical methods

Lignin content, acid detergent fiber and neutral detergent fiber were determined on an automatic FiberAnalyzer, ANKOM A2000. The difference between these values established the proportion of hemicellulose, while cellulose concentration was obtained by difference between acid detergent fiber and lignin content. Humidity content was determined according to the AOAC method (925.45:1990, Association of Official Analytical Chemists AOAC (1990) Official Methods of Analysis. 15th Edition).

The dry base proportion of ash, volatile matter and fix carbon were determined by thermogravimetric analysis in a Shimadzu DTG-60/60H equipment, with a reactive gas flow of 100 ml/min and 40.174 mg of dry sample. The heating program had a constant speed of 10°C/min, for 60 min to evaporate the humidity taken from the environment, continued with a heating ramp at 50°C/min up to 110°C and subsequently at 90°C/min up to 950°C.

Total sugars were determined using the Dubois method (DuBois et al., 1956), while the quantification of reducing sugars was conducted by applying the DNS method (Miller 1959). Hydroxymethylfurfural determination was made following the 980.23:2012 AOAC method (Association of Official Analytical Chemists, AOAC (1990) Official Methods of Analysis. 15th Edition), Alcohol content was determined using an FTIR Analyzer Alpha Bruker, from the National Viticulture Institute (INV-San Juan).

RESULTS AND DISCUSSION

Melon characterization

The results of the melon characterization are shown in Table 2. The high percentage of water present in these fruits leads to increase cares for their conservation since they are highly vulnerable to degradation by micro-organisms and, consequently, chemical changes (Celestino, 2010). Cellulose values are comparable to citrus fruits such as orange, while the contents of lignin and hemicellulose present greater discrepancies.

Table 2: Proximate analysis of melon husks and seeds.

Property	Value
Humidity (%w/w)	89.42
Ash (%w/w)*	9.58
Volatile matter (%w/w)*	63.14
Fix carbon(%w/w)*	24.20
Lignin (%w/w)*	7.32
Cellulose (%w/w)*	13.98
Hemicellulose (%w/w)*	3.04
Total Sugars (%mg/g)*	502.11
pH	5.55

* Dry basis.

Pretreatments

The conditions of the factors studied and the effects of their interactions on total sugars content were determined using a single block 2^3 factorial design. The responses at various pretreatment conditions are presented in Table 3. Determinations were performed by triplicate, informing the average value. Total sugars contents were expressed in mg/g dry material.

The results of the analysis of variance (ANOVA) applied to the experimental design is shown in Table 4. The low p-value (lower than 0.05) obtained for the liquid:solid ratio and for the interaction between acid concentration and liquid:solid ratio indicates the significant influence they have on the process. In terms of the lack of fit test, their disagreement indicates that the selected model successfully describes the observed data at a 95% confidence level.

Figure 1 shows the surface in response to acid treatment. During the comparison tests, the same effect of the interaction between acid concentration and liquid:solid ratio was easily detected. It can be observed that total sugars content reaches its highest value (328.55 mg/g dry) when working with high L:S ratio (11 ml/g) and low-acid concentration (0.5%), while when increasing only the H_2SO_4 concentration, the response variable decreases to 274.03mg/gdry matter. For trials 7 and 8, carried out for 90 min and acid concentration of 2%, the response variable reduced from 325.18 to 235.04 mg/g dry matter when L:S ratio changed from 8 to 11 ml/g. The effect of this interaction is shown in Figure 2.

The influence of the time on total sugars content increase was analysed by comparing trials 3 and 4. The results were very similar, indicating nonsignificant for acid pretreatment

Table 3: Assays conditions and response variable values for the pretreatment.

Assay	Time (min)	L:S ratio (ml/g)	Acid concentration (%)	Total sugars (mg/g)	Total sugar predicted
1	30	8	0.5	114.91	100.53
2	60	9.5	1.25	287.75	246.30
3	30	11	0,5	328.55	350.33
4	90	11	0,5	320.69	307.79
5	30	11	2	274.70	260.32
6	60	9.5	1.25	240.37	246.30
7	90	8	2	325.18	312.28
8	90	11	2	235.04	258.31
9	30	8	2	222.33	244.11
10	90	8	0.5	104.93	128.19

Table 4: ANOVA for pretreatment with sulfuric acid.

Source	Sum of squares	Degrees of freedom	Middle square	F-Reason	p-value
A:acid	4946.97	1	4946.97	3.28	0.17
B:LS	16491.60	1	16491.60	10.92	0.04
C:Time	330.47	1	330.47	0.22	0.67
AB	27278.90	1	27278.90	18.06	0.02
AC	820.93	1	820.935	0.54	0.51
BC	2463.60	1	2463.60	1.63	0.29
Lack of fit	3408.25	2	1704.12	1.52	0.490
Pure error	1122.48	1	1122.48		
Total (corr.)	59022.900	9			

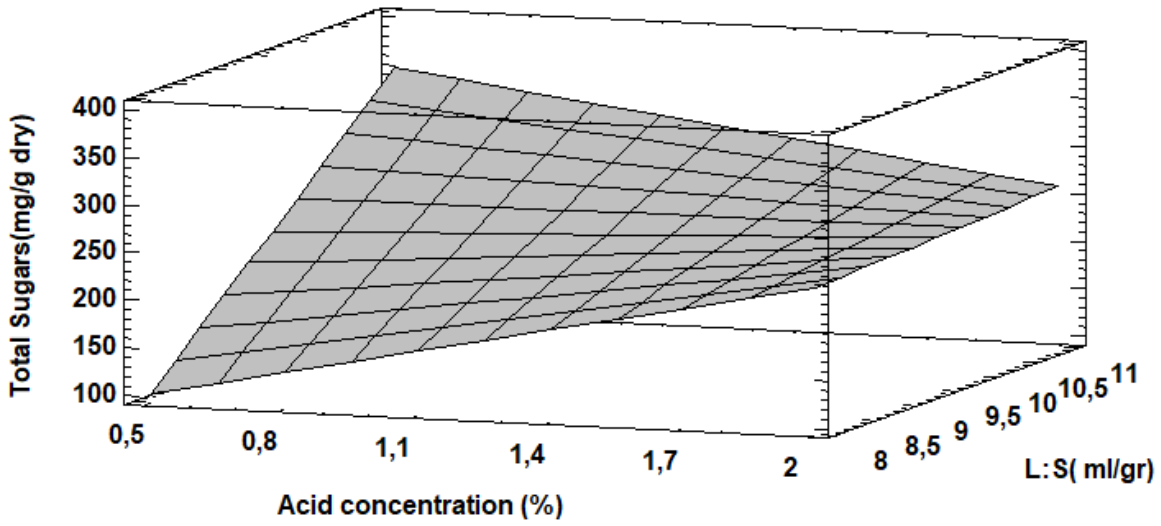

Figure 1: Total sugar yields surface as a function of acid concentration and L:S ratio at 55°C and 30 min.

under the experimental conditions evaluated in this study. These results are graphically displayed by the Pareto diagram (Figure 3).

Based on the experimental results, the model that fits the data with R^2 =92.32% (R^2_{ajus} 76.97%) is given by Equation 1:

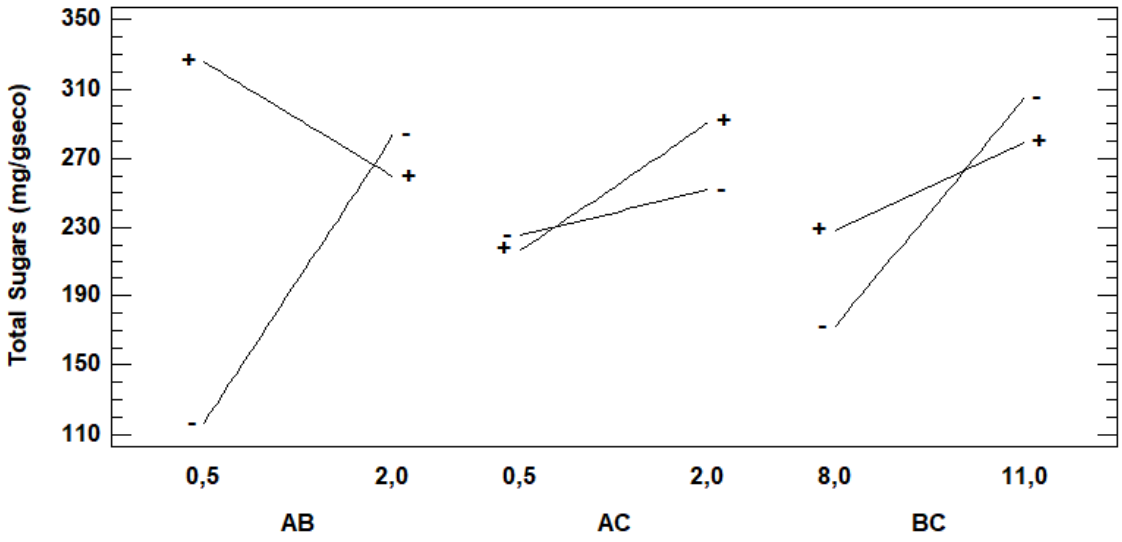

Figure 2: Interaction for significant variables.

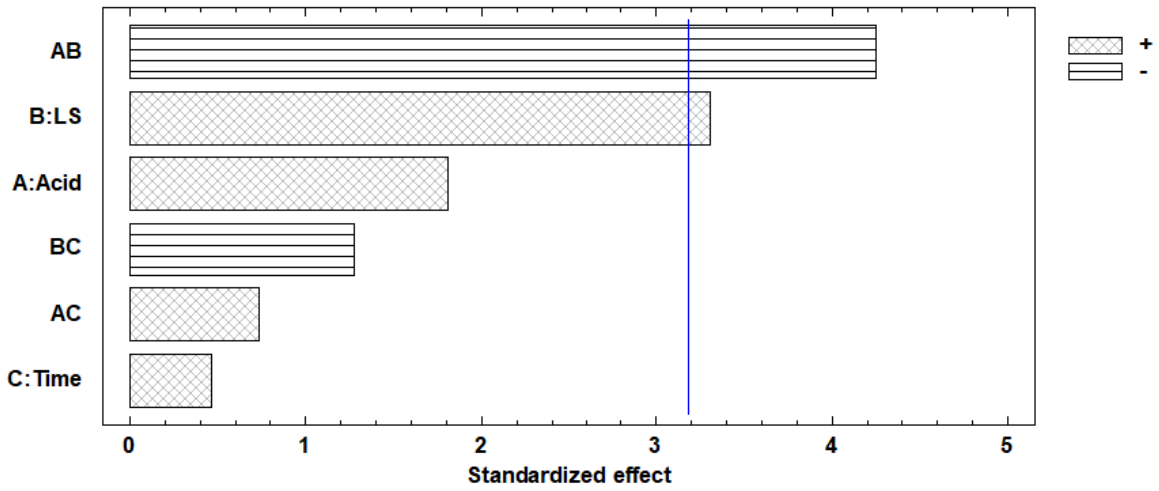

Figure 3: Pareto diagram for acid treatment.

$$Y = -921.728 + 497.454 * A + 120{,}916 * B + 3.355 * C - 51.906 * A * B + 0{,}4502 * A * C - 0.989 * B * C \quad (1)$$

Where Y_a is the total sugars content in mg/g dry matter, A the acid concentration (% w/w), B the L: S ratio(ml/gr) and C time (minutes). The optimal concentration of total sugars predicted by Equation 1 is 350.28 mg/g dry, when pretreatment is performed for 30 min with an acid concentration of 0.5% and a L:S ratio of 11 ml/g. These conditions were validated by duplicate, taking the average of the response variable values. Validation tests samples had an average total sugars content of 345.5 mg/g dry matter, which presents a tiny discrepancy regarding the optimal predicted.

Determination of inhibitory compounds: Components such as hydroxymethylfurfural (HMF) can be produced as a result of the acid treatment applied to the biomass and can inhibit the fermentation stage or potentially decrease ethanol yields as a consequence of a lower rate of fermentation (Modig, 2002; Boucher et al., 2015), requiring a detoxification period for the material. According to Lee and Jeffries (2011), values equal or greater than 5 g/l are harmful to *S. cerevisiae* yeasts.

For this reason, a sample obtained under optimal pre-treatment conditions was analyzed in order to determine

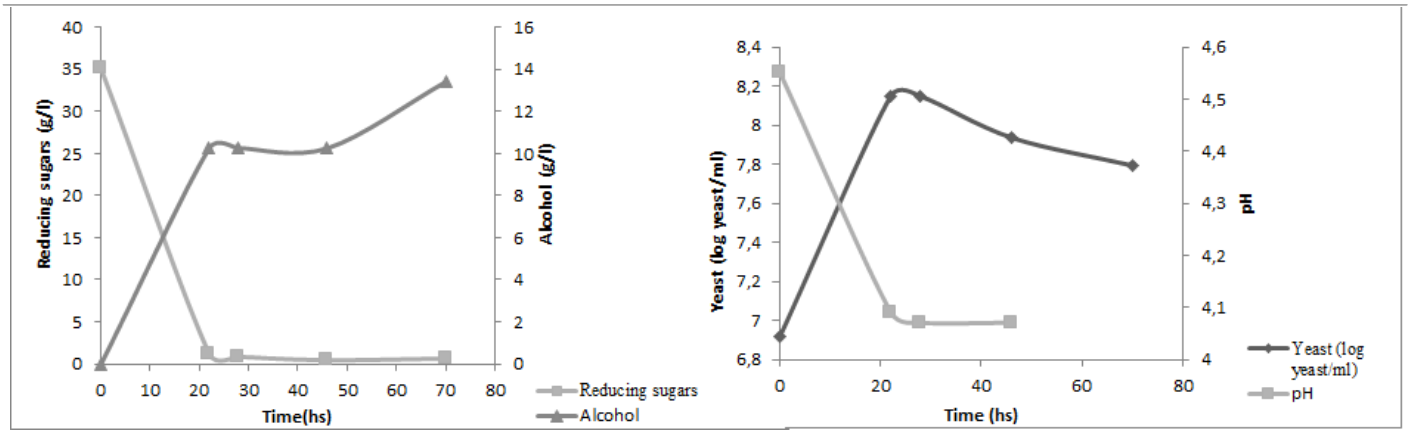

Figure 4: a) Growth of yeast *S. cerevisiae* PM16 and decrease in pH; b) Variation in the content of sugars and ethanol over time.

HMF concentration. The value obtained was 0.0246 g/l. The content of hydroxymethylfurfural determined in the pre-treated sample ensures a detoxification period is not necessary prior to enzymatic hydrolysis and fermentation stages.

Enzymatic hydrolysis and fermentation

This study focuses on the maximization of total sugars content during the pre-treatment with sulfuric acid. The performance of the enzymatic hydrolysis was quantified using the reducing sugars content, since they allow estimating the amount of fermentable sugars (glucose and fructose) (Hernández, 2017). The acid pretreatment, performed under optimal conditions increased the reducing sugars content from 3872.5 to 5312.02 mg/g dry untreated biomass. Enzymatic hydrolysis lifted this value to 34953.02 mg/g, indicating that acid treatment followed by a hydrolysis step improves almost 10 times the content of fermentable sugars as compared with the raw material. This is attributed to the improved bioavailability of carbohydrates achieved by acid treatment, which were later degraded to monomeric sugars by enzymatic hydrolysis, increasing ethanol yields. Figure 4 shows cell growth and pH evolution as a function of time. It can be observed, the evolution of these variables during the first 24 h of fermentation. These results agree with the reported by other authors for fruits such as banana and mango (Arumugam and Manikandan, 2011).

After 72 h of fermentation, a concentration of ethanol of 13.41 g/l was reached. This represents a yield of 18.32 ml of alcohol per kilogram of treated biomass and a fermentation efficiency of 75%. Bhandari et al. (2013) reported 3.08 g/l of ethanol obtained after fermentation of melon without any previous treatment. The results presented in this study demonstrates that it is possible to increase almost 6 times ethanol yields by adding a pretreatment step, improving hydrolysable sugars availability, representing an important benefit for the process economy.

CONCLUSSION

This study shows that it is possible to increase the concentration of fermentable sugars from melon husks and seeds by application of an acid pretreatment to degrade complex carbohydrates. The optimal conditions for this treatment, statistically determined, were 2% w/w of sulfuric acid, a solid to liquid relation of 11:1 g/ml, 55°C and 30 min. Under these conditions, total sugars content increased to 15.27%.

An enzymatic hydrolysis performed after acid treatment improved almost 10 times the reducing sugars content in the dry melon husks and seed without any treatment. The role of the acid pretreatment was to increase complex carbohydrates bioavailability and consequently improving enzymatic hydrolysis yields. The fermentation of this hydrolysate produced 13.41 g/L of ethanol, which represents a 75% yield, almost 6 times over the value obtained for the fruit without treatment.

These results indicate that biomass accessibility to enzymes is a key controlling factor. The application of pretreatments prior to enzymatic hydrolysis degrades complex carbohydrates present in husk and melon seeds, indicating that this residue is a potential raw material for the production of alcohol.

ACKNOWLEDGMENTS

This study was financed by the following Argentine institutions: National Council of Scientific and Technological Research (CONICET) and National University of San Juan (UNSJ).

REFERENCES

Aguayo E, Escalona V, Artés H (2001). Industrialización del Melón procesado fresco. Rev. Hort. 155: 48-60.

Ajanovic A (2011). Biofuels versus food production: does biofuels production increase food prices? Energy, 36(4): 2070-2076.

Arumugam R, Manikandan M (2011). Fermentation of pretreated hydrolyzates of banana and mango fruit wastes for ethanol production. Asian J. Exp. Biol. Sci. 2(2): 246-256.

Association of Official Analytical ChemistsAOAC (1990) Official Methods of Analysis. 15th Edition, A.O.A.C., Arlington.

Bañuelos JA, Velázquez-Hernández I, Guerra-Balcázar M, Arjona N (2018). Production, characterization and evaluation of the energetic capability of bioethanol from *Salicornia bigelovii* as a renewable energy source. Renew. Energy 123: 125-134.

Bhandari SV, Panchapakesan A, Shankar N, Kumar HA (2013). Production of bioethanol from fruit rinds by saccharification and fermentation. Int. J. Sci. Res. Eng. Technol. 2(6): 362-365.

Boucher J, Chirat C, Lachenal D (2015). Comparison between acid hydrolysis and two-step autohydrolysis for hemicellulosic ethanol production. Cellul. Chem. Technol. 49: 303-308.

DuBois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28(3): 350-356.

Gupta A, Verma JP (2015). Sustainable bio-ethanol production from agro-residues: a review. Renew. Sustain. Energy Rev. 41(C): 550-567.

Hernández GC (2017). Obtención de bioetanol a partir de hidrolizados de residuos de fruta.

Lee J-W, Jeffries TW (2011). Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. Bioresource Technol. 102(10): 5884-5890.

Modig T (2002). Kinetics and inhibition effects of furfural and hydroxymethyl furfural on enzymes in yeast. Biochem. J. 4(2): 323-328.

Mussatto SI, Dragone G, Guimarães PM, Silva JPA, Carneiro LM, Roberto IC, Vicente A, Domingues L, Teixeira JA (2010). Technological trends, global market, and challenges of bio-ethanol production. Biotechnol. Adv. 28(6): 817-830.

Nyakuma BB (2015). Thermogravimetric and kinetic analysis of melon (Citrullus colocynthis L.) seed husk using the distributed activation energy model. Environ. Clim. Technol. 15(1): 77-89.

Orozco RS, Hernández PB, Morales GR, Núñez FU, Villafuerte JO, Lugo VL, Ramírez NF, Díaz CEB, Vázquez PC (2014). Characterization of lignocellulosic fruit waste as an alternative feedstock for bioethanol production. BioResources, 9(2): 1873-1885.

Sánchez Orozco R., Balderas Hernández P, Flores Ramírez N, Roa Morales G, Saucedo Luna J, Castro Montoya AJ (2012). Gamma irradiation induced degradation of orange peels. Energies, 5(8), 3051-3063.

Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012). Bioethanol production from agricultural wastes: an overview. Renewable Energy, 37(1): 19-27.

Staff IEA (2008). CO_2 Emissions from Fuel Combustion: 1972. Organization for Economic.

Zhang K, Pei Z, Wang D (2016). Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review. Bioresourc. Technol. 199: 21-33.

Cite this article as:

Montoro ML, Mamaní A, Herrero ML, Sardella MF, Deiana AC (2018). Melon husks and seeds as potential energy source. Acad. J. Environ. Sci. 6(12): 310-316.

Submit your manuscript at
http://www.academiapublishing.org/journals/ajes