Effects of pretreatments on the bioavailability of sugars on discarded melons to obtain bioethanol.

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Abstract

Melon is one of the main horticultural crops in the province of San Juan, Argentina. An excess of 20% of its production is not marketed for various reasons. To take advantage of this biomass, acid pretreatments (PA), acid combined with ultrasound (PAU), were applied to evaluate increases in the bioavailability of sugars for the production of 2G bioethanol.

The results showed that under the optimal conditions of both pretreatments: concentration of 2% sulfuric acid, time of 30 minutes, the temperature of 55 °C and solid: liquid ratio11:1, followed by enzymatic hydrolysis using cellulase, hemicellulose and pectinase, increases of 111 and 576% are achieved in the content of fermentable sugars, compared to untreated melon.

Acid pretreatment combined with ultrasound and enzymatically hydrolyzed, using cellulase, hemicellulase, and pectinase, turned out to be the best alternative in terms of increasing fermentable sugars, obtaining 38.99 mL of bioethanol per kg of melon.

1. Introduction

The great dependence on oil to obtain fuels that still exists, leads to search for new sources of renewable energy. Bioethanol produced from lignocellulosic biomass is a fuel that reduces the environmental effects of greenhouse gas emissions, in addition to reducing the energy dependence on non-renewable sources.

Various policies promote the development of biofuels by assigning mandatory goals and quotas for blending with conventional fuels. Besides its use as an additive for fossil fuels, due to its ability to improve octane rating and reduce polluting emissions, bioethanol is seen as a great alternative, because it can be obtained from a wide variety of vegetables from different climates around the world Hamelinck et al. [1].

Currently at the industrial level, the largest production of bioethanol is done from saccharides and amylase raw materials, which is known as first generation ethanol. The main problem derived from this production is the high cost of raw material, since these biomasses are linked to the food market, which affects the final price of the product in addition to competing with food supplies. Meanwhile, bioethanol obtained from agro-food waste, considered a second generation fuel, is presented as a future alternative to first-generation biofuels [2]. In this way, it does not interfere on human or animal food supplies availability, while contributes to a sustainable development [3].

The direct conversion of lignocellulosic biomass into ethanol is difficult due to the complexity in the structure of plants cell walls. Therefore, it is necessary to apply specific treatments to these materials, prior to the hydrolysis and fermentation stages, to modify the chemical and structural composition of the lignocellulosic biomass, improving the access of the hydrolytic agents to cellulose fibers [4], [5]. There are several kinds of pre-treatments: physical (crushing, hydrothermolysis) [6], chemical (acid, alkaline,

solvents and ozone) [7], physicochemical (steam explosion, ammonium fiber explosion) [8] and biological [9], or a combination of these [10] .The choice of the treatment to be applied depends on the biomass to be treated Farias [11].

Melon belongs to the family *of Cucurbitaceae* and it is the fourth most consumed fruit in the world, after oranges, bananas and grapes [12]. This is one of the distinguished horticultural crops of the Province of San Juan, Argentina. For various reasons, such as oversupply, low quality of the fruit that does not meet the market requirements for fresh consumption and nonexistent industrialization alternatives for this fruit, have led to a large percentage of loss (around 20% of its annual production).

The composition of melon varies according to the variety, on average it contains 85–90% water, 7–8% sugar and about 10% cellulose and hemicellulose [13]. Its use to generate added value products implies economic and environmental benefits for both, the producers and the community.

This work presents the results obtained from the application of pre-treatments to melons discarded from the production of this fruit, in order to evaluate the feasibility of its use to produce bioethanol. The applied methodology and the results of the combination of physical, chemical and enzymatic pretreatments on this horticultural fruit are shown, this is done in order to maximize the bioavailability of sugars for the subsequent obtaining of ethanol.

2. Materials

The material object of the studies is melon (*Cucumis melo*) discarded, surplus of the non-commercialized production of the 2016–2017 season. The fruit corresponds to the *Sundew* variety, provided by producers of the Sarmiento Department, Province of San Juan of Argentina. The sample was disintegrated in blender of 2 liter capacity, Metvisa model LAR2220CC2, and stored at a temperature below − 15ºC until its use.

All chemicals used in pretreatments and analytical determinations were reactive grade.

3. Methods

Melon samples were subjected to different pretreatments, acid, acid combined with ultrasound, and enzymatic. This last one applied to the pretreated samples in the optimal conditions and to the untreated melon. This is in order to evaluate the impact of pretreatments on the bioavailability of fermentable sugars to produce ethanol.

3.1 Pretreatment with dilute sulfuric acid (PA)

Melon samples were placed in contact with sulfuric acid solutions at different concentrations of 0.5, 1.25 and 2% (w/w). Liquid:solid ratio was set at 8:1, 9.5:1 and 11:1 (v/w), maintaining the temperature constant at 55 °C (± 3 °C), in a thermostated stirred reactor.

3.2 Pretreatment with ultrasound (PAU)

Melon samples were treated with sulfuric acid solutions of variable concentrations 0.5, 1.25 and 2% (w/w), with liquid:solid ratios of 8:1, 9.5:1 and 11:1 (v/w), at constant temperature 55 °C (± 3 °C), in an ultrasonic equipment Testlab SRL model "+B10 + Aca", with an operating power of 400 W and a frequency of 40 kHz.

3.3 Enzymatic treatment (TE)

Cellulase from *Trichoderma reesei* ATCC26921 (Sigma-Aldrich) and hemicellulase of *Aspergillus niger* (Sigma-Aldrich) were applied to the samples from the previous treatments and to the untreated melon.

Tests were conducted for 24 hours, using 20 units of enzymes per gram of substrate. In all cases the pH was adjusted to 4.5, adding 0.1N NaOH. The experiments were carried out in 500 ml erlenmeyers in an orbital shaker (Lab. Companion SI-600) at 45 °C and 150 rpm.

3.4 Fermentation

Alcoholic fermentation was carried out under anaerobic conditions, using Saccharomyces cerevisiae yeast (Thermosacc® DRY). The inoculum was prepared by dissolving 0.136 g of yeast in 1.3 mL of melon juice at 35 °C, which was previously sterilized by tindalization.

In 1000 mL Erlenmeyers flasks, the inoculum and 700 mL of the material to be fermented and previously sterilized were added by tindalization.

Incubation time was at 35 °C for 72 hours at 100 rpm, in a thermostated orbital shaker (Lab.Companion SI-600).The samples were taken at different times, centrifuged at 1500 rpm for 5 minutes. The supernatant was filtered with a 0.45 µm nylon filter and stored at -5 °C for future analysis.The microbiological analysis was performed by microscopic counting in the Neubaüer chamber.

3.5. Experimental Design

In order to study the influence of the operation variables in the PA and PAU, an experimental design 2^3 single block with two central points was used. The Stagraphics Centurion 16.1.15 software was used. The effects of three factors were analyzed by 10 experimental runs. According to this design, each factor or variable was placed at two levels, being the variables proposed for this study: acid concentration, time and liquid:solid ratio; while the temperature remained invariable at 55 °C. The lower level (-) greater (+) and the central points of the factors for the pretreatments are detailed in Table 1.

Table 1
Variables and levels adopted for PA y PAU of melon

Variables	Niveles		Central Point
	-	+	
H_2SO_4 conc. (% w/w)	0.50	2.00	1.25
Time (min)	30	90	60
Liquid:solid ratio (mL:g)	8:1	11:1	9.5:1

The response variable selected to evaluate the influencing factors in the proposed design and its subsequent optimization was the content of total sugars.

3.6 Analytical methods

Moisture content was determined using a vacuum oven (Vacuum Oven model DZF 6020) according to the [14] method (1990). The dry base proportion of ash, volatile matter and fix carbon was 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 minutes to evaporate the humidity taken from the environment, continuing with a heating ramp at 50 °C/min up to 110 °C and subsequently at 90 °C/min up to 950 °C.

The lignin content was analyzed under the ASTM D1107 technique, while cellulose and hemicellulose were determined using an automatic fiber analyzer, ANKOM A2000.

The total sugars were analyzed by the Dubois method [15], while the quantification of reducing sugars was carried out using the DNS technique [16].

Ethanol quantification was performed by GC (gas chromatography), using a Shimadzu 2010 plus equipment, equipped with a 20is automatic injector and a flame ionization detector (FID). H and airflow were established at 40 and 400 mL/min, respectively. The detector temperature was 240 °C and the injection port was 180 °C, the latter operating in split mode with a ratio of 10 and a volume of 1µL. Nitrogen was used as a carrier, with a flow rate of 30 mL/min. A CPSIL 8Cb 50mt x 0.25 mm column (Chrompack) was used, operating under a temperature program of 35 °C, for 1 minute, 35–100 °C at 10 °C/min.

4. Results And Discussion

4.1 Melon characterization

Table 2 presents the characterization results of untreated melon (UM). The high moisture content of melon is comparable to fruits such as watermelon and mango, the proportion of ash similar to that of

pomegranate and watermelon, as reported by [17] ,while lignin and hemicellulose content are lower and similar to citrus fruits such as oranges, respectively [18] .

Table 2
Melon characterization

Propiedad	Value
Humidity (%w/w)	89,39 ± 0,54
Ash (%w/w)*	7,06 ± 0,09
Volatile matter (%w/w)*	63,38 ± 0,32
Fix carbon (%w/w)*	24,5 ± 0,42
Lignin (%w/w)*	1,07 ± 1,07
Cellulose (%w/w)*	0,78 ± 0,7
Hemicellulose (%w/w)*	0,29 ± 0,64
Total Sugars (%mg/g)*	15,45 ± 1,18
Reducing sugars (g/L)	9,45 ± 0,8
pH	5,63 ± 0,11
* dry base	

Figure 1 shows the loss of mass as a function of temperature, in its normal and derived form. The loss of mass observed at temperatures lower than 200°C corresponds to the humidity present in the material [19], [20]. From this temperature value mass losses are associated to the organic compounds decomposition and the generation of volatile substances from inorganic compounds [21]. Between 200 and 400 °C occurs the greatest loss of mass due to the decomposition of cellulose and hemicelluloses and, above 580°C, the decomposition of lignin is the main phenomena [22] .

4.2 Pretreatments

Table 3 shows the conditions in which the PA and PAU pretreatment tests were carried out and the total sugar values of the response variable adopted to evaluate the effectiveness of each one. Determinations were made by triplicate, reporting the average value. Total sugars contents are expressed in mg/g of dried melon.

Table 3
Assays conditions and response variable values for the pretreatments

Assay	Time (min)	L:S ratio	Acid concentration (%)	Total sugars (mg/g)	
				PA	PAU
1	30	8	0.5	314.765	281.496
2	60	9.5	1.25	239.744	368.608
3	30	11	0,5	259.615	401.349
4	90	11	0,5	301.644	228.633
5	30	11	2	736.458	605.633
6	60	9.5	1.25	329.027	320.694
7	90	8	2	336.435	591.864
8	90	11	2	491.414	439.058
9	30	8	2	473.546	298.004
10	90	8	0.5	365.105	437.040

4.2.1 Statistical analysis for acid pretreatment

Table 4 describes the results from the analysis of variance (ANOVA) of the factorial design 2^3. In this case, the effect of the acid concentration has a *p-value* less than 0.05, indicating that its influence is significant on the process, with a confidence level of 95.0%. Graphically, these results are evidenced through the Pareto diagram (Fig. 2).

Table 4
ANOVA for PA

Source	Sum of squares	Degrees of freedom	Middle square	F-Reason	p-value
A:acid	62,635.4	1	62,635.4	8.46	0.0437
B:LS	11,196.1	1	11,196.1	1.51	0.2862
C:Time	12,989.1	1	12,989.1	1.75	0.2560
AB	35,979.3	1	35,979.3	4.86	0.0922
AC	28,146.6	1	28,146.6	3.80	0.1230
Total error	29,618.5	4	7,404.64		

Acid concentration shows a positive effect, indicating that an increase in this variable improves the response variable, while time and L:S ratio reveal a negative effect. This is coincident with the results obtained in the experimental tests (Table 3). Comparing tests 1 and 9, both in similar conditions of time (30 minutes) and L:S ratio (8 mL/gr), it can be observed that when acid concentration was raised from 0.5 to 2%, total sugar content also increased, from 314.765 to 473.546 mg/g. The same effect can be observed for trials 4 and 8.

Equation 1 shows the model obtained for acid pretreatment effect, which indicates the relationship between the variables analyzed and the response variable, as well as the interactions between the effects. Data were well correlated with an R^2 of 85%.

$$Y_a = 573.733 - 275.346 * A - 49.5742 * B + 1.95587 * C + 59.6113 * A * B - 2.63624 * A * C \quad (1)$$

Where Y_a is the content of total sugars in mg/g dry matter, A the acid concentration (% wt/wt), B the L:S ratio (mL/gr) and C time (minutes).

The values of the variables provided by the model (Ec.1) that maximize the sugar content (688 mg/gr dry), were 2% H_2SO_4, 30 minutes and 11 mL/gr. The validation test was carried out by duplicate, obtaining an average value of the response variable of 731.64 mg/g dry, which differs by only 6% with the value predicted by the model. The composition of melon is given mainly by cellulose, hemicelluloses and pectins. The last one is a complex mixture of polysaccharides, whose main components are α-D-galacturonic acid subunits with side chains of neutral sugars, such as arabinose, galactose, glucose, xylose and mannose [23].Considering that factors such as time and temperature were kept constant at average values during the pre-treatment stage, the effect of the high acidity of the medium may have directly impacted on the content of the neutral sugars. Too low pH values cause the breakdown of hydrogen bonds between cellulose and pectic acids, hydrolyzing pectin and releasing the neutral sugars [24].

4.2.2 Statistical analysis for ultrasound-assisted acid pretreatment

From the statistical analysis for the ultrasound-assisted acid treatment (Table 5), only two significant effects were obtained: acid concentration and L:S-time interaction, with a p-value of less than 0.05.

Table 5
ANOVA for PAU

Source	Sum of squares	Degrees of freedom	Middle square	F-Reason	p-value
A:acid	45,272.8	1	45,272.8	12.70	0.0377
B:LS	516.704	1	516.704	0.14	0.7288
C:Time	1,065.56	1	1,065.56	0.30	0.6226
AB	7,404.05	1	7,404.05	2.08	0.2452
AC	2,608.48	1	2,608.48	0.73	0.4552
BC	77,755.0	1	77,755.0	21.81	0.0185
Total error	10,695.2	3	3,565.05		

The Pareto diagram (Fig. 3) shows that the response variable in the PAU is favored by the use of high concentrations of acid, otherwise it happens with the interaction time-relationship L:S. This is evidenced by comparing the results of tests 7 and 10 (Table 3), carried out for 90 minutes with an L:S ratio of 8 mL/gr, varying only the acid concentration from 0.5 to 2%, resulting in an increase in the sugar concentration from 437.04 to 591.86 mg/g dry matter. With an R^2 92.5%, the model was adjusted to the data, which is given by Eq. 2:

$$Y_u = 668.173 - 207.377 * A - 103.169 * B + 20.1932 * C + 27.0419 * A * B - 0.802539 * A * C - 2.19082 * B * C \quad (2)$$

The model predicts an optimum concentration of total sugars of 602.05 mg/g dry, working for 30 minutes with an L:S ratio of 8 mL/gr and 2% sulfuric acid. Validation tests were carried at these conditions by duplicate and total sugars concentration reached a value of 741.90 mg/g dry matter, surpassing the predicted value in 18.8%. At the frequency of 40 kHz the delignification of the material is increased, while the mechanoacoustic effects on the impact of sulfuric acid on the biomass are accentuated [25].

4.3. Comparison of pretreatments

To compare the effect of each previous treatment, the content of lignin, cellulose and hemicellulose was analyzed. Table 6 shows the content of these components after applying acid treatment to each fraction of the materials and the combination of acid with ultrasound.

Table 6
Effects of pretreatment on the structural components of the melon

		Lignina (%w/w)*	Cellulose (%w/w)*	Hemicellulose (%w/w)*
	Raw	1,14 ± 0,09a	0,82 ± 0,06a	0,32 ± 0,04a
	PA	0,87 ± 0,05a,b	0,43 ± 0,04a	0,27 ± 0,02b
	PAU	0,69 ± 0,06b	0,3 ± 0,03b	0,12 ± 0,02b
*% dry base.				
a,b results with different superscript letters within each column are significantly different				

From these results it can be seen that both treatments caused a decrease in the values of the structural components of the melon. The values indicate that the cell structure of the fruit has been altered, allowing the hydrolysis of 42% of the cellulose present initially. By treating the melon with acid only and 64% applying acid pretreatment plus ultrasound, generating glucose, a monomer of the greatest interest for fermentation. Furthermore, the partial hydrolysis of hemicellulose was 5% for the first case and 55% for the second, while lignin decreased by 23% and 32%, respectively. The significant effect of ultrasound on the lignocellulosic components of melon is evident.

Comparison of both pretreatments shows a synergistic effect of ultrasound with acid, as a result of shear forces that improve mass transfer and surface erosion, together with the production of oxidizing radicals. These effects facilitate the homolytic division within the lignin macromolecule, improving the breaking of bonds between lignin and hemicellulose. On the other hand, ultrasound also tends to contribute to the separation and depolymerization of polysaccharides as a consequence of cavitation phenomena, improving hydrolysis and facilitating the accessibility of enzymes. Therefore, the mechanical acoustic effects accentuate the action of the acid on the biomass [25]. During the pretreatment stage, reactions occur that give rise to the formation of inhibitory compounds that affect the fermentation process. The combination of temperature and acid in high concentrations causes the degradation of carbohydrates to non-fermentable forms or compounds such as furfural (F) and hydroxymethylfurfural (HMF) resulting from the degradation of pentoses and hexoses, which can be further degraded forming formic, levulinic, acetic acid, among others. There are several investigations about the effects of F and HMF on growth rate, fermentation speed and cell composition in Saccharomyces cerevisiae. According to Wan et al. [26], elevated concentrations of furfural (1.5 g/L) interfere with the respiration and growth of microorganisms such as Saccharomyces cerevisiae, resulting in lower yield and productivity of ethanol. Lee et al. [27],reported that, during fermentations with S. cerevisiae, the presence in equal concentrations (5 g/L) of furfural and HMF, hydroxymethylfurfural turned out to have a toxic effect on yeast, while furfural had no action harmful. On the other hand, Taherzadeh et al.[28],observed a reduction in the fermentation rate when the furfural concentration exceeded 4 g/L. Various authors confirm that furfural and HMF concentrations below 2 g/L are not considered detrimental to the development of S. cerevisiae yeast [29].

For this reason, the content of these toxic compounds (furfural and HMF) was analyzed according to the ISO 1388-5 /ISO 1388-11 and AOAC 980.23: 2012 methods, after applying pretreatment under optimal conditions. Table 7 shows the content of these compounds found in each of the treated samples.

Table 7
Furfural and hydroxymethylfurfural content in PA and PAU

	Furfural (mg/L)	**Hidroximetilfurfural (mg/L)**
PA	*ND	0,43 ± 02
PAU	92 ± 0,1	0,21 ± 0,2
*ND: does not detect		

The results obtained show that the low concentrations of furfural and HMF, found after subjecting each melon fraction to each of the pretreatments, are below the values that could cause harmful effects on the yeast. This is attributed to the fact that the treatments were carried out under conditions of low temperature and low acid concentration, in addition to short reaction times. The presence of a low concentration of inhibitors avoids significant costs to the process, making it unnecessary to add a detoxification stage before fermentation.

4.4. Enzymatic treatment (TE)

This treatment was applied to the pre-treated melon and also to the raw melon. The working temperature was 45 °C and the pH corrected to 4.5, values suggested as optimal for the enzymatic reaction .The reaction time, determined in preliminary trials, was 24 hours which indicated that it is sufficient to consume the hemicellulase and cellulase present in the system. It is also necessary to consider that in very prolonged periods the enzymes are inactivated, either by thermal action or by irreversible adsorption onto lignin or the crystalline region of cellulose [30] .

Table 8 summarizes the increase concerning the untreated melon in the content of reducing sugars, for the two fractions, melon pretreated with acid and with acid assisted by ultrasound, to which they applied physicochemical and enzymatic treatment. The results showed that the combination of acid treatment with enzymatic hydrolysis using cellulase and hemicellulase doubled the content of reducing sugars, while the addition of cellulase, hemicellulase, and pectinase increased this variable by 2.2 times.

The same experimental scheme used for the melon treated with acid only was applied to the melon pretreated with acid plus ultrasound, the results showed an increase of 6.4 times when only cellulase and hemicellulase were used and 6.8 times when pectinase was added, cellulase and hemicellulase. This important improvement is attributed to the effect of ultrasound on the cellulose structure and its crystalline disposition. That is, the cellulose granules contain ordered crystalline regions and amorphous regions, by the action of ultrasound the polymer chains are less ordered and are more susceptible to attack by enzymes [31]. Furthermore, pectinase allows the hydrolysis of (1,4) polygalacturonic acid by

breaking down pectin molecules into smaller ones [32]. However, the action of pectinase on the decomposition of melon polysaccharides is not very relevant, compared to the effect produced by ultrasound

Table 8
Effects of physicochemical and enzymatic treatment on reducing sugars in melon

	Reducing sugars (g/kg melon)			
	PA	Increase	PAU	Increase
Untreated melon	8,85 ± 0,25	---	8,85 ± 0,25	---
Treated	15,38 ± 0,3	1,7	19,22 ± 0,3	2,2
Treated hydrol. 2 enzymes	17,75 ± 0,2	2	56,27 ± 0,25	6,4
Treated hydrol. 3 enzymes	18,74 ± 0,2	2,2	59,89 ± 0,27	6,8

Figure 4 summarizes the impact of the enzymatic treatment on untreated melon, on pretreated with acid, and acid assisted by ultrasound, hydrolyzate with two and three enzymes. On the bars the percentage increase is represented with respect to the untreated material.

5. Fermentation

Fermentation of the pretreated and hydrolyzed melon with cellulase, hemicellulase and these combined with pectinase, was carried out using commercial yeast Sacharomyces cerevisiae Thermosacc © Dry (Lallemand, Denmark), during a period of 72 hours at 35 °C ± 0, 6.

In order to compare the results achieved in this stage, direct melon fermentation was carried out, without prior treatment using it as control fermentation. For this, the solid-liquid ratio was placed at the same value used in the material pretreated with acid and its combination with ultrasound (0.11 mL/g), as well as the pH value at 4.9. The fermentation and yeast conditions used are identical to those stated above.

The results show that the first 24 hours of fermentation were, in all cases, the most significant for the production of ethanol, showing slight changes beyond this time period, as presented in Fig. 5.

The results of the tests indicated that the alcoholic fermentation of the UM reached 5.17 g/L, while for the melon pretreated with acid and hydrolyzed only with cellulase and hemicellulase, 9.6 g of ethanol were obtained per liter of melon. For the same treatment, hydrolyzing with cellulase, hemicellulase, and pectinase, 13.14 g/L was achieved. In the case of melon pretreated with acid and ultrasound and hydrolyzed with the enzymatic complexes formed by two and three enzymes, ethanol concentrations of 16.01 g/L and 32.86 g/L, respectively, were obtained. It can be seen that there is a marked difference in the ethanol content for each of the pretreatments, after hydrolyzing with two and three enzymes.

However, the concentration of reducing sugars after the hydrolysis step using each group of enzymes is not so different. This is due to the ability of the yeast used to metabolize the pectolytic enzymes present in the material.

6. Conclusion

The use of chemical pretreatments with dilute sulfuric acid and the combination of this with ultrasound, improve the availability of total sugars in the melon. For acid treatment and its combination with ultrasound will achieve increases of 31.37 and 32.34% compared to the same untreated.

The application of enzymatic hydrolysis using cellulase, hemicellulose, and pectinase, as a post-pretreatment stage, exhibited increases of 111 and 576% in the content of fermentable sugars, compared to the raw material.

The alcoholic fermentation of the melon fraction, pretreated with acid combined with ultrasound and enzymatically hydrolyzed, using cellulase, hemicellulase, and pectinase, turned out to be the alternative with the highest increase in fermentable sugars and the bioethanol yield of 38.99 mL/Kg.

Abbreviations

PA
Acid pretreatments
PAU
Acid combined with ultrasound

Declarations

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials**:**All data generated or analyzed during this study are included in this article.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions

M.L. Montoro performed the experiments and the analyzing of the products. M.L.Herrero and A.Mamaní contributed in analyzing the results and writing the manuscript. M.F. Sardella,M.D. Vallejo and A.C. Deiana contributed to the assessment of the results and conclusion. All authors read and approved the final manuscript.

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Figures

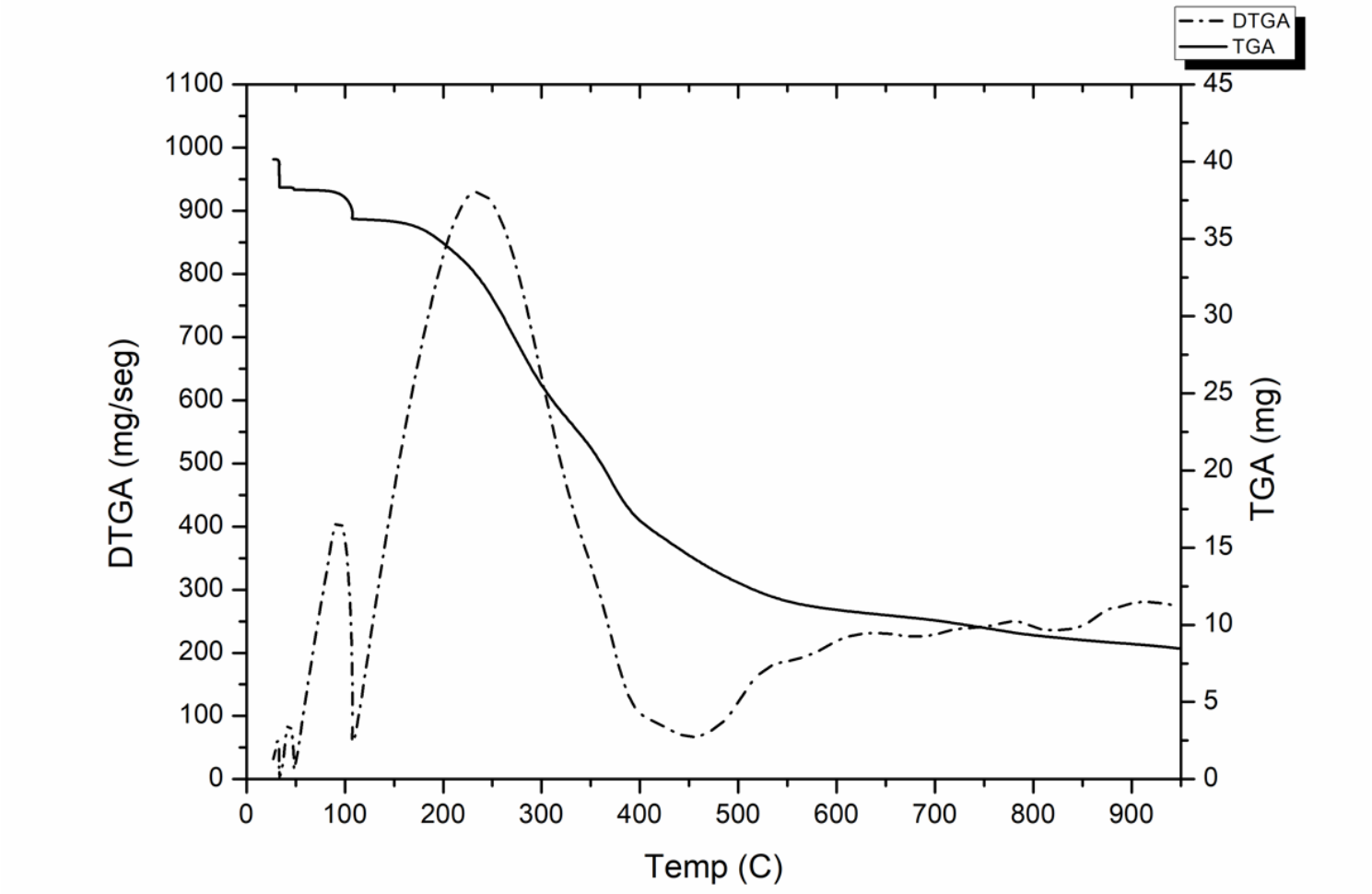

Figure 1

Melon thermogravimetric analysis

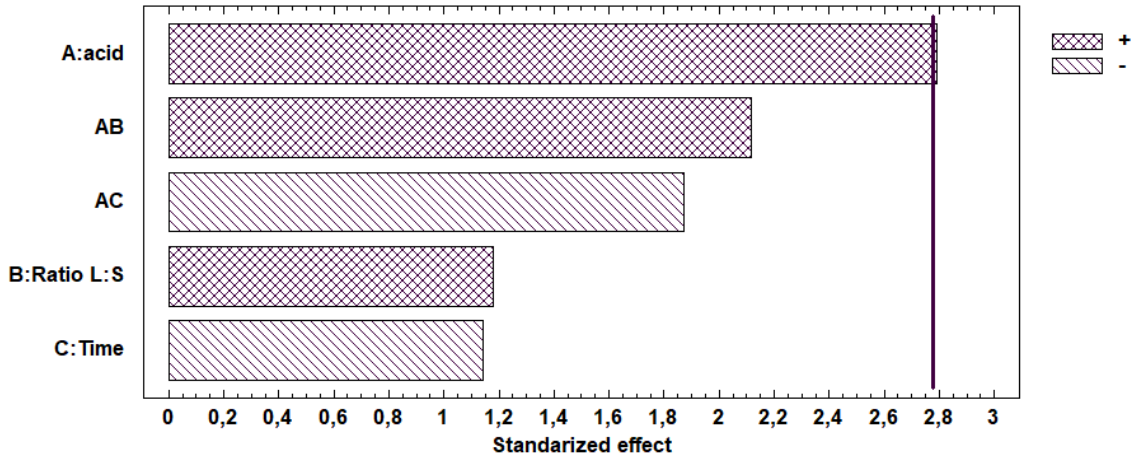

Figure 2

Pareto diagram for acid pretreatment

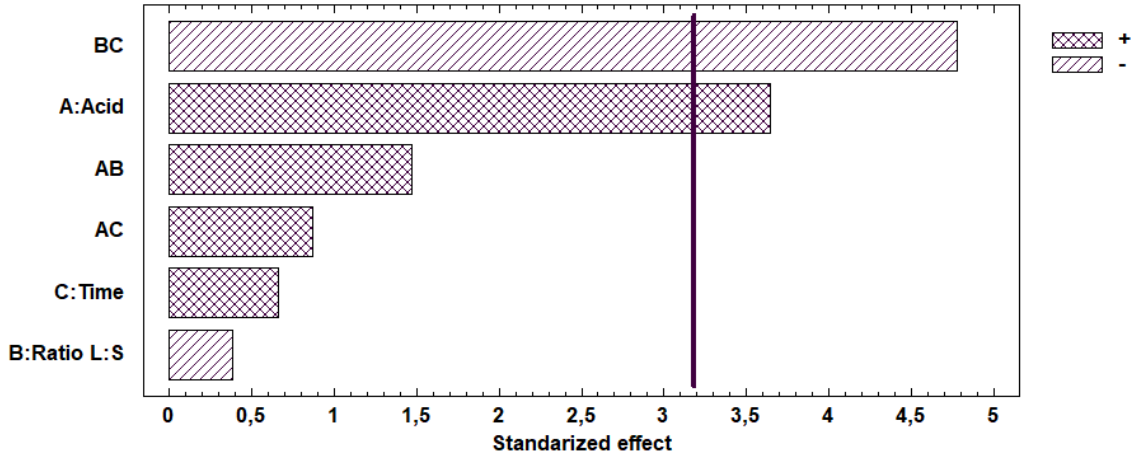

Figure 3

Pareto diagram for the acid treatment combined with ultrasound

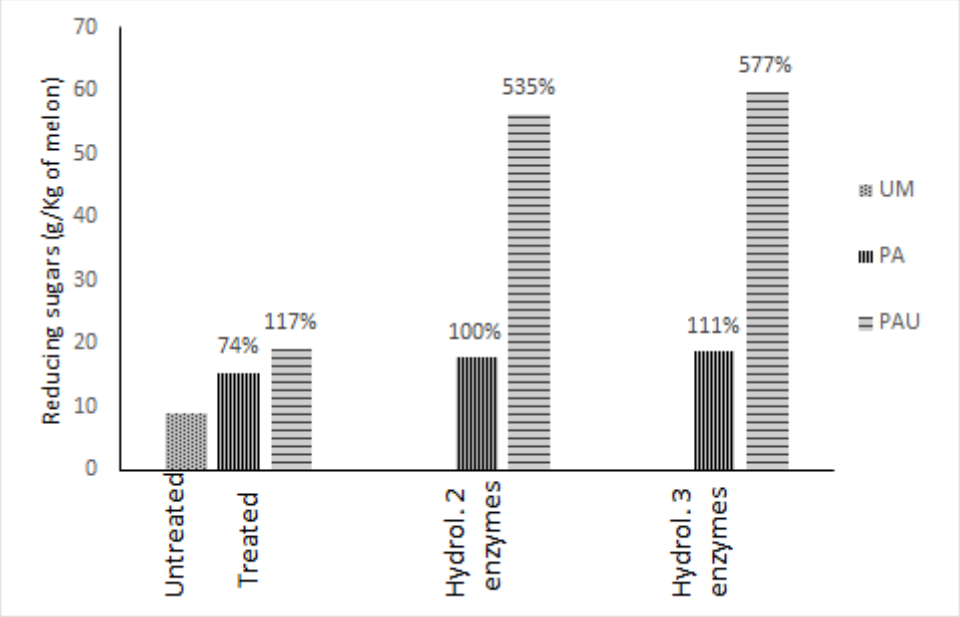

Figure 4

Comparison of reducing sugars for UM, PA and PAU, hydrolyzate with two and three enzymes.

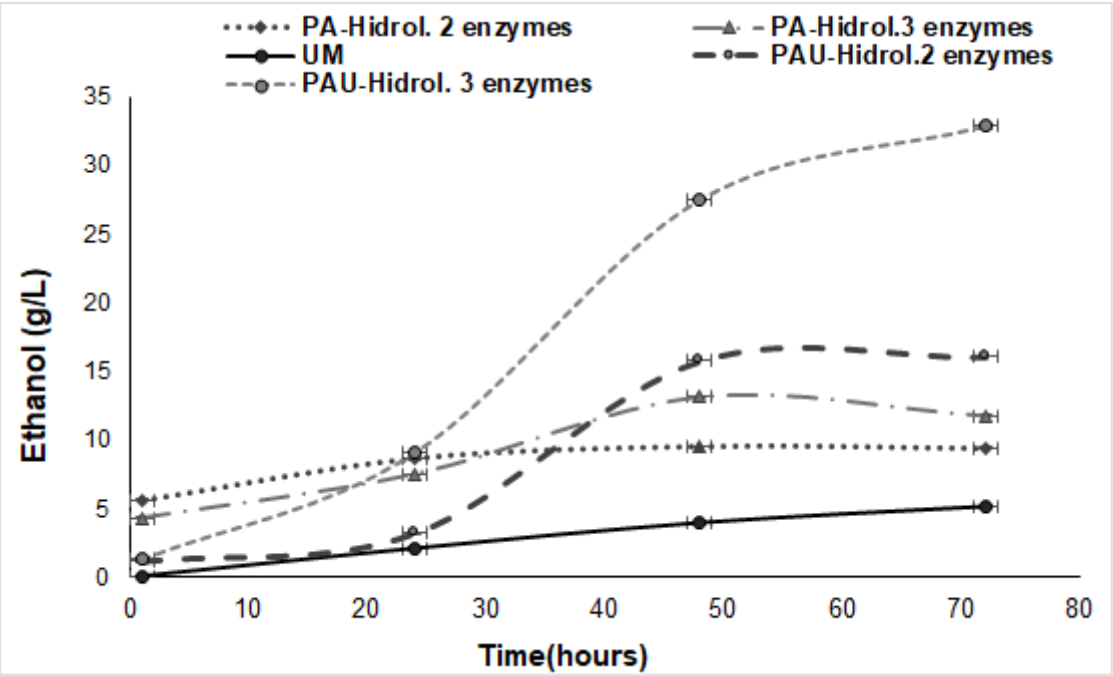

Figure 5

Evolution of ethanol during the fermentation of the melon

Supplementary Files

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- AbstractGraphics.jpg