Hydrothermal Treatment of Empty Fruit Bunch (EFB) Aimed at Increased Production of Reducing Sugars

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The waste generated from the palm oil production chain is increasing. The purpose of this work is to enable more effective use of empty fruit bunches (EFB) to produce reducing sugars by a hydrothermal treatment process (hydrothermolysis) at elevated pressures and temperatures. The EFB was dried and milled to obtain three different granulometries: thin (> 60 mesh), medium (28 to 60 mesh), and thick (< 28 mesh). The operating conditions were defined using a complete factorial design of 2^5, while considering the variables as particle size (thin, medium, and thick), solid/liquid ratio (1/13.33 and 1/20), temperature (130 and 170 °C), reactor pressure using CO_2 (150 bar and 200 bar), and reaction time (10 and 20 min). The reactional system converted the EFB into 17.5% and 57.9% of reducing sugars, for thin and medium samples, respectively, which were performed under the same conditions. The statistical analysis indicated that the main effects for hydrothermal treatment are time and temperature.

Keywords: Hydrothermal treatment; Biomass; Reducing sugar; Empty fruit bunch

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INTRODUCTION

The oil palm production chain produces numerous byproducts and waste. While some are directly usable, others require treatment, which entails an investment of resources, both for use and disposal. Such byproducts include empty fruit bunches (EFB), which are used as biomass, and often are used as a source of energy in the boiler of palm oil extraction plants (Shinoj *et al.* 2011).

The term biomass is defined by ASTM International (2015) as a "substance wholly comprised of living or recently living (non-fossil) material. Sometimes referred to as renewable organic material, examples of biomass include whole, or parts of, plants, trees, aquatic organisms, animals, algae, and microorganisms". The cellulosic biomass is composed of cellulose chains (polysaccharides consisting of glucose molecules linked *via* β-1,4-glycosidic bonds) joined by hydrogen bonds. These long cellulosic fibers are coated with hemicellulose and lignin. The EFB typically contain 42.7 to 65% cellulose, 17.1 to 33.5% hemicellulose, and 13.2 to 25.31% lignin; thus, these polysaccharides can be hydrolyzed to produce fermentable sugars (Khalil *et al.* 2007; Law *et al.* 2007; Shinoj *et al.* 2011).

Hydrolyses experiments conducted at high temperatures that keep the liquid as water in the reaction are known as hydrothermolysis or uncatalyzed solvolysis. Hydrothermolysis is a technique that does not need to use solvents or strong acids during the process, unlike common hydrolytic techniques, which use different organic reactants

or strong and extremely polluting acids. The process happens in a reactor that is at a high temperature and high pressure, and water as the reactor medium. Because of this methodology, absent of any toxic substance, hydrothermolysis is a more environmentally friendly alternative combined with the reuse of waste (Bonn *et al.* 1983; Kallury *et al.* 1986; Mok and Antal 1992; Bobleter 1994; Pińkowska *et al.* 2014).

In this study, carbon dioxide (CO_2), a weak acid, was used as the main agent responsible for raising the pressure. A reaction system was employed for hydrolysis of biomass composed mostly of C, H, and O, and numerous other species such as CO_2, H_2O, CO, O_2, H_2, CH_4, H_2CO_3, and complex hydrocarbons (Mader 1991). The increase in the water temperature causes an increase in the dissociation constant, making water usable as an acid or a base. In such chemical reactions, the water is the catalyst, reactant, and solvent. This means that the water in the subcritical range accelerates the biomass depolymerization by hydrolysis. Considering that the glycosidic bonds of the cellulose and hemicellulose are polar, they are broken very quickly and form monosaccharides, and such a process can potentially degrade the entire biomass structure (Katritzky *et al.* 1996; Kruse and Dinjus 2007a,b; Narayanaswamy *et al.* 2011; Pińkowska *et al.* 2014).

The objective of this work, based on bibliographic references, is to produce reducing sugars by a hydrothermal treatment at high pressures and temperatures to evaluate how the main process variables influence the final product yield.

EXPERIMENTAL

Materials

Empty fruit bunch (EFB)

The EFB was obtained from Biopalma (a palm oil company), located in the city of Moju (Pará, Brazil) and was air dried in a recirculating oven for 72 h at 75 ºC, to prevent the fungal proliferation. The dried EFB was stored in a freezer at -25 ºC.

Reactor

The reactor used was located at Laboratório de Operações de Separação (LAOS) in the Institute of Technology (ITEC) of the Federal University of Pará (UFPA), Pará, Brazil, and was adapted for this study. The reactor was made of stainless steel, had an internal volume of 100 mL, and safely reached the maximum conditions proposed in this study (200 bar and 170 ºC). A heating tape (FISATOM,5 Standard Model - Class 300; 1.3 cm width, 120 cm length, São Paulo, São Paulo, Brazil) coupled to an electronic power regulator (Model 407 FISATOM) was used for heating the reactor. The CO_2 was pressurized, inside the reactor by a membrane compressor (Hofer Mülheim, Model 120-40 MKZ, Mülheim an der Ruhr, Germany). The carbon dioxide used was provided by the White Martins Gases Industriais Norte S/A (Belém, Pará, Brazil) with 99.9% purity. All chemicals were analytical grade and purchased from Sigma-Aldrich Brasil Ltda (São Paulo, São Paulo, Brazil).

Methods

Experimental design

The 2^5 experimental design was partially based on Moreschi *et al.* (2004) during the hydrothermolysis of ginger bagasse. The operational parameters were: particle size (thin and medium sample), solid/liquid ratio (1/13.33 and 1/20), reactor temperature (130

and 170 ºC), pressure system (150 and 200 bar), and reaction time (10 and 20 min). The response variable was yield, because it represents the amount (g) of biomass converted into reducing sugar, totaling 32 experiments plus all repetitions were in triplicate. All experiments used approximately 3 g of EFB.

EFB preparation

The EFB was milled using a Wiley mill (TECNAL, Model TE650/1, Piracicaba, São Paulo, Brazil) and classified using an electromagnetic sieve shaker (Bertel, Model BT-001, Caieiras, São Paulo, Brazil) in three particle sizes: thin sample (> 60 mesh), medium sample (28 to 60 mesh), and thick sample (≤ 28 mesh).

Hydrothermal treatment

Initially, it was necessary to hold the reactor temperature at 90 ºC to prevent water from boiling when added. Then, the EFB and distilled water were added into the reactor, following the experimental plan (keeping the EFB below water level). The system was then pressurized and heated. When the reactor was heated, it caused the system pressure to increase. It was essential that the system was well controlled at the stabilizing pressure, not allowing significant variations. After the temperature and pressure were stabilized, the reaction timer started. The reactor was shaken during the reaction to mix the sample.

When the reaction ended, the liquid sample (converted EFB) was collected to quantify how much of the reducing sugar was produced. The solid sample (unconverted EFB) was analyzed to evaluate the amount of cellulose, hemicellulose, and lignin that remained.

Analysis of cellulose, hemicellulose, and lignin content

The analysis of cellulose, hemicellulose, and lignin content from the EFB samples (thin, medium, and thick) were determined using the acid detergent fiber (ADF) and neutral detergent fiber (NDF) following the methodology proposed by Goering and Van Soest (1970). The heating value was obtained using a calorimetry bomb (PAAR Instrument, 6200, Moline, IL, USA). The reducing sugars content was quantified using the method proposed by Miller (1959) that uses the 3,5-dinitrosalicylic acid (DNS) as the reagent. The moisture, fat, and ash content were determined following the AOAC (2016) methods. All analyzes were performed in triplicate.

Reducing sugars content

The method proposed by Miller (1959) for the quantification of the reducing sugars provides values expressed in g of reducing sugars dissolved in 1 L of solution (g/L). This method considers the sample being solubilized in water, because it is a spectrophotometric analysis. As shown in the experimental planning, the amount of water used in each reaction assay ranged from 40 to 60 mL, and used 3 g of EFB in all assays, so without a method of compensation, the results would be underestimated or overestimated. Thus, the values given in g/L may not report the actual amount of reducing sugars produced, and therefore the results are expressed in "Yield %". This means that the amount of the sample that became a reducing sugar were compared to the initial sample mass before the reaction.

The calculation was initiated by the value obtained from the DNS analysis, which was in g/L, and then changed the expression values to g/40 mL (ratio 1/13.33) or g/60 mL (ratio 1/20), according to the volume of water. This allowed the raw amount of reducing sugars produced in each test to be obtained. The yield is obtained using Eq. 1,

$$Yield\ (\%) = \frac{MRS \times 100}{MS} \quad (1)$$

where *MRS* is the amount of reducing sugars produced in each assay and *MS* is the initial amount of sample used in each assay.

Autoclaving pretreatment

The main purpose for autoclaving the EFB was to evaluate its influence in the yield of reducing sugars. According to Medina *et al.* (2016) and Wang *et al.* (2012), water vapor at high pressure and high temperature reduces the crystallinity and increase the porosity of the EFB, due to rupture of its internal structures. These structural changes may facilitate the hydrolysis of lignocellulosic biomass. About 3 grams of the EFB fraction was added in a glass container, then placed in the autoclave for 45 min at 121 °C. After this step the hydrothermal treatment of the pre-treated fraction was performed.

Statistical analysis

All the experiments were performed in triplicate, and the results were analyzed using the STATISTICA 12 Software (StatSoft Inc., Palo Alto, CA, USA) to generate the response surface and the Pareto chart to determine which independent variable has the highest influence in the reducing sugar production.

RESULTS AND DISCUSSION

Obtaining and Separation of EFB Samples

Although the samples obtained were separated by particle size, the equipment used in this step classified the milled EFB according to fiber length. The longer fiber was more difficult to pass through the sieve with smaller apertures. The samples obtained from the milled EFB were 25.16%, 38.93%, and 35.9% of the thin, medium, and thick sample, respectively.

The sample retained in the bottom plate (> 60 mesh) was dark colored, as shown in Fig. 1A. The dark color is a characteristic of the EFB inner part (Wang *et al.* 2012).

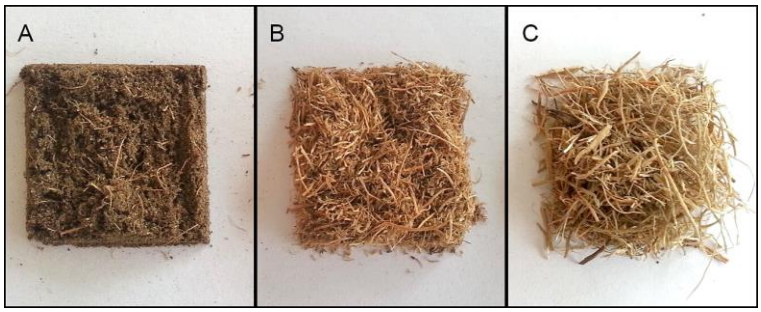

Fig. 1. Different granulometries from the milled EFB. A - Thin sample (> 60 mesh). B - Medium sample (28 to 60 mesh). C - Thick sample (< 28 mesh)

Figure 1B shows the EFB at 28 mesh. The color of this sample had a dark yellow color from the lignocellulosic fiber, with an average length of less than 1 cm, which is much larger than the fibers present in the thin sample. The thick sample had the same color as the medium sample but with a larger length, as shown in Fig. 1C.

As shown in Fig. 1A, the color of the thin sample differs from the other samples because the darker color is characteristic of the innermost part of the EFB and, according to the analysis, consisted of mostly lignin and presented levels of ash and minerals higher than the other samples, *i.e.*, the innermost part of the cluster is more susceptible to breakage by mechanical force (Khalil *et al.* 2007; Rao and Rao 2007; Xu 2010).

Chemical Composition of EFB Samples

The analyses were performed using the thin, medium, and thick samples. The results are shown in Table 1.

Table 1. Physical and Chemical Characteristics of the EFB Samples

Analysis	Thin Sample	Medium Sample	Thick Sample
Moisture content (%)	6.36[b]	5.23[a]	5.31[a]
Lipids (%)	0.52[b]	0.98[a]	0.99[a]
Ash (%)	8.34[b]	3.81[a]	3.81[a]
Calorific value (kcal/kg)	4,162.3[a]	4,613.9[b]	4,623.1[c]
Cellulose (%)	18.37[b]	42.39[a]	41.74[a]
Hemicellulose (%)	13.28[b]	18.26[a]	17.77[a]
Lignin (%)	38.18[b]	13.70[a]	13.41[a]
Reducing sugar (g/L)	0.0167[b]	0.0122[a]	0.0113[a]
The average results above are expressed on dry basis, except for water content analysis; the average results followed by the same letter in the same line, do not differ each other by Tukey test at 5% significance level.			

The ash content was similar for the medium and thick samples, but the thin sample had twice as much ash as the other samples. To the authors' knowledge, no results have been reported for the ash values for EFB with different particle sizes. Mohammed *et al.* (2012), Medina *et al.* (2016), and Hong *et al.* (2013) showed ash content values of 3.45%, 3.19%, and 1.2%, respectively, similar to the medium and thick samples.

Fan *et al.* (2011) obtained 4630 kcal/kg for the heat value using a 20 mesh sieve, which is close to the value of the medium and thick samples. The thin sample showed the value of 4162 kcal/kg, which is lower compared to other results, and is similar to that found by Yang *et al.* (2004) and Mohammed *et al.* (2012), which found 4080 kcal/kg and 4160 kcal/kg as heating values, but did not report EFB particle size the analysis was performed on. The low value for the thin sample can be explained by the high concentration of ash, which promotes a negative effect on the calorific value (Demirbas and Arin 2002).

In general, the results obtained from the analysis of cellulose, hemicellulose, and lignin content were in the ranges found in the literature. Since references were not found that evaluated the EFB in different particles sizes, the material was only fragmented and analyzed, excluding the information about the particle size separation step. On this basis, it is possible to reaffirm the fact that lignin is more susceptible to breaking by mechanical force (milling), causing it to be broken in sizes small enough to pass through the > 60 mesh sieve, thereby increasing the lignin content of the thin sample.

The Tukey test showed that the thick and medium samples were the same in chemical constitution and differing from each other by only in the heat value analysis. These small differences may be attributed to the granulometries and relate to how combustion occurred.

The thin sample was statistically different in all analyses, as shown in Table 1, from the medium and thick samples. Thus, it is possible to infer that the particle size distribution

obtained from the milling of the EFB, had a strong influence on the differentiation of the thin sample in comparison to the medium and thick samples, as each physical element present in the EFB had different resistances to the cutting force applied during milling.

Evaluation of the Hydrothermal Treatment Parameters

Due the medium and thick samples being chemically the same, it was decided to perform the experiment using just the medium sample instead of the thick, because it had a larger contact surface and allowed a better fit inside the reactor, ensuring that the material was always in contact with water.

The results of the hydrothermal treatment for thin and medium samples are in Tables 2 and 3, respectively. The yield is the percentage of biomass converted into reducing sugars.

Table 2. Hydrothermal Treatment Yield of the Thin Sample

Run	Ratio (Sol./Liq.)	Temperature (ºC)	Pressure (bar)	Time (min)	Yield (%)
1	1/13.33	130	150	10	2.30 ^{a} ± 0.70
2	1/20	130	150	10	3.01 ^{ab} ± 0.12
3	1/13.33	170	150	10	8.73 ^{de} ± 0.60
4	1/20	170	150	10	8.21 ^{de} ± 0.24
5	1/13.33	130	200	10	4.25 ^{b} ± 0.43
6	1/20	130	200	10	3.32 ^{ab} ± 0.31
7	1/13.33	170	200	10	10.65 ^{f} ± 0.42
8	1/20	170	200	10	9.84 ^{f} ± 0.52
9	1/13.33	130	150	20	6.20 ^{c} ± 1.19
10	1/20	130	150	20	7.88 ^{cd} ± 0.34
11	1/13.33	170	150	20	16.23 ^{g} ± 0.42
12	1/20	170	150	20	16.10 ^{g} ± 0.24
13	1/13.33	130	200	20	8.14 ^{de} ± 0.34
14	1/20	130	200	20	8.72 ^{de} ± 0.67
15	1/13.33	170	200	20	17.29 ^{g} ± 0.87
16	1/20	170	200	20	17.48 ^{g} ± 0.9
The average results followed by the same letter in the same line do not differ each other by the Tukey Test at a 5% significance level.					

Run 7 showed a high yield at 10 min, which was 10.6%, and run 16 at 20 min was 17.5%, showing the high influence of the time on the efficiency of the hydrothermal treatment. The results showed that there was compensation between the time and temperature variables, because the runs with a higher temperature and less time had similar yields with runs with lower temperatures and longer reaction times. Runs lasting 20 min and 170 ºC showed that the pressure had no significant effect, although once there was pressure variation from 150 to 200 bar, the results were statistically the same.

In general, the runs that occurred at 10 min promoted higher yields when performed at higher temperatures, which were affected by the system pressure. This is because the yield at 150 bar was statistically different than the yield obtained at 200 bar, which are represented by letters A and F, respectively.

The hydrothermal treatment of the medium sample produced different results compared with the thin sample results, as shown in Table 3. Run 16 had the highest yield, which was 57.9%. There was a possibility of the hydrolysis still occurring after 20 min, because even after doubling the reaction time, the reducing sugars yield did not double, but it did show a significant increase.

Comparing the best yields of Table 2 and 3, it was verified that the medium sample had an efficiency that was 228% higher than the thin sample. The Tukey test revealed that the reactions that occurred at 130 ºC and 10 min were not influenced by the water and pressure variations, thus, presenting yields statistically equal, which are represented by the letter A. The same behavior occurred for runs carried out for 20 min under the same conditions, which are shown in the table by the letter B.

Comparatively, it is observed that the difference in results between the two particle sizes was more related to the chemical composition than the particle size itself, because, as shown in Table 1, the samples showed significant chemical differences, even though they were derived from the same raw material.

Table 3. Hydrothermal Treatment Yield of the Medium Sample

Run	Ratio (Sol./Liq.)	Temperature (ºC)	Pressure (bar)	Time (min)	Yield (%)
1	1/13.33	130	150	10	1.032 [a] ± 0.27
2	1/20	130	150	10	0.79 [a] ± 0.20
3	1/13.33	170	150	10	30.12 [c] ± 0.80
4	1/20	170	150	10	33.43 [c] ± 1.93
5	1/13.33	130	200	10	1.03 [a] ± 0.08
6	1/20	130	200	10	1.28 [a] ± 0.15
7	1/13.33	170	200	10	39.43 [d] ± 0.98
8	1/20	170	200	10	40.48 [d] ± 2.06
9	1/13.33	130	150	20	3.26 [b] ± 0.18
10	1/20	130	150	20	5.23 [b] ± 0.50
11	1/13.33	170	150	20	50.49 [e] ± 0.84
12	1/20	170	150	20	52.89 [e] ± 2.32
13	1/13.33	130	200	20	4.23 [b] ± 0.24
14	1/20	130	200	20	5.23 [b] ± 0.97
15	1/13.33	170	200	20	54.95 [f] ± 1.98
16	1/20	170	200	20	57.89 [f] ± 2.13
The average results followed by the same letter in the same line do not differ each other by the Tukey Test at 5% significance level.					

Statistical Evaluation

Thin sample

The Pareto chart (Fig. 2A) was generated to show which variables were most significant in the experimental design at a 95% confidence level. The results showed that only the solid/ratio variable was not significant, *i.e.*, the amount of water, under the studied conditions, did not influence the yield of reducing sugars, but the interactions between water versus temperature and water versus time were significant, implying that the influence of water was always associated with another variable.

The time and temperature variables had the same significance, as well as the interaction between itself. The pressure was statistically significant when evaluated without any interaction, which was most likely due to the amount of CO_2 compressed at 150 bar was sufficient to generate the carbonic acid inside the reactor and influenced the conversion of the sample into reducing sugar.

The Pareto chart shows that time and temperature were the variables with larger effects, thus, a response surface graph (Fig. 2-B) using these variables was generated. The graph confirmed previous results that the most intense conditions of the experiment provided higher yield of reducing sugar over time with increasing temperature.

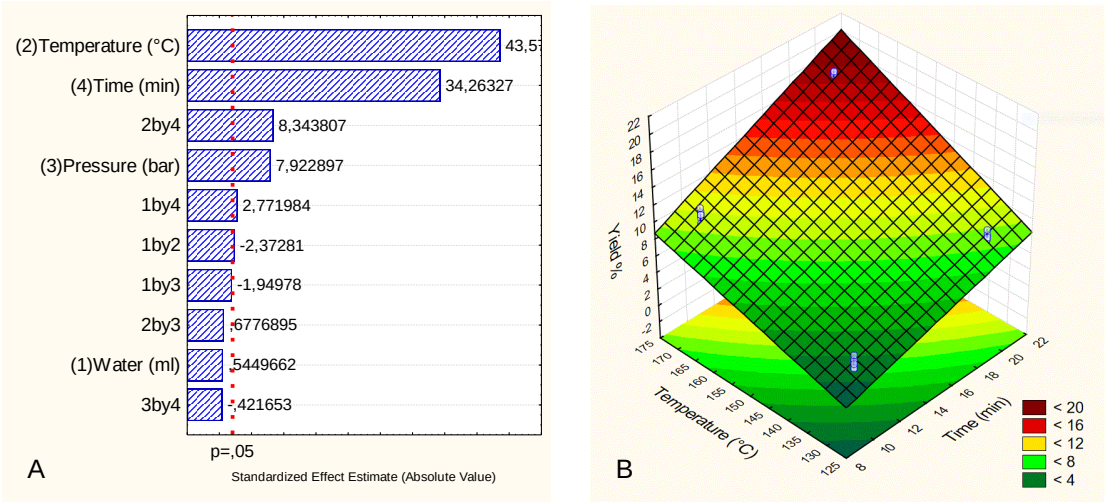

Fig. 2. A - Pareto chart for the results from the hydrothermal treatment of the thin sample. B - Response surface from the hydrothermal treatment yield of the thin sample

Medium sample

All variables had a significant influence on the hydrothermal treatment of the medium sample, as shown in the Pareto chart (Fig. 3A).

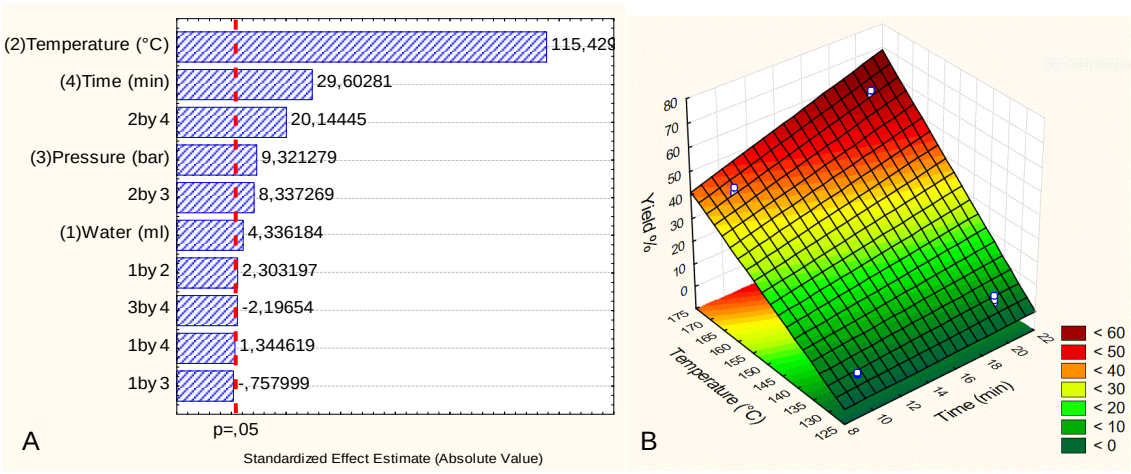

Fig. 3. A - Pareto chart for the results from the hydrothermal treatment of the medium sample. B - Response surface from the hydrothermal treatment yield of the medium sample

Hydrothermal Treatments Effects

To verify how the cellulose, hemicellulose, and lignin were consumed, a new analysis was performed using the remaining solid material from the reactor. Run 16 was chosen for both samples because it had obtained the highest yield of reducing sugars. The results are in Table 4.

Table 4. Fiber Content in the Samples Before and After Hydrothermal Treatment

Fiber Fraction (%)	Thin Sample		Medium Sample	
	Before	After	Before	After
Cellulose	18.38	14.28	43.23	14.72
Hemicellulose	13.53	12.01	18.11	5.92
Lignin	38.28	36.92	13.92	9.79

The hydrolysis process deeply affected the sample structure, which means that other components that were not on focused on in this study may have been produced or degraded, considering that the fiber fractions do not have a sum of 100%. The consumption of fiber fractions during the process occurred differently for each run. The thin and medium samples had the cellulose as the most consumed fiber fraction. This implies that the cellulose is the more degradable material was independent of its concentration, or, in other words, the cellulose fiber fraction was more susceptible to conversion.

Another analysis was carried out to verify if an autoclave pretreatment favored the increase of the reducing sugars yield. This step consisted of autoclaving the medium sample for 45 min at 121 ºC, and then performing the hydrolysis in the reactor under the conditions of run 16. The results are in Table 5.

Table 5. Influence of Autoclaving in the Reducing Sugars Yield

Sample	Yield (%)
Medium sample *in natura*	0.0121 [a] ± 0.0038
Medium sample (only autoclave)	0.0264 [a] ± 0.0042
Medium Sample (autoclave + hydrothermal treatment)	60.83 [b] ± 2.3781
Medium Sample (only hydrothermal treatment)	57.89 [b] ± 2.1343
Analyses were performed in triplicate; The average results followed by the same letter in the same line, do not differ each other by Tukey test at 5% significance level.	

The results demonstrated that the autoclaving step did not promote a significant increase in the yield, but the pretreated sample promoted an increase yield of 2.94% higher than the best experiment without the autoclaving step (run 16 with a 57.9% yield). Therefore, the pretreatment exposed the inner fibers of the sample, facilitating the later hydrothermal treatment. However, this step did not promote the production of a significant amount of reducing sugars. In fact, this step would be more of a cost than an effective artifice to increase the yield.

CONCLUSIONS

1. The hydrolysis of fractions (lignin, cellulose, and hemicellulose) during hydrothermal processing for empty fruit bunch occurred for all the samples, but the cellulose and hemicellulose were the most consumed.
2. The medium sample provided the highest yields of reducing sugar.
3. Statistical analysis showed that longer times (20 min) and higher temperatures (170 ºC) provided higher yields of reducing sugars.
4. The autoclaving step did not promote an increase in yield.

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