Oxidative treatments of citric industrial by-product towards biotechnological valorization

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ABSTRACT

Brazil, globally renowned for citrus production, faces the challenge of underutilizing agricultural waste generated in its prolific agroindustry. Since the 1972 Stockholm Conference and the rise of green chemistry, the pursuit of waste reuse has become crucial, aligning with the generation of higher value-added products. This study aimed to assess the enzymatic hydrolysis of citrus by-products, exploring pre-treatments with ultraviolet light (UV), hydrogen peroxide (H_2O_2), and iron ion (Fe). Experiments analyzed substrate variation and the effects of different pre-treatments in the enzymatic hydrolysis performance. The results indicated that pre-treatment using UV combined with H_2O_2 followed by enzymatic hydrolysis, are effectives in generating fermentable sugars from citrus by-products, which can be employed to produce high value-added biomolecules through a circular economy concept.
Keywords: enzymatic hydrolysis, pre-treatment, citrus by-product, circular economy.

1 INTRODUCTION

Brazil is part of the world's top five agricultural exporters and has been consolidating its positions as a prominent producer of agricultural products[11]. The agricultural sector plays a vital role in Brazil, accounting for 24.8% of the country's Gross Domestic Product (GDP), production stands out with crops such as corn, soybeans, sugarcane, and oranges[1]. In 2023 Brazil was the leading producer of oranges, producing 16 million tons of oranges[11]. The citrus production generates large amounts of agro-industrial subproducts, this product is rich in cellulose (11%), lignin (21%), and pectin (5%), which makes this product ideal for the biotechnology industry[6].

Biorefinery and biotechnology have emerged as a “greener” solution to industrial problems being able to replace chemical reactions to bioprocess, an example is biocatalytic alternatives for oxidative conversions[12]. Furthermore, biotechnology was responsible for bringing a new perspective to industrial residues, allowing the creation of new subproducts of these residues that would go to waste. In the Brazilian context, biorefinery and biotechnology has evolved to better use of agricultural residues, in this context enzymatic hydrolysis is a promising method to value citrus waste, replacing non-renewable substrates in microbial processes, and generating fermentable glucose[6].

The enzymatic hydrolysis reactions consist in the breaking of chemical bonds through water catalyzed by an enzyme, used mainly as an alternative to acid catalysis[9]. When analyzing the chemical structure of citrus by-products, the primary component is lignin and cellulose, forming lignocellulosic compounds that when hydrolysed releases glucose[6]. But, lignocellulosic compounds have rigid structure, that means that strong chemical bonds hinder the access of the enzyme to the subtract, lowering the efficiency of the reaction. As a solution to this problem pre-treatment step can be added, before undergoing hydrolysis, this process aims to enhance the hydrolysis reaction by weakening chemical bonds, which means less force of attraction between molecules[7]. This process allows a better enzyme-substrate bound, resulting in higher yield of the hydrolytic reaction.

In conclusion, enzymatic hydrolysis emerged as a solution to agricultural waste, allowing the citrus by-product to become a new product (glucose) with higher added value. Beyond that, the fermentable sugars generated from hydrolysis can be used for many different applications such as the production of biofuels, bio inputs and natural colorants[9]. The main objective of this work is to use an agricultural residue that has no longer use for industry, and give a new and greener vision to by-product, doing that by studying how different pre-treatments can affect the efficiency of the enzymatic hydrolysis.

2 MATERIAL & METHODS

The citrus by-product (CB) used in this work was kindly provided by *Cutrale ®*. The material was ground to standardize granulometry (molecules diameter equal to 0.22 cm), and dried to standardize humidity. After that the CB underwent a soluble sugar extraction process in water at 150 rpm/25 °C/1 h. The solution was vacuum-filtered and the filtrate was stored for future analyses, while the retained material proceeded to subsequent experiments. For the pre-treatment stage[2], different reactions were studied, using ultraviolet light (UV), hydrogen peroxide (H_2O_2), iron (Fe). The first reaction studied was the control group, or the group that had no treatment (NT). The second group studied the effects of UV light, in this group was added 4 sub groups, so first sub-group analyzed the effects of only UV light, second subgroup studied Uv light combined with hydrogen peroxide (UV+H_2O_2), third subgroup studied Uv light combined with iron (UV+Fe), and the last subgroup analyzed Uv light combined with hydrogen peroxide and iron (UV+Fe+H_2O_2). The last reaction studied was the effects of pre-treatments without Uv light being divided in three small groups, only iron (Fe), only hydrogen peroxide (H_2O_2), and hydrogen peroxide and iron (Fe+H_2O_2). The assays were prepared with a fixed consistency of dried solid content of 4.5% (w/v). The material from the UV assay was kept in a beaker in an oxidative chamber under constant agitation at 25 °C/30 min, in the presence of a UV-C Germicidal lamp. The control untreated condition involved CB, buffer solution without iron sulfate and hydrogen peroxide, and the absence of UV light, under the same conditions. Subsequently, the material was autoclaved (121 °C.1 atm.15 min), filtered, and prepared for subsequent assays and analyses.

Enzymatic reaction was standardized at 10 filter paper units (FPU)[7]. Hydrolysis was done in Erlenmeyer flasks with CB and 20 mL of 50 mM acetate buffer at pH 4.8, on an orbital shaker at 150 rpm and 45°C for 8 h, in triplicate. Cellic-CTec-3 HS, donated by Professor Fernando Masarin of UNESP Araraquara. The sugars released by hydrolysis were analyzed using High-Performance Liquid Chromatography (HPLC). Glucose concentration was determined on a Shimadzu-LC 20AD chromatograph with cation exchange column AMINEX HPX-87H BIO-RAD©, in isocratic mode; flow rate of 0.6 mL/min at 60°C[3]. Statistical analyses of the assays were conducted using a Student t-test.

3 RESULTS & DISCUSSION

The citrus by-product (CB) from juice production contains peels, seeds, and orange membrane residues, rich in cellulose, lignin, and pectin. This composition enables its use in bioprocesses like fermentable sugar generation[6]. The aim of this work is to extract soluble sugars by washing the residue and bound sugars through pre-treatments followed by enzymatic hydrolysis (table 1). Initially, the soluble sugar extraction process released 13.28 g.L^{-1} of total sugars (glucose, fructose, and sucrose).

Table 1. Kinetics of glucose release using substrates pretreated with the addition of complexes and combinations (Fe + H_2O_2, H_2O_2 and Fe.), with UV-C light (UVFe + H_2O_2, UVH_2O_2 and UVFe) and NT as a negative control at a consistency of 4.5 % m.v^{-1}.

Assays	Time (h)				
	0	2	4	6	8
	Total Sugar (g.L^{-1})				
UV	0.08	0.38	0.89	1.56	1.22
UV+Fe	0.13	0.73	1.09	1.34	1.73
UV+ H_2O_2	0.19	1.21	2.08	2.17	2.90
UV+Fe +H_2O_2	0.07	1.40	1.99	2.34	2.94
Fe	0.11	0.42	0.30	1.66	0.69
H_2O_2	0.15	1.88	1.20	2.17	2.45
Fe +H_2O_2	0.19	1.22	1.55	2.09	2.81
NT	0.00	0.83	0.72	0.79	1.82

Source: Author data.

Legend: The hydrolysis tests were carried out in triplicate on orbital shaker for 150 rpm/45°C/8 hours. The values described are the means (g. L^{-1}) of the triplicates and the error bar indicates the sample standard deviation.

Table 1 highlights that CB treated with UV+Fe+H2O2 and UV+H2O2 exhibited superior efficiency in total sugar release compared to other studied conditions. This phenomenon is attributed to photocatalysis, which excites electrons from the valence band to the conduction band, enabling charge transfer and transport, thereby altering the lignocellulosic structure, weakening fibers, and shortening chains[9]. There was no statistically significant difference between UV+Fe+H2O2 and UV+H2O2. Finally, an experimental hydrolysis at a consistency of 10% w/v aimed to evaluate the influence of consistency variation on the amount of released sugars (Table 2).

Table 2. Glucose concentration after pre-treatment and enzymatic hydrolysis conducted at a consistency of 10% w. v^{-1}

Sample	Glucose (g.L^{-1})
Washing water	2.52
UV + H_2O_2 treatment	4.69
Results of hydrolysis in the condition of 10% in the time of 6 hrs	2.41

Source: Author data.

When comparing the consistencies of 4.5% and 10% (w/v), it becomes evident that, as anticipated, the higher consistency (10%) led to a greater availability of sugar (61% more glucose released) . Furthermore, upon comparing these findings with those reported[10], it is noteworthy that the enzymatic hydrolysis of the substrate following UV+H2O2 pretreatment released approximately 200% more glucose than documented in a previous study of enzymatic hydrolysis of citrus by-product by Cypriano, 2017[10].

4 CONCLUSION

The results obtained demonstrate the effectiveness of enzymatic hydrolysis for the generation of fermentable sugars, which showed remarkable efficacy in glucose release, particularly when the substrate consistency was 10% w/v. Additionally, the study addressed the importance of pre-treatments in the sugar release process. Specific pre-treatments, such as exposure to ultraviolet light (UV) and the addition of Fe+H_2O_2 and H_2O_2, demonstrated improvements in glucose release efficiency, being able to obtain glucose from hydrolysis and soluble sugars.

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