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Full Length Article

Effect of Pretreatments on Physicochemical Characteristics of Olive Pomace and on Production of Cellulases from *Trichoderma reesei* RUT C30 under Solid-State Fermentation

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Abstract

Olive pomace (OP) is a cheap and abundant agricultural by-product that could be valorized by different biotechnological processes. The present study was conducted to better understand the effects of alkaline, milling and thermal pretreatments on OP for obtaining high value-added products (cellulases). *Trichoderma reesei* RUT C-30 fungus was used for cellulases production on OP substrate under Solid-State Fermentation (SSF) process and cellulases activity was assessed by the filter paper method (*FPase*). The effect of the three pretreatments and their combinations on physico-chemical composition and cellulases production was investigated. Results showed that untreated OP was a favorable environment for the growth of *T. reesei* and a good fermentation substrate that gave *FPase* activity of 0.83 IU/g DS. The chemical composition (lipids, proteins, carbohydrates and ash) was significantly *($P < 0.05$)* affected by the different pretreatments as well as their combinations. Regarding the fiber fraction, alkaline and thermal pretreatments did not affect the NDF content, while a remarkable decrease (29.88%) was recorded after milling pretreatment. Alkaline pretreatment decreased significantly the cellulose contents whereas milling increased it of 35%. ADL fraction was only decreased by the milling treatment of 42%. No significant effect of thermal pretreatment was noticed on ADL and cellulose. The alkaline pretreatment with 1% NaOH improved the cellulase activity to a value of 1.28 IU/g DS, while lower yields were obtained after milling (0.2 IU/g DS) and thermal (0.15 IU/g DS) pretreatments. This study showed that only alkaline treatment improved the production of cellulase from OP without being combined with milling and thermal treatment.

Keywords: Olive pomace; *Trichoderma reesei*; Pretreatments; Cellulases; Solid-state Fermentation

Introduction

The world production of olive oil is valued to be more than 18 million tones/year (Coimbra *et al.* 2010) and Algeria covers more than 1.5% of this production (Stamatelatou *et al.* 2012). 42% of the national production of olive oil is located in the central region: Béjaia, Bouira, TiziOuzou and Jijel (Rives 2021). However, olive oil extraction process produces great amount of wastes. According to Nefzaoui (1991), 100 kg of olives produced about 35 kg of crude olive pomace and 100 liters of vegetation water. Depending on the extraction method, olive pomace (OP) can reach up to 30–40% of olive oil production (Aliakbarian *et al.* 2011). Olive pomace by-product contains fragments of skin, pulp, stones and oil (Mirabella *et al.* 2014). OP is composed of lignin (31%), hemicellulose (24%), cellulose (14%), fat (11%), soluble sugars (6.5%), protein (6%) and many mineral salts (Roig *et al.* 2006). This physico-chemical composition of OP depends principally on the type and origin of olives, environmental conditions and storage time (Papaioannou *et al.* 2013). Several studies have proven the negative effects of this solid by-product on the microbial flora of the soil and even on the aerial environment (Aranda *et al.* 2007). Therefore, it is important to manage these wastes in order to minimize their negative effects on the environment. In fact, olive pomace contains valuable raw material such as a great proportion of organic matter and a varied range of nutrients, which could be used for energy generation, as an animal feed or as a fermentation substrate in biotechnological means for bio-fuels biofuels or enzymes

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production (Roig *et al.* 2006; Roussos *et al.* 2009). Still, some obstacles are associated with effective utilization of lignocellulosic residues for enzymes production. The main constraint is the recalcitrance of the plant cell walls of the lignocellulosic fractions (Kumar and Sharma 2017) to develop effective and low-cost pretreatments as potential ways of altering the structure and improving the degradability of lignocellulose biomass (Hendriks and Zeeman 2009). In literature, several pretreatments were described taking into account several aspects: (1) mechanisms concerned (2) advantages and disadvantages and (3) economic valuation (Menon and Rao 2012). According to the National Research Council (1999), an effective pretreatment must preserve the hemicellulose fraction, minimize the production of growth inhibitors of fermentation microorganisms and reduce energy costs.

Different pretreatments have been investigated on olive solid wastes, including physical (Neifar *et al.* 2013; Leite *et al.* 2016), chemical (López-Linares *et al.* 2013; Pellera *et al.* 2016; Erdocia *et al.* 2017), thermal (Fernández-Bolaños *et al.* 2001; Aliakbarian *et al.* 2011) and biological (Haddadin *et al.* 2009) methods and various combinations thereof (El-Ghonemy *et al.* 2014; Ouyang *et al.* 2018). Physical pretreatment means include mechanical deterioration and irradiation that lead to structural disruption and reduction of the particle size, degree of polymerization and crystallinity of the raw material (Cara *et al.* 2007; Ravindran and Jaiswal 2016), which increases the enzymatic digestibility of cellulose and hemicelluloses in the lignocellulosic biomass (Mtui 2009). Chemical pretreatments are performed using alkalis (NaOH, Ammonia), acids (H_2SO_4), oxidants (Ozone, Oxygen, H_2O_2), organic solvents (Alcohols, Organic acids) or ionic liquids (Organic salts) (Gandla *et al.* 2018). Chemical pretreatments favors hydrolysis of lignocellulosic biomass by eliminating hemicelluloses, disrupting lignin or reducing the crystallinity of cellulose during processing (Mosier *et al.* 2005; Zheng *et al.* 2009). Among these, alkali has been most extensively investigated. The use of alkali causes the degradation of ester and glycosidic side chains causing in structural modification of lignin fraction, separation of structural bonds between lignin and carbohydrates, cellulose swelling and its partial decrystallinization, in addition to a dissolution of hemicellulose (Zheng *et al.* 2009; Brodeur *et al.* 2011). Thermal pretreatments are effectively used on an industrial scale for lignocellulosic residue processing: hydrothermal, steam-explosion and hydro-chemical pretreatments are attested to cause elimination of hemicelluloses without being hydrolyzed, re-localization of lignin and hydration of cellulose and at the same time, swelling the pore size of the fibers which facilitate the enzyme accessibility (Gandla *et al.* 2018). In biological pretreatments, lignocellulosic degrading fungi are used to reduce the lignin barrier from the biomass prior to fermentation. Although, this pretreatment is only significant if combined with other pretreatments (Vasco-Correa *et al.* 2016). Filamentous fungi species are recognized for their high aptitude to secrete large amount of enzymes into their environment, making them very interesting for industrial enzyme production (Gudynaite-Savitch and White 2016; Srivastava *et al.* 2018). *Trichoderma reesei* is the most used fungus in enzyme industry, particularly for cellulases (Jun *et al.* 2011; Hinterdobler *et al.* 2021). *Trichoderma* cellulases are presently used in many industries such as textile, food, biofuel production, agriculture, animal feed, paper and pulp industries (Linke *et al.* 2015; Imran *et al.* 2016). Cellulases enzymes catalyze the bioconversion of cellulose into fermentable sugars. Cellulases complex are formed of three types of enzymes: endo-1,4-*β*-D-glucanase, exo-1,4-*β*-D-glucanase and *β*-glucosidase (Paloheimo *et al.* 2016). The production of cellulolytic enzymes by *T. reesei* has been the subject of various studies using different substrates (Belal 2013; Pirota *et al.* 2014; Abdullah *et al.* 2016). Among the several mutants of *T. reesei*, *T. reesei RUT-C30* is known to be one of the best producing cellulolytic strain studied (Aftab and Vermette 2008; Dhillon *et al.* 2011; Fonseca *et al.* 2020). Solid-state fermentation (SSF) process has been used for the cultivation of filamentous fungi because it simulates their living conditions in their natural habitat (Ugwuanyi *et al.* 2009; Ray and Behera 2017). This process includes absence or near absence of free water. The SSF is an attractive way to produce cellulases from microorganisms because of its lower capital cost investment, simpler equipment and higher productivity (Ray and Behera 2017; Soccol *et al.* 2017).

The use of olive pomace as substrate in fermentation processes for cellulases production requires pretreatments due to the heterogeneity and complexity of this lignocellulosic biomass. Several studies have been previously carried out but, to the best of our knowledge, no study has included at the same time, three pretreatments with their combinations, and provides elements of answer to their effects on the physicochemical parameters, the fiber fraction and the kinetics of cellulases production. Consequently, the main objective of this research was to valorize the OP biomass from Jijel region (Northen-east of Algeria) as a naturel medium for cellulases production using *Trichoderma reesi* RUT-C30 using three major combined pretreatments namely: alkaline pretreatment with different concentrations of NaOH (1, 3, 5 and 7%), mechanical milling and thermal pretreatment.

Materials and Methods

Substrate

In this study, olive pomace, used as substrate for the solid-state fermentation, was provided by a traditional oil mill located in Jijel region (Northern Algeria). After the oil pressing operation, fresh olive pomace was immediately collected, transported to the laboratory and kept in sealed bags at -20°C for further analysis. Then, divided into four

batches, three of them have undergone different pretreatments, namely mechanical milling, alkaline and thermal treatments, while the last batch has been reserved as is for the comparison of the results.

Substrate pretreatments

Mechanical pretreatment (milling): Olive pomace (OP) samples from the batch 1 were pretreated according to the method of Haddadin *et al.* (2009). Samples were oven-dried at 65°C for 48 h. After that, dried olive pomace samples were crushed into fine particles using a mechanical grinder with three-phase motor and then separated through a sieve with a porosity of 1.25 mm. The fine powder was recovered and stored at -20°C in closed containers.

Alkali pretreatment: OP samples of the batch 2 were chemically treated with alkaline solution of sodium hydroxide (NaOH) according to Bansal *et al.* (2012). 20 g of substrate was weighed in Erlenmeyer flasks, then 100 mL of NaOH solution prepared at different concentrations (1, 3, 5 and 7% w/v) were added and left at room temperature standing for 2 h. After soaking, the treated substrates were filtered through a metal sieve and carefully washed with distillated water until the pH of the washing water become neutral. The washed residues were then oven-dried at 65°C during 48 h. Treated samples were then kept frozen at -20°C prior to fermentation.

Thermal pretreatment: OP samples of the batch 3 were treated thermally by using boiled water. Aliquots of 20 g of each sample were boiled in 100 mL of distilled water for 2 h on hotplates in thermo resistant flasks. The treated samples were then drained in a metal sieve and left to cool. After that, the residues were oven-dried at 65°C prior to analysis.

Physicochemical characterization of olive pomace

In order to evaluate the chemical composition of all OP samples before and after treatment, physico-chemical analyses were carried out. The pH of OP samples was measured by pH-meter (Hanna, pH 210, Romania) following the method of Haddadin *et al.* (2009), OP samples were extracted by mixing 5 g of OP with 50 mL of distilled water on magnetic stirrer for 30 min. Moisture content and dry matter (DM) of OP samples were determined gravimetrically after drying at 105°C to constant weight (Moftah *et al.* 2012). Moisture and Dry matter were expressed in percentage (%). The ash content was assessed through incineration in a muffle furnace (Nabertherm GmbH, Germany) at 550°C, from aliquots of 5 g of each OP sample until obtaining white ashes of constant weight. Ash content is expressed in percentage. Total nitrogen of OP samples was analyzed by the Kjeldahl method (AOAC method number 954.18-B, 1990) using a semi-automatic Kjeltech apparatus (BUCHI, Digest Automat + Distillation Unit, Germany). Nitrogen content was expressed in percentage on dry weight basis. The crude protein content of OP samples was calculated by multiplying the total nitrogen values by 6.25 to obtain percentages. Lipid content of OP samples was determined by solvent extraction from 1g aliquots using hexane (25 mL) at 130°C during 45 min using the Soxhlet Apparatus (FOSS, Soxtec^{TM} 2043 Sweden). Lipid content was expressed as percentage of dry matter. The fiber contents of OP samples namely, neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL) were evaluated by the method of Soest and Robertson (1979), using the semi-automated Fibertec apparatus (FOSS Fibertec2010, Sweden). Fiber contents were expressed in percentage on dry weight basis. The total carbohydrates content was determined by the phenol-sulfuric acid method described by Dubois *et al*. (1956), after hot extraction of 1 g of OP samples using 16 mL of ethanol 80% at 100°C during 30 min. Extracts containing carbohydrates were recovered by centrifugation (5000 rpm/10 min) and pellets were re-extracted twice in the same conditions, then the volume of the extracts was made up to 100 mL with ethanol 80%. Reducing sugars content of OP extracts was determined according to the method described by Miller (1959) using the colored dinitro-3, 5-salicylic acid (DNS) reagent. Carbohydrates and sugars contents were expressed as percentage on dry weight basis. All treatments and measurements were carried out in triplicate.

Fungal strain and spore suspension preparation

The fungal strain *Trichoderma reesei* RUT C30 was provided by the Industrial Microbiology Laboratory of the University of Reims Champagne-Ardenne (France). Suspension of spore was prepared by incubating the cultures of fungus on PDA plates at 30°C for about 5 or 7 days, then spores were collected by washing with 10 mL of sterile water containing 0.1% (v/v) of Tween 80 and the prepared suspension was adjusted to a concentration of approximately 3×10^7 spores/mL, using Malassez counting chamber.

Enzymes production under solid-state fermentation

The solid fermentation was carried out in 250 mL Erlenmeyer containing 5 g of substrate (fresh or pretreated OP) and then humidified by distilled water to a proportion of 1:1 (w/v) taking into account the initial moisture of each substrate. The preparations were sterilized at 121°C for 20 min. Once cooled, the sterilized substrates were inoculated with spore suspension previously prepared (3×10^7 spores/mL) then incubated at a temperature of 30°C for 6, 12, 18, 24 and 30 days of static fermentation. The operation was performed in triplicate.

Extraction of crude enzyme

Cellulases enzymes were extracted by mixing the fermented

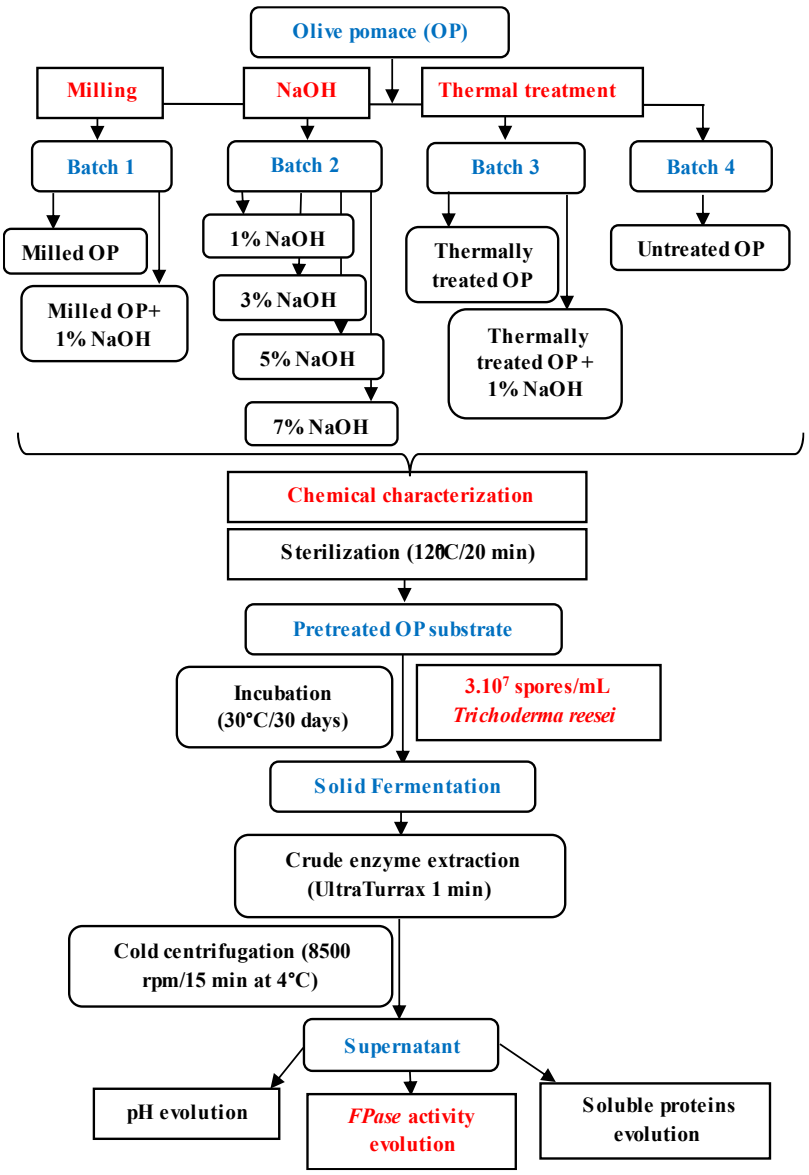

Fig. 1: Scheme of the principal steps of the experimental work including substrate pretreatments and fermentation process.

OP with 50 mL of distilled water and homogenized by UltraTurrax (IKA, T25 digital, Germany) for 1 min. The supernatants were recovered after cold centrifugation at 8500 rpm for 20 min at a temperature of 4°C. Supernatants will be used for the determination of the pH, soluble proteins and for measuring the total cellulolytic activity of the extracts.

Determination of total cellulase activity using filter paper (*FPase* activity)

Cellulase activity against Whatman No. 1 filter paper (W1FP) was measured as described by Silveira *et al.* (2014). *FPase* activity was determined by mixing 0.5 mL of citrate-phosphate buffer (0.05 *M*, pH 4.8) with 0.5 mL of the enzyme extract. After 10 min at 50°C, the W1FP strips, each weighing approximately 50 mg (1.0 × 6.0 cm), were added to the test tubes. The mixture was incubated in a water bath at 50°C for 60 min. The reducing sugars released after the enzymatic reaction were revealed by adding 1.5 mL of DNS reagent, placing the tubes in a boiling water bath for 5 min and adding 10 mL of distilled water and then measuring the absorbances at 540 nm by a UV/visible spectrophotometer (Agilent Technologies Cary 60 UV-Vis, Germany). The cellulolytic activity was expressed by the international unit, corresponding to one micromole of glucose released per minute and per mL of enzymatic extract under the assay conditions. The *APFase* is calculated by converting sample absorbances to released glucose concentration by linear interpolation from a standard curve of D-glucose used as reference.

Table 1: Chemical characterization of olive pomace substrates before and after milling, alkaline, thermal pretreatments and their combinations

Pretreatment	Moisture (%)	DM (%)	Ash (%)	Lipid (%)	TN (%)	Proteins (%)	TC (%)	RS (%)
Untreated OP	37.64 ± 1.47^{a}	62.36 ± 1.47^{d}	0.70 ± 0.05^{g}	7.68 ± 0.44^{b}	0.43 ± 0.03^{b}	2.68 ± 0.18^{b}	0.86 ± 0.10^{b}	0.68 ± 0.08^{b}
1% NaOH	2.51 ± 0.94^{c}	97.49 ± 0.94^{b}	1.11 ± 0.02^{e}	1.48 ± 0.15^{d}	0.25 ± 0.01^{d}	1.56 ± 0.01^{d}	0.21 ± 0.01^{cde}	ND
3% NaOH	0.69 ± 0.55^{d}	99.31 ± 0.55^{a}	1.25 ± 0.10^{de}	ND	0.32 ± 0.07^{c}	2.00 ± 0.46^{c}	0.23 ± 0.01^{cd}	ND
5% NaOH	0.83 ± 0.72^{d}	99.17 ± 0.72^{a}	1.51 ± 0.07^{bc}	ND	0.37 ± 0.04^{bc}	2.31 ± 0.27^{bc}	0.22 ± 0.03^{cd}	ND
7% NaOH	0.91 ± 0.72^{d}	99.09 ± 0.72^{a}	1.68 ± 0.10^{b}	ND	0.32 ± 0.03^{c}	2.02 ± 0.19^{c}	0.18 ± 0.01^{de}	ND
Milled OP	4.62 ± 0.04^{b}	95.38 ± 0.04^{c}	2.27 ± 0.03^{a}	18.30 ± 0.26^{a}	0.81 ± 0.04^{a}	5.08 ± 0.23^{a}	2.01 ± 0.05^{a}	1.33 ± 0.14^{a}
Milling + 1% NaOH	1.44 ± 0.01^{cd}	98.56 ± 0.01^{ab}	0.87 ± 0.07^{f}	0.63 ± 0.05^{e}	0.18 ± 0.02^{e}	1.14 ± 0.12^{e}	0.27 ± 0.02^{c}	0.22 ± 0.01^{c}
T° OP	2.51 ± 0.15^{c}	97.49 ± 0.15^{b}	0.16 ± 0.06^{h}	3.48 ± 1.38^{c}	0.41 ± 0.03^{b}	2.58 ± 0.20^{b}	0.18 ± 0.01^{de}	ND
T° OP + 1% NaOH	1.48 ± 0.11^{cd}	98.52 ± 0.11^{ab}	1.36 ± 0.21^{cd}	ND	0.13 ± 0.02^{e}	0.79 ± 0.15^{e}	0.15 ± 0.02^{e}	ND

All values were expressed in percentage (%) on dry substrate basis. Values with different letters in the same column (a, b, c, d, e, f, g, h) are significantly different ($P < 0.05$). DM, Dry matter; ND, Not detected; OP, Olive pomace substrate; T°, Thermal pretreatment; TN, total nitrogen; TC, Total carbohydrates; RS, reducing sugars.

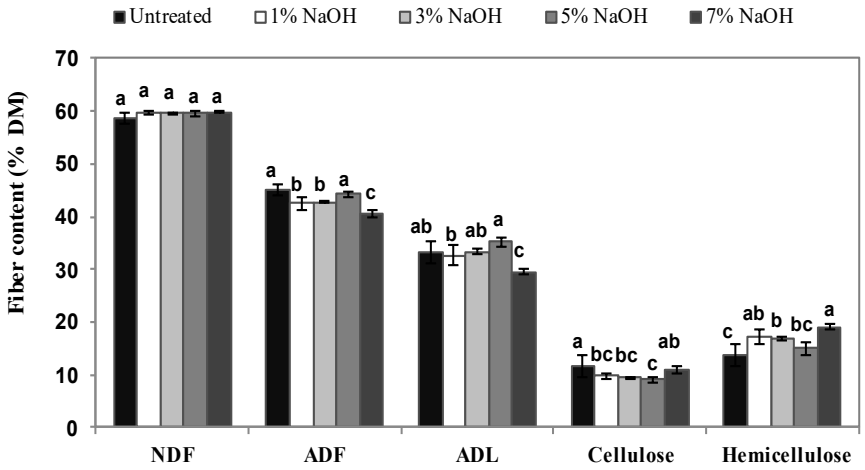

Fig. 2: Effect of Alkaline pretreatment (1, 3, 5 and 7% NaOH) on fiber composition of olive pomace substrate
Vertical bars indicate standard error of three replicates. Different letters for the same parameter (a, b, c) indicate significant differences ($P < 0.05$). DM, Dry matter; NDF, Neutral detergent fibers; ADF, Acid detergent fibers; ADL, Acid detergent lignin

Soluble proteins

The amount of soluble proteins in the crude extract was quantified according to the method of Bradford (1976), which is a colorimetric method based on the reaction of proteins with Coomassie brilliant blue G250 reagent (Bradford reagent). 100 μL of enzymatic extract are mixed with 2 mL of Bradford reagent. After stabilization of the color for 10 min, the absorbance of the reaction mixture is determined at 595 nm using a spectrophotometer (Agilent Technologies Cary 60 UV-Vis, Germany). Protein concentration was determined by a calibration curve using bovine serum albumin (BSA) as standard. The different pretreatment and fermentation steps performed above are summarized in Fig. 1.

Statistical analysis

All data were obtained from at least three independent assays. Results of the physico-chemical analysis are expressed in the form of mean ± standard deviation. The significance of the effect of pretreatments on each physico-chemical characteristic of OP samples was tested by the analysis of variance (ANOVA) followed by a post-hoc multiple comparison of means using LSD test using the software STATISTICA (version 5.5, Stat Soft Inc., USA). Differences with *P*-values < *0.05* were considered as statistically significant.

Results

Effect of different pretreatments on OP chemical composition

The effects of all pretreatment used in this study on the composition of OP are shown in Table 1. All treatments showed a significant decrease in the moisture content of OP substrates ($P < 0.05$). A significant increase ($P < 0.05$) in ash content is noticed for all pretreatments, except for thermal pretreatment where a significant decrease was observed. Regarding the lipid content, significant decrease was obtained after all pretreatments except the milling pretreatment which caused a significant increase ($P < 0.05$). Thermal pretreatment did not affect the protein content of OP substrate, however, milling

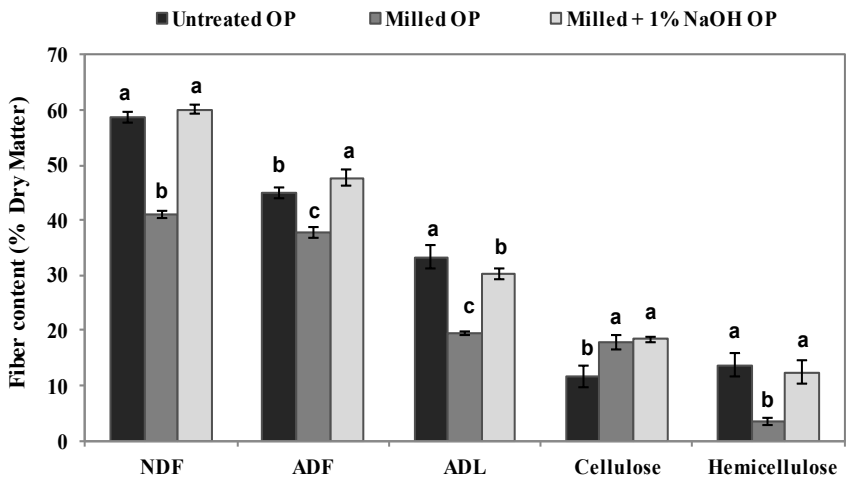

Fig. 3: Effect of milling pretreatment (combined or not with 1% NaOH) on fiber composition of olive pomace substrate
Vertical bars indicate standard error of three replicates. Different letters for the same parameter (a, b, c) indicate significant differences ($P < 0.05$). DM, Dry matter; NDF, Neutral detergent fibers; ADF, Acid detergent fibers; ADL, Acid detergent lignin

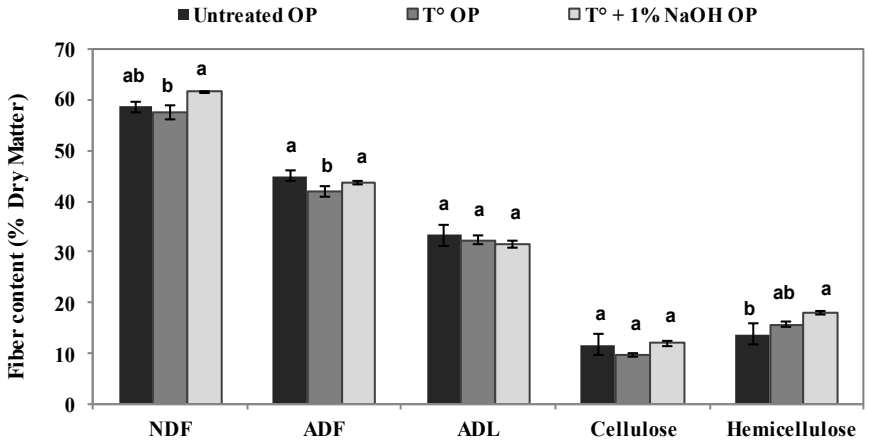

Fig. 4: Effects of thermal pretreatment (combined or not with 1% NaOH) on fiber composition of olive pomace substrate
Vertical bars indicate standard error of three replicates. Different letters for the same parameter (a, b, c) indicate significant differences ($P < 0.05$). DM, Dry matter; NDF, Neutral detergent fibers; ADF, Acid detergent fibers; ADL, Acid detergent lignin

increased significantly the proteins in OP substrate, while the other pretreatments caused its decrease ($P < 0.05$). Total carbohydrates and reducing sugar contents of OP substrates were significantly increased by milling, while the other pretreatments caused their decrease ($P < 0.05$), in addition to a total loss of reducing sugars.

Effect of different pretreatments on OP fiber composition

As shown in Fig. 2, NaOH pretreatment did not affect the NDF content. In parallel, a significant decrease was observed on ADF and cellulose contents, in contrary to hemicellulose fraction, where a significant increase was observed after alkaline pretreatments. Milling pretreatment caused significant decrease of NDF (29.88%), ADF (16.22%) and hemicellulose (74.60%) fractions compared to untreated OP (Fig. 3), whereas, combined pretreatment augmented significantly NDF (31.51%), ADF (21.01%) and hemicellulose (69%) fractions compared with the milled OP. It is interesting to note that after milling pretreatment, ADL fraction decreased of about 42% compared to the untreated OP, and a slight decrease of 9.3% was observed after combined pretreatment with 1% NaOH. Contrary to the trends noted for the previous fractions, the milling has augmented significantly the cellulose fraction of OP of almost 35%, the same effect was observed for the combined pretreatment. Regarding the thermal pretreatment, no clear significant effect was observed in the NDF and hemicellulose fractions after both pretreatments, while ADF fraction decreased slightly but significantly after thermal process (Fig. 4). Regarding ADL and cellulose fractions, no significant effect of

thermal pretreatment on olive pomace was noticed whether combined or not with 1% NaOH compared to untreated one.

Effect of alkaline pretreatment on the *Fpase* activity

As shown in Fig. 5a, the highest *FPase* activity (0.83 IU/g DS) was recorded at the 24th day of fermentation on untreated OP. This activity was improved by 35.15% after the alkaline pretreatment with 1% NaOH. In contrast, the activity decreased after alkaline pretreatment with 3, 5 and 7% NaOH by 38.55, 10.84 and 60.24%, respectively. It is worth noting that olive pomace used in this study was only moistened with distilled water, without nutrient medium addition for both untreated and pretreated OP substrates. Despite this, rapid and remarkable growth of the mycelium of *T. reesei* RUT C30 was observed from the third day of fermentation for untreated and pretreated OP with 1% NaOH compared to those pretreated with 3, 5 and 7% NaOH.

The evolution of soluble proteins (Fig. 5b) for all used substrates follows the same progression as the cellulolytic activity. The maximum content of soluble proteins (6.99 mg/g DS) corresponds to the optimum enzymatic activity for 1% NaOH pretreated OP (24th day of fermentation). It was also seen that extracts from the untreated substrate presented the highest soluble protein content in comparison with the other pretreated samples.

The pH evolution (Fig. 5c) showed the same shape for all the samples analyzed. From different initial values, the pH has decreased during the 12th first days of fermentation to reach values between 5.8 and 6.8, which represents the adequate pH for the production of cellulolytic enzymes by *T. reesei*. Taking into account the results of the alkaline pretreatment, 1% NaOH was the most effective pretreatment on OP substrate for cellulases production (*FPase* activity). Therefore, it was chosen for all further combinations with milling and thermal pretreatments.

Effect of milling pretreatment on the *Fpase* activity

The first notable result is that a slow fungal colonization was observed on milled OP substrate during the fermentation assay compared to OP pretreated with 1% NaOH. It was clearly shown in Fig. 6a, that the optimal cellulolytic activities obtained on milled and combined pretreatments were lower than the activity obtained on 1% NaOH pretreated OP. However, an improvement of the *FPase* activity was recorded when milling was combined with 1% NaOH.

It can also be noted, that protein contents (Fig. 6b) of milled OP combined or not with alkaline pretreatment during the fermentation period were much lower than those recorded in OP pretreated with 1% NaOH, which were proportional to the cellulolytic activity.

As shown in Fig. 6c, pH evolution of milled OP

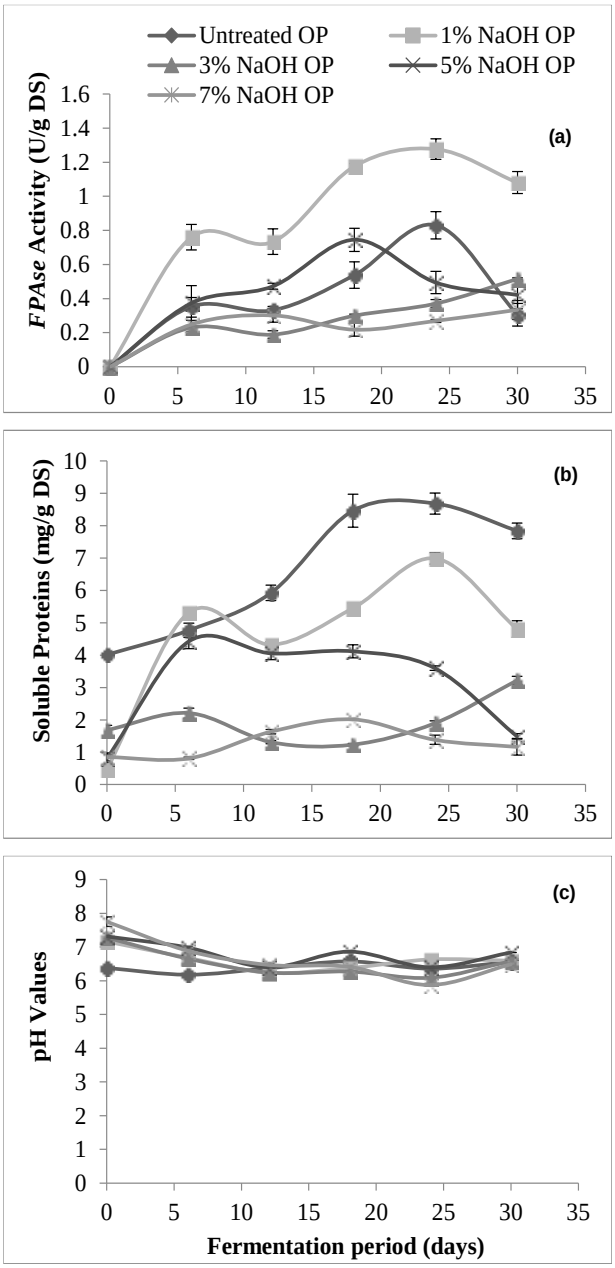

Fig. 5: Evolution of *PFase* activity (**a**), protein content (**b**) and pH values (**c**) of extracts obtained from *T. reesei* culture on alkaline pretreated plive pomace during 30 days of fermentation
Vertical bars indicate standard error of three replicates. OP, Olive pomace substrate; DS, Dry substrate; *FPase* activity, Filter paper cellulase activity.

during fermentation followed the same trend as soluble proteins, with a remarkable drop in pH value at the 12th day.

Effect of thermal pretreatment on *Fpase* activity

Thermal pretreatments either alone or in combination with 1% NaOH have negative effects on the cellulolytic enzyme production (Fig. 7a). A very slow mycelial growth was observed during the fermentation period on OP samples

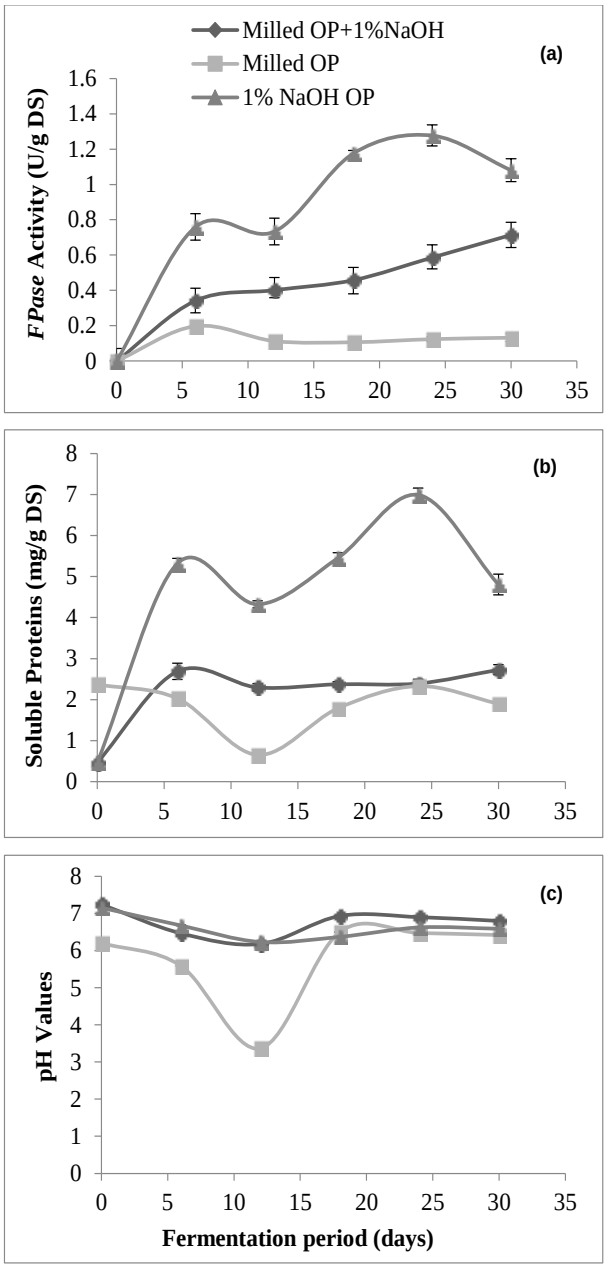

Fig. 6: Evolution of *PFase* activity (**a**), protein content (**b**) and pH values (**c**) of extracts obtained from *T. reesei* culture on milled olive pomace substrate during 30 days of fermentation
Vertical bars indicate standard error of three replicates. OP, Olive pomace substrate; DS, Dry substrate; *FPase* activity, Filter paper cellulase activity

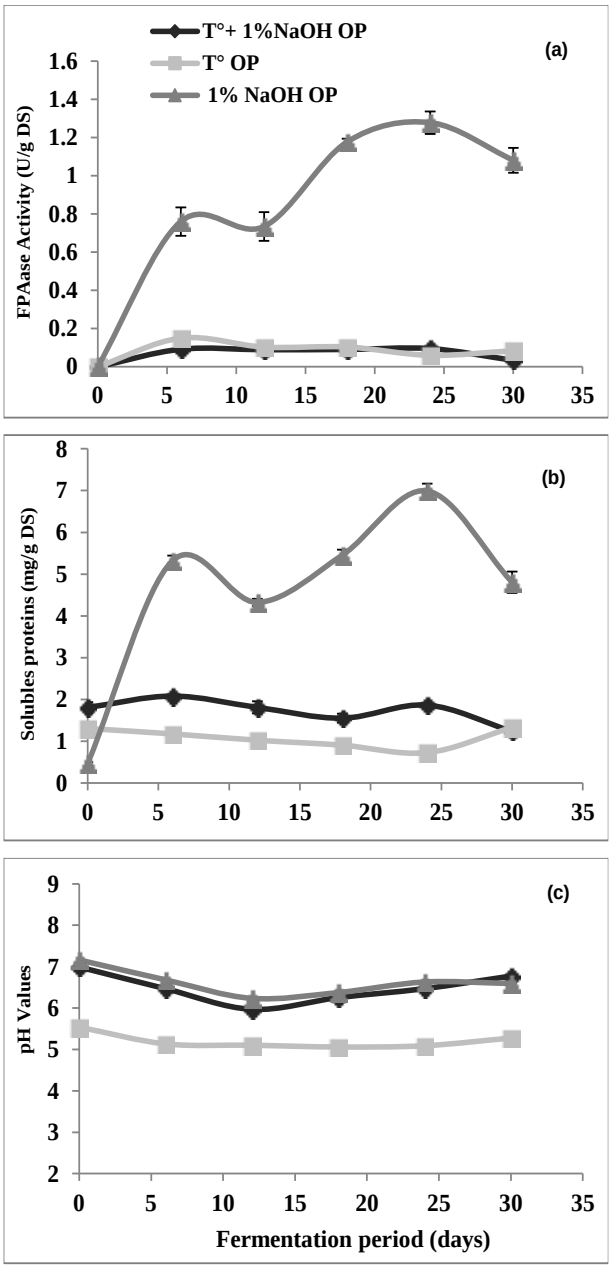

Fig. 7: Evolution of *FPase* activity (**a**), protein content (**b**) and pH values (**c**) of extracts obtained from *T. reesei* culture on thermal pretreated olive pomace substrate during 30 days of fermentation
Vertical bars indicate standard error of three replicates. OP, Olive pomace substrate; DS, Dry substrate; *FPase* activity, Filter paper cellulase activity

thermally pretreated, combined or not with 1% NaOH. The results of *FPase* activities showed that the highest values were obtained on the 6th day of fermentation on thermal pretreated OP alone or combined with 1% NaOH, but these values were widely lower than the obtained activity with alkali pretreated OP.

As illustrated in Fig. 7b, the soluble protein contents during the fermentation period followed the same evolution as *FPase* activities. Soluble protein contents recorded for both thermal and combined pretreatments were much lower than those noted for alkaline pretreatment.

The pH evolution (Fig. 7c) showed stability during the fermentation period maintaining the initial pH values. As expected, the pH values obtained for combined pretreatment (temperature + 1% NaOH) were close to that of alkaline pretreatment, but higher than the thermal pretreatment alone.

Discussion

This study revealed that the alkaline pretreatment at different concentrations caused a significant decrease in lipid content of the OP (Table 1) which can be explained by the saponification process. The loss of carbohydrates is usually due to peeling and hydrolytic reactions (Hendriks and Zeeman 2009).

In order to better demonstrate the effect of alkaline pretreatment on fiber contents, the NDF, ADF, ADL, cellulose and hemicellulose contents were calculated taking into account the dry matter of untreated olive pomace (Fig. 2). It is important to note that NDF comprises lignin, cellulose and hemicellulose, ADF includes lignin and cellulose, while ADL represents only the lignin content. Alkaline pretreatment at different concentrations had no effect on NDF content. Unlike our findings, Pellera *et al*. (2016) found a significant increase of NDF content of OP after alkaline pretreatment with different concentrations of NaOH (1–16%). It can also be seen that ADF and cellulose contents decreased slightly but significantly The decrease of ADF is mainly the consequence of cellulose diminution after pretreatment.

The different pretreatments used in this study were designed to improve cellulose production by increasing the accessibility of *T. reesei RUT C30* fungi to the cellulosic fraction of the substrate. Alkaline pretreatment is frequently used as a mean to modify the originally complex and recalcitrant chemical structure of lignocellulosic biomass (Yoon *et al.* 2014).

Bali *et al*. (2015), in a comparison of various alkaline pretreatments, demonstrated that the highest increase in cellulose accessibility was found with dilute NaOH solution (2%). Moreover, El-Ghonemy *et al*. (2014) found that pretreated substrates by 1% NaOH were much more efficient on enzymatic hydrolysis compared to that treated by 4% NaOH. The same trend was reported also by Rodríguez-Zúñiga *et al*. (2015). In another study, Sun *et al.* (2008) reported that the highest *FPase* activity was found on alkali-treated rice straw compared to the untreated one using *T. reesei* Rut C-30 fungi. The improvement of cellulolytic activity after pretreatment with 1% NaOH (Fig. 3a), may be due to the swelling of the biomass that becomes more accessible for enzymes after solvation and saponification reactions caused by the alkaline treatment (Galbe and Zacchi 2007; Hendriks and Zeeman 2009). In fact, cellulose can be swelled or dissolved in NaOH solutions, leading to the decrease of lignocellulosic biomass crystallinity (Sun *et al.* 2016). Besides, the improvement of enzyme activity after pretreatment may be due to the fact that alkaline treatment did not make changes in the fibers composition but, it depleted the medium of available carbon source readily accessible for the strain used, like the lipids and carbohydrates. This situation promoted the induction of cellulolytic enzyme synthesis by the cellulose of the fermentation medium. Several authors have already affirmed this suggestion. Ballerini (2006) explained that the production of cellulases is regulated by the processes of induction and catabolic repression: induction linked to the presence of substrates (in this case cellulose) and repression by the sources of carbon molecules (such as glucose *via* catabolic repression mechanisms). Kaur *et al.* (2006) reported that it is difficult to deduce the nature of the inducers that will be valid for the known cellulolytic microorganisms but it is evident that the insoluble native cellulosic material is certainly the best substrate for the production of cellulases. Candace and Weimer (1991) suggested that close physical contact between fungus and cellulose can stimulate induction and assumed that there is an appropriate sites recognized in cellulose. It is also possible that this improvement of *FPase* activity after pretreatment with 1% NaOH is due to the nature of the cellulose used by *T. reesei* RUT C30. Since, there are two types of cellulose in OP composition. The cellulose located in pulp of OP easily accessible for *T. reesei* compared to that located in the OP stones (woody endocarp) which contains, in addition to cellulose, high levels of lignin. Knowing that the pure pulp of OP, contains about 20% only of the total crude cellulose as reported by Sansoucy *et al*. (1984). Besides the high amount of lignin in biomass, the enzymatic hydrolysis is also controlled by lignin location in biomass and its surface area which play a significant role (Kim *et al.* 2016). In addition, it was shown that alkali pretreatments are more effective on agricultural residues than on woody materials (Kumar *et al.* 2009). In the other hand, Oke *et al*. (2016) observed that the untreated mixed lignocellulosic substrates (MS) supported the highest cellulase production, in comparison to MS treated with 1% NaOH. Similar results were also found by Brijwani and Vadlani (2011) when soybean hulls were used as substrate.

The pH diminution showed in Fig. 3c can be explained by NH4 cations assimilation in the form of NH_3 which induce H^+ ions accumulation (Roussos *et al.* 1983). This diminution can be also related to the production of acidic metabolites that neutralize the NaOH to give lower pH. It was found in this study that a higher concentration of NaOH leads to a higher initial pH, and consequently, a high residual NaOH content in the substrate pores, since an important characteristic of alkali pretreatment is that the lignocellulosic biomass on itself absorbed some of the alkali: approximately 3 g NaOH/100 g of total substrate (Hendriks and Zeeman 2009). This affects the good growth of *T. reesei* strain and resulted in a slow mycelial growth on the solid residue and explained the low celluolytic activities obtained for OP pretreated with concentrations higher than 1% NaOH. Deswal *et al*. (2011) observed that increasing the initial pH of the medium from 5.5 to 10.0 leads to a significant decrease in cellulases production.

It appears that the milling pretreatment was more efficient on ADL fraction than the combined one (Fig. 4). This contradiction can be explained by the possible recondensation and reorganization of soluble lignin

compounds, solubilized during the alkaline pretreatment (Hendriks and Zeeman 2009; Pellera *et al.* 2016). Rodhe *et al.* (2011) observed about 75% loss of lignin on milled sorghum straw pretreated with 0.2 *M* of NaOH. The same fact was also found by Haddadin *et al*. (1999) when milled OP pretreated at 3%NaOH was used. After milling, the NDF and ADF diminutions are mainly the consequence of ADL decrease. Mtui (2009) reported that ADL decrease may be due to reduction of the degree of lignin depolymerization *via* the cleavage of uncondensed-aryl ether linkages. After combined pretreatment in reference to untreated OP, a significant increase in ADF and cellulose fractions was recorded, whereas NDF and hemicellulose remained unchanged, only ADL fraction was decreased. Our results are in agreement with the findings of Oke *et al*. (2016) who found that cellulose content of lignocellulosic substrates increased after milling pretreatment combined with alkali (1% NaOH). In contrast, Haddadin *et al.* (1999) observed a significant decrease in cellulose content of milled OP pretreated at 3% NaOH, these findings may be the result of the NaOH high concentration, compared to the dosage used in our research. The slow growth of *T. reesei* strain observed with milled OP during the fermentation period may be probably due to the heterogenic granulometry of milled sample, which contains particles with different sizes (below 1.25 mm diameter). In fact, the fine particles formed after milling of olive pomace in presence of high lipid contents (18.30%) causes a clogging that prevents an effective transfer of oxygen in the culture medium, unlike larger particles that improve breathing and aeration efficiency by increasing the inter-particle space. Moreover, Sanchez (2009) thought that the mycelial development is associated to the effective degradation of the lignocellulosic biomass that constitutes its carbon source. In fact, the best solid substrate should contain all the essential nutrients to the growing microorganism for optimal function (Bansal *et al*. 2012; Gordillo-Fuenzalida *et al*. 2019).

Although milling resulted in a decrease in lignin content, cellulase activity was not improved (Fig. 5a). According to Bali *et al*. (2015), lignin removal has been shown to increase the yield of enzymatic hydrolysis, however, the direct effect of lignin removal on cellulose accessibility is still not fully clear because lignin is also associated with cellulases inhibition, and the relative contributions of these two roles of lignin have not yet been fully defined. In addition, Berlin *et al.* (2006) stated that lignin depolymerization has been considered as an effective inhibitor of cellulases. The negative effect of milling process on cellulolytic activity may be due to the formation of inhibitor compounds after degradation of lignocellulose during pretreatment such as soluble phenolic compounds as reported by Vancov and McIntosh (2011) and Sun *et al*. (2016). In addition, Pellera *et al*. (2016) reported that phenolic compounds are produced by the degradation of lignocellulosic materials, and generally by hemicellulose and lignin solubilization. The low *PFAse* activity obtained on milled OP may also be due to the particle size of this substrate. In fact, several authors concluded that the pore size of the substrate is in relation to the size of the enzymes which constitute an important limiting factor in the enzymatic hydrolysis of lignocellulosic biomass (Chandra *et al.* 2007; Alvira *et al.* 2010). Therefore, Grethlein (1985) found linear correlations between the initial hydrolysis rate of pretreated biomass and the pore size accessible to a molecule with a diameter of 51 A° similar to the size of *T. reesei* cellulase components. Consequently, cellulase can get captivated in the pores of substrate if the internal area is much superior than the external area which is the case for many lignocellulosic substrates (Zhang and Lynd 2004).

Several authors reported that when milling is combined with alkaline pretreatment an improvement in cellulolytic activity was noticed. Bansal *et al*. (2012) found an enhancement of the cellulase production after alkali pretreatment (1% NaOH) using *Aspergillus niger* fungi cultured on milled agricultural and kitchen wastes. Belal (2013) have also observed a positive effect of combined pretreatment (milling + 5% NaOH) on conversion of polysaccharide into sugar by *T. reesei* cellulases on milled rice straw substrate. In another study, Wu *et al.* (2011) reported that milled bagasse pretreated with 2.5 *M* NaOH has given an enzymatic hydrolysis yield of 98.7%.

The remarkable decrease in soluble protein content of milled OP (Fig. 5b) was noted on the 12th day; this may be due to the assimilation of the medium proteins during mycelium growth (Roussos *et al.* 1983). The thermal pretreatment of olive pomace did not affect the ADL and cellulose fractions in comparison to the untreated one (Fig. 6). Aiello *et al.* (1996), in a study on sugarcane bagasse substrate, found unchanged lignin and cellulose fractions after combined pretreatment (100°C + 5% NaOH). The same finding was reported by Rodríguez-Zúñiga *et al*. (2015) when hydrothermal pretreatment (190°C, 10 min) was applied on sugarcane bagasse substrate, in term of lignin fraction. Moreover, Xiao *et al.* (2017) reported that cellulose and lignin are unaffected by the hydrothermal pretreatment. It seems that, this pretreatment has no effect on lignin fraction, this can be due to the spatial re-localization or reorganization of this later, which can occur with hydrothermal pretreatment (Kristensen *et al.* 2008). In addition, Agbor *et al.* (2011) explained that not all pretreatments result in substantial delignification: the structure of lignin may be altered without extraction due to changes in the chemical properties of the lignin. Thermal pretreatment combined with 1% NaOH caused a slight increase in hemicellulose fraction compared to the untreated OP, whereas, theoretically, thermal pretreatment causes a removal of hemicellulose of the solid fraction. In fact, this increase of hemicellulose was the result of alkaline pretreatment only, and as a consequence, thermal pretreatment had no effect on fiber fractions because of the hardness of olive pomace and the mild temperature applied during pretreatment. The study of Wu *et al.* (2011) revealed

that low thermal pretreatment (50°C) combined with 2.5 *M* NaOH significantly accelerated the removal of hemicellulose and lignin on sorghum bagasse substrate. Further, Hendriks and Zeeman (2009) reported that after thermal process, a certain part of the hemicellulose is hydrolyzed and produce acids. This leads to conclude that the improvement of the digestibility of lignocellulosic biomass depends on the nature of the substrate and the operating conditions (pretreatments combination). The low *FPase* activities obtained after thermal pretreatment represented in Fig. 7a curve may possibly be the result of changes in the chemical structure of olive pomace after thermal pretreatment because the enzymatic hydrolysis of lignocellulosic substrate could be influenced not only by the efficiency of the enzymes, but also by the physical, chemical and morphological characteristics of these biomass, as reported by Sun *et al.* (2016). It could also be explained by the removal of certain nutrients from the substrate after pretreatments (Abdullah *et al.* 2016).

In our study, a slight production of cellulase was noted, while Aiello *et al.* (1996) found no detectable activity during the fermentation using *Trichoderma reesei* QM 9414 on sugarcane bagasse treated with alkali (5% NaOH at 100°C). The authors suggested that the loss of activity could be the result of absorption of cellulase on cellulose and lignin or the inhibition of enzymes by glucose and cellobiose of the fermentation medium. This is an indication that in some cases, pretreatment of substrate prior to cellulose production might not be necessarily efficient, it could make a substrate less accessible and less suitable for microbial growth and fermentation when compared with the untreated one (Yoon *et al.* 2014). Several studies confirmed this trend, when higher cellulolytic enzyme production was obtained with untreated sugarcane bagasse (Rodríguez-Zúñiga *et al.* 2014; Ducom *et al.* 2019), wheat straw (Sharma *et al.* 2015), municipal solid wastes (Abdullah *et al.* 2016) and mixed lignocellulosic substrates (Oke *et al.* 2016) among others.

Gordillo-Fuenzalida *et al.* (2019), confirm the ability of the *Trichoderma* spp. to grow and produce cellulolytic enzymes in the presence of FMWs could enable the onsite production of cellulase enzymes in lignocellulosic bioethanol biorefineries. This would improve biorefinery economics by avoiding the purchase of commercial enzymes, while improving their environmental sustainability by utilizing waste materials, which goes in the line with our finding.

Conclusion

In the present study, local olive pomace (OP) was investigated as a substrate for solid-fermentation to produce cellulases enzymes from *Trichoderma reesei* fungi. Three different pretreatments and their combinations were applied on OP for the first time to improve the cellulases production. Results showed that alkaline pretreatment with 1% NaOH improved significantly the enzyme production from OP substrate. The other pretreatments (milling, thermal and their combinations) showed a negative effect on the improvement of cellulases production, besides being expensive and require high energy consumption. Therefore, olive pomace could be considered as a valuable substrate for fermentations process, especially for cellulases production given its chemical composition and fiber content.

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Author Contributions

Malika Boutiche: Conceptualization, Experimental work (enzymatic activity), Writing original draft, Writing review and editing. Fatma Sahir-Halouane: Supervision, Writing review and editing. Leila Meziant: Experimental work (enzymatic activity), Statistical analysis, Writing original draft. Fairouz Saci: Writing original draft. Kahina Oudjedi: Experimental work (pretreatments + enzymatic activity). Mouna Derdour: Experimental work (microbiology + physicochemical analysis). Karima Ouffroukh: Experimentral work (physicochemical analysis). Ibtissem Maghboune: Experimental work (physicochemical analysis). Samah Fiala: Experimental work (physicochemical analysis). Amel Bekrar: Experimental work (physicochemical analysis).

Conflicts of Interest

The authors decrare that they have no conflicting interests.

Data Availability

Data related to this article are not confidential and could be supplied on request to the authors.

Ethics Approval

Not applicable in this study.

References

Abdullah JJ, D Greetham, N Pensupa, A Gregory, GA Tucker, C Du (2016). Optimizing cellulase production from municipal solid waste (MSW) using solid state fermentation (SSF). *J Fund Renew Ener Appl* 6:3

Aftab A, P Vermette (2008). Culture-based strategies to enhance cellulose enzyme production from *Trichoderma reesei* RUT C30 in bioreactor culture conditions. *Biochem Eng J* 40:399–407

Agbor VB, N Cicek, R Sparling, A Berlin, DB Levin (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnol Adv* 29:675–685

Aiello C, A Ferrer, A Ledesma (1996). Effect of alkaline treatments at various temperatures on cellulase and biomass production using submerged sugarcane bagasse fermentation with *Trichoderma reesei QM 9414*. *Bioresour Technol* 57:13–18

Aliakbarian B, AA Casazza, P Perego (2011). Valorization of olive oil solid waste using high pressure–high temperature reactor. *Food Chem* 128:704–710

Alvira P, E Tomás-Pejó, M Ballesteros, MJ Negro (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis. *Bioresour Technol* 101:4851–4861

AOAC Official Methods of Analysis (1990). Method 945, 18–B. In: *Association of Official Analytical Chemists*, 15th edn. Arlington, Virginia, USA.

Aranda E, I Garcia-Romera, JA Ocampo, V Carbone, A Mari, A Malorni, F Sannino, A De Martino, R Capasso (2007). Chemical characterization and effects on *Lepidium sativum* of the native and bioremediated components of dry olive mill residue. *Chemosphere* 69:229–239

Bali G, X Meng, JI Deneff, Q Sun, AJ Ragauskas (2015). The effect of alkaline pretreatment methods on cellulose structure and accessibility. *Chem Sustain Chem* 8:275–279

Ballerini D (2006). Les Biocarburants: Etat des Lieux, Perspectives Etenjeux du Développement, Editions, Technip, Paris, France

Bansal N, R Tewari, R Soni, SK Soni (2012). Production of cellulases from *Aspergillus niger* NS-2 in solid-state fermentation on agricultural and kitchen waste residues. *Waste Manage* 32:1341–1346

Belal EB (2013). Bioethanol production from rice straw residues. *Braz J Microbiol* 44:225–234

Berlin A, M Balakshin, N Gilkes, J Kadla, V Maximenko, S Kubo, J Saddler (2006). Inhibition of cellulase, xylanase and *β*-glucosidase activities by soft wood lignin preparations. *J Biotechnol* 125:198–209

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254

Brijwani K, PV Vadlani (2011). Cellulolytic enzymes production *via* solid-state fermentation: Effect of pretreatment methods on physicochemical characteristics of substrate. *Enz Res* 2011:1–10

Brodeur G, E Yau, K Badal, J Collier, KB Ramachandran, S Ramakrishnan (2011). Chemical and physicochemical pretreatments of lignocellulosic biomass: A review. *Enz Res* 2011:1-17

Candace HH, PJ Weimer (1991). *Biosynthesis and Biodegradation of Cellulose*, Edition. Marcel Dekker, New York, USA

Cara C, I Romero, JM Oliva, F Sáez, E Castro (2007). Liquid Hot Water Pretreatment of Olive Tree Pruning Residues. *In: Applied Biochemistry and Biotecnology, ABAB Symposium*, pp:136–140. Mielenz JR, KT Klasson, WS Adney, JD McMillan (Eds). Humana Press, New York, USA

Chandra RP, R Bura, WE Mabee, A Berlin, X Pan, JN Saddler (2007). Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Adv Biochem Eng Biotechnol* 108:67–93

Coimbra MA, SM Cardoso, JALD Silva (2010). Olive Pomace, a Source for Valuable Arabinan-Rich Pectic Polysaccharides. *In: Carbohydrates in Sustainable Development I. Topics in Current Chemistry*. pp:129–141. Rauter A, P Vogel, Y Queneau (Eds). Springer, Berlin, Heidelberg, Germany

Deswal D, YP Khasa, RC Kuhad (2011). Optimization of cellulase production by a brown rot fungus *Fomitopsis* spp. *RCK2010* under solid state fermentation. *Bioresour Technol* 102:6065–6072

Dhillon GS, HS Oberoi, S Kaur, S Bansal, SK Brar (2011). Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray 632 fermentation employing mixed-culture of fungi. *Ind Crops Prod* 34:1160–1167

Dubois M, KA Gilles, TK Hamilton, PA Rebers, E Smith (1956). Colorimetric method for determination of sugar and related substances. *Anal Chem* 28:350–356

Ducom G, M Gautier, M Pietraccini, JP Tagutchou, D Lebouil, N Dumont, R Gourdon (2019). Caractérisation de grignons d'olives en vue d'une valorisation thermochimique 637 par gazéification. *Déch Sci Tech* 82:41–54

El-Ghonemy DHI, TH Ali, ME Moharam (2014). Optimization of culture conditions 639 for the production of extracellular cellulases *via* solid state fermentation. *Braz Microbiol Res J* 4:698–714

Erdocia X, E Ruiz, I Romero, MJ Diaz, E Castro, J Labidi (2017). Lignin characterization from two different pretreatments in bioethanol production processes from olive tree pruning. *Chem Eng Trans* 61:421–426

Fernández-Bolaños J, B Felizon, A Heredia, R Rodriguez, R Guillen, A Jimenez (2001). Steam-explosion of olive stones: Hemicellulose solubilization and enhancement of enzymatic 64 hydrolysis of cellulose. *Bioresour Technol* 79:53–61

Fonseca LM, LS Parreiras, MT Murakami (2020). Rational engineering of the *Trichoderma reesei* RUT-C30 strain into an industrially relevant platform for cellulase production. *Biotechnol Biof* 13:93–107

Galbe M, G Zacch (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv Biochem Eng Biotechnol* 108:41–65

Gandla ML, C Martin, LJ Jonsson (2018). Analytical enzymatic saccharification of lignocellulosic biomass for conversion to biofuel and bio-based chemicals. *Energies* 11:2936–2955

Gordillo-Fuenzalida F, A Echeverria-Vega, S Cuadros-Orellana, C Faundez, T Kähne, R Morales-Vera (2019). Cellulases production by a *Trichoderma* spp. using food manufacturing wastes. *Appl Sci* 9:4419–4420

Grethlein HE (1985). The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulose substrates. *Biol Technol* 3:155–160

Gudynaite-Savitch L, TC White (2016). Fungal biotechnology for industrial enzyme production: Focus on (hemi) cellulose production strategies, advances and challenges. *In: Gene Expression Systems in Fungi: Advancements and Applications, Fungal Biology*, pp:395–439. Schmoll M, C, Dattenböck (Eds). Springer, Switzerland

Haddadin MS, J Haddadin, OI Arabiyat, B Hattar (2009). Biological conversion of olive pomace into compost by using *Trichoderma harzianum* and *Phanerochaete chrysosporium. Bioresour Technol* 100:4773–4782

Haddadin MS, SM Abdulrahim, GY Al-Khawaldeh, RK Robinson (1999). Solid-state fermentation of waste pomace from olive processing. *J Chem Technol Biotechnol Intl Res Process Environ Clean Technol* 74:613–618

Hendriks ATWM, G Zeeman (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour Technol* 100:10–18

Hinterdobler W, G Li, K Spiege, S Basyouni-Khamis, M Gorfer, M Schmoll (2021). *Trichoderma reesei* isolated from Austrian soil with high potential for biotechnological 670 application. *Front Microbiol* 12:552301

Imran M, Z Anwar, M Irshad, MJ Asad, H Ashfaq (2016). Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization industry. *Adv Enz Res* 4:44–55

Jun H, T Kieselbach, LJ Jönsson (2011). Enzyme production by filamentous fungi: Analysis of the secretome of *Trichoderma reesei* grown on unconventional carbon source. *Microb Cell Fact* 10:1–10

Kaur J, SC Bhupinder, SS Harvinder (2006). Regulation of cellulase production in two thermophilic fungi *Melanocarpus* spp. MTCC 3922 and *Scytalidium thermophilum* MTCC 4520. *Enz Microb Technol* 38:931–936

Kim JS, YY Lee, TH Kim (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour Technol* 199:42–48

Kristensen JB, LG Thygesen, C Felby, H Jørgensen, T Elder (2008). Cell–wall structural changes in wheat straw pretreated for bioethanol production. *Biotechnol Biof* 1:1–9

Kumar AK, S Sharma (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review. *Bioresour Bioproc* 4:7

Kumar P, DM Barrett, MJ Delwiche, P Stroeve (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res* 48:3713–3729

Leite P, JM Salgado, A Venâncio, JM Domínguez, I Belo (2016). Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase production by solid-state fermentation. *Bioresour Technol* 214:737–746

Linke R, GT Gerhard, T Haarmann, J Eidner, M Schreiter, P Lorenz (2015). Restoration of female fertility in *Trichoderma reesei QM6a* provides the basis for inbreeding in this industrial cellulose producing fungus. *Biotechnol Biofuels* 8:155

López-Linares JC, I Romero, M Moya, C Cara, E Ruiz, E Castro (2013). Pretreatment of olive tree biomass with FeCl3 prior enzymatic hydrolysis. *Bioresour Technol* 128:180–187

Menon V, M Rao (2012). Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. *Progr Ener Combust Sci* 38:522–550

Miller GH (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 31:426–429

Mirabella N, V Castellani, S Sala (2014). Current options for the valorization of food manufacturing waste. *J Clean Prod* 65:28–41

Moftah OAS, S Grbavčić, M Žuža, N Luković, D Bezbradica, Z Knežević-Jugović (2012). Adding value to the oil cake as a waste from oil processing industry: Production of lipase and protease by *Candida utilis* in solid state fermentation. *Appl Biochem Biotechnol* 166:348–364

Mosier N, C Wyman, B Dale, R Elander, YY Lee, M Holtzapple, M Ladisch (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* 96:673–686

Mtui GYS (2009). Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *Afr J Biotechnol* 8:1398–1415

National Research Council (1999). *Committee on Biobased Industrial Products. Biobased Industrial Products-Priorities for Research and Commercialization*. National Academy Press, Washington DC, USA

Nefzaoui A (1991). Valorisation des sous-produits de l'olivier. *In: Fourrages et Sous-produits Méditerranéens*, pp:101–108. Tisserant JL, X Alibés (Eds). CIHEAM *Options Méditerranéennes*, Zaragoza

Neifar M, A Jaouani, A Ayari, O Abid, HB Salem, A Boudabous (2013). Improving the nutritive value of olive cake by solid-state cultivation of the medicinal mushroom *Fomes fomentarius*. *Chemosphere* 91:110–114

Oke MA, MM Ishola, MJ Taherzadeh, MSM Annuard, K Simarani (2016). Effects of pretreatments of single and mixed lignocellulosic substrates on production of endoglucanase by *Bacillus aerius* S5-2. *Bioresources* 11:6708–6726

Ouyang X, L Chen, S Zhang, Q Yuan, W Wang, LJ Linhardt (2018). Effect of simultaneous steam explosion and alkaline depolymerization on Corncob lignin and cellulose structure. *Chem Biochem Eng Quart* 32:177–189

Paloheimo M, T Haarmann, S Mäkinen, J Vehmaanperä (2016). Production of industrial enzymes in *Trichoderma reesei. In: Gene Expression Systems in Fungi: Advancements and Applications, Fungal Biology*, pp:23–58. Schmoll M, C Dattenböck (Eds). Springer International Publishing, Cham, Switzerland

Papaioannou EH, SI Patsios, AJ Karabelas, NA Philippopoulos (2013). Characterization of condensates from an indirect olive oil pomace drying process: The effect of drying temperature. *J Environ Chem Eng* 1:831–837

Pellera FM, S Santori, R Pomi, A Polettini, E Gidarakos (2016). Effect of alkaline pretreatment on anaerobic digestion of olive mill solid waste. *Waste Manage* 58:160–168

Pirota RDPB, PS Delabona, CS Farinas (2014). Simplification of the biomass to ethanol conversion process by using the whole medium of filamentous fungi cultivated under solid-state fermentation. *Bioener Res* 7:744–752

Ravindran R, AK Jaiswal (2016). A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. *Bioresour Technol* 199:92–102

Ray RC, SS Behera (2017). Biotechnology of Microbial Enzymes. *In: Solid-state Fermentation for Production of Microbial Cellulases*, pp:45–55. Goutam B, AL Demain, JL Adrio (Eds). Academic Press, New York, USA

Rives O (2021). Consommation et production de l'huile d'olive en Algérie. *In: Programmed'Appui au Secteur de l'Agriculture,* p:39. Pasa Ue (Ed.), Algérie

Rodhe AV, B Venkateswarlu, L Sateesh, J Sridevi, LV Rao (2011). Enzymatic 798 hydrolysis of sorghum straw using native cellulose produced by *T. reesei NCIM 992* under solid 799 state fermentation using rice straw. *3 Biotech* 1:207–215

Rodríguez-Zúñiga UF, D Cannella, RDC Giordano, RDLC Giordano, H Jørgensen, C Felby (2015). Lignocellulose pretreatment technologies affect the level of 749 enzymatic cellulose oxidation by LPMO. *Green Chem* 16:2896–2903

Rodríguez-Zúñiga UF, VB Neto, S Couri, S Crestana, CS Farinas (2014). Use of spectroscopic and imaging techniques to evaluate pretreated sugarcane bagasse as a substrate 752 for cellulase production under solid-state fermentation. *Appl Biochem Biotechnol* 172:2348–2362

Roig A, ML Cayuela, MA Sánchez-Monedero (2006). An overview on olive mill wastes and their valorization methods. *Waste Manage* 26:960–969

Roussos S, I Perraud-Gaime, H Lakhtar, F Aouidi, Y Labrousse, N Belkacem, H Macarie, J Artaud (2009). Valorisation biotechnologique des sous-produits de l'olivier par Fermentation en Milieu Solide. *Olivebioteq* 26:52–59

Roussos SG, GL Garcia, M Raimbault (1983). Valorisation de la cossette de betterave par culture de *Trichoderma harzianum* en milieu liquide et solide. *Industr Aliment Agric* 100:449–452

Sanchez C (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnol Adv* 27:185–194

Sansoucy R, X Alibes, F Martilotti, A Nefzaoui, P Zoïopoulos (1984). Utilisation des sous-produits de l'olivier en alimentation animale dans le bassin méditerranéen. *Etude FAO Prod Anim* 121:23–43

Sharma B, R Agrawal, RR Singhania, A Satlewal, A Mathur, D Tuli, M Adsul (2015). Untreated wheat straw: Potential source for diverse cellulolytic enzyme secretion by *Penicillium janthinellum* EMS-UV-8 mutant. *Bioresour Technol* 196:518–524

Silveira MHL, RS Aguiar, M Siika-Aho, LP Ramos (2014). Assessment of the enzymatic hydrolysis profile of cellulosic substrates based on reducing sugar release. *Bioresour Technol* 151:392–396

Soccol CR, ESFD Costa, LAJ Letti, SG Karp, AL Woiciechowski, LPDS Vandenberghe (2017). Recent developments and innovations in solid state fermentation. *Biotechnol Res Innov* 1:52–71

Soest PJV, JB Robertson (1979). *Systems of analysis for evaluating fibrous feeds*. Cornell University Press. New York, USA

Srivastava N, M Srivastava, PK Mishra, VK Gupta, S Rodriguez-Couto, A Manikanta, PW Ramteke, G Molina (2018). Applications of fungal cellulases in biofuel production: Advances and limitations. *Renew Sustain Ener Rev* 82:2379–2386

Stamatelatou K, PS Blika, I Ntaikou, G Lyberatos (2012). Integrated Management Methods for the Treatment and/or Valorization of Olive Mill Wastes. *In: Novel Technologies in Food Science. Integrating Food Science and Engineering Knowledge into the Food Chain*, pp:65–118. McElhatton A, P Sobral (Eds). Springer, New York USA

Sun S, S Sun, X Cao, R Sun (2016). The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour Technol* 199:49–58

Sun WC, CH Cheng, WC Lee (2008). Protein expression and enzymatic activity of cellulases produced by *Trichoderma reesei Rut C-30* on rice straw. *Proc Biochem* 43:1083–1087

Ugwuanyi JO, B McNeil, LM Harvey (2009). Production of protein-enriched feed using agro-industrial residues as substrates. *In: Biotechnology for Agro-Industrial Residues Utilization*, pp:77–103. Nigam PSN, A Pandey (Eds). Springer, Dordrecht, Netherlands

Vancov T, S McIntosh (2011). Alkali pretreatment of cereal crop residues for second-generation biofuels. *Ener Fuels* 25:2754–2763

Vasco-Correa J, X Ge, Y Li (2016). Biological Pretreatment of Lignocellulosic Biomass. *In: Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery*, pp:561–585. Mussatto SI (Ed). Elsevier Amsterdam, Netherlands

Wu L, M Arakane, M Ike, M Wada, T Takai, K Tokuyasu, M Gau, K Tokuyasu (2011). Low temperature alkali pretreatment for improving enzymatic digestibility of sweet sorghum 802 bagasse for ethanol production. *Bioresour Technol* 102:4793–4799

Xiao LP, GY Song, RC Sun (2017). Effect of Hydrothermal Processing on 804 Hemicellulose Structure. *In: Hydrothermal Processing in Biorefineries*, pp:45–94. Ruiz HA, MH Thomsen, HL Trajano (Eds.). Springer International Publishing, Cham, Switzerland

Yoon LW, TN Ang, GC Ngoh, ASM Chua (2014). Fungal solid-state fermentation and various methods of enhancement in cellulase production. *Biomass Bioener* 67:319–338

Zhang HY, LR Lynd (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnol Bioeng* 88:779–797

Zheng Y, Z Pan, R Zhang (2009). Overview of biomass pretreatment for cellulosic ethanol production. *Intl J Agric Biol Eng* 2:51