



# Article High-Pressure Water Jet System Treatment of Argan Nut Shell and Enzymatic Hydrolysis for Bioethanol Production

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**Abstract:** Argan nut shell represents the most generated by-product during the process of the extraction of argan oil. For the first time, argan nut shell was characterized and assessed as a new potential feedstock for bioethanol production using a combination of mechanical and enzymatic pretreatment. Argan shell samples were first disintegrated using the Star Burst system, which involves a high-pressure water jet system. Then, the pretreated argan nut shell was subjected to enzymatic hydrolysis using Viscozyme L (30 FBGU/g). Afterwards, the fermentation of the hydrolysate by *Saccharomyces cerevisiae* was investigated. Argan nut shell, as a feedstock plentiful in carbohydrates, conferred a high yield of saccharification (90%) and an optimal ethanol bioconversion (45.25%) using Viscozyme L (30 FBGU/g) at  $2\%_{w/v}$  of argan feedstock.

**Keywords:** argan nut shell; mechanical treatment; high-pressure water jet system; enzymatic hydrolysis; fermentation; bioethanol

## 1. Introduction

Nowadays, increasing demand for sustainable biofuel has gained considerable attention all over the world [1–3]. Biofuels provide engaging solutions for reducing dependence on fossil resources, lowering greenhouse gas emissions, and elaborating the sustainability of the global transportation system [4]. Bioethanol is the dominant biofuel produced, as it supplies 80% of global biofuel production [5,6]. Furthermore, this shows that it has potential in dealing with the global energy dilemma and current environmental issues [7]. Besides its renewable and green characteristics, bioethanol has been proven to have positive traits for the environment, socio-economic status, and energy safety. Moreover, highly abundant renewable feedstocks are the main contributor to large-scale bioethanol production [3,8,9].

Among the several bioenergy resources, biomass as raw material is earning more and more attention, as it is the most dependable and environmentally friendly resource of energy and is available with a wide range of products [10]. Nevertheless, lignocellulosic biomass represents a precious source for the sustainable and economical production of bioethanol [11]. Moreover, it permits the low-priced production of biofuel, since the cost of this biomass is evaluated to be affordable in comparison with starch, which is the most utilized for bioethanol production [12–14]. Nevertheless, obtaining fermentable sugar fractions from these substrates is challenging because of the complex structure of lignocellulosic biomass. Several pretreatments, hydrolysis, and fermentation steps are required to improve the yield. The principal objectives of the pretreatment step are the



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). degradation of hemicellulose and/or lignin, a reduction in the particle size, and the recovery of the cellulose from the biomass [15].

*Argania spinosa* (L.) Skeels represents a miraculous tree in North Africa and the secondmost joint forest species from southwestern Morocco. This tree fills about 871,210 ha, with an estimated planting density of 20 million trees and an annual production of 500 kg of fruit per hectare [16,17]. The tree is essentially known for its oil and endowing a wide range of nutritional, therapeutic, and cosmetic benefits [18–20], as well as by its socio-economic and environmental role [21]. However, during the process of cosmetic or food argan oil production, every year, huge quantities of by-products are generated (52.6% argan shells, 43% argan pulp, and 2% argan cake) and remain without added value at present [22]. Several researchers are currently looking for innovative uses for these by-products to contribute to the valorization and preservation of the argan ecosystem.

Our previous study investigated the optimization of argan pulp enzymatic hydrolysis with the aim of achieving a high content of fermentable carbohydrates. Then, the viability of sugar conversion to bioethanol by *Saccharomyces cerevisiae* demonstrated that argan pulp is a successful, cheap, and abundant crop for bioethanol production [23]. Hence, in the present study, argan shell was chosen as a lignocellulosic biomass generated in large quantities, estimated at 60,000 tons per year [24], for bioethanol production for the first time. To enhance the reducing sugars production, high-pressure water jet system technology (the Star Burst system) was subsequently added to the enzymatic pretreatment. The efficiency of argan shell hydrolysis and bioethanol production by the new combined system was investigated.

## 2. Materials and Methods

## 2.1. Argan Shell Feedstock Collection

Argan shell used was sourced by the NECTAROME company (https://www.nectarome. com/, accessed on 20 December 2019), located 35 km from Marrakech (Morocco). The samples studied were taken from a stock of the 2019 production. They were already dried and crushed into small particles ( $\leq$ 500 µm) by the company. After that, the recovered feedstock was stored for further use.

## 2.2. Argan Shell Chemical Analysis

First, the ash content and dry weight were determined using the Association of Official Analytical Chemists method [25]. Lipid content was assessed by the Soxhlet method [25] and protein content was determined using the Bradford assay [26]. Sugar contents were measured using the phenol–sulfuric acid technique [27]. Reducing sugars were estimated utilizing the procedure described by Miller [28]. Lignin, cellulose, and hemicellulose determination was conducted using the method described by Van Soest [29]. The reported results are given as percentages (%) corresponding to dry mass (DM). All of these analyses were the average of at least three replicates.

#### 2.3. Disintegration of Argan Shell

In tap water, a  $2\%_{wt}$  slurry of argan shell powder was prepared and homogenized for 5 min with an Ultra Turrax homogenizer. The argan shell mixture was thereafter treated with a Star Burst Mini system (HJP-25001CE, Sugino Machine Co., Ltd., Toyama, Japan) outfitted with a ball-collision chamber, as described by Ogura et al. (2022) [30]. Argan shell samples processed in one, five, ten, and twenty passes were collected for analysis.

## 2.4. Particle Size Distribution Analysis of Argan Shell Suspension

Based on previous work [31], the granulometry of the argan shell suspension was determined using the laser-scattering particle size distribution analyzer Partica LA-960 (Horiba, Ltd., Kyoto, Japan).

## 2.5. Enzymes and Other Reagents

Argan shell enzymatic hydrolysis was conducted using Viscozyme L, which represents an enzymatic cocktail from *Aspergillus* spp. with numerous carbohydrases, counting cellulase, hemicellulase, arabinase,  $\beta$ -glucanase, and xylanase. Viscozyme L activity was  $\geq$ 100 Fungal Beta-Glucanase Units (FBGU)/g and 1FBGU correspond to 1 µmol glucose released/min. Sigma-Aldrich (France) acquired all enzymes, reagents, solvents and standards.

## 2.6. Enzymatic hydrolysis of Argan Shell

The enzymatic hydrolysis of argan shell  $(2\%_{w/v})$  was executed in a Radleys reactor (Carousel 6 Plus Reaction Station) (100 mL; 200 rpm). Enzymatic saccharification was carried out on 50 mL of distilled water using Viscozyme L (30 FBGU/g of substrate) at 44 °C and pH 4.5. The saccharification was maintained for 24 h. Immediately after each sampling, the enzymatic reaction was stopped using a boiling water bath (95 °C, 10 min) followed by centrifugation (13,000× g, 10 min). The estimation of the reducing sugars of the hydrolysate was carried out as outlined below in Section 2.2. A control was realized by excluding the enzyme in similar conditions.

## 2.7. Pretreated Argan Shell Fermentation

## 2.7.1. Inoculum Preparation

Saccharomyces cerevisiae ATCC 7754 culture was performed according to the protocol highlighted in our previous study [23]. Briefly; the lyophilized strain was kept in yeast medium agar (Difco 0712-01-8), then incubated for 24 h at 28 °C. Afterwards, the strain was kept at 4 °C before use in culture vessels [32]. Additional elements were added to the culture medium as determined by Kristiansen (1994) [33], and incubated for 18 h (35 °C, 150 rpm). Yeast growth was monitored by measuring its absorbance at  $A_{(550nm)}$  (Biomate 3S, UV–Vis Spectrophotometer, Thermo Scientific, France), and dry weight was assessed gravimetrically.

#### 2.7.2. Saccharification and Fermentation of Pretreated Argan Shell

The saccharification step was managed as reported in Section 2.6, using argan shell  $(2\%_{w/v})$  and an enzyme concentration of 30 FBGU/g. Simultaneously, the fermentation was executed in a 500 mL bioreactor (Infors HT, Multitron 2). The glucose medium was used as a control under the same conditions with the complete medium (Section 2.7.1) of the inoculum (A<sub>(550nm)</sub> = 2; 100 mL) provided ( $50\%_{v/v}$ ). Glucose at  $2\%_{w/v}$ , 30 °C, 100 rpm, and pH 6 were our culture parameters. The fermentation kinetics were maintained for 48 h. Samples were collected at 0, 2, 4, 8, 12, 24, and 48 h for reducing sugars and bioethanol evaluation.

The conditions of enzymatic hydrolysis and fermentation represent the optimal conditions obtained in our earlier study using argan pulp and standard glucose [23]. All experiments were performed in three replicates, and the results were presented as the mean value  $\pm$  standard deviations.

## 2.8. Bioethanol Quantification

Prior to bioethanol quantification, all samples were first deproteinized using 125 µL of zinc sulfate  $(5\%_{w/v})$ , with 125 µL of barium hydroxide (0.3 M) to avoid the clogging of the chromatography column, centrifuged  $(5 \text{ min}, 10,000 \times g)$  (Thermo Scientific, Bourgoin-Jallieu, France), and filtered, then the recovered solution was evaluated. Bioethanol was chromatographically measured using a high-performance liquid chromatography (HPLC) device (1260 Infinity Quaternary LC system, Agilent Technology, Palo Alto, CA, USA), using 2 columns (Rezex ROA  $300 \times 7.8 \text{ mm}$ , Phenomenex, Torrance, CA, USA) and a refractometer (HP 1100 series, Agilent Technologies, Palo Alto, CA, USA). Sulfuric acid (5 mM) was the mobile phase, with a flow rate of 0.7 mL/min.

#### 2.9. Data Analysis

All experiments for argan shell saccharification and fermentation were conducted in triplicate under the same conditions, and the reported results represent their mean values.

## 3. Results and Discussion

#### 3.1. Argan Shell Chemical Properties

Argan shell is mainly used as a traditional fuel for heating and cooking in rural areas due to its ability to keep the fire burning for an extended time. Researchers have given a lot of importance to argan oil, its composition, and its benefits [20,34-36]; however, there is little information on the physicochemical composition of argan shell. The majority of studies have been dedicated to its combustion capacity. Rahib et al. (2020) investigated the thermo-kinetic characteristics of argan shell in combustion in order to assess the potential for its recovery into a source of energy. The authors highlighted that argan shell presents a potential feedstock for producing energy by combustion [37]. The search for potential new avenues for argan shell valorization contributes to extending the market for biomass and biofuels and could also help to solve energy problems. Currently, several researchers have taken interest in the valorization of argan shell in several sectors. Bouqbis et al. (2021) studied the physicochemical characteristics of argan shell bio-char, intended for use in soil enrichment and nutrient and water retention [38]. Additionally, argan shell was used for the preparation of activated carbons by physical and chemical activation processes [39]. In addition, Essabir et al. studied the physical, mechanical, and thermal properties of bio-composite materials based on polypropylene, polyethylene, and polylactic acid reinforced with argan shell [40-42]. Nevertheless, to our knowledge, no reports are available or published in the literature evaluating the potential use of argan shell as a new feedstock for bioethanol conversion.

The composition of the lignocellulosic substrate must be determined before starting the conversion process to ethanol. Table 1 describes the content of the main chemical constituents in argan shell. An antecedent study on argan shell highlighted that it contains 3% fat, 1.75% proteins, 2% ash, and 91.95% sugar [43]. Similarly, a report by the Social Development Agency (Morocco) and Agropolis International showed that argan shell contain 3.3% proteins, 2.7% fat, and 73.6% neutral digestible fiber [44].

Composition  $%_{w/w}$ Argan Shell Dry matter %<sub>w/w</sub>  $5.02\pm0.16$  $3.29\pm0.01$ Ash %<sub>w/w</sub> Protein %<sub>w/w</sub>  $3.46\pm0.16$ Lipid %<sub>w/w</sub>  $1.02\pm0.16$ Cellulose %<sub>w/w</sub>  $46.15\pm0.12$ Hemicellulose %w/w  $19.06 \pm 0.09$ \* NDF/total fibers %<sub>w/w</sub>  $94.58 \pm 0.15$ \*\* ADF Lignocellulose %<sub>w/w</sub>  $75.52\pm0.12$ Lignin %<sub>w/w</sub>  $29.17\pm0.14$ Total sugars (mg/g DW)  $893.05 \pm 0.21$ Reducing sugars (mg/g DW)  $70.32\pm0.01$ 

 Table 1. Chemical composition of argan shell.

\* NDF: neutral detergent fiber; \*\* ADF: acid detergent fiber.

In general, lignocellulosic feedstocks are mainly composed of cellulose (35–55% of the total dry weight) and hemicellulose (20–40%), which are bound with pectin, as well as proteins, enzymes, and lignin (10–25%) [45]. In our study, the contents of cellulose (46.15%), hemicellulose (19.06%), and lignin (29.17%) were slightly comparable to those reported by Essabir et al. (2014), who obtained 48.8%, 16%, and 30% for cellulose, hemicellulose, and lignin, respectively [46]. Additionally, Akhzouz et al. (2021) studied the physical characterization of a bio-composite stabilized with argan nut shell and cement. As a result, the authors highlighted that argan shell is composed of 25% cellulose, 34.3% hemicellulose, and

34.5% lignin [47]. This difference can be interpreted by environmental conditions, which can affect cellulose synthesis including light, temperature, osmotic conditions, etc. [48].

#### 3.2. Mechanical Pretreatment of Argan Shell

The argan shell is the hardest and most complex part of the argan fruit. It protects the almond from any external factors such as humidity and heat. In the present work, high-pressure water jet system technology was studied for the very first time (Figure 1) as a mechanical treatment to disintegrate argan shell to small particles.



Figure 1. High-pressure water jet system for argan nut shell disintegration.

Mechanical pretreatments are the most accessible, simple, and economic methods of pretreatment for agri-residues. In general, these methods can enhance the efficiency of lignocellulosic wastes [49] and usually do not generate toxic by-products [50].

In our procedure, we executed one to twenty passes of mechanical treatment with the Star Burst system ( $2\%_{wt}$  of argan shell) to obtain small particles and to reduce the degree of polymerization. Supplementary passes of mechanical treatment significantly increased the dispersion of argan shell. The average particle diameter was 64 µm, 30 µm, 16.7 µm, 13.2 µm, and 11.4 µm (D50) in the case of 0, 1, 5, 10, and 20 passes of treatment, respectively (Figure 2).

The reduced argan particle size through physical pretreatment may help to increase its enzyme accessibility. Physical pretreatment methods are mainly used to decrease the particle size of the lignocellulosic biomass. This can be achieved in different ways such as fragmentation, grinding, hacking, milling, rolling, mechanical interactions, microwave radiation, sonication, spray drying, gamma radiation, and pyrolysis. It is well reported that the objective of size reduction is to increase the specific surface area, thus facilitating enzymatic accessibility. Mechanical pretreatment allows a reduction in particle size and crystallinity degree, and improves enzymatic accessibility and bioconversion efficiency [51,52]. Additionally, it is known that the smallest particle size of biomass has the largest specific surface area, which is a critical factor for enzymatic hydrolysis [53]. Kumar et al. (2020) studied a combination of physical, chemical, and microbial treatments for bioethanol production using sesame wastes. These authors observed that the particle size was the most influential factor in the pretreatment of sesame plant residue. Indeed, for the smallest particle size, the maximum surface area was exposed to microbial cells and acid solution, which catalyzed the pretreatment reactions [54]. In addition, they reported that a larger particle size may have fewer freely available reactive sites, which slow down the pretreatment process. Moreover, an attrition mill device was used to decrease corn stover particle size for biofuel production. After one hour of milling, the portion of corn stover

particles of less than 100  $\mu$ m was 73.5%, while the mean diameter of the corn stover was 61.3  $\mu$ m. The authors reported that this drastic reduction in the size of corn stover particles increased the accessibility of cellulase to the cellulose in corn stover [55]. In another study, the milling of corn stover was investigated. The authors reported that the median particle size dropped from 285.80  $\mu$ m to 22.46 and 18.8  $\mu$ m after ball milling for 20 and 60 min, respectively. These results showed that ball milling effectively alters the structure of corn stover by reducing the particle size and destroying the intact surface, which could in part account for the enhanced substrate reactivity [56]. Additionally, the ball-milling of an oil palm empty fruit bunch resulted in a reduction in its crystallinity index from 56.1% to 9.3% compared to the control. As a result, higher yields of glucose (67.5%) and xylose (80.1%) were reported after enzymatic hydrolysis for ball-milled samples [57]. Additionally, pulverized banana peels were compared with unmilled peels for cellulose yields, and it has been highlighted that pulverized banana peels result in higher cellulose yields [58].



**Figure 2.** Argan shell granulometry after one, five, ten, and twenty passes of the high-pressure water jet system treatment.

## 3.3. Enzymatic Hydrolysis of Argan Shell

The enzymatic hydrolysis of pretreated and untreated argan shell using Viscozyme L for the production of bioethanol has been studied. Figure 3 represents the evolutions of reducing sugars, which mainly represent glucose, according to the time using Viscozyme L. The choice of the enzyme concentration (30 FBGU/g), as well as the reaction period (48 h), was made according to our previous study, which dealt with the optimization of the enzymatic hydrolysis process of argan pulp for bioethanol production [23]. In addition, a  $2\%_{w/v}$  argan shell fraction mechanically pretreated (20 passes) with the high-pressure water jet system process was chosen for enzymatic hydrolysis.

The mechanical pretreatment of argan shell increased the initial content of reducing sugars from 70.62  $\pm$  0.02 mg/g DW to 201.4  $\pm$  0.6 mg/g DW for untreated and pretreated argan shell, respectively. Coupling enzymatic and size reduction pretreatments produced a significant and intense increase in reducing sugars content (Figure 3), with 810.60  $\pm$  0.13 mg/g DW compared to the control (114  $\pm$  0.09 mg/g DW) after 48 h for tests with Viscozyme L. This can largely be attributed to the fact that particle size affects the diffusion kinetics, effectiveness of pretreatment, sugar yield, lignin removal, and rate of reaction. Indeed, the pretreatment of argan shell combined with enzymatic saccharification were used to disrupt the lignocellulosic structure of the argan shell and to enhance the enzyme accessibility to cellulose by increasing the accessible surface area of the argan shell; the lignocellulosic biomass pretreatment strongly affects the enzymatic conversion by increasing

the accessible surface. Surface area is generally used to indicate the amount of surface area available to enzymes. Nevertheless, a higher specific surface area is a consequence of size reduction in the lignocellulosic particles [58,59]. A study by Lu et al. (2019) investigated the effect of the physicochemical characteristics of milled cellulose on enzyme activity and glucose production. The results show that the milling process had an effect on the specific surface area, granulometry, crystallinity, and polymerization degree. Cellulase enzyme adsorption capacity decreased and initial hydrolysis rate increased with prolonged ball milling [59]. Additionally, the authors reported that the specific surface area and crystallinity are the pillar factors affecting glucose yield, and the use of ball milling as a pretreatment can considerably reduce the enzyme application. Thus, by increasing enzyme accessibility, higher levels of fermentable carbohydrate are recorded and a higher content of ethanol can be reached. In this work, in the case of disintegrated argan shell by a jet water system (20 passes) combined with enzymatic hydrolysis using Viscozyme L, we reached a higher conversion yield of 90% regarding total sugars concentration before hydrolysis. Likewise, in our previous work, ground argan pulp was investigated by enzymatic hydrolysis for bioethanol production [23]. We obtained a conversion yield of 91% using Viscozyme L after 48 h. Moreover, Ji et al. (2016) studied the impact of mechanical fragmentation of corncob on enzymatic hydrolysis. They reported that ultrafine grinding modified the properties of samples significantly compared with typically ground samples, which significantly improved the enzymatic hydrolysis yield. Glucose conversion resulted in a 98.3% yield of cellulose to glucose using Celluclast 1.5 L and Novozyme, which is the highest conversion ever reported [60]. Additionally, Zhang et al. (2016) stated that ultrafine grinding can significantly decrease the particle size (from 218.5  $\mu$ m to 17.45  $\mu$ m), enhance the cellulose surface area, and increase the pore size in comparison to conventional mechanical crushing—which had an important boosting effect on the enzyme accessibility to cellulose and the hydrolysis rate [61].



**Figure 3.** Analysis of enzymatic hydrolysis of argan shell at  $2\%_{w/v}$  using Viscozyme L (30 FBGU/g).

#### 3.4. Bioethanol Production

After enzymatic hydrolysis, the four hydrolysates obtained from the enzymatic hydrolysis of pretreated and untreated argan shell at  $2\%_{w/v}$  using Viscozyme L (30 FBGU/g) (see Section 3.3.) were used as a substrate for ethanol production using *Saccharomyces cerevisiae* (ATCC 7754) (as described in Section 2.7.2).

This step was employed to convert reducing sugars to ethanol by their own specific metabolism [62]. In addition, it represents a consecrated and worldwide distribution for

bioethanol industry owing to its high tolerance to the inhibitors, effective ethanol production, and extensive availability compared to alternative yeast strains and bacteria [63].

The initial reducing sugars content was 16.01 g/L, 10 g/L, 6 g/L, and 3.42 g/L for the pretreated argan shell + Viscozyme L (30 FBGU/g), untreated argan shell + Viscozyme L (30 FBGU/g), pretreated argan shell, and untreated argan shell (control), respectively, and these were almost completely consumed after 24 h of fermentation. (Figure 4).



**Figure 4.** Reducing sugar and ethanol content (g/L) during 48 h of fermentation for the five samples studied: (**a**) glucose (control), (**b**) pretreated argan shell + Viscozyme L (30FBGU/g), (**c**) pretreated argan shell, (**d**) untreated argan shell + Viscozyme L (30 FBGU/g), and (**e**) untreated argan shell (control).

As mentioned above, the untreated argan shell and pretreated argan shell in the absence of Viscozyme L reactors constituted a similar medium as the hydrolysate of argan shell with a lack of glucose. Nevertheless, the glucose reactor represented an identical medium with pure glucose  $(2\%_{w/v})$ .

Figure 4 shows the changes in the concentrations of both reducing sugars and ethanol for the pretreated argan shell in the presence of Viscozyme L (30 FBGU/g) (Figure 4b), pretreated argan shell in the absence of Viscozyme L (Figure 4c), untreated argan shell in the presence of Viscozyme L (30 FBGU/g) (Figure 4d), untreated argan shell in the absence of Viscozyme L (Figure 4e), and the glucose medium control (20 g/L) (Figure 4a). In terms of the outcomes of the five samples studied, sugars conversion to ethanol was noticed in all treatments, but the difference lay in the glucose conversion rates and ethanol production. For the glucose medium (Figure 4a), the higher ethanol content was revealed to be  $0.47 \pm 0.02$  g/g fermentable sugars after 48 h, which constituted 47.2% of the conversion yield and 94.4% of the theoretical conversion efficiency. Similarly, the maximal ethanol concentration was obtained in the case of pretreated argan shell in presence of Viscozyme L, resulting in  $0.45 \pm 0.01$  g/g fermentable sugars (Figure 4b), which represented a 45.25% conversion yield. Additionally, in accordance with the Gay–Lussac yield law, this conversion consti-

tuted 90.5% of the theoretical conversion yield. Furthermore, the maximum content of ethanol was  $0.35 \pm 0.01$  and  $0.29 \pm 0.01$  g/g fermentable sugars simultaneously for the pretreated argan shell and untreated argan shell, respectively, in the absence of Viscozyme L—thus the need for enzymatic hydrolysis.

Recently, sugarcane bagasse was investigated for bioethanol production using an *Aspergillus tubingensis* enzymatic cocktail. An ethanol yield of 0.415 g/g fermentable sugars and a productivity of 0.161 g/L/h were obtained [2]. Potato peel wastes were also investigated for bioethanol production via different saccharification and fermentation schemes using commercial cellulase and amylase. The results showed that an enzymatic treatment conducted to a higher saccharification rate resulted in 72.38%, which corresponds to 96% of the theoretical maximum [64]. Moreover, another potential feedstock for bioethanol production is sisal waste, which underwent enzymatic hydrolysis using cellulase C1794 and yielded a 92% glucose conversion [65].

In this study, the conversion yield shifted from 39% to 45.25% in the case of pretreated argan shell and untreated argan shell (both in the presence of Viscozyme L (30 FBGU/g), which demonstrates the need to combine physical pretreatment and enzymatic hydrolysis to improve bioethanol production. In parallel, the lignocellulosic matrix of wheat hulls was destroyed using sonication pretreatment, then hydrolyzed using crude enzyme solutions of two white-rot fungi, Phanerochaete chrysosporium and Trametes sp. A considerable amount of the enzymatic sugar produced was observed in the case of combined pretreatments [66]. In addition, in a recent study, a microwave-assisted hydrothermal pretreatment in combination with fungi were used for the enzymatic treatment of cereal straw. The reducing sugars productivity (25.51 g/100 g) and saccharification rate (66.28%) of the pretreatment proved to perform better than a single pretreatment [67]. Additionally, the valorization of cultivated tobacco stalks was investigated by combined pretreatment before enzymatic hydrolysis for potential bioethanol production. The highest result in ethanol yields (75.74 g/L) was obtained after 48 h of fermentation. As a consequence, this combined pretreatment technique could be an encouraging way to enhance the overall bioethanol yield of dried tobacco stalks. Furthermore, three combined pretreatments including physical, microbial, and chemical were investigated for bioethanol production from sesame plant residue. The results showed that a 400 µm size of sesame biomass particles provided the highest yield of reducing sugars after microbe-assisted diluted acid pretreatment with an attractive yield of bioethanol (1.90 g/L) using Saccharomyces cerevisiae [54].

Finally, in our study, we produced 362 kg of ethanol per ton of argan shell after pretreatment with the water jet pressure system, and enzymatic hydrolysis using Viscozyme L (30 FBGU/g), which represented a very significant and promising bioethanol production yield. Similarly, in a recent study, residual banana bulbs were investigated as a future raw material for bioethanol production [68]. To optimize their enzymatic digestibility, the hydrothermal pretreatment of residual banana bulbs was assessed before simultaneous saccharification and fermentation with *Saccharomyces cerevisiae*. The highest ethanol yield resulted in 310 kg of ethanol per ton of banana bulb residue, which constituted 93% of theoretical production using both amylase and cellulase.

## 4. Conclusions

For the first time, argan shell was investigated as a low-priced feedstock for ethanol production. In the present study, a high-pressure water jet system pretreatment (20 passes) applied to the argan shell was needed to efficiently hydrolyze the shell. On the basis of total sugar concentration prior to hydrolysis using Viscozyme L with a  $2\%_{w/v}$  substrate concentration condition, a higher conversion yield of 90% was reached. At this substrate loading, the maximum ethanol yield (90.5% of theoretical production) was obtained using *Saccharomyces cerevisiae*, which is equivalent to 362 kg of ethanol per ton of argan shell. As a result, this process is an auspicious area of activity to meet the energy requirements of human activities in the Arganery territory, that would also correspond to the advancement of a greener environment and energy transition policy.

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