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APRICOT KERNEL SHELLS AS A FEEDSTOCK FOR SECOND-GENERATION BIOFUEL

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This study investigated an oxidative treatment method for apricot kernel shells (AKS) to extract their polysaccharide component for subsequent conversion into liquid biofuel. The aim was to overcome the inherent recalcitrance of lignocellulosic biomass by selectively removing lignin and enriching cellulose content - critical steps for efficient enzymatic hydrolysis. The AKS were treated in an acetic acid medium with hydrogen peroxide under varied conditions, and the resulting substrates were analyzed for yield, cellulose, and lignin content. Mathematical modeling, using regression equations with statistically significant coefficients, accurately described the changes in substrate properties depending on the treatment parameters. Optimization of this multi-criteria process using a generalized Harrington desirability function identified 9 % hydrogen peroxide and 120 minutes of treatment as optimal parameters, yielding a composite desirability value of 0.7966. Under these conditions, the obtained substrate exhibited a 50.1 % recovery, 54.4 % cellulose, and 12.4 % lignin, closely matching theoretical predictions. Visual and IR spectroscopic analyses confirmed the efficiency of the treatment: the processed substrate (AKS-S) displayed a lighter color, indicating reduced lignin and increased cellulose content compared to raw AKS. IR spectra further revealed significant lignin removal and degradation of low-molecular-weight hemicelluloses, which is advantageous as it minimizes the formation of inhibitory byproducts during enzymatic hydrolysis. The treatment also doubled the specific surface area and increased porosity six-fold, improving enzyme accessibility. These findings underscore the potential of this oxidative treatment as an effective pretreatment strategy for converting agricultural waste into a high-quality substrate for biofuel production.

Keywords: *apricot kernel shells, bioconversion, pretreatment, cellulose, biofuel*

INTRODUCTION

Global energy demand continues to grow due to population growth and industrial expansion. At the same time, concerns about climate change and the depletion of fossil fuel reserves are increasing. All this requires a rapid transition to sustainable and renewable energy sources[1]. Among the various alternatives, biofuels, particularly second-generation biofuels derived from non-food biomass, offer a promising pathway to reduce reliance on fossil fuels and mitigate greenhouse gas emissions [2, 3]. These advanced biofuels, unlike their first-generation counterparts, do not compete with food crops for land and resources, thereby offering a more sustainable solution [4].

Agricultural waste and by-products are an abundant and largely untapped resource for the production of second-generation biofuels. These materials are often underutilized, incinerated or left to decompose, leading to environmental problems. Converting such waste into high-value products such as biofuels not only addresses energy security issues but also promotes waste management and the principles of a circular economy. The chemical composition of plant waste, especially lignocellulosic biomass, is a key feature that allows its consideration as a potential raw material for biofuel production, particularly bioethanol. Lignocellulosic biomass primarily consists of three main polymers: cellulose, hemicellulose, and lignin, along with minor amounts of proteins, pectin, and ash [5–9]. The presence of cellulose and hemicellulose makes lignocellulosic biomass a promising feedstock for bioethanol production. However, the recalcitrant nature of lignocellulosic materials, primarily due to their intricate matrix of cellulose, hemicellulose, and lignin, presents a significant challenge to efficient biochemical conversion. Lignin, in particular, acts as a physical barrier, protecting cellulose and hemicellulose from enzymatic attack and inhibiting microbial activity during fermentation. Therefore, effective pretreatment strategies are paramount to disrupt this recalcitrant structure, enhance enzymatic accessibility, and maximize sugar yields [10].

There are numerous methods for the pretreatment of plant biomass, ranging from physical and chemical to biological and physicochemical approaches. Among these, organosolvent treatment is considered particularly promising. This method utilizes organic solvents or their aqueous mixtures to remove lignin selectively from the lignocellulosic matrix. A distinct advantage of organosolvent pretreatment is its ability not only to isolate a solid polysaccharide-rich residue, highly suitable for subsequent bioconversion into fermentable sugars, but also to recover high-quality lignin from the spent liquors [11]. This recovered lignin, unlike lignin typically obtained by traditional delignification methods, possesses higher purity and reactivity, making it a valuable co-product that can be further utilized for the production of advanced materials, chemicals, and fuels, thereby enhancing the overall economic viability and sustainability of the biorefinery concept [12].

To further broaden the sustainability and availability of feedstock, it has become increasingly important to diversify raw material sources beyond traditional fibrous biomass. This includes exploring the potential of non-fibrous plant raw materials, such as various fruit and berry seed shells [13]. Apricot is a widely cultivated fruit in various regions of the world, the processing of which results in the generation of significant quantities of apricot kernel shells (AKS) as a by-product. Currently, AKS are primarily used for low-value applications, such as abrasives or direct combustion, or are simply discarded. This represents a significant opportunity to expand the liquid biofuel resource.

The biochemical conversion of lignocellulosic biomass, such as AKS, into biofuels must involve several critical steps: pretreatment, enzymatic hydrolysis, and fermentation. Each step plays a crucial role in breaking down the complex structure of the biomass into fermentable sugars, which are then converted into biofuels [14–16].

In this study, we focused on investigating an oxidative treatment method for apricot kernel shells to extract their polysaccharide component for subsequent conversion into liquid biofuel.

MATERIALS AND METHODS

Materials. AKS, characterized by their cellulose (28.1 %), lignin (48.3 %), ash (0.8 %), and resin/fat/wax (7.6 %) content, were ground to 0.5 mm and served as the primary feedstock. To ensure consistent moisture content and chemical composition throughout the study, the crushed material was stored in hermetically sealed bags.

The glacial acetic acid and 50 % hydrogen peroxide used for biomass treatment were of pure analytical grade.

Treatment procedure. To optimize the experimental process and thoroughly assess the impact of oxidative treatment conditions on substrate properties, a 2^2 full factorial experimental design was implemented [17]. The oxidative treatment of AKS was conducted under controlled laboratory conditions at 95 °C. The process involved treatment the raw material in an acetic acid medium with hydrogen peroxide, maintaining a liquid-to-raw material ratio of 5 : 1.

The primary independent variables (x_i) chosen for this study, which significantly influence the substrate indicators during oxidative treatment in an acetic acid medium, were: hydrogen peroxide concentration (x_1, %) and treatment time (x_2, min). The experimental factors were systematically varied within defined ranges: x_1 from 3 to 9 %, and x_2 from 30 to 150 min. These ranges represent the full scope of conditions explored in the study (Table 1).

Table 1. Variation ranges of x_i factors

Factors x_i	**Variation levels**			**Variation interval**
	(-1)	**(0)**	**(+1)**	**(Δx_i)**
x_1	3	6	9	3
x_2	30	90	150	30

Once treated, the resulting solid substrate was thoroughly washed with distilled water and air-dried. Its yield and composition were then determined. The key response variables (y_i)

measured for the substrate were: solid substrate yield (y_1, %), cellulose content (y_2, %) and lignin content (y_3, %).

Substrate Characterization and Data Analysis. Product yield was quantified gravimetrically, while lignin and cellulose content were determined using standard procedures [18].

During the oxidative treatment of biomass, both polysaccharide and aromatic structural components simultaneously undergo various chemical transformations, for instance, lignin is oxidized and solubilized, while hemicelluloses and low-molecular-weight cellulose fractions are hydrolyzed [16, 17]. The extent of these processes is directly influenced by the oxidative treatment conditions. The combined effect of multiple treatment factors on substrate properties can be complex. Therefore, a third-order polynomial statistical model was employed to accurately reproduce the experimental data and enhance prediction reliability:

$$y_i = a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 + a_4x_1^2 + a_5x_2^2 + a_6x_1^2x_2 + a_7x_1x_2^2 + a_8x_1^3 + a_9x_2^3.$$

where y_i signifies the measured substrate indicators; a_0–a_9 are the coefficients of the polynomial statistical model; x_1 and x_2 represent the independent factors in their encoded, dimensionless form.

The optimization of the substrate production was achieved using a multicriteria evaluation method based on the generalized Harrington's desirability function [17]. Each substrate indicator (y_i) was converted into a corresponding desirability value (d_i) on a scale ranging from 0 ("very bad") to 1 ("very good") and the generalized desirability function was calculated. To pinpoint the optimal oxidative treatment parameters for the substrate production, one-sided Harrington's desirability profiles were employed. The search for the optimum within the generalized desirability function was conducted using a scanning method with a 0.001 step.

All values y_i represent the mean of three experimental replicates. The relative error consistently remained below 10 %.

Infrared (IR) spectra of the samples were acquired using a Specord M80 spectrophotometer (Carl Zeiss, Germany), scanning the range of 4000–300 cm^{-1}. Samples were prepared by mixing the initial and treated biomass with potassium bromide (KBr) in a 1 : 9 ratio and pressing the mixture into tablets.

The specific surface area of both initial and modified materials was measured via nitrogen adsorption at 77 K using a NOVA 2200 analyzer (Quantachrome, USA). Mercury porosimetry was conducted on a PoreMaster 33 (Quantachrome, USA). Additionally, the pore volume of the materials was determined through benzene vapor adsorption in a desiccator [18].

RESULTS AND DISCUSSION

Investigating the impact of treatment parameters on substrate properties. The performed experimental work successfully produced substrate samples characterized by distinct yields, cellulose content, and lignin content. These characteristics indicate the potential utility of such substrates for downstream processing applications, specifically for the generation of liquid biofuel.

Mathematical processing of the experimental data yielded regression equations that adequately describe how the substrate's properties change with varying parameters of the AKS oxidative treatment process:

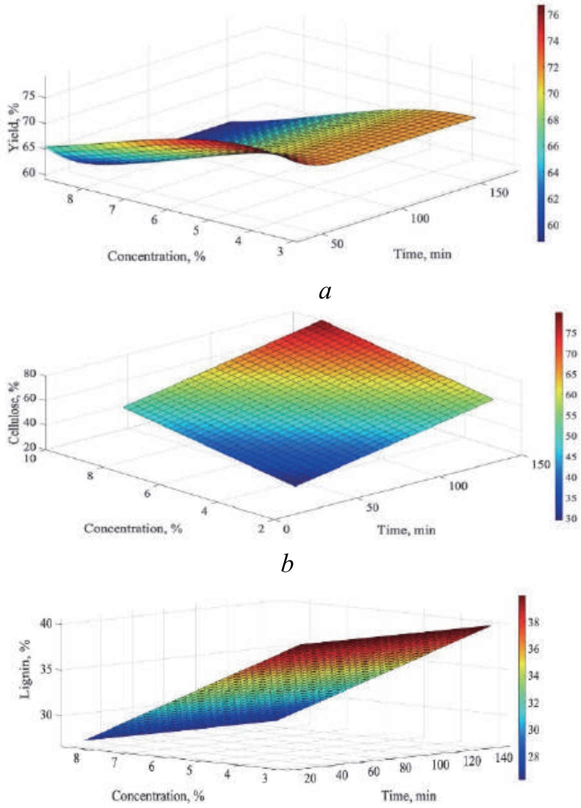
$$\begin{aligned} y_1 = 66{,}75 - 9{,}67x_1 - 6{,}16x_2 - 3{,}02x_1x_2 \\ + 3{,}01x_2^2 + 3{,}13x_1^2x_2 \\ + 0{,}24x_1x_2^2 + 1{,}99x_1^3 - 3{,}35x_2^3 \\ y_2 = 44{,}18 + 7{,}33x_1 + 5{,}04x_2 + 3{,}87x_1x_2 \\ + 0{,}25x_1^2 - 1{,}11x_2^2 + 0{,}89x_1^2x_2 \\ - 1{,}83x_1x_2^2 + 1{,}58x_2^3 \\ y_3 = 27{,}98 - 11{,}33x_1 - 6{,}79x_2 - 3{,}02x_1x_2 \\ - 0{,}71x_1^2 + 4{,}01x_2^2 + 0{,}47x_1^2x_2 \\ + 2{,}60x_1x_2^2 - 1{,}17x_2^3 \end{aligned}$$

The regression equations present the regression equation coefficients (a_i) for each y_i indicator without those found to be statistically insignificant. The derived mathematical models were evaluated using Student's t-criterion. For this analysis, the critical t-value was 2.36.

The substrate properties were visualized as 3D surface plots using MATLAB (Fig. 1).

Based on the presented data, we can conclude that the oxidative treatment of AKS effectively removes a portion of the material's structural components, particularly the aromatic constituent, lignin. This process, known as delignification, becomes more intense with increased hydrogen peroxide concentration and extended treatment duration. Both of these variables positively influence lignin removal [21].

Simultaneously, a clear positive correlation is observed between both oxidant consumption and increased treatment time and the rise in the polysaccharide content of the resulting substrate. This indicates that while lignin is being removed, the valuable polysaccharide fraction is becoming more concentrated within the material.



a

b

c

Fig. 1. 3D surface visualizations of the influence of treatment parameters on substrate properties: *a* – substrate yield, *b* – cellulose content, *c* – lignin content

Process parameter optimization. Optimizing the oxidative treatment of AKS presents a multicriteria challenge. To identify a compromise optimal solution, the pooling of quality indicators was employed through the use of a generalized Harrington desirability function. The individual desirability scales for each indicator are detailed in Table 2.

Table 2. Substrate indicator desirability scales and optimization outcomes

Indicator y_i	**Desirability scale**		**Optimal value**
	Very good	**Very bad**	
Y1	82.0	47.1	52.0
Y2	59.4	33.2	55.7
Y3	11.9	41.2	12.3

Calculations revealed that the optimal parameters for the oxidative treatment of AKS i are a hydrogen peroxide concentration of 9 % and a treatment time of 120 min. At these specific conditions, the generalized Harrington desirability function reaches its maximum value of 0.7966, signifying the best overall compromise among the various desired substrate properties.

Conducting the experiment under the determined optimal parameters yielded a substrate, labeled AKS-S, with a 50.1 % recovery, a cellulose content of 54.4 %, and a lignin content of 12.4 %. A comparison of these experimentally obtained values with the theoretically calculated figures demonstrates a strong agreement, confirming the accuracy and predictive capability of our optimized model.

Substrate Property Analysis. The substrate AKS-S was investigated in comparison to the initial biomass AKS to establish the effectiveness of the oxidative treatment (Fig. 2). The lighter color of the AKS-S sample indicates a higher cellulose content and lower lignin content when compared to AKS.

Maximizing lignin removal from the biomass is crucial for reducing the inherent recalcitrant nature of the AKS, which is essential for efficient enzymatic hydrolysis. Oxidative treatment of AKS successfully increased the cellulose content to over 54 %. This significant enrichment of cellulose highlights the effectiveness of the chosen method in modifying the biomass composition, making it more amenable for downstream applications like enzymatic hydrolysis.

a *b*

Fig. 2. Visual characterization of AKS (*a*) and AKS-S (*b*) samples

Fig. 3 displays the IR spectra of the original AKS and the substrate AKS-S. A broad absorption band between 3000–3700 cm^{-1} in both samples confirms the presence of O-H stretching vibrations associated with hydrogen bonding [22, 23]. The characteristic peak at 1640 cm^{-1}, indicative of water bending vibrations, shows that both materials contain absorbed moisture. Both AKS and AKS-S exhibit absorption bands in the 2800–3000 cm^{-1} range, corresponding to asymmetric and symmetric C-H stretching vibrations from methyl ($–CH_3$) and methylene ($–CH_2$) groups. Notably, the increased intensity of these bands in the AKS-S spectrum suggests an elevated polysaccharide content following the treatment. A band at 1215 cm^{-1}, representing C-C and C-O bonds in lignin, is present in both samples but is more pronounced in the AKS, indicating lignin removal from AKS-S.

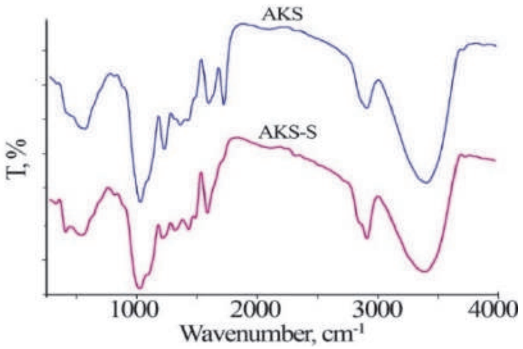


Fig. 3. IR spectra of AKS and AKS-S

The absence of an absorption band at 1730 cm^{-1} (assigned to ketone/aldehyde C=O stretch in hemicelluloses) in the AKS-S spectrum points towards the degradation of low-molecular-weight polysaccharides during the oxidative process. Hydrolyzing hemicelluloses during treatment and reducing their content in AKS-S will positively affect enzymatic hydrolysis. This eliminates the potential formation of inhibitory byproducts like furfural and hydroxymethylfurfural, which are known to hinder enzymatic activity [24].

It's well-established that the specific surface area of a biomass substrate is crucial for the efficiency of enzymatic hydrolysis. This is because enzymatic reactions heavily rely on direct physical contact between the enzymes and their target substrates, primarily cellulose. Sorption processes play a critical role in the enzymatic hydrolysis of plant biomass, as this is a heterogeneous reaction occurring at the solid-liquid interface. The efficiency of hydrolysis directly depends on the interaction between enzymes (in the liquid phase) and biomass polysaccharides (in the solid phase). Therefore, the structural features of the initial biomass before and after treatment were also investigated. The results are presented in Table 3.

Table 3. Structural characteristics of initial and treated biomass

Method	AKS	AKS-S
Specific surface area, m^2/g		
Nitrogen adsorption	6	12
Pore volume, cm^3/g		
Benzene vapour sorption	0.04	0.21

Analysis of the data further reveals that the proposed treatment doubled the specific surface area. More significantly, the porosity, measured by benzene vapor sorption, increased six-fold (from 0.04 to 0.121 cm^3/g). This notable difference between the pore volume increases can be attributed to the AKS-S' ability to swell in organic solvents, which enhances vapor sorption.

Overall, the presented results demonstrate the positive impact of oxidative treatment of apricot kernel shells (AKS) in an acetic acid medium. This process yields a substrate with reduced lignin content and an increased hexose (cellulose) component. Consequently, the treatment effectively mitigates the limitations and barriers that hinder enzymatic hydrolysis of plant biomass, thus facilitating the subsequent production of second-generation biofuels.

CONCLUSIONS

This research successfully demonstrates an effective oxidative treatment strategy for AKS, yielding a substrate highly suitable for enzymatic hydrolysis and subsequent liquid biofuel production. The oxidative process significantly delignified AKS, concurrently enriching its cellulose content to over 54 % and resulting in a lighter-colored substrate. This modification is critical for overcoming the inherent recalcitrance of the biomass.

Mathematical regression models accurately predict the impact of treatment parameters on substrate properties, showing that higher hydrogen peroxide concentration and extended treatment time positively influence both lignin removal and polysaccharide enrichment. Multi-criteria optimization using the Harrington desirability function identified optimal treatment parameters (9 % hydrogen peroxide and 120 minutes), which maximized overall desirable substrate characteristics. Experimental validation under these optimal conditions confirmed the model’s predictive accuracy, yielding AKS-S with 50.1 % recovery, 54.4 % cellulose, and 12.4 % lignin.

IR spectroscopy provided molecular-level evidence of the treatment's efficacy, confirming lignin degradation and removal, and notably, the hydrolysis of low-molecular-weight hemicelluloses. This is a key benefit, as it mitigates the formation of enzymatic inhibitors. Beyond chemical changes, the treatment induced significant structural modifications, doubling the specific surface area and increasing porosity six-fold, which directly enhances the accessibility of polysaccharides for enzymatic action.

The proposed oxidative treatment method effectively transforms AKS into a highly digestible substrate, paving the way for more efficient and cost-effective production of second-generation biofuels.

Шкаралупи абрикосових кісточок як сировина для біопалива другого покоління

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У цьому дослідженні було вивчено метод окисної обробки шкаралупи абрикосових кісточок (ШАК) з метою вилучення її полісахаридних компонентів для подальшої конверсії в рідке біопаливо. Метою було подолання притаманної лігноцелюлозній біомасі стійкості за рахунок селективного видалення лігніну та збагачення целюлозою – критично важливих етапів для ефективного ферментного гідролізу. ШАК обробляли в середовищі оцтової кислоти з перекисом водню за різних умов, після чого отримані субстрати аналізували на вихід, вміст целюлози та лігніну. Математичне моделювання із застосуванням рівнянь регресії зі статистично значущими коефіцієнтами точно описують зміни властивостей субстрату залежно від параметрів обробки. Оптимізація багатокритеріального процесу за допомогою узагальненої функції бажаності Харінгтона дозволила визначити оптимальні параметри: 9 % перекис водню та 120 хвилин обробки, що дало узагальнену бажаність на рівні 0.7966. За цих умов отриманий субстрат мав вихід 50.1 %, вміст целюлози 54.4 % і лігніну 12.4 %, що добре узгоджується з теоретичними прогнозами. Візуальний аналіз і ІЧ-спектроскопія підтвердили ефективність обробки: оброблений субстрат (ШАК-S) мав світліше забарвлення, що свідчить про зниження вмісту лігніну та підвищення вмісту целюлози порівняно з необробленим ШАК. ІЧ-спектри також засвідчили значне видалення лігніну та деградацію низькомолекулярних геміцелюлоз, що є позитивним фактором, оскільки зменшує утворення інгібуючих побічних продуктів під час ферментативного гідролізу. Обробка також подвоїла питому площу поверхні та збільшила пористість у шість разів, що покращує доступність субстрату для ферментів. Отримані результати підтверджують перспективність застосування окиснювальної обробки як ефективної стратегії підготовки лігноцелюлозної біомаси до виробництва біопалива.

***Ключові слова**: шкаралупи абрикосових кісточок, біоконверсія, попередня обробка, целюлоза, біопаливо*

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