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PRODUCTION, PHYSICOCHEMICAL AND THERMODYNAMIC CHARACTERIZATION OF BIOETHANOL DERIVED FROM CORK: A PROMISING VALORIZATION PATHWAY FOR A NON-FOOD LIGNOCELLULOSIC WASTE

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ABSTRACT

This study investigates the potential of cork biomass, an underutilized lignocellulosic residue, to produce second-generation bioethanol. The process involved biomass preparation, Thermochemical pretreatment, concentrated acid hydrolysis, and alcoholic fermentation to convert the carbohydrate fraction of cork into fermentable sugars. The resulting bioethanol, obtained through distillation, reached a purity of 30 % (v/v). Physicochemical and thermodynamic caracteristics of the produced bioethanol included measurements of density, viscosity, flash point, calorific value, and vapor pressure. Spectroscopic analyses (FT-IR and Raman) were also conducted to confirm ethanol composition and purity. Despite its moderate purity, the bioethanol exhibited properties in line with initial fermentation - stage fuels, confirming the feasibility of cork as a raw material for biofuel production. These findings demonstrate a promising valorization route for cork waste within the framework of circular bioeconomy and sustainable energy development.

Keywords: bioethanol, cork biomass, lignocellulosic waste, alcoholic fermentation, physicochemical and thermodynamic caracteristics.

INTRODUCTION

The global energy demand is continuously increasing and remains heavily reliant on fossil fuels. This dependency not only accelerates the depletion of non - renewable resources but also significantly contributes to environmental degradation [1, 2]. Consequently, the search for sustainable and eco - friendly alternatives, such as bioethanol, has gained significant momentum in recent years [3, 4]. Bioethanol production is particularly promising due to its compatibility with sustainable development goals and its potential to reduce greenhouse gas emissions [5, 6].

Fermentable sugars, such as glucose and sucrose, can be biologically converted into ethanol through alcoholic fermentation [7, 8]. These sugars, found in polymerized forms in many plant species, serve as a key substrate for ethanol production [9]. The first generation of bioethanol from edible resources primarily involves the utilization of starch - based crops like maize, wheat, barley, cassava, and potatoes, as well as sucrose - rich plants such as sugarcane, sugar beet, and sweet sorghum. However, this reliance on food - grade biomass has raised major concerns about its long - term sustainability, both economically and ecologically [10]. The cultivation of these crops for fuel purposes competes directly with food production, placing additional stress on freshwater supplies and reducing the availability of fertile land. Furthermore, intensive agricultural practices used in such systems may accelerate environmental issues including water scarcity, soil erosion, and contamination from excessive fertilizer use [11]. Additionally, this competition can drive up the costs of staple foods and animal feed, impacting food security and market stability. To address these limitations, research has shifted toward second - generation bioethanol, which is derived from lignocellulosic biomass - non - food, renewable materials available in large quantities. These include residues like bagasse [12], and agricultural wastes, such as cereal straw (wheat straw [13], rice husk [14], corn cob [15]). One such underexplored lignocellulosic source is cork, a by - product of the cork oak tree industry. It has the potential to serve as a sustainable and abundant raw material for bioethanol production, especially in cork - producing countries like Algeria.

Lignocellulosic biomass, including cork, is mainly composed of cellulose, hemicellulose, and lignin [16, 17].

To produce fermentable sugars, the biomass must undergo a pretreatment stage to disrupt the hydrogen bonds between these components and make the carbohydrates more accessible. This can be achieved through physical, chemical, or biological methods [18]. Among these, Thermal pressure pretreatment which consists of exposing the lignocellulosic material to elevated temperature and pressure conditions, then suddenly releasing the pressure. This abrupt change causes structural disruption in the biomass, effectively loosening the complex matrix and enhancing the accessibility of cellulose and hemicellulose components for subsequent conversion [19]. The pretreated biomass is then subjected to concentrated acid hydrolysis using sulfuric acid, performed at low temperatures (around 40°C) with high acid concentrations (30 - 70 %), is a highly efficient method for saccharification of lignocellulosic biomass, meaning it effectively converts complex carbohydrates into simple sugars [20].

The subsequent step in the bioethanol production process is fermentation, during which fermentable sugars are anaerobically converted into ethanol by microorganisms. The yeast strain *Saccharomyces cerevisiae* was well - established efficiency in ethanol production [21]. This species is widely used in industrial fermentation because of its high ethanol yield, strong ethanol tolerance, rapid fermentation rate, and resilience to environmental stresses and fermentation inhibitors. Its metabolic adaptability and genetic stability make it particularly suitable for converting hexose sugars derived from lignocellulosic biomass into ethanol [22].

The selection of raw material plays a vital role in determining the efficiency and economic feasibility of the bioethanol production process. Lignocellulosic biomasses derived from agricultural and industrial residues are particularly attractive due to their abundance and low cost [23].

Cork, a renewable and non-food lignocellulosic material, is a notable by - product of the cork industry, especially prominent in Mediterranean regions such as Portugal, Spain, and Algeria [24]. In Algeria, despite substantial cork reserves, the valorization of cork waste remains limited, and waste management practices are often underdeveloped [25]. Therefore, harnessing cork biomass for bioethanol production represents not only a strategy for value addition to an underutilized resource but also supports local circular economy and sustainable development goals.

This study focuses on the feasibility of using cork as a substrate for bioethanol production. The aim is to reduce processing costs in the production of second - generation bioethanol, making the process more viable for industrial applications. Chemical composition of the biomass and total sugar variation during alcoholic fermentation were performed, and the produced bioethanol was characterized using Raman and Fourier Transform Infrared Spectroscopy (FT-IR) as well as its physico - chemical and thermodynamic properties were measured to compare the bioethanol obtained from cork with conventional ethanol. A schematic flow diagram of the process is shown in Fig. 1.

EXPERIMENTAL

Reagents and chemicals

All chemicals used in this study were of analytical grade. Concentrated sulfuric acid (70 %), sodium hydroxide, sodium chlorite, ethanol (≥ 99.8 %), dichloromethane, phenol, ammonium sulfate ($(NH_4)_2SO_4$), monopotassium phosphate (KH_2PO_4), and magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) were purchased from Sigma - Aldrich (Germany). Distilled water was used throughout the experiments.

Raw material preparation

Cork biomass was sourced from industrial cork waste obtained from local cork processing society "E.P.E/ Jijel Étanchéité S.P.A.". The raw material consisted of post -industrial cork trimmings and fragments. The biomass was first manually sorted to remove any foreign materials, including adhesives or coatings, then washed with distilled water to eliminate surface impurities. After washing, the cork was oven -

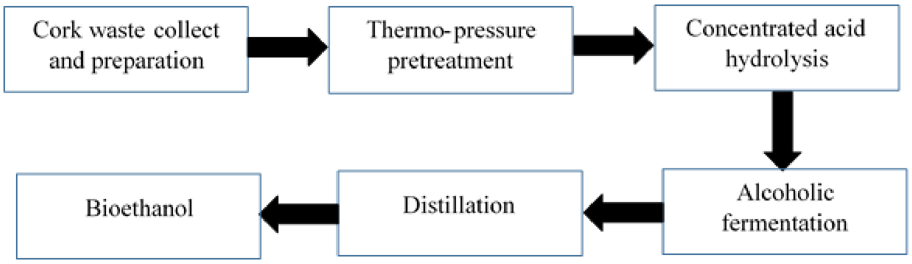

Fig. 1. Bioethanol production process from cork.

dried at 110°C for 24 h to reduce its moisture content and facilitate grinding. The dried material was ground and sieved to obtain a uniform particle size (0.1 < d < 0.16 mm). Physical pretreatment enhances the efficiency of hydrolysis by reducing particle size, thereby exposing more reactive surface area of the lignocellulosic material [26]. The powdered cork was then stored in airtight glass containers at room temperature in a dark environment to prevent moisture uptake prior to be subjected to the various stapes of pretreatments.

Chemical composition of the biomass

The chemical composition of cork was determined through standardized analytical procedures widely applied to lignocellulosic biomass. Organic extractives were quantified by successive Soxhlet extractions with dichloromethane, ethanol, and hot water, each performed for 6 h on finely milled cork powder (40 - 60 mesh), according to the TAPPI T 204 cm - 07 protocol. Mineral content (ash) was measured by incinerating 2 g of oven-dried cork in a muffle furnace at 525 ± 25°C for 4 h, following the ASTM D1102 - 84 method. Lignin content was determined using the Klason method (TAPPI T 222 om - 15), where extractive-free cork was treated with 72 % sulfuric acid at 30°C for 2 h, diluted to 3 % acid, and autoclaved at 121°C for 1 h. The acid - insoluble residue was reported as Klason lignin, while acid-soluble lignin was quantified using UV - Vis spectrophotometry at 205 nm [27]. Holocellulose was isolated by delignifying the biomass with 1.5 % sodium chlorite at 70°C under acidic conditions (pH 4 - 5) for 4 h, as described by Wise et al., [28]. Cellulose was then extracted from holocellulose using 17.5 % sodium hydroxide at room temperature for 30 min, following ASTM D1103 - 60. Hemicellulose content was calculated as the difference between holocellulose and cellulose. All measurements were conducted in triplicate, and results were reported as percentages of the oven - dried cork weight.

Thermo-pressure pretreatment

Thermo - pressure or vapor explosion is an effective thermochemical pretreatment technique used to disrupt the rigid structure of lignocellulosic biomass. It enhances the accessibility of cellulose and hemicellulose by breaking the complex lignin - carbohydrate matrix and partially solubilizing hemicelluloses and lignin [29]. In this process, the prepared cork biomass was loaded into an autoclave (Systec D) and subjected to high - temperature steam treatment at 270°C under a pressure ranging from 10 to 50 bar for 20 min. Every 5 min, a rapid depressurization was applied (lasting 1 - 2 s), inducing a mechanical disruption of the biomass through a thermal shock effect. This treatment caused significant structural disorganization of the plant cell wall [30]. A solid - to - liquid ratio of 1.5 % (w/v) was used to ensure optimal steam penetration and treatment efficiency.

Concentrated acid hydrolysis pretreatment

Lignocellulosic materials are commonly broken down using sulfuric acid due to its strong ability to hydrolyze cellulose effectively [31]. Concentrated acid hydrolysis is employed to cleave the glycosidic bonds in polysaccharides (cellulose and hemicellulose), releasing fermentable sugars. The use of a strong acid, such as sulfuric acid at high concentration (70 %), ensures a high hydrolysis efficiency [32]. Concentrated acid hydrolysis eliminates the need for enzymatic treatment during saccharification, presenting notable advantages such as broad applicability to diverse lignocellulosic feedstocks, elevated yields of fermentable monosaccharides, and the ability to operate under moderate thermal conditions [31].

In this procedure, cork powder was first dried at

105°C for 24 h to minimize residual moisture. A 10 g sample of the dried biomass was accurately weighed and mixed with 100 ml of 70 % (w/w) sulfuric acid. The mixture was stirred at room temperature for 1 h using a magnetic stirrer to achieve uniform dispersion. Hydrolysis was then carried out by incubating the mixture under slow agitation for 24 h, allowing gradual depolymerization of the polysaccharide matrix [33].

To mitigate the harshness of the concentrated sulfuric acid used in the previous step, a gradual dilution was performed until a final concentration of approximately 5 % H_2SO_4 was achieved. This controlled dilution facilitates the selective hydrolysis of structural polysaccharides while minimizing the degradation of liberated monosaccharides [34]. Following dilution, the mixture was subjected to a pressurized thermal treatment at a temperature ranging from 120 to 130°C and a pressure of 1 - 2 bar for 1 h. This heating step accelerates the depolymerization of cellulose and hemicellulose fractions into fermentable sugars such as glucose, xylose, and arabinose [35]. This mild hydrolysis approach, conducted under moderate conditions, helps reduce the formation of undesirable by - products such as furfural and hydroxymethylfurfural (HMF), both of which are known to inhibit yeast metabolism and compromise the efficiency of alcoholic fermentation [36].

Inoculum preparation

Inoculum preparation is a critical step in the fermentation process, aimed at introducing a metabolically active and exponentially growing yeast population to ensure a rapid fermentation onset, reduce the lag phase, and enhance process robustness and efficiency [37]. The yeast *Saccharomyces cerevisiae* used in this study was commercially sourced. To initiate the culture, *S. cerevisiae* was first cultivated in YEPD medium - comprising yeast extract, peptone, and dextrose-in a shake flask incubated at 30°C for 24 h with agitation at 100 rpm [38, 39]. The YEPD medium provides essential nutrients for yeast propagation and is commonly used for biomass build - up prior to fermentation [40].

Alcoholic fermentataion

The alcoholic fermentation process was carried out using *Saccharomyces cerevisiae* in a cork - derived hydrolysate supplemented with essential nutrients, including 1 g of ammonium sulfate ($(NH_4)_2SO_4$), 1 g of monopotassium phosphate (KH_2PO_4), and 0.5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), as these compounds play a vital role in supporting yeast metabolism and optimizing ethanol yield [41]. Prior to fermentation, detoxification of the hydrolysate was achieved by treatment with activated carbon to reduce the concentration of fermentation inhibitors such as acetic acid, furfural, and hydroxymethylfurfural (HMF), known to inhibit yeast growth and fermentation performance [42, 43]. Fermentation was carried out in batch mode in a laboratory - scale glass bioreactor equipped with temperature control, agitation, and sampling ports, aligning with protocols commonly used in second-generation bioethanol production [44]. The fermentation temperature was maintained at 30°C, and the pH was adjusted to 4.9 before inoculation, conditions reported as optimal for ethanol production by *S. cerevisiae* [45]. The fermentation lasted approximately 72 h [46], and sugar consumption was monitored using the phenol -sulfuric acid method, a standard colorimetric technique for quantifying total carbohydrates in fermentation samples [47]. In this assay, 0.5 ml of sample was mixed with 0.5 ml of 5 % phenol and 2.5 ml of concentrated sulfuric acid, followed by a 30 min incubation at room temperature. The absorbance was measured at 490 nm to determine the residual sugar content and estimate fermentation progress [48].

Alcoholic distillation

At the end of fermentation, the broth was filtered to remove solid residues. The resulting liquid, containing ethanol, was subjected to distillation at 78°C, the boiling point of ethanol [49]. The ethanol vapors were condensed and collected in liquid form, enabling both isolation and quantification of the produced bioethanol to assess the process efficiency.

Evaluation of physicochemical and thermodynamic attributes of cork-derived bioethanol

The energy content of the bioethanol was assessed through a bomb calorimeter (IKA C 200) complies with international standards like ASTM D 5865, while its viscosity was measured using a Brookfield DVNext viscometer adhered to standardized protocol, namely ASTM D 2196. Reid vapor pressure was determined via the MINIVAP VP Vision system, following the ASTM D6378/D323. Density measurements were carried out with a DA-850 densimeter, based on the ASTM D 4052 standard. The flash point was identified using the MINIFLASH FP Vision device in compliance with ASTM D 6450. Ethanol content in the sample was estimated using a precision alcoholmeter. Additionally, the elemental composition (C, H, and O) was analyzed through elemental analyzer PerkinElmer 2400 Series II

FT-IR spectroscopy

Fourier Transform Infrared Spectroscopy (FT-IR) of bioethanol-cork derived was performed using a Shimadzu IRSpirit-T spectrometer. Samples were scanned in the range of 4500 - 450 cm^{-1} with a resolution of 4 cm^{-1}. The spectra were analyzed for characteristic absorption bands of ethanol and compared to a commercial ethanol standard.

RESULTS AND DISCUSSIONS

Chemical composition of cork

The chemical composition analysis of cork showed a notably high suberin content (42.84 %), followed by lignin (21.66 %) and cellulose (15.58 %). Organic extractives represented 15.32 %, hemicellulose 4.71 %, and ash 0.75 % (Table 1). The predominance of suberin, a complex polyester of long - chain fatty acids and alcohols, is typical of cork and imparts hydrophobicity and biological resistance but also poses a significant challenge in biomass deconstruction [50]. This waxy and inert layer must be disrupted effectively to enable access to the polysaccharide components. Given the relatively high lignin content, cork exhibits considerable resistance to degradation. Lignin acts as a physical barrier and forms lignin - carbohydrate complexes that hinder hydrolytic accessibility. However, the presence of cellulose and hemicellulose confirms the presence of fermentable sugars after suitable treatment. Unlike enzymatic hydrolysis, concentrated acid hydrolysis effectively breaks down these polymers, by cleaving glycosidic bonds and releasing monosaccharides such as glucose and xylose [51]. The low mineral extractives content is advantageous for thermochemical processing, minimizing potential ash formation and equipment fouling. However, the high organic extractives fraction, which may include phenolic and triterpenoid compounds, could generate fermentation inhibitors during acid hydrolysis if not adequately removed or neutralized [52].

Total sugar variation during alcoholic fermentation

Following acid hydrolysis, the fermentable sugar fraction was subjected to alcoholic fermentation using *Saccharomyces cerevisiae*, a widely used yeast strain known for its high ethanol yield from hexose sugars, particularly glucose [53, 54]. However, *S. cerevisiae* lacks the metabolic capacity to convert pentose sugars such as xylose and arabinose into ethanol, limiting its efficiency in fully utilizing lignocellulosic hydrolysates [55, 56]. Pentose fermentation poses challenges due to the absence of robust, naturally occurring microorganisms that can efficiently ferment these sugars under industrial conditions, particularly in the presence of inhibitory compounds generated during pretreatment steps [57].

During fermentation, yeast metabolizes sugars to generate energy for growth, releasing ethanol and carbon dioxide as metabolic byproducts. The dynamic relationship between sugar depletion and ethanol formation can be tracked over time [58]. In the initial phase of fermentation (0 - 12 h), as can be seen in Fig. 2, sugar uptake is relatively limited, likely due to the lag phase, where the yeast undergoes physiological adaptation and regulatory

Table 1. Chemical composition of cork.

Composition	Value, %
Organic extractives	15.32
Ash	0.75
Suberin	42.84
Lignin	21.66
Cellulose	15.58
Hemicellulose	4.71

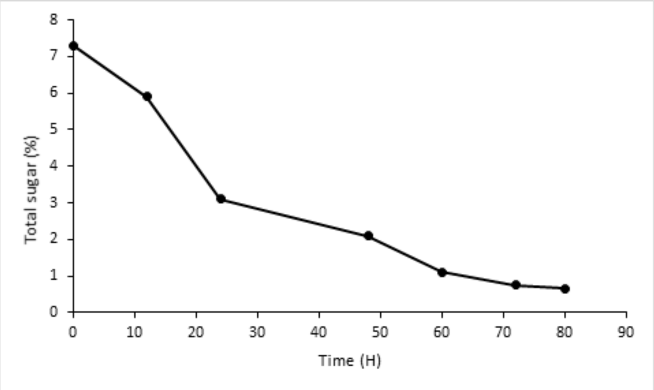

Fig. 2. Variation of total sugars throughout alcoholic fermentation.

adjustments to the new environment [59]. After 12 h, sugar consumption accelerates as the yeast enters exponential growth, driven by favorable conditions and increased biomass [60]. At 48 h, a decline in sugar utilization is observed, suggesting the accumulation of organic acids and possible nutrient limitation. This phase typically signals the onset of stationary growth, associated with the depletion of essential substrates and the build-up of inhibitory metabolites [61]. By 72 h, the fermentation rate significantly slows, indicating that the yeast has reached metabolic arrest, with cell division halted and structural changes occurring because of environmental stress and ethanol toxicity [62].

Distillation

Following the completion of alcoholic fermentation, the resulting fermented mixture underwent distillation to isolate the bioethanol. The recovered ethanol, exhibiting a purity of 30 % (v/v), was subsequently analyzed using Raman spectroscopy and Fourier Transform Infrared (FT-IR).

Characterization of bioethanol

Raman spectroscopy

The Raman spectrum of the produced bioethanol (Fig. 3) displays characteristic vibrational bands confirming the presence of ethanol in water - rich mixture. A broad and intense peak centered around 3300 - 3500 cm^{-1} is attributed to O - H stretching vibrations, indicative of strong hydrogen bonding and a high-water content in the sample [63]. This observation is consistent with the relatively low purity of bioethanol measured at 30 % (v/v). The presence of ethanol is confirmed by distinct C - H stretching vibrations observed near 2100 cm^{-1}, corresponding to the methyl and methylene groups of ethanol [64]. Additional bands in the region of 1000 - 1500 cm^{-1} can be assigned to C - C and C - O stretching, as well as CH_3 and CH_2 bending modes, which are typical of aliphatic alcohols like ethanol [65]. The combination of these spectral features supports the successful conversion of cork biomass into bioethanol, albeit with significant water content due to incomplete separation or azeotropic behavior during recovery.

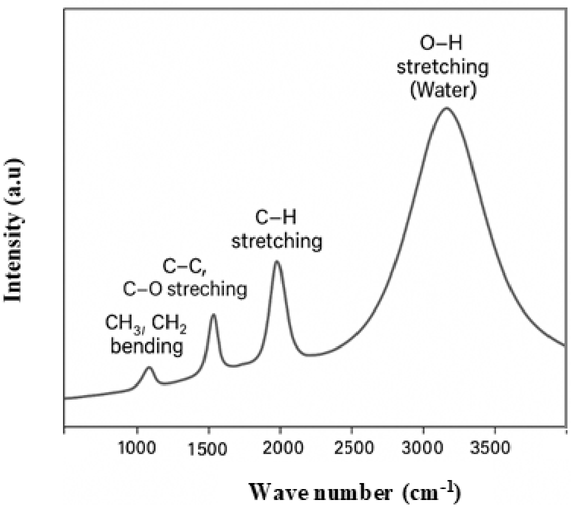

Fig. 3. Raman spectrum of bioethanol - cork derived.

FT-IR analysis

The FT-IR spectra illustrated in Fig. 4 compare commercial bioethanol and cork-derived bioethanol, highlighting characteristic absorption bands associated with functional groups typical of ethanol and potential impurities. Both spectra exhibit a broad absorption band in the region of 3000 - 3600 cm^{-1}, which corresponds to the O - H stretching vibration, indicative of hydroxyl groups in ethanol [66]. This band is broader and more intense in commercial ethanol, suggesting higher hydroxyl-containing compound content. The C-H stretching vibrations, observed between 2850 and 3000 cm^{-1}, appear in both samples, confirming the presence of alkyl chains [67]. The peaks around 1450 - 1375 cm^{-1} can be attributed to C - H bending vibrations. Notably, the cork - derived ethanol displays slight shifts and intensity variations in the 1000 - 1300 cm^{-1} region, associated with C - O stretching vibrations, potentially reflecting impurities or residual fermentation by - products |68]. These spectral features suggest that, while both ethanol types share a similar molecular fingerprint, cork - derived ethanol may contain trace compounds from the lignocellulosic biomass, resulting in subtle differences in peak shapes and intensities. Such deviations are common in bioethanol obtained from lignocellulosic materials due to the complex nature of the biomass and the potential for incomplete purification [69].

Physicochemical and thermodynamic properties of bioethanol

The physicochemical and thermodynamic characterization of bioethanol derived from cork are

Table 2. Physicochemical and thermodynamic properties of bioethanol cork - derived vs. commercial ethanol.

Property	Bioethanol cork derived	Commercial ethanol
Density, g cm^{-3} at 20°C	0.970	0.789
Dynamic viscosity, MPa at 20°C	1.9	1.2
Flash point, °C	26	13
Lower calorific value, MJ kg^{-1}	20.1	26.8
Reid vapor pressure, kPa at 37.8°C	7.60	16
Carbon content, %	23.4	52
Hydrogen content, %	10.8	13
Oxygen content, %	53.70	35

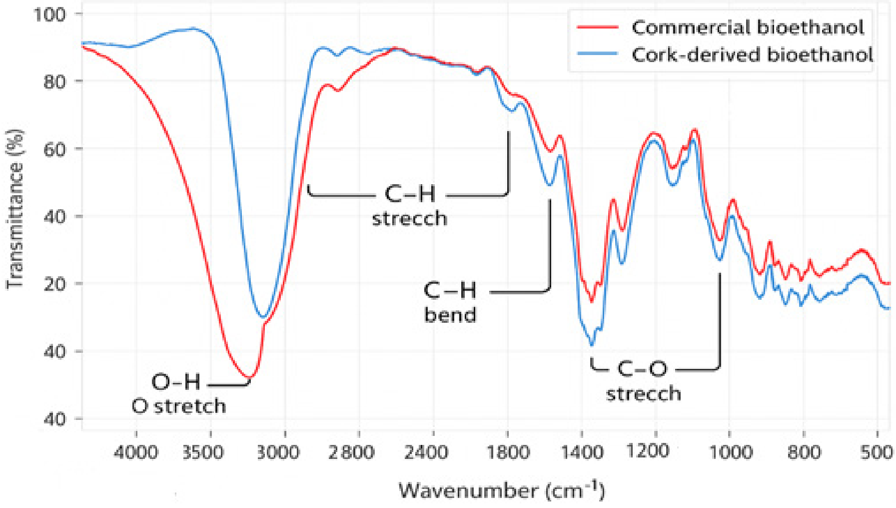

Fig. 4. FT-IR spectrum of commercial and cork - derived bioethanol.

presented in Table 2. Results revealed significant differences when compared to commercial - grade ethanol. Due to the relatively low ethanol content, the cork - derived bioethanol exhibited a higher density and dynamic viscosity, which can be attributed to the elevated water content. This dilution also led to a higher flash point, indicating reduced flammability compared to commercial ethanol. Furthermore, the lower calorific value and reduced Reid vapor pressure suggest lower energy efficiency and volatility, respectively. Elemental analysis indicated a lower carbon content and higher oxygen content, which is consistent with the composition of diluted ethanol-water mixtures. These findings highlight the necessity of further purification to improve the fuel quality of cork-derived bioethanol and align its properties with those of standard commercial ethanol.

CONCLUSIONS

The present work highlights the feasibility of utilizing cork biomass - an abundant, non-food lignocellulosic waste, to produce second-generation bioethanol. Through a combination of thermochemical pretreatment, concentrated acid hydrolysis, and alcoholic fermentation, fermentable sugars were successfully extracted and converted into ethanol. The final distillate reached a purity of 30 % (v/v), which, although moderate, reflects a typical outcome of limited distillation step. The produced bioethanol exhibited key physicochemical properties such as density, viscosity, flash point, calorific value, and vapor pressure, consistent with its composition and use in low - purity fuel applications. Furthermore, spectroscopic analyses (FT-

IR and Raman) confirmed the presence of characteristic functional groups of ethanol and water, validating the composition and production process. Overall, the study provides a strong foundation for the valorization of cork biomass in biofuel production, supporting sustainable waste management and contributing to the broader goals of the circular bioeconomy.

Authors' contributions

All experiments and analyses presented in this manuscript were conducted and prepared by F.K.

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