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Research Article

Sustainable Biofuel Production from Agricultural By-Products: Citrus Peels and Pumpkin Seeds as Feedstocks for Bioethanol and Biodiesel

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Abstract: Energy is fundamental to development, and in correlation with the rapid population growth, its demand is escalating. The issues associated with fossil fuels, their depletion, and negative environmental effects have led to the exploration of alternative energy sources. Biofuels such as biodiesel and bioethanol have emerged as promising, renewable, and environmentally friendly alternatives. This study explores the sustainable production of bioethanol from *Citrus* peels and biodiesel from *Pumpkin* seed oil, assessing their potential contributions to energy efficiency and societal benefits. *Citrus* peels, underwent acid hydrolysis (0.5% H_2SO_4, 2 h) to release fermentable sugars, followed by anaerobic fermentation using 10% (v/v) *Saccharomyces cerevisiae*a. The resulting bioethanol was confirmed through qualitative analysis via Jones reagent. GC-MS of the hydrodistilled *Citrus* peel revealed 90.53% limonene in *Citrus* oil Biodiesel was produced via transesterification of pumpkin seed oil with methanol and potassium hydroxide (KOH) catalyst. GC-MS identified 65.9% unsaturated fatty acids (linoleic and oleic acids dominant), with a 97.5% yield, 0.7% ash content, and calorific value of 39,586 kJ/kg, meeting ASTM/EN standards. The study highlights the potential of utilizing agricultural waste and by-products in biofuel production, contributing to a circular economy and reducing environmental impact. The study demonstrates the viability of *Citrus* peel waste and *Pumpkin* seed oil as feedstocks, reducing reliance on fossil fuels while valorizing agricultural by-products. Challenges in scaling production require further exploration to maximize societal and environmental benefits, particularly in resource-rich regions.

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1. Introduction

According to the most recent data available from Forbes for 2024, "Renewable energy expanded at a rate six times faster than that of total primary energy, accounting for 14.6% of overall consumption, while primary energy consumption rose by 2% from 2022 and exceeded pre-COVID levels by more than 5%". Despite this growth, fossil fuels still dominate, comprising 81.5% of primary energy

consumption, though their share is slightly declining. Fossil fuels, including petrol, diesel, coal, and natural gas, remain the world's primary energy sources but are plagued by several issues such as finite supply, fluctuating prices, and uneven geographical distribution. More critically, they are the leading contributors to greenhouse gas (GHG) emissions, driving global climate change and threatening ecosystems worldwide (Rapier, 2024).

To limit the average global temperature increase to below 1.5°C, a minimum reduction of 40% in greenhouse gas (GHG) emissions by 2050 is required. Therefore, it is crucial to seek alternative solutions for energy generation as a substitute for fossil fuel to meet the Sustainable Development Goals by 2030 and combat environmental pollution and natural resource shortages (Samaras, 2019; Manakas et al., 2024).

To create a sustainable energy framework and lower CO_2 emissions, biofuels have emerged as a highly promising energy source. Biofuels are produced from various feedstocks, including animal fats, agricultural waste, and used oils, and can be classified into solid, liquid, and gaseous forms. They are biodegradable, non-toxic, and carbon-neutral, emitting fewer carbon and greenhouse gases, as well as lower levels of CO, nitrogen oxides, sulfur oxides, unburnt hydrocarbons, and particulate matter compared to fossil fuels and conventional energy sources (Correa et al., 2019).

Biomass, derived from agricultural waste, including citrus peels, pumpkin seeds, rice husks, and sugarcane bagasse, represents a vast, underutilized resource. It consists of readily available, renewable, and eco-friendly raw materials that do not contribute to increased CO_2 levels and produce lower amounts of sulfur and nitrogen emissions. Conventional biofuels (bioethanol and biodiesel), which rely on crops, have well-established production processes, with bioethanol derived from sugars and starchy feedstocks through fermentation, and biodiesel produced via the transesterification of vegetable or edible oils. They only account for approximately 4% of total transport fuel consumption because of several limitations, including feedstock shortages, limited CO_2 reduction, blending constraints, and cost issues. These biofuels not only offer a reduction in GHG emissions but also provide a pathway to a more circular economy by utilizing waste materials (Correa et al., 2019).

Citrus is the most widely cultivated fruit crop worldwide, with an annual production of about 110–124 million tons, whereas the food sector, widely processed for juice and flavoring agents, usually discards 45–55% of the entire fruit as waste (peels, seeds, and pomace) after processing. These peels, rich in carbohydrates and essential oils, often become industrial waste despite their fermentable sugar content. Globally, food processing industries generate millions of tons of such organic residues annually, which pose disposal challenges but offer potential as biofuel feedstock due to their biochemical composition. The waste's bioactive components (carotenoids, vitamins, pigments, and essential oils) can be used in packaging, medications, cosmetics, food additives, and synthetic fuels.(Mahato et al., 2021; Suri et al., 2022). The carbohydrates and fermentable sugars found in *Citrus* biomass are harnessed to produce biogas and ethanol. These biofuels can cut CO_2 emissions by up to 80% compared to gasoline or petroleum fuels (Mahato et al., 2021). In the realm of biofuel production, *Citrus* waste is classified as a second-generation feedstock. Although it is technically viable for bioethanol production, it is not yet widely established in industrial-scale operations, despite not competing with food resources (Medina et al., 2020).

Biodiesel represents a major alternative to traditional fuels that has acquired significant attention. The American Society for Testing and Materials (ASTM) defines biodiesel as "mono-alkyl esters of long-chain fatty acids derived from animal fats or vegetable oils." Therefore, the main reactions in current biodiesel production are the esterification and transesterification of free fatty acids and triglycerides with alcohol, applying both catalytic (chemical and biological catalysts) and non-catalytic methods. Recent studies have explored the combustion performance of biodiesel blends in direct injection diesel engines, including biodiesel derived from palm, *Garcinia gummi-gutta,* and tamarind, as well as alternative fuels blended with diesel (Lam and Lee, 2011; Kant Bhatia et al., 2021; Muhammad et al., 2021). To find new low-cost alternative feedstocks for biodiesel production, efforts are ongoing (Schinas et al., 2009). Different factors affect the selection process of the feedstocks used for biodiesel production that include cost, quality and chemical content variability, consistent availability, supply scalability, and transportation and pretreatment costs (Schinas et al., 2009).

Egypt ranked 28th in the production of gourds, squashes, and pumpkins. Pumpkin is a type of winter squash with large fruits, belonging to genus *Cucurbita*. The pulp is commonly used in dishes like pies and soups, while the seeds are enjoyed as snacks. Additionally, pumpkin seed oil is used for cooking

and as a salad dressing. Moreover, pumpkins have long been used for their medicinal properties for being rich in nutritional components and phytochemicals. Pumpkin seeds are known for their significant immunomodulatory effects, antioxidant, nephroprotective as well as gastroprotective. Pumpkin seed oils are a notable source of phenolics, including ferulic acid, syringic acid, chlorogenic acid, *p*-coumaric acid, tyrosol, vanillic acid, vanillin, luteolin, and sinapic acid, with high phenolic content, besides the presence of minerals (such as potassium, iron, zinc, copper, magnesium, selenium, and phosphorus), sterols, terpenes and hydrocarbons as β-sitosterol, stigmasterol, squalene, and *β*-carotene.(Eleiwa et al., 2014; Ezzat et al., 2022; Hussain et al., 2023).

Over 140 billion metric tons of agro-industrial waste are generated yearly, yet less than 25% are valorized. The persistence of linear "take-make-dispose" models in agri-food systems underscores the urgency to adopt circular economy frameworks, where waste is transformed into energy, materials, or high-value products. This study addresses this gap by exploring citrus peels and pumpkin seeds—two abundant, non-food-competing feedstocks—for biofuel production, thereby mitigating waste and fossil fuel dependence. By focusing on these underutilized feedstocks, this research contributes to the ongoing efforts to develop sustainable, cost-effective, and environmentally friendly energy solutions. The findings could play a crucial role in shaping future biofuel production strategies, particularly in regions with abundant agricultural resources.

2. Material and Methods

2.1. Bioethanol production and characterization

2.1.1. Citrus peels collection and volatile oil extraction for bioethanol production

Orange peels from *Citrus sinensis* were gathered from Egyptian Market in October 2023, sun-dried, and then manually ground into a powder. 500 grams of dried ground peel were immersed in 2 L of distilled water for hydrodistillation. The extraction was conducted on a **Clevenger Apparatus** for essential oil extraction and kept for 4 h until the amount of essential oils stabilized. The essential oils were then stored in a refrigerator at +4°C until analyzed by gas chromatography coupled with mass spectrometry (GC-MS).

2.1.2. GC/MS analysis for Citrus peels essential oil

To determine the primary volatile oil isolate, the extracted essential oil was subjected to GC/MS analysis at Ain Shams University's Faculty of Pharmacy. A Shimadzu GC MS-QP2010 equipped with a Rtx-5MS column (30 m x 0.25 mm x 0.25 µm; Restek, USA) was used for the analysis. The carrier gas, helium, was used at a flow rate of 2 milliliters per minute. Using the PeakSimple q2000 chromatography data system (SRI Instruments, Torrance, USA), chromatograms were captured and integrated. The average areas under the peaks from three separate independent chromatographic runs were used to compute the percentage makeup of each component. The temperature of the column was set to rise at a rate of 5 °C per minute, from 45 to 300 °C. The temperatures of the injector and detector were adjusted to 250 °C and 300 °C, respectively. The oils were subjected to GC/MS analysis in split mode with a split ratio of 1:15. Using the PeakSimple q2000 chromatography data system (SRI Instruments, Torrance, USA), chromatograms were captured and integrated. The average areas under the peaks from three separate independent chromatographic runs were used to compute the percentage makeup of each component. Under the same circumstances, an identical series of n-alkanes (C8–C18; Aldrich Chemical Company, USA) was injected. In addition to comparing the calculated Kovats retention indices (KIs) with those in the literature and the Adams library (Adams, 2007), the essential oil components were determined by comparing their mass spectra with the database spectra that was accessible on the apparatus. Without using any correction factors, the relative contents of each component were determined as a percentage based only on the GC peak regions.

2.1.3. Acid hydrolysis

The remaining 300 grams of dried *Citrus* peels were hydrolyzed with 0.5% H_2SO_4 and refluxed for two hours. The liquid was cooled, and then 30% NaOH was added to neutralize it to get the samples ready for fermentation. Next, the *Citrus* peels were extracted from the mixture. Lastly, a rotary evaporator was used to concentrate the hydrolysate until it was completely dry. The dinitrosalicylic acid

(DNS) approach was utilized to ascertain the hydrolysate's reducing sugar content (Agu et al., 1997; Ahmad et al., 2022).

2.1.4. Estimation of reducing sugars in the hydrolyzed solution of Citrus peels using dinitrosalicylic acid (DNS)

One gram of DNS acid was dissolved in twenty milliliters (2 M NaOH) and fifty milliliters (distilled water) to create the DNS reagent. After adding thirty grams of Rochelle salt, the amount was raised to 100 milliliters using purified water. 30 (g) of potassium sodium tartrate tetrahydrate were dissolved in 20 (mL) of distilled water to create the Rochelle salt solution. Rochelle salt is added to assist in stopping the reagent from absorbing oxygen (Agu et al., 1997; Hu et al., 2008).

In the assay, a test tube was filled with 0.2 mL of analytical glucose at a specified concentration, 1.8 mL of distilled water, and 2 mL of DNS reagent. After that, the mixture was brought to a boiling water bath for five min., which turned it from light brown to brick red. The sample was diluted to 24 mL after cooling. A series of dilutions ranging from 0.5 to 2.5 mg mL^{-1} was made. The absorbance of the diluted samples was measured using a UV spectrophotometer at 540 nm. The data obtained was used to construct a calibration curve, which was employed to determine the reducing sugar concentration in all the hydrolysate samples.

2.1.5. Fermentation and Distillation

The acid hydrolysate of the *Citrus* peel extract was fermented in the following medium (per liter): 1g $MgSO_4 \cdot 7H_2O$, 2g $(NH_4)_2SO_4$, and 5g KH_2PO_4, with the pH adjusted to 5.5 using 2M NaOH. This medium was placed in a 250 mL Erlenmeyer flask and sterilized by autoclaving at 121 °C and 15 psi for 30 min. After sterilization, an inoculum suspension of *Saccharomyces cerevisiae* S288 cells (10% v/v) was added to the medium. The fermentation process was conducted at 30 °C under static conditions for 96 hours. Samples of the fermented broth were collected every 12 h to monitor ethanol production and yield (Akaracharanya et al., 2011).

Citrus peel extract acid hydrolysate was fermented in 1 g $MgSO_{44} \cdot 7H_2O$, 2 g $(NH_4)_2SO_4$, and 5 g KH_2PO_4 per liter. The pH was then adjusted to 5.5 employing 2M NaOH. This medium was autoclaved for 30 min. at 121°C and 15 psi after being put into a 250 mL Erlenmeyer flask. Following sterilization, the medium was supplemented with a 10% v/v inoculum suspension of *Saccharomyces cerevisiae* S288 cells. For 96 h, the fermentation process was carried out in static circumstances at 30 °C. Every twelve hours, samples of the fermented broth were taken to track the yield of the produced ethanol.

Each aliquot of the fermented broth was quantified, subsequently transferred into a round-bottom flask, and positioned upon a heating mantle that was connected to a distillation column. A secondary flask was affixed to the opposing extremity of the distillation column to facilitate the collection of the distillate at a temperature of 60 °C. This methodology was systematically repeated for every individual sample of the fermented broth. The volumetric measurement of the distillate collected was conducted utilizing a measuring cylinder, and this volume was subsequently converted into the yield of ethanol produced (g/L) by multiplying the distillate volume by the density of ethanol (0.8033 g/cm^3).(Somda et al., 2010; Cheng et al., 2017).

2.1.6. Qualitative estimation of Bioethanol

The production of bioethanol was assessed utilizing Jones reagent (composed of K_2Cr_2O7 and H_2SO_4). A total of one milliliter of $K_2Cr_2O_7$ (at a concentration of 2%), five milliliters of concentrated H_2SO_4, and three milliliters of the sample were incorporated into the Jones reagent. The ethanol underwent oxidation to form acetic acid in the presence of potassium dichromate and sulfuric acid, resulting in a blue-green coloration. The manifestation of a green hue signifies a positive result. (Caputi et al., 1968). This test was repeated with samples of day 1, day 2, day 3, and day 4.

2.2. Biodiesel production and characterization

2.2.1. Pumpkin seed oil extraction

The seeds of *Cucurbita moschata* (commonly known as pumpkin) were meticulously separated from the fruit, subsequently subjected to drying under solar exposure, during which 190 g of the pumpkin seeds were processed by grinding and maceration in hexane, with the solvent being replenished

daily until depletion. The resultant hexane extract was then subjected to concentration via a rotary evaporator maintained at a temperature of 50 °C and stored for biodiesel synthesis.

2.2.2. Transesterification of the pumpkin fixed oil and biodiesel formation

A mass of 0.25 g of potassium hydroxide was accurately measured and subsequently introduced into 63 mL of methanol, which was then subjected to a water bath maintained at 60 °C until complete dissolution, resulting in the formation of potassium methoxide. A volume of 0.5 mL of the fixed oil was incorporated into the potassium methoxide within a round-bottom flask, and this resultant mixture was agitated for 50 min. employing a magnetic stirrer set at 1100 rpm, after which it was permitted to settle for 24 hours, followed by the separation of the methyl ester and glycerol utilizing a separating funnel. After the separation, the upper phase was thoroughly rinsed by the addition of distilled water and subjected to gentle agitation to eliminate impurities, and then it was allowed to settle, yielding a biphasic mixture from which the biodiesel was extracted. This procedure was reiterated and subjected to centrifugation until a more translucent biodiesel was achieved (Eleiwa et al., 2014) .

2.2.3. GC/MS analysis of the obtained biodiesel

0.2 mL of the trans-esterified oil underwent analysis utilizing Gas Chromatography-Mass Spectrometry (GC/MS). The mass spectra were acquired employing a Shimadzu GCMS-2010 Plus (Kyoto, Japan) that was outfitted with an Rxi-1MS fused-silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) from Restek, USA, accompanied by a split-splitless injector. The initial temperature of the column was established at 50 °C for 3 min. (isothermal), subsequently scheduled to rise to 300 °C at an increment rate of 5 °C min^{-1}, and sustained at 300 °C for 10 minutes (isothermal). The injector was maintained at a temperature of 280 °C, while the flow rate of the helium carrier gas was regulated at 1.37 mL min^{-1}. All mass spectra were recorded under the following specifications: filament emission current set to 60 mA; ionization voltage maintained at 70 eV; and ion source temperature fixed at 220 °C. Diluted samples (1% v/v) were introduced in split mode with a split ratio established at 1:30.

2.2.4. Determination of ash content

A 1 mL sample of biodiesel was dispatched to the Egyptian Petroleum Research Institute (EPRI) for evaluation employing the Ash Standard Method applicable to petroleum products (ASTM D-482) (D482, 2019). The analytical process commenced with the heating of an evaporating dish to a temperature range of 700-800 °C for a minimum duration of 10 minutes, followed by its subsequent cooling to ambient temperature and weighing to the nearest 0.1 mg. The biodiesel specimen underwent thorough agitation for 10 minutes to guarantee uniformity prior to being weighed into the dish with a precision of up to 0.1 g. Subsequently, the dish was subjected to heating via a Meeker burner until the specimen ignited and combusted at a controlled rate, resulting in the production of solely carbonaceous residue. Any crust that formed during the combustion process was fragmented and returned to the dish utilizing ashless filter paper. The resultant residue was subjected to further heating within a muffle furnace at a temperature of 775±25 °C until the complete elimination of all carbonaceous material was achieved. The dish was then allowed to cool to ambient temperature and was reweighed, with the heating, cooling, and weighing procedures being reiterated until consecutive weight measurements exhibited a variance of no greater than 0.5 mg (Moser, 2009). The ash content percentage was calculated using the formula:

$$\text{Ash sample } \% = (w/W) \times 100 \quad (1)$$

Where w is the mass of the ash (g) and W is the mass of the sample (g).

2.2.5. Determination of calorific value

Biodiesel produced from pumpkin seeds was prepared for combustion analysis utilizing the Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels via Bomb Calorimeter (ASTM D240-19) (D240, 2019.) which involved a filtration process to eliminate free water and insoluble ash. Subsequently, the biodiesel specimen was accurately weighed to achieve a combustion temperature elevation corresponding to 0.9-1.1 g of benzoic acid. An aliquot of 1 ml of water was

introduced into the combustion bomb, which was subsequently charged with oxygen to a gauge pressure of 3.0 MPa at ambient temperature. The combustion process was terminated when the oxygen pressure surpassed 4.0 MPa. Before the weighing procedure, the calorimeter water temperature was meticulously adjusted, ensuring that the final temperature was marginally above the jacket temperature. The calorimeter assembly was encased within a jacket, and the stirring mechanism was activated. Temperature measurements were systematically recorded at one-minute intervals over five minutes to ascertain equilibrium.

Upon achieving equilibrium within the calorimeter, the jacket temperature was meticulously adjusted to either match or remain slightly below that of the calorimeter. Following a stabilization period of three minutes, the ignition charge was activated, and the initial temperature was documented. During the period of temperature elevation, the jacket was precisely adjusted to sustain equivalent temperatures with the calorimeter. The final equilibrium temperature was recorded after confirming stable readings over three consecutive minutes.

After the combustion bomb was detached, the pressure was systematically released, and a thorough examination of the interior was conducted to assess any signs of incomplete combustion (Ezema et al., 2023). The gross energy content was calculated using the equation:

$$\text{Energy content} = \frac{E\Delta T - 2.3L - V}{g} \text{ (KJ / Kg)} \quad (2)$$

where ΔT is the temperature rise, L is the length of burnt wire, V is the volume, g is the sample weight, and E is the calorimeter's energy equivalent.

3. Results and discussion

The extraction and isolation of *Citrus* peels essential oil is a fundamental step, as it is imperative to eliminate limonene which can inhibit *S. cerevisiae* activity during the saccharification and fermentation processes (Wilkins et al., 2007). Hydro-distillation represents a traditional and extensively employed methodology for the extraction of essential oils. During this process, the sample is directly submerged in distilled water and heated within a Clevenger apparatus, wherein the thermal energy facilitates the release of volatile compounds from the plant cells and water that can evaporate concurrently at the same pressure, subsequently, these compounds are then condensed and separated based on their densities and immiscibility in a Florentine flask. (Li et al., 2014). The velocity of oil liberation from the botanical material is modulated by the accessibility of volatile oils within the cellular pores, which constitute the oil, and is predominantly dictated by their solubility in the liquid medium (Nader et al., 2022).

The yield of the essential oil extracted was 0.6 mL g^{-1} of dry biomass. GC-MS analysis of the *Citrus* Essential oils revealed a complex mixture dominated by monoterpenes and sesquiterpenes. Limonene, a transparent and colorless liquid hydrocarbon categorized as a cyclic monoterpene, serves as the principal constituent within the essential oils of *Citrus* fruit peels accounting for 90.53% of the identified constituents (Table 1). In comparison, the monoterpene β-myrcene constituted 2.65%, and linalool (a terpene alcohol) represented 1.3%. Across all investigations concerning *Citrus* peels, limonene emerged as the predominant volatile oil isolate, though its concentration may vary depending on the specific Citrus species and extraction conditions (Hardjono et al., 2021; Teke et al., 2023).

Hydrolysis of the *Citrus* peel biomass is crucial for releasing reducing sugars that are necessary for fermentation. Sulfuric acid was used in this study due to its ability to enhance hydrolysis without significantly degrading the cellulose components. The hydrolysate contained 1.6 g L^{-1} of total reducing sugars, as determined from a calibration curve (Figure 1). This concentration is consistent with previous reports, indicating the effectiveness of the hydrolysis process (Ayala et al., 2021; Ahmad et al., 2022).

The hydrolysate was then subjected to anaerobic fermentation employing 10% v/v *S. cerevisiae* at a temperature of 30 °C over 96 hours for the production of bioethanol (Figure 2). Ethanol concentration was assessed at 12-hour intervals, with peak production observed at 441.81 g L^{-1} at the 60-hour mark, before declining to 221 g L^{-1} by the end of the fermentation period. The bioethanol yield from *Citrus* peels (441.81 g L^{-1} at 60 h) surpasses reported values from similar studies using orange peel hydrolysates (e.g., (Wilkins et al., 2007): 38 g/L). This enhancement likely stems from optimized acid

hydrolysis (0.5% H_2SO_4), which effectively liberates reducing sugars while minimizing inhibitor formation. Conversely, the decline in ethanol concentration post-60 h aligns with observations by (Castro and Roberto, 2015), attributing this to nutrient depletion, including nitrogen, vitamins, and minerals, resulting in diminished yeast activity. Besides, ethanol-induced yeast inhibition where elevated ethanol levels may hinder yeast growth and fermentation, thereby further diminishing ethanol yield. The accumulation of by-products, such as organic acids or other fermentation inhibitors, can also negatively affect yeast performance and reduce ethanol output. Over time, factors such as autolysis (self-digestion) or the buildup of toxic substances may compromise yeast cell viability, leading to a decline in ethanol production rates (Brown et al., 1981; Castro and Roberto, 2015; Tse et al., 2021). To confirm the presence of bioethanol, the Jones reagent test was utilized. The samples collected from days 1 to 4 exhibited a green coloration, indicating the oxidation of ethanol into acetic acid through the action of potassium dichromate in the presence of sulfuric acid, in contrast to the yellow coloration observed in the control sample. This qualitative test corroborated the successful production of bioethanol from the Citrus hydrolysate.

Table 1. GC/ MS Analysis of *Citrus* peel oil

Name	Retention Time	Calculated Retention Index	Theoretical Retention İndex	Molecular Weight	*m/z*	Percentage Identified %
1)*α*-pinene	7.575	923	923	136	41,77,**93**,105, 121,136	0.69
2) *α*-thujene	8.645	959	969	136	41,69,77,**93**,121,136	0.34
3) Octanal	9.165	976	977	128	41,**43**,56,84,100	0.37
4) *β*- Myrcene	9.241	979	992	136	**41,**69,93	2.65
5) Carene	9.829	998	1011	136	41,77,**93**,105,121,136	0.13
6) Limonene	10.411	1017	1031	136	41,53,68,79,93, **107,121**	**90.53**
7)γ terpinene	11.235	1043	1062	136	65,77,**93,**105, 121	0.15
8) Linalool	12.385	1080	1098	154	41,55,69,**71**,91, 121,136,154	1.3
9)Terpinen-4 ol	14.655	1153	1177	154	43,**71**, 93, 107	0.71
10) *α*-Terpineol	14.995	1164	1197	154	43,**59**,81,93,121136	0.57
11) Decanal	15.45	1179	1207	156	**41,**57,70,82,95, 110	0.25
12) Ciral	17.14	1236	1266	152	**41,69,**84	0.21
Total						97.9

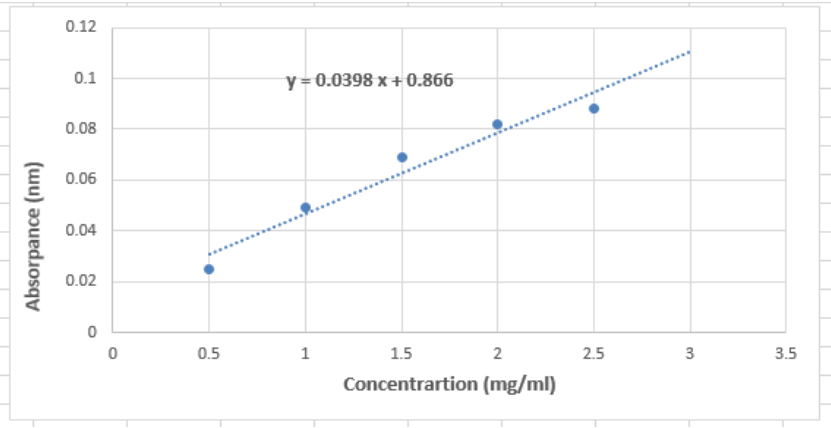

Figure 1. Calibration curve of reducing sugar (glucose).

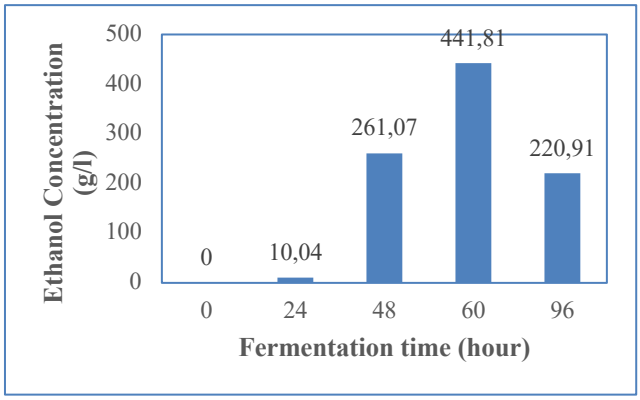

Figure 2. Bioethanol production from *Citrus* peels from 96h fermentation Biodiesel from Pumpkin seeds.

While fossil diesel currently benefits from lower production costs due to established infrastructure and economies of scale, pumpkin seed oil biodiesel offers environmental advantages such as renewability and reduced greenhouse gas emissions. For biodiesel production, pumpkin seed oil was transesterified, and GC-MS analyzed the resulting methyl esters with a total identification percentage of 99.69%. Among the identified components, the total percentage of unsaturated fatty acids was 65.9%, with linoleic acid (33.66%) and methyl oleate (31.72%) being the most abundant unsaturated fatty acid. The elevated proportion of unsaturated fatty acids contributes to the favorable flow characteristics of the fuel. Saturated fatty acids accounted for 33.79% of the biodiesel, with methyl palmitate (19%) and methyl stearate (12.78%) as the major components. These results are consistent with previous studies, which have reported similar fatty acid profiles in biodiesel derived from various seed oils (Hagos et al., 2023).

The biodiesel was further evaluated using ASTM D-482 method, revealing an ash content of 0.7 wt.%. The ash content (0.7%) currently exceeds ASTM standards, likely due to [possible reasons: incomplete transesterification, catalyst residues, or unwashed biodiesel]. Moreover, the energy output from this biodiesel sample was assessed per ASTM D240, yielding a calorific value of 39.586 KJ/kg, reflecting its substantial energy output and potential as a high-performance fuel. These properties meet the quality standards specified by EN 14214, affirming the suitability of pumpkin seed oil as a feedstock for biodiesel production. Prior studies have noted the substantial calorific yield associated with pumpkin seeds (Schinas et al., 2009; Ahtesham and Hebbal, 2020).

While pumpkin seed biodiesel has a lower calorific value (39.5 kJ/g) than fossil diesel (~45 kJ/g), it aligns with other vegetable oil-based biodiesels. The marginally lower energy output may be compensated by its carbon-neutral lifecycle and potential use of agricultural waste (e.g., pumpkin seed by products) (Knothe and Razon, 2017). Future work will optimize post-production purification (e.g., water washing, adsorbents) to reduce ash content for engine compatibility

Diverting citrus and pumpkin waste to biofuel production could reduce greenhouse gas emissions by up to 80% compared to fossil fuels, as estimated by (Mahato et al., 2021). This aligns with SDG 7 (Affordable and Clean Energy) and SDG 12 (Responsible Consumption). However, scalability hinges on addressing logistical challenges, such as seasonal feedstock availability and decentralized processing infrastructure. For instance, citrus waste is generated year-round in tropical regions but peaks during harvest seasons in temperate zones, necessitating storage solutions. While this study focuses on citrus and pumpkin waste, its methodology is applicable to other lignocellulosic residues (e.g., mango peels, coffee grounds). Diversifying feedstock portfolios can buffer against supply chain disruptions and cater to regional agricultural profiles, fostering resilient bioeconomies.

Table 2. GC/MS Analysis of Pumpkin seed oil biodiesel

Name	Retention time (min)	Calculated retention index	Theoretical retention index	Molecular weight	*m/z*	Area %
1) Methyl tetradecanoate (Myristic acid)	29.215	1698	1710	242	43, 55, 74, 87,101,129	0.08
2) Methyl hexadecanoate (palmitic acid methyl ester)	33.610	1900	1921	270	43, 55, 74, 87, 101, 129, 143, 227, 270	19.00
3) Heptadecanoic acid, methyl ester (margaric acid methyl ester)	35.580	1998	2009	284	43, 55, 74, 87, 101, 129, 143, 185, 199, 241, 284	0.09
4) Methyl linoleate	36.865	2064	2100	294	41, 55, 67, 81, 95, 109,123,135	33.66
5) 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	37.055	2074	2104	296	55, 69, 83, 97	31.72
6) Methyl octadecanoate (methyl stearate)	37.560	2100	2124	298	43, 57, 74, 87, 143, 157, ,185, 199, 255, 298	12.78
7) Cis Methyl 11-eicosenoate	40.615	2267	2279	324	41, 55, 69, 83, 97, 98	0.15
8)Eicosanoic acid, methyl ester	41.140	2297	2299	326	41, 43, 74, 87, 143	1.20
9) Docosanoic acid, methyl ester $$ Behenic acid, methyl ester	44.482	2496	2498	354	43, 57, 74, 87, 143, 199, 255, 354	0.38
10) Tetracosanoic acid	47.575	2696	2715	382	41, 43, 57, 74, 87, 143	0.21
11) Squalene	48.993	2793	2833	410	41, 69, 81, 95, 121,137	0.37
12) Hexacosanoic acid methyl ester	50.455	2896	2913	410	43, 57, 74, 87, 101, 143	0.05
Total identified						99.69
Total unsaturated fatty acids						65.9
Total saturated fatty acids						33.79

Conclusion

This study demonstrated the feasibility of producing biofuels from agricultural waste materials, specifically *Citrus* peels and pumpkin seeds. The conversion of *Citrus* peels into ethanol using *S. cerevisiae* proved it to be a viable and environmentally sustainable alternative to fossil fuels. The bioethanol yield increased by 2.2 times after three days of fermentation. Additionally, the production of biodiesel from pumpkin seed oil showed promise, with a high content of beneficial fatty acids, low ash content, and a high calorific value, indicating its potential as a sustainable biofuel. Future research should focus on optimizing these processes to enhance biofuel yields and explore scalable production methods for broader application.

Ethical Statement

Ethical approval is not required for this study because no animal or human samples were used.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

Conceptualization, methodology, supervision, writing, and editing: R.O.B; data curation, formal analysis: M.S.F, M.J.A, B.G.F, R.G.S.

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References

Adams, R. P., 2007. Identification of essential oil components by gas chromatography/mass spectorscopy. Allured Pub. Corp.

Agu, R. C., Amadife, A. E., Ude, C. M., Onyia, A., Ogu, E. O., Okafor, M., & Ezejiofor, E. (1997). Combined heat treatment and acid hydrolysis of cassava grate waste (CGW) biomass for ethanol production. *Waste Management, 17*, 91–96. https://doi.org/10.1016/S0956-053X(97)00027-5

Ahmad, A., Rahmad, Rita, N., & Noorjannah, L. (2022). Effect of acid hydrolysis on bioethanol production from oil palm fruit bunches. *Materials Today: Proceedings*, *63,* S276–S281. https://doi.org/10.1016/J.MATPR.2022.02.461

Ahtesham, S. A., & Hebbal, O. (2020). Pumpkin seed oil biodiesel an alternate fuel to diesel: A review. *International Research Journal of Engineering and Technology*, *7(10),* 700–707. www.irjet.net

Akaracharanya, A., Kesornsit, J., Leepipatpiboon, N., Srinorakutara, T., Kitpreechavanich, V., & Tolieng, V. (2011). Evaluation of the waste from cassava starch production as a substrate for ethanol fermentation by *Saccharomyces cerevisiae*. *Annals of Microbiology*, *61,* 431–436. https://doi.org/10.1007/S13213-010-0155-8

Ayala, J. R., Montero, G., Coronado, M. A., García, C., Curiel-Alvarez, M. A., León, J. A., Sagaste, C. A., & Montes, D. G. (2021). Characterization of orange peel waste and valorization to obtain reducing sugars. *Molecules, 26(5),* 1348. https://doi.org/10.3390/MOLECULES26051348

Brown, S. W., Oliver, S. G., Harrison, D. E. F., & Righelato, R. C. (1981). Ethanol inhibition of yeast growth and fermentation: Differences in the magnitude and complexity of the effect. *European*

Journal of Applied Microbiology and Biotechnology, *11,* 151–155. https://doi.org/10.1007/BF00511253/METRICS

Caputi, A., Ueda, M., & Brown, T. (1968). Spectrophotometric determination of ethanol in wine. *American Journal of Enology and Viticulture, 19,* 160–165. https://doi.org/10.5344/AJEV.1968.19.3.160

Castro, R. C. de A., & Roberto, I. C. (2015). Effect of nutrient supplementation on ethanol production in different strategies of saccharification and fermentation from acid-pretreated rice straw. *Biomass Bioenergy, 78,* 156–163. https://doi.org/10.1016/J.BIOMBIOE.2015.04.019

Cheng, N., Koda, K., Tamai, Y., Yamamoto, Y., Takasuka, T. E., & Uraki, Y. (2017). Optimization of simultaneous saccharification and fermentation conditions with amphipathic lignin derivatives for concentrated bioethanol production. *Bioresource Technology, 232*, 126–132. https://doi.org/10.1016/J.BIORTECH.2017.02.018

Correa, D. F., Beyer, H. L., Fargione, J. E., Hill, J. D., Possingham, H. P., Thomas-Hall, S. R., & Schenk, P. M. (2019). Towards the implementation of sustainable biofuel production systems. *Renewable and Sustainable Energy Reviews, 107,* 250–263. https://doi.org/10.1016/J.RSER.2019.03.005

D240 Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb Calorimeter. (2019.). Retrieved August 8, 2024, from https://www.astm.org/d0240-19.html

D482 Standard Test Method for Ash from Petroleum Products. (2019.). Retrieved August 8, 2024, from https://www.astm.org/d0482-19.html

Eleiwa, N. Z. H., Bakr, R. O., & Mohamed, S. A. (2014). Phytochemical and Pharmacological Screening of Seeds and Fruits Pulp of Cucurbita moschata Duchesne Cultivated in Egypt. *International Journal of Pharmacognosy and Phytochemistry*, *29,* 12261–1236. https://doi.org/10.12817/20517858.29.1.27703371

Ezema, B. O., Omeje, K. O., Ozioko, J. N., Fernandez-Castane, A., Oscar O., & Eze, S. (2023). Biodiesel potential of Cucumeropsis mannii (white melon) seed oil: A neglected and underutilized resource in Nigeria. *Heliyon, 9,* e16799. https://doi.org/10.1016/J.HELIYON.2023.E16799

Ezzat, S. M., Adel, R., & Abdel-Sattar, E. (2022). Pumpkin Bio-Wastes as Source of Functional Ingredients. *Mediterranean Fruits Bio-wastes: Chemistry, Functionality and Technological Applications*, 667–696. https://doi.org/10.1007/978-3-030-84436-3_29

Hagos, M., Yaya, E. E., Chandravanshi, B. S., & Redi-Abshiro, M. (2023). Determination of fatty acids composition by GC-MS and physicochemical parameters of pumpkin (*Cucurbita maxima*) seed oil cultivated in Ethiopia. *Bulletin of the Chemical Society of Ethiopia*, *37(3),* 565–577. https://doi.org/10.4314/BCSE.V37I3.3

Hardjono, H., Dewi, E. N., Lusiani, C. E., Febriansyah, I., & Bachtiar, R. I. (2021). D-limonene from orange (*Citrus Maxima*) peel extraction as destructive agent of styrofoam waste. *IOP Conference Series: Materials Science and Engineering*, *1073*(1), 012013. https://doi.org/10.1088/1757-899X/1073/1/012013

Hu, R., Lin, L., Liu, T., Ouyang, P., He, B., & Liu, S. (2008). Reducing Sugar Content in Hemicellulose Hydrolysate by DNS Method: A Revisit. *Journal of Biobased Materials and Bioenergy*, *2*(2), 156–161.https://doi.org/10.1166/JBMB.2008.306

Hussain, A., Kausar, T., Sehar, S., Sarwar, A., Quddoos, M. Y., Aslam, J., Liaqat, A., Siddique, T., An, Q. U., Kauser, S., Rehman, A., & Nisar, R. (2023). A review on biochemical constituents of pumpkin and their role as pharma foods; a key strategy to improve health in post COVID 19 period. *Food Production, Processing and Nutrition, 5*(1). https://doi.org/10.1186/S43014-023-00138-Z

Kant Bhatia, S., Kant Bhatia, R., Jeon, J. M., Pugazhendhi, A., Kumar Awasthi, M., Kumar, D., Kumar, G., Yoon, J. J., & Yang, Y. H. (2021). An overview on advancements in biobased transesterification methods for biodiesel production: Oil resources, extraction, biocatalysts, and process intensification technologies. *Fuel, 285,* 119117. https://doi.org/10.1016/J.FUEL.2020.119117

Knothe, G., & Razon, L. F. (2017). Biodiesel fuels. *Progress in Energy and Combustion Science, 58,* 36–59. https://doi.org/10.1016/J.PECS.2016.08.001

Lam, M. K., & Lee, K.T. (2011). Production of biodiesel using palm oil. In: Pandey, A., Larroche, C., Ricke, S.C., Dussap, C. & Gnansounou, E. (eds) *Biofuels: Alternative Feedstocks and Conversion Processes,* 353–374. *https://doi.org/10.1016/B978-0-12-385099-7.00016-4*

Li, Y., Fabiano-Tixier, A.-S., & Chemat, F. (2014). *Essential Oils as Reagents in Green Chemistry.* SpringerBriefs in Molecular Science. https://doi.org/10.1007/978-3-319-08449-7

Mahato, N., Sharma, K., Sinha, M., Dhyani, A., Pathak, B., Jang, H., Park, S., Pashikanti, S., & Cho, S. (2021). Biotransformation of Citrus waste-I: Production of biofuel and valuable compounds by fermentation. *Processes, 9,* 220 -229, https://doi.org/10.3390/PR9020220

Manakas, P., Balafoutis, A.T., Kottaridi, C., & Vlysidis, A. (2024). Sustainability assessment of orange peel waste valorization pathways from juice industries. *Biomass Conversion and Biorefinery*, 1–20. https://doi.org/10.1007/S13399-024-05626-X/FIGURES/6

Medina, J. D. C., Jr, A. I. M., & Medina, J. D. C., Jr, A.I.M. (2020). Ethanol production, current facts, future scenarios, and techno-economic assessment of different biorefinery configurations. *Bioethanol Technologies*. https://doi.org/10.5772/INTECHOPEN.95081

Moser, B.R. (2009). Biodiesel production, properties, and feedstocks. *In Vitro Cellular & Developmental Biology – Plant, 45(3),* 229–266. https://doi.org/10.1007/S11627-009-9204-Z

Muhammad, G., Alam, M. A., Mofijur, M., Jahirul, M. I., Lv, Y., Xiong, W., Ong, H. C., & Xu, J., (2021). Modern developmental aspects in the field of economical harvesting and biodiesel production from microalgae biomass. *Renewable and Sustainable Energy Reviews, 135,* 110209. https://doi.org/10.1016/J.RSER.2020.110209

Nader, J., Allaf, T., & Allaf, K. (2022). *Instant Controlled Pressure Drop (DIC) as an Emerging Food Processing Technology*. In: Gavahian, M. (eds) Emerging Food Processing Technologies. Methods and Protocols in Food Science . Humana, New York, NY. 229–246. https://doi.org/10.1007/978-1-0716-2136-3_16

Rapier, R. (2024). Breaking Records: 2024 Statistical Review Of World Energy Highlights. Forbes. https://www.forbes.com/sites/rrapier/2024/06/22/breaking-records-2024-statistical-review-of-world-energy-highlights/.

Samaras, C. (2019). Wasting less electricity before use. *Nature Climate Change, 9,* 648–649. https://doi.org/10.1038/s41558-019-0558-x

Schinas, P., Karavalakis, G., Davaris, C., Anastopoulos, G., Karonis, D., Zannikos, F., Stournas, S., & Lois, E. (2009). Pumpkin (*Cucurbita pepo* L.) seed oil as an alternative feedstock for the production of biodiesel in Greece. *Biomass Bioenergy*, *33,* 44–49. https://doi.org/10.1016/J.BIOMBIOE.2008.04.008

Somda, M. K., Savadogo, A., Ouattara, C. A. T., Ouattara, A. S., & Traore, A.S. (2010). Thermotolerant and alcohol-tolerant yeasts targeted to optimize hydrolyzation from mango peel for high bioethanol production. *Asian Journal of Biotechnology, 3,* 77–83. https://doi.org/10.3923/AJBKR.2011.77.83

Suri, S., Singh, A., & Nema, P.K. (2022). Current applications of citrus fruit processing waste: A scientific outlook. *Applied Food Research 2,* 100050. https://doi.org/10.1016/J.AFRES.2022.100050

Teke, G. M., De Vos, L., Smith, I., Kleyn, T., & Mapholi, Z. (2023). Development of an ultrasound-assisted pre-treatment strategy for the extraction of d-Limonene toward the production of bioethanol from citrus peel waste (CPW). *Bioprocess. Biosyst. Eng., 46*, 1627–1637. https://doi.org/10.1007/S00449-023-02924-Y/TABLES/4

Tse, T. J., Wiens, D.J ., Chicilo, F., Purdy, S. K., & Reaney, M. J. T. (2021). value-added products from ethanol fermentation—a review. *Fermentation, 7*, 267-273. https://doi.org/10.3390/FERMENTATION7040267

Wilkins, M. R., Widmer, W. W., & Grohmann, K. (2007). Simultaneous saccharification and fermentation of citrus peel waste by Saccharomyces cerevisiae to produce ethanol. *Process Biochemistry, 42,* 1614–1619. https://doi.org/10.1016/J.PROCBIO.2007.09.006