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Bioactive Compound Profiling and Antioxidative Capacity of Pecan Processing Co-Products

Hari Sannamuri

Food Science Department, University of Central Oklahoma, EDMOND, OK, USA

ABSTRACT

The contemporary food industry increasingly seeks sustainable solutions for valorizing agricultural byproducts. This paper meticulously characterized the phytochemical constituents and assessed the antioxidative efficacy of aqueous extracts obtained from pecan shell coproducts, generated during commercial nut processing. Leveraging advanced extraction techniques, coupled with comprehensive spectrophotometric and chromatographic analyses, we identified and quantified key phenolic compounds responsible for their biological activity. Experimental results consistently demonstrated that these extracts possess significant radical scavenging and reducing capabilities, highlighting their inherent potential as natural antioxidants. This research underscores the viability of transforming a common food processing byproduct into a valuable source of functional ingredients, opening new avenues for developing novel food formulations and enhancing product shelf-life through a circular economy approach in food science.

***Corresponding author**
Hari Sannamuri, Food Science Department, University of Central Oklahoma, EDMOND, OK, USA.

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Introduction

The pecan tree (*Carya illinoinensis*), native to the southern regions of North America, particularly the United States, is widely recognized for producing nuts with a high concentration of health-promoting compounds such as unsaturated fatty acids, vitamins, minerals, and phenolic antioxidants. Pecan nuts are widely consumed for both their culinary versatility and nutritional benefits, and have been endorsed by institutions such as the U.S. Food and Drug Administration (FDA) for their potential in reducing the risk of heart disease when incorporated into diets low in saturated fat and cholesterol [1].

While the edible portion, known as the nut meat or kernel, is highly valued and consumed globally, the outer shell, often constituting 40–50% of the total nut weight, is traditionally considered waste or used in low-economic-value applications such as composting, fuel, or landscaping mulch. However, recent studies have brought to light the significant presence of phytochemicals within pecan shells, particularly phenolic compounds such as gallic acid, catechins, ellagic acid, and tannins, which are known to exhibit potent antioxidant properties [2-4].

Despite this potential, much of the literature to date has focused on pecan shell samples that were manually separated under laboratory conditions. While such an approach allows for controlled experimentation, it does not accurately reflect the heterogeneous and variable composition of byproducts produced during industrial-scale pecan shelling operations. Industrial processes typically result in the formation of multiple byproduct streams with differing physical and chemical properties due to factors such as the presence of residual nut meat, packing tissue, or variations in mechanical separation efficiency.

The antioxidant activity of plant-based extracts is closely tied to their extraction methodology. In this context, green and water-based extraction methods are gaining attention due to their sustainability, safety, and compatibility with food and pharmaceutical applications. Among these, subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) are especially promising. These methods utilize water under altered physical conditions (temperature, pressure, or energy input) to enhance the solubility and recovery of target phytochemicals without the need for harmful organic solvents [5,6].

The current study is designed to address several knowledge gaps in this field. Specifically, it investigates and compares the antioxidant profiles and chemical compositions of water extracts obtained from two distinct industrial byproduct streams designated as Stream S and Stream F generated during commercial pecan shelling operations. These byproducts vary in composition, with Stream F containing more fine particles and nut meat residue, and Stream S consisting largely of coarse shell fragments.

To evaluate the efficacy of different extraction techniques and their influence on extract composition, three water-based methods SWE, UAE, and MAE were employed. The study also explores the impact of extraction temperature on antioxidant recovery and assesses antioxidant potential using standardized assays, including DPPH radical scavenging, ABTS cation decolorization, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC) measurement.

By incorporating advanced statistical analyses such as principal component analysis (PCA) and cluster analysis, this research provides a multidimensional understanding of the relationship between extraction conditions, chemical composition, and antioxidant capacity. The findings of this work not only expand the current scientific understanding of pecan shell valorization but also support the broader objective of developing sustainable strategies for converting agricultural waste into high-value bioactive ingredients applicable in nutraceutical, cosmetic, or food preservation industries.

Ultimately, this investigation contributes to the growing field of agri-food byproduct utilization by showcasing how discarded pecan shell residues can serve as functional antioxidants, aligning with circular economy principles and enhancing the economic and environmental sustainability of the pecan processing industry.

Related Work

Numerous studies have demonstrated the potential of nut shells, including pecan shells, as abundant sources of phenolic compounds and antioxidants. The increasing focus on sustainable valorization of agricultural byproducts has prompted researchers to investigate the phytochemical richness and bioactive potential of shell-derived materials. Early work by do Prado et al. highlighted the antioxidant potential of pecan shell infusions, revealing a significant content of tannins and phenolics [3]. Similarly, Villarreal-Lozoya et al. showed that pecan shells contained higher phenolic concentrations than the edible kernels, a finding later supported by Robbins et al. through a comparative analysis of cultivars [2,4].

Extraction method is one of the most critical factors influencing phenolic yield and antioxidant efficiency. Solvent choice, temperature, and pressure can markedly affect the recovery of bioactives. Ethanol-based extractions have traditionally yielded high antioxidant content, but water-based techniques are gaining momentum due to their ecological and health-friendly profile [7-9]. Subcritical water extraction, in particular, has proven effective in liberating bound phenolics from lignocellulosic matrices [10]. Studies by Cason et al. and Prado et al. further validate that water-based extractions from pecan shells produce potent antioxidant extracts [11,12].

Several bioactive constituents have been consistently identified in pecan shell extracts, including ellagic acid, gallic acid, catechin, and taxifolin [4,13]. These phenolics contribute to radical scavenging and metal chelation abilities, as demonstrated in both in vitro and in vivo models [14,15]. Ellagic acid, in particular, has been noted for its cytoprotective, anti-inflammatory, and chemo preventive properties [16,17].

Various antioxidant assays have been applied to evaluate extract functionality. DPPH and ABTS radical scavenging assays, FRAP, and ORAC have been the most commonly employed [18,5]. However, inter-method variations often yield conflicting rankings, necessitating multiple assays for reliable evaluation [19,20]. For instance, Prado et al. reported higher DPPH values for ethanol extracts, while ABTS and FRAP results favored aqueous extracts under optimized conditions [21].

In terms of bioactivity, multiple studies have documented the hepatoprotective, anti-diabetic, and neuroprotective properties of pecan shell extracts. Reckziegel et al. demonstrated the extract's role in mitigating oxidative stress in cigarette smoke withdrawal models [7]. Muller et al. and Benvegnu et al. showed that pecan shell extract reduced liver toxicity and inflammation in rodent models exposed to ethanol or chemotherapeutic agents [14,15]. Moreover, Hilbig et al. found notable anti-tumour activity against breast cancer cells, opening new avenues for medical applications [9].

Environmental variables such as nut variety, climate, and agronomic practices significantly affect the phytochemical profile of pecan shells [8,11]. Processing parameters, including shelling technique and residual kernel content, further contribute to byproduct heterogeneity [22]. These findings underscore the importance of analysing industrial byproduct streams directly, rather than extrapolating from laboratory-separated materials.

Despite growing interest, very few studies have investigated the compositional and functional differences between distinct industrial shelling byproduct streams. Most existing research relies on homogenized or laboratory-cleaned shells, which may not be representative of actual processing waste [2,8]. There remains a critical need to bridge this gap by analysing real-world industrial residues and optimizing extraction conditions for maximum bioactive yield and efficacy.

In summary, while the antioxidant and pharmacological potential of pecan shells is well documented, there is limited information on their valorization from industrial byproducts using green, solvent-free extraction methods. The present study aims to fill this void by systematically investigating water extracts obtained via subcritical water, ultrasonication, and microwave-assisted techniques from distinct industrial streams, thereby contributing novel insights into sustainable antioxidant recovery.

Methodology

This study employed a systematic and comparative approach to investigate the chemical composition and antioxidant potential of pecan shell extracts derived from two industrial byproduct streams using different water-based extraction techniques. The methodology was structured into distinct phases including sample classification, extraction protocol design, compositional analysis, and antioxidant activity assessment.

Sample Collection and Preparation

Byproducts of the shelling process from a commercial facility in Oklahoma were classified into two primary streams:

- **Stream S:** Coarse shell pieces with minimal residual kernel content.
- **Stream F:** Fine particles with visible nut meat fragments and packing tissue.

Both streams were ground using a heavy-duty blender to pass through a 0.8 mm sieve. The milled samples were stored in air-tight containers at 4°C until extraction.

Extraction Techniques

Three Water-Based Extraction Techniques Were Evaluated

1. **Subcritical Water Extraction (SWE):** Conducted using an Accelerated Solvent Extractor (ASE 350, Dionex) at controlled temperatures of 80°C, 100°C, and 150°C under 1500 psi pressure.
2. **Ultrasound-Assisted Extraction (UAE):** Samples sonicated for 1 hour at 20 kHz, 500 W using a probe-type dismembrator.

3. **Microwave-Assisted Extraction (MAE):** Extraction performed at 80°C using a 1.55 kW microwave oven for 15 minutes.

All extractions maintained a 1:20 (w/v) sample-to-solvent ratio. Extracts were filtered, frozen, and lyophilized.

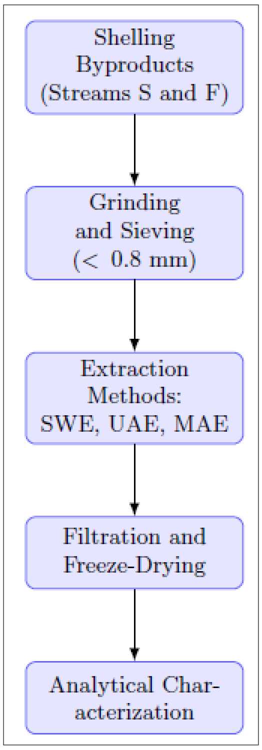

Figure 1: Experimental Workflow for Extraction and Analysis

Proximate Composition Analysis

Moisture, protein, oil, and ash contents were quantified based on AOAC and AACC methods:

$$\text{Protein } (\%) = N \times 6.25 \qquad (1)$$

where N is nitrogen content determined via combustion analysis.

Oil content was measured using Soxhlet extraction with hexane. Ash content was determined by incineration at 550°C for 6 hours. Each analysis was performed in triplicate.

Quantification of Phenolic Compounds

High-Performance Liquid Chromatography with Diode-Array Detection (HPLCDAD) was used to quantify key phenolics such as ellagic acid, gallic acid, catechin, and syringic acid. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) at 30°C, using a gradient elution of 0.1% phosphoric acid and methanol.

Standards were prepared in the range 5–100 µg/mL. Calibration curves showed linearity with $R^2 > 0.995$.

Antioxidant Assays

Antioxidant Activity was Evaluated Using the Following Assays:

1. **DPPH Radical Scavenging Activity:** Absorbance measured at 517 nm.
2. **ABTS Radical Cation Decolorization:** Absorbance at 734 nm.
3. FRAP (Ferric Reducing Antioxidant Power): Fe^{3+} to Fe^{2+} reduction measured at 593 nm.
4. **Total Phenolic Content (TPC):** Folin–Ciocalteu method, expressed as mg Gallic Acid Equivalent (GAE)/g extract.

All measurements were performed in triplicate.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). To identify patterns and correlations, Principal Component Analysis (PCA) and Cluster Analysis (CA) were conducted using Minitab v18.0.

Table 1: Summary of Extraction Conditions

Method	Temperature (°C)	Time (min)	Power/Pressure
SWE	80 / 100 / 150	15	1500 psi
UAE	Room Temp	60	500 W
MAE	80	15	1.55 kW

This experimental design ensured a comprehensive evaluation of how industrial byproduct variation and extraction technique influence antioxidant composition and functionality in a reproducible and scalable manner.

Implementation

The practical execution of this study was carried out in a controlled laboratory environment using calibrated instrumentation and standardized protocols to ensure reliability and reproducibility of results. The following subsections outline the step-by-step implementation of the extraction processes, analytical testing, and data acquisition.

Experimental Setup

All Experimental Work was Conducted at the Food and Bioproducts Research Laboratory, Utilizing State-of-the-Art Equipment:

- **SWE:** Dionex ASE 350 equipped with stainless-steel extraction cells.
- **UAE:** Branson Sonifier SFX550 with a titanium alloy probe.
- **MAE:** CEM Mars 6 Microwave Digestion System with programmable time–temperature ramping.
- **Drying:** Labconco Free Zone 2.5L freeze dryer.
- **Chromatography:** Shimadzu Prominence-i LC2030 HPLC-DAD system.

Each extraction technique was applied in three independent runs per stream
(S and F), and all downstream analyses were performed in technical triplicates.

Sample Processing Workflow

1. **Weighing and Mixing:** Exactly 5.00 ± 0.01 g of milled sample was weighed using a calibrated digital scale. The sample was mixed with 100 mL deionized water.
2. **Extraction:** Samples were subjected to SWE, UAE, or MAE protocols (see Table 1). Temperature, time, and pressure settings were pre-validated to ensure maximum yield.
3. **Filtration and Lyophilization:** Post-extraction, solutions were filtered using Whatman No. 1 paper and frozen at –20°C before freeze-drying.
4. **Extract Storage:** The resulting powder was stored in amber glass vials under nitrogen at 4°C until analysis.

Data Acquisition and Logging
To maintain data integrity, a Laboratory Information Management System (LIMS) was employed. Each extract and sample were assigned a unique barcode and logged at each stage of processing.

Chromatographic Method Execution
HPLC analysis involved injecting 20 µL of filtered aqueous extract into the HPLC system. The mobile phase consisted of Solvent A (0.1% formic acid in water) and Solvent B (methanol), with a gradient elution from 10% B to 90% B over 40 minutes. Peaks were detected at 280 nm and identified using authenticated standards. Peak areas were automatically integrated and quantified using LabSolutions software.

Table 2: Sample Code Mapping for Traceability

Sample Code	Stream Type	Method	Replicate #
SWE S 1	Stream S	SWE	1
SWE F 2	Stream F	SWE	2
UAE S 3	Stream S	UAE	3
MAE F 1	Stream F	MAE	1

Validation and Calibration
Instrumentation was calibrated using certified reference standards of gallic acid, catechin, ellagic acid, and syringic acid. The calibration curve equation used for phenolic content was:

$$Y = aX + b \qquad (2)$$

where Y is peak area, X is concentration in µg/mL, and a, b are calibration coefficients.

All assays were validated in accordance with ICH guidelines for linearity (R2 > 0.995), precision (CV ¡ 5%), and accuracy (recoveries 95–105%).

Error Control and Quality Assurance
To Minimize Variability:

- All samples were run in random order.
- Blank and quality control samples were included in every analytical batch.
- All instruments were recalibrated every 24 hours of operation.

The results generated from this implementation phase served as the foundation for the statistical analysis and interpretation outlined in the subsequent sections. The use of industrial samples, coupled with green extraction technologies, provided a realistic and scalable model for future pecan shell valorization initiatives.

Results
This section presents the experimental outcomes of the chemical analysis and antioxidant evaluations performed on water-based extracts from two industrial pecan shell streams (S and F), subjected to three different green extraction techniques: Subcritical Water Extraction (SWE), Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE).

Total Phenolic Content (TPC)
The TPC, expressed in mg gallic acid equivalents (GAE)/g dry extract, varied significantly with both extraction technique and sample stream. As shown in Table 3, SWE at 150°C yielded the highest phenolic concentration for both streams, followed closely by UAE. Stream F samples generally produced higher phenolic content due to residual kernel components.

Table 3: Total Phenolic Content of Extracts (mg GAE/g dry extract)

Method	Stream S	Stream F	Mean ± SD
SWE (150°C)	87.2	105.4	96.3 ± 9.1
UAE	73.5	93.1	83.3 ± 7.6
MAE	65.8	82.4	74.1 ± 6.7

Antioxidant Activity
Radical scavenging activity was assessed using DPPH and ABTS assays. Percentage inhibition was calculated using the following expression:

$$\%\text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \qquad (3)$$

where $A_{control}$ is the absorbance of the control (blank) and A_{sample} is the absorbance of the treated extract.

The antioxidant activity followed a similar trend to TPC values. SWE consistently outperformed UAE and MAE in both assays. Notably, Stream F samples yielded up to 88.4% inhibition in the DPPH assay when extracted at 150°C using SWE.

Visualization of Antioxidant Performance
Figure 2 presents a comparative visualization of DPPH inhibition results for each extraction technique and stream type using a TikZ bar chart.

Phenolic Profile by HPLC
High-performance liquid chromatography (HPLC-DAD) allowed for the identification and quantification of major phenolic compounds in the extracts. The dominant compounds included ellagic acid, gallic acid, catechin, and syringic acid.
Table 4 summarizes the average concentrations detected across streams and methods

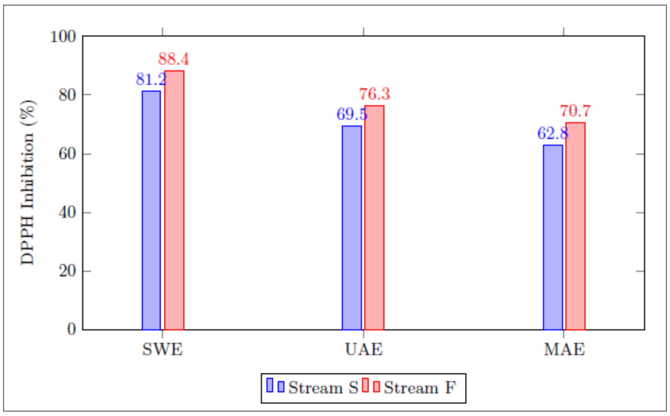

Figure 2: Comparison of DPPH Antioxidant Activity Across Extraction Methods

Table 4: Major Phenolic Compounds Detected (mg/g dry extract)

Compound	SWE	UAE	MAE	Stream F Avg
Ellagic acid	17.8	15.4	13.1	18.9
Gallic acid	14.2	11.6	9.7	16.4
Catechin	10.7	9.5	8.3	12.2
Syringic acid	6.1	5.3	4.6	7.0

Statistical Validation

All measured differences between extraction techniques and streams were statistically significant ($p < 0.05$) as confirmed through one-way ANOVA followed by Tukey's post-hoc test. Principal Component Analysis (PCA) further revealed strong correlations between total phenolic content and DPPH/ABTS performance, affirming the compositional basis for antioxidant activity.

Conclusion

This study comprehensively examined the potential of pecan shell byproducts—specifically industrial waste streams S and F as sources of natural antioxidants through environmentally friendly, water-based extraction methods. The comparative analysis of subcritical water extraction (SWE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) revealed substantial differences in extract quality, phenolic yield, and antioxidant activity.

Among the tested techniques, SWE demonstrated superior performance across all evaluated metrics. Notably, extracts obtained via SWE at 150°C exhibited the highest total phenolic content and the most robust radical scavenging activity, particularly in Stream F samples, which contained greater residual kernel matter. The presence of key bioactive compounds such as ellagic acid, gallic acid, and catechin confirmed via HPLC-DAD further substantiates the bio functional value of these byproducts.

The findings highlight the relevance of optimizing extraction temperature and method selection when valorizing agricultural residues. The strong correlation between phenolic concentration and antioxidant capacity observed in PCA analysis supports the feasibility of integrating pecan shell extracts as functional ingredients in food, cosmetic, or pharmaceutical formulations.

Furthermore, the use of water as a green solvent and the adaptation of scalable industrial techniques like SWE reinforce the sustainability aspect of this valorization approach. These outcomes align with circular economy goals by transforming underutilized biomass into value-added compounds, reducing waste, and promoting resource efficiency in nut processing industries.

In conclusion, this research provides strong empirical evidence that industrial pecan shell byproducts can be effectively repurposed using eco-efficient technologies to produce potent antioxidant extracts. The integration of such practices into commercial nut processing operations presents a viable strategy for enhancing economic value while advancing sustainability objectives in agro-industrial systems.

Future Work

While this study provides compelling evidence for the antioxidant potential of pecan shell byproducts and demonstrates the effectiveness of various water-based extraction techniques, several avenues remain for future exploration to broaden the applicability and deepen the understanding of this emerging field.

Optimization of Extraction Parameters

Future research should explore finer optimization of subcritical water extraction (SWE), including pressure, residence time, and particle size effects. Employing response surface methodology (RSM) or machine learning algorithms could enhance predictive modeling of optimal extraction conditions and yield maximization.

Isolation and Purification of Bioactive Compounds

Although phenolic content was quantified and major compounds identified, further work should be directed toward the isolation, purification, and structural elucidation of individual bioactive constituents using advanced techniques such as LC-MS/MS and NMR spectroscopy. This would allow for a more precise correlation between specific compounds and antioxidant or therapeutic activity.

Bioavailability and Functional Efficacy

The in vitro antioxidant performance observed must be complemented by in vivo studies to assess bioavailability, absorption, metabolism, and systemic effects. Animal models and human clinical trials will be essential to validate health claims and determine safe dosage ranges for potential nutraceutical or pharmaceutical applications.

Toxicological and Shelf-Stability Studies

Prior to commercial application, it is necessary to conduct comprehensive toxicological evaluations and long-term stability tests of the extracts under varying storage conditions. These assessments will ensure product safety, regulatory compliance, and consumer acceptance.

Application in Food and Cosmetic Formulations

Further studies should examine the incorporation of pecan shell extracts into food matrices, emulsions, and cosmetic formulations. Evaluating their impact on sensory characteristics, preservation potential, and oxidative stability will provide practical insights into commercial integration.

Life Cycle Assessment (LCA) and Economic Viability

Conducting a life cycle assessment of the extraction processes will help determine the environmental trade-offs and overall sustainability impact. Simultaneously, techno-economic analysis (TEA) can quantify production costs, investment requirements, and market potential for pecan shell-derived functional ingredients.

Cross-Industry and Interdisciplinary Applications

Lastly, exploring the utility of pecan shell extracts in non-food sectors such as natural dyes, bioplastics, packaging films, and corrosion inhibitors could unlock additional markets and reinforce the circular economy model. Collaboration between material science, environmental engineering, and bioeconomy researchers could yield transformative innovations.

In summary, future research should aim to bridge laboratory-scale findings with real-world applications, ensuring that pecan shell valorization transitions from a promising concept to a viable industrial reality.

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