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Cashew Nut Shell Liquid as Natural Antimicrobial Preservative for Beef: Characterization, Formulation, Efficacy and Application

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ABSTRACT: Food spoilage caused by microbial contamination has remained a major challenge in meat preservation, especially in regions with limited refrigeration infrastructure. The potential of cashew nut shell liquid (CNSL) as a natural antimicrobial preservative for beef, which is culturally significant and a highly consumed meat product worldwide, was investigated. The CNSL was extracted using ethanol and characterized by Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier-Transform Infrared Spectroscopy (FTIR). Results revealed a high abundance of phenolic lipids, cresols, cardanol and resorcinol derivatives, with active O-H and C-O functional groups. The antimicrobial efficacy against *Pseudomonas*, *Clostridium* spp. and *Proteus* spp., which are major bacteria implicated in meat spoilage, was assayed by applying different concentrations (0.5%, 1.0% and 2.0%) of CNSL to the meat samples and evaluating microbial loads over a 14-day storage period. Results indicate a significant reduction in total viable counts and pathogenic bacteria, with optimal preservation observed at 2.0% CNSL. The study demonstrates that CNSL exhibits potential to act as an effective natural preservative and sets the foundation for its application in sustainable meat preservation strategies.

Keywords: Beef; Cashew nut shell liquid; Food preservative; FTIR; GC-MS



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1. Introduction

Food preservation is associated with many hurdles in some local communities, mainly due to climatic conditions and limited access to cold-chain storage systems. Highly perishable meats often spoil rapidly, posing risks to food safety and economic progress. Traditionally, beef is consumed in every part of the world, making it an important target for preservation efforts. Recently, there has been a growing demand for clean-label foods and natural preservatives. This has stimulated interest in bio-based alternatives to synthetic antimicrobial agents. Cashew nut shell liquid (CNSL), a by-product of cashew processing, is rich in phenolic compounds such as anacardic acids, cardol and cardanol [1,2], which have been reported to exhibit potent antimicrobial and antioxidant properties [3–5]. This study considers the

potential of ethanol-extracted CNSL as an antimicrobial additive for preserving beef, with the aim of providing a culturally relevant, sustainable alternative to conventional preservatives.

Although CNSL has been extensively investigated for its application in industrial and medicinal purposes [5–8], its application in food preservation, particularly meat, remains underexplored. Previous studies have explored the use of CNSL in polymers or pharmacological contexts, with limited emphasis on food-grade applications [4,9]. To the best of our knowledge, no studies have examined the antimicrobial efficacy of CNSL across different meat types within the same experimental design, nor have any focused specifically on traditional beef. In addition, the optimal CNSL concentrations for meat preservation, formulation and application methods, and sensory impact remain poorly understood.

In recent research involving natural food preservation, the effectiveness of some plant-derived antimicrobials in extending the shelf life of perishable products has been explored [10]. For instance, extracts from clove, rosemary, thyme, and oregano have been reported to suppress spoilage microorganisms while maintaining desirable sensory attributes of meat products [11,12]. Similarly, plant-based polyphenols and essential oils have gained attention as clean-label alternatives due to their multifunctional roles as antioxidants, antimicrobials, and flavour enhancers [13,14]. These findings emphasize the growing interest in natural preservation strategies which also align with consumer demand for minimally processed foods. However, challenges such as variability in bioactive composition, formulation stability and cost remain significant, necessitating exploration of more sustainable and locally available plant by-products.

Cashew by-products, especially cashew nut shell liquid (CNSL), present a promising yet underutilized resource within this context. Some of the phenolic lipids in CNSL have demonstrated antimicrobial and antioxidant activities in pharmacological and industrial applications [2,5]. Recent studies have confirmed its potential against foodborne pathogens and spoilage organisms, including *Enterococcus faecalis* and *Staphylococcus aureus* [6]. Moreover, the valorization of CNSL as a preservative aligns with current global emphasis on sustainable waste management and circular bioeconomy, providing value addition to cashew-processing industries [6]. Despite this, reports on the application of CNSL in food preservation are scarce. By situating CNSL within the broader discourse on plant-based antimicrobials, this study contributes novel insights into its potential as a natural preservative specifically for beef, thereby addressing food safety and sustainability goals.

Apart from expanding the knowledge base on the bioactive profile of CNSL and its antimicrobial potential in food matrices, this study also offers several scientific and socio-cultural benefits. Focusing on traditional meats aligns preservation efforts with indigenous food practices. It also promotes value addition to cashew by-products, supports local economies, and provides an eco-friendly alternative to synthetic preservatives. The use of natural antimicrobial agents will help to mitigate risks associated with chemical preservatives and foodborne disease outbreaks.

2. Materials and Methods

2.1. Collection and Extraction of CNSL

Already de-kernelled cashew nut shells were obtained from the Cashew plantation at Pacajus, Ceará, Brazil and conveyed to the Laboratory of Thermodynamics and Supercritical Technology (LATESC), UFSC, in Florianópolis, Brazil. The shells were washed several times in distilled water, air-dried for three days and pulverized using a mechanical grinder. The picture of the dried and pulverized samples is shown in Figure 1. Pulverized sample was subjected to cold extraction using 70% ethanol at a material-to-solvent ratio of 1:5 (w/v). The extraction was carried out at 30 °C for 24 h under continuous agitation. The resulting extract was filtered using 0.22 µm membrane filters to remove particulate matter. A portion of the filtered extract was concentrated to remove solvent paste using a rotary evaporator under reduced pressure, while the remaining portion was retained in liquid form. Both forms of the extract were stored in airtight containers at 4 °C until use, and labelled e-CNSL.

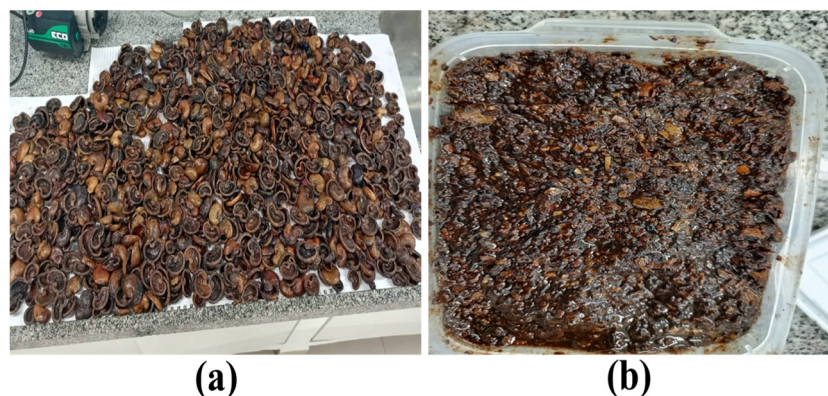


Figure 1. (a) Air-dried sample and (b) pulverized sample of cashew nut shells.

2.2. CNSL Characterization

The microfiltered e-CNSL extract was collected in 2 mL glass vials and analyzed for its chemical composition and functional groups by GC-MS and FTIR respectively. The GC-MS analysis was performed using an Agilent GC 7890A instrument coupled with an Agilent 5975C MS detector, following standard protocols [15]. The column used was an HP-5MS (Agilent, Santa Clara, CA, USA) fused silica capillary column (30 m length $\times$ 250 μ m i.d. $\times$ 0.25 μ m film thickness, composed of 5% phenyl-95% methylpolysiloxane) connected to an EI (Electron Impact Ionization) source operating at 70 eV with a quadrupole mass analyzer. The mass scan range was set from 40 to 550 m/z . Helium was used as the carrier gas at a 1.2 mL min^{-1} flow rate. The injector, operated in split mode (10:1), was fitted with a split glass liner containing deactivated quartz wool (Agilent) to facilitate efficient vaporization and reproducible transfer of analytes into the column and the interface was maintained at a temperature of 300 $^{\circ}\text{C}$. The solvent delay was set to 5.0 min. The injection volume was 0.2 μL , performed using an Agilent GC Sampler 80 equipped with a 10 μL syringe. The oven temperature program consisted of an initial hold at 60 $^{\circ}\text{C}$ for 3 min, followed by a ramp of 4 $^{\circ}\text{C}/\text{min}$ to 270 $^{\circ}\text{C}$ with a hold for 2 min, and then a ramp of 30 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ with a final hold for 5 min, resulting in a total run time of 63.5 min. Compounds were identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST) library (2011) [16]. The GC-MS data were acquired in full-scan mode for qualitative compositional profiling. Relative abundances were calculated by area normalization without an internal standard; the values are semi-quantitative and intended for within-run comparison only. Match quality refers to the similarity index (SI) generated by the NIST library search. Only compounds with clearly resolved peaks and acceptable SI values were reported; lower SI matches are presented as tentative identifications.

Additionally, Attenuated Total Reflectance—Fourier Transform Infrared (ATR-FTIR) spectroscopy was conducted to identify functional groups associated with the antimicrobial activity of the extract. The analysis was performed using a PerkinElmer FTIR Spectrometer (Model: Spectrum Two, PerkinElmer, Shelton, CT, USA) equipped with a diamond ATR accessory. The spectral range was set between 4500 cm^{-1} and 550 cm^{-1} , with a resolution of 4 cm^{-1} and 8 scans per sample. Peaks corresponding to phenolic hydroxyl groups, alkenes, carboxylic acids, and aromatic rings were particularly interesting.

2.3. Meat Samples Preparation

Fresh boneless beef muscle samples were purchased from different abattoirs in different locations, depending on the laboratory where a given aspect of the test was conducted. The samples were thoroughly washed under running potable water, rinsed with de-ionized water and standardized to uniform weights of 100 g per portion. The meat was randomly divided into four groups: one control group (no treatment) and three treatment groups. The treatment groups were immersed in ethanol extract CNSL at concentrations of 0.5%, 1.0%, and 2.0% (v/v) for 10 min at ambient temperature. Following immersion, samples were drained and prepared for subsequent analyses.

2.4. Preparation of the CNSL Preservative

For use as an antimicrobial preservative, the ethanol extract of cashew nut shell liquid (e-CNSL) was utilized in its liquid form. Following microfiltration and cold storage, a stock solution of CNSL was prepared by dissolving the 10 mg paste extract (obtained via rotary evaporation) in 100 mL food-grade ethanol obtained from local suppliers to

achieve a working solution with a known concentration of active phenolic components. The liquid extract was standardized for application based on total phenolic content and observed antimicrobial potency from preliminary trials.

To facilitate uniform application and enhance dispersibility, the CNSL extract was emulsified in a sterile aqueous ethanol solution (10% v/v ethanol in distilled water), which served as the carrier medium. The final preservative solutions were prepared at three different concentrations: 0.5%, 1.0%, and 2.0% v/v of CNSL in the dispersing medium. These concentrations were selected based on minimum inhibitory concentration (MIC) data from previous *in vitro* screening. This formulation approach was designed to reduce the required e-CNSL dosage while maintaining effectiveness, improving safety, and minimizing solvent residue on meat surfaces.

2.5. Application of CNSL Preservative

Meat samples (100 g each) were immersed in 100 mL of the prepared e-CNSL solutions for 10 min at room temperature to ensure thorough surface contact. After the immersion treatment, samples were allowed to drain under sterile conditions to remove excess liquid and were then transferred to sterile containers for storage. Control samples received no e-CNSL treatment and were immersed in the carrier solution (10% ethanol-water) alone to account for any antimicrobial effect of the solvent.

2.6. Microbial Analysis

Samples were stored at ambient temperature (25 ± 2 °C) and refrigerated conditions (4 °C) for 14 days. Microbial load was assayed on days 0, 3, 7, and 14 using standard plate count methods. Standard plate count methods were used to determine total viable counts (TVC) and the specific enumeration of *Pseudomonas* spp., *Clostridium* spp., and *Proteus* spp. Following ISO guidelines, the American Public Health Association (APHA, 2015), and the FDA Bacteriological Analytical Manual (2020) [17].

2.6.1. Sample Preparation

From each sample, 10 g of meat was aseptically transferred into sterile stomacher bags containing 90 mL of sterile 0.1% peptone water. The mixture was homogenized for 2 min using a stomacher (Seward Stomacher 400 Circulator, Seward Ltd, Worthing, West Sussex, UK). Serial ten-fold dilutions (10^{-1} to 10^{-6}) were prepared in sterile 0.1% peptone water for microbial enumeration.

2.6.2. Total Viable Count (TVC)

Aliquots (0.1 mL) from appropriate dilutions were plated in triplicate on Plate Count Agar (PCA, Oxoid, Hampshire, UK) using the pour plate method. Plates were incubated at 37 °C for 24–48 h. Colonies were manually counted and expressed as $\log_{10}$ colony-forming units per gram (CFU/g) of meat.

2.6.3. Bacterial Detection and Enumeration

Pseudomonas spp. were selectively enumerated using Pseudomonas Agar Base (Oxoid) supplemented with CFC selective supplement (Cetrimide–Fucidin–Cephaloridine). Aliquots (0.1 mL) were surface plated and incubated at 25 to 30 °C for 48 h. Colonies appearing pale green to bluish-green with a fruity odor were presumptively identified as *Pseudomonas* spp. Confirmation was carried out using the oxidase test and further growth on King A and King B media for pigment production as per ISO 13720:2010 guidelines [18].

Enumeration of *Clostridium* spp. was performed using Tryptose Sulfite Cycloserine (TSC) agar under anaerobic conditions. Plates were inoculated with 1 mL aliquots (or spread-plated for higher accuracy) and incubated at 37 °C for 24 to 48 h in anaerobic jars containing gas-generating kits. Black colonies due to sulfite reduction were presumptively identified as *Clostridium* spp. Confirmatory tests included Gram staining, catalase test (negative), and spore staining. All steps were performed following the ISO 7937:2004 protocol [19].

Proteus spp. were isolated using MacConkey Agar and XLD Agar as initial screening media. Diluted samples (0.1 mL) were plated using the surface spread technique and incubated at 35 to 37 °C for 24 h. Colonies with swarming motility or characteristic appearance (non-lactose fermenting pale colonies on MacConkey and yellow with black centers on XLD) were presumptively identified as *Proteus* spp. Confirmation involved motility test, urease activity, phenylalanine deaminase (PDA) test, and Triple Sugar Iron (TSI) agar reactions according to standard biochemical procedures and BAM Chapter 9 [20].

For all microbial analyses, serial ten-fold dilutions (10^{-1} to 10^{-6}) were prepared, and aliquots of 0.1 mL (spread-plate on selective media for the enumeration of target organisms, whereas 1.0 mL aliquot were used in the pour-plate method for total viable counts. Colony counts were expressed as $\log_{10}$ CFU per gram of sample, standardizing to the initial 10 g homogenized portion. The detection limit of the enumeration procedure was approximately 1.0×10^1 CFU/g, corresponding to growth observed from the lowest dilution plated. Quantification limits were considered reliable within the range of 30–300 colonies per plate. Incubation parameters (temperature and duration) followed ISO and BAM guidelines as specified for each organism, with anaerobic cultures conducted in gas-tight jars containing gas-generating kits to ensure strict anaerobiosis. Confirmatory biochemical tests were performed for each bacterial group to validate presumptive identifications.

2.7. Physicochemical and Mycological Analyses

To complement the microbial evaluations, physicochemical parameters and fungal counts were monitored during the 14-day storage period under ambient and refrigerated conditions. These analyses provided additional insights into meat spoilage progression and preservative efficacy of CNSL.

2.7.1. Fungal and Mold Count

Fungal populations were assayed using Sabouraud Dextrose Agar (SDA) supplemented with 0.01% chloramphenicol to inhibit bacterial growth. Using the spread plate method, 0.1 mL aliquots were plated in triplicate from appropriate serial dilutions. Plates were incubated at 25 °C for 5–7 days. Fungal colonies were counted and expressed as $\log_{10}$ CFU/g. Morphological features were noted to distinguish yeast-like colonies from filamentous molds.

2.7.2. pH Determination

The pH of each meat sample was measured on days 0, 3, 7, and 14 using a calibrated digital pH meter. For each measurement, 10 g of the meat sample was homogenized in 100 mL of distilled water and left to equilibrate for 15 min at room temperature. The electrode was rinsed with distilled water between readings to prevent cross-contamination. The pH values were recorded in triplicate, and the average value reported.

2.7.3. Water Activity (a_w) Measurement

Water activity (a_w) was determined using a bench-top digital water activity meter (AquaLab 4TE, Decagon Devices, Pullman, WA, USA). The instrument was calibrated prior to analysis using standard salt solutions (0.760 and 0.920 a_w , supplied by Decagon Devices) according to the manufacturer's instructions. Approximately 5 g of the meat sample was placed in a disposable measurement cup and allowed to equilibrate in the sealed chamber of the instrument for 5–7 min until a stable reading was achieved. Measurements were conducted at a laboratory temperature of 30 ± 2 °C, with about 50–55% relative humidity. To minimize condensation or temperature-induced variation, samples were allowed to equilibrate to the measurement temperature before placement in the device. All measurements were performed in duplicate, and the mean value was recorded and reported.

2.8. Statistical Analysis

Statistical analyses were carried out using Microsoft Excel 2019 with the Data Analysis ToolPak. Data were analyzed using one-way analysis of variance (ANOVA) to assess differences between treatments (control, 0.5, 1.0, and 2.0% e-CNSL) at each storage time point. Values in the reported tables within the same row followed by different superscript letters (a, b, c, d) are significantly different at $p < 0.05$.

3. Results and Discussion

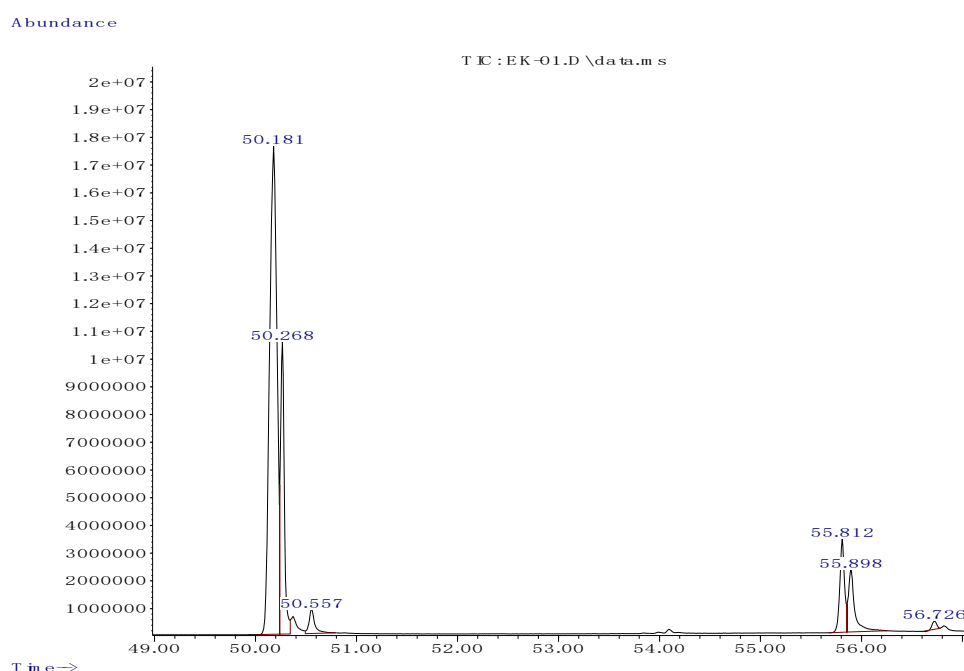
3.1. Characterization of e-CNSL

The data obtained from GC-MS analysis of the e-CNSL revealed six major constituents, predominantly phenolic in nature. These are depicted in Table 1 and Figure 2. The phenolics so revealed are consistent with known chemical composition of CNSL [1]. Table 1 presents the retention times, relative peak areas, compound identities, and match qualities from the NIST spectral library.

Table 1. Some compounds identified in e-CNSL from GC-MS analysis.

Peak	Retention Time (min)	Area (%)	Compound Name	Library Match (Qmal.)
1	50.18	62.32	m-cresol (3-methylphenol)	99
2	50.27	19.99	o-cresol (3-methylphenol)	70
3	50.56	2.47	3-pentadecylphenol (cardanol-type phenol)	93
4	55.81	7.59	1-(6-methyl-2-pyrazinyl)-3-methyl-1-butanol (pyrazine derivative)	64
5	55.90	7.03	5-pentylresorcinol	50
6	56.73	0.60	4,5-dimethylresorcinol	52

The percentages reported in Table 1 represent normalized peak areas and should not be interpreted as absolute concentrations of the compounds.

**Figure 2.** Chromatogram of e-CNSL obtained by GC-MS analysis.

From the results, phenol, 3-methyl-(m-cresol) was detected at a retention time of 50.18 min and it accounts for about 62.32% of the total peak area. It also exhibited a high spectral match (Qual. = 99%) which confirms its identity with high confidence. The m-cresol is a mono-substituted phenol that has been reported to exhibit impressive antimicrobial, antiseptic, and preservative properties making it a common bioactive in pharmaceutical and agrochemical formulations [21]. The second major component, phenol, 2-methyl- (o-cresol), was detected at 19.99% area contribution and exhibits antimicrobial characteristics [2]. Together, these cresol isomers constituted over 82% of the e-CNSL. This indicates that the ethanol extraction process effectively concentrated small-to-moderate polarity phenolic compounds, which are generally associated with strong bioactivity.

Also identified was 3-pentadecyl-phenol (2.47%), a notable phenolic lipid which is structurally similar to cardanol. Cardanol is a long-chain alkyl phenol which has been recognized for its use in biocide formulation [3]. Although it was a minor component, its presence supports the relevance of e-CNSL in antimicrobial industrial products development. The other compounds were 1,3-benzenediol derivatives, namely, 1,3-benzenediol, 5-pentyl- (7.03%) and 1,3-benzenediol, 4,5-dimethyl- (0.60%) 1,3-denzenediol compounds. These compounds belong to the alkylresorcinol class, known for their antioxidant, antimicrobial, and cytotoxic properties [4]. Their detection presupposes the pharmacological and industrial value of the extract, especially in applications requiring natural preservation systems.

Interestingly, 1-(6-methyl-2-pyrazinyl)-3-methyl-1-butanol, a nitrogen-containing compound, was detected at 7.59%. Although less commonly reported in CNSL, pyrazine derivatives are noted for their aromatic and flavor-enhancing roles, and some exhibit antibacterial and antitumor properties. Its presence may result from either minor biosynthetic pathways or thermal transformations during extraction or GC-MS analysis.

On the other hand, the chromatogram obtained (Figure 2) depicts a well-resolved separation of distinct peaks within the 50 to 57 min retention time window, indicating an efficient GC-MS run with minimal baseline noise. The dominant peak, which eluted at about 50.18 min, corresponds to phenol, 3-methyl-, accounting for over 60% of the total peak area. This sharp and symmetrical peak highlights its high abundance and purity. Other peaks, around 50.26 min and 55.81 to 55.90 min, represented secondary components such as phenol, 2-methyl- and 1,3-benzenediol derivatives, each contributing between 7 to 20% of the total area. The presence of minor peaks with lower intensity and narrower shapes reflects trace constituents that may still possess relevant bioactivity.

Overall, the chemical profile of the e-CNSL provides a clue that the e-CNSL is composed of diverse bioactives dominated by cresol isomers and enriched with functionally active phenolics. This profile demonstrates its potential for antimicrobial and biocidal applications. The chromatographic profile (Figure 2) also supports a complex yet predominantly phenolic composition, typical of CNSL extracts, and validates the effectiveness of the extraction and analytical procedures used.

The FTIR spectrum of the e-CNSL (Figure 3) revealed several characteristic absorption bands corresponding to functional groups associated with bioactive phenolic lipids. A broad, intense band between 3200 and 3525 cm^{-1} , with a maximum of 3427 cm^{-1} , may be attributed to O–H stretching vibrations typical of hydrogen-bonded hydroxyl groups present in phenols and carboxylic acids [22]. This broadness signifies the possibility of extensive intermolecular hydrogen bonding, consistent with cardanol, which is abundant in CNSL and known for its antimicrobial activity. Prior reports using HPLC analysis had also identified anacardic acid and cardol as bioactives in CNSL. These compounds, which are also known to exhibit antimicrobial properties, may also have contributed to the intermolecular hydrogen bonding interactions. A very sharp and intense peak at 2911 cm^{-1} and 2920 cm^{-1} may be assigned to asymmetric C–H stretching vibrations of long alkyl chains, supporting the lipophilic character of e-CNSL. This hydrophobic tail region enhances the potential of e-CNSL components to interact with and disrupt microbial cell membranes, which could be a key mechanism in their antimicrobial action [23].

The strong peak around 1640 cm^{-1} can be assigned to C=C stretching in aromatic rings, affirming the aromatic nature of the phenolic compounds. It can also be assigned to C–H bending vibrations in methylene and methyl groups of the aliphatic chains. Of particular interest is the very intense and prominent peak at 1260 cm^{-1} , which is assigned to C–O stretching vibrations of phenols and ether linkages. This band is a strong spectral fingerprint for cardanol and cardol compounds in CNSL, which have been widely associated with antioxidant and antimicrobial functions [2]. The lower frequency bands at 890, 730, and 590 cm^{-1} are attributed to aromatic C–H out-of-plane bending modes, confirming the presence of substituted benzene rings, which further supports the identification of cresol and resorcinol derivatives in the extract.

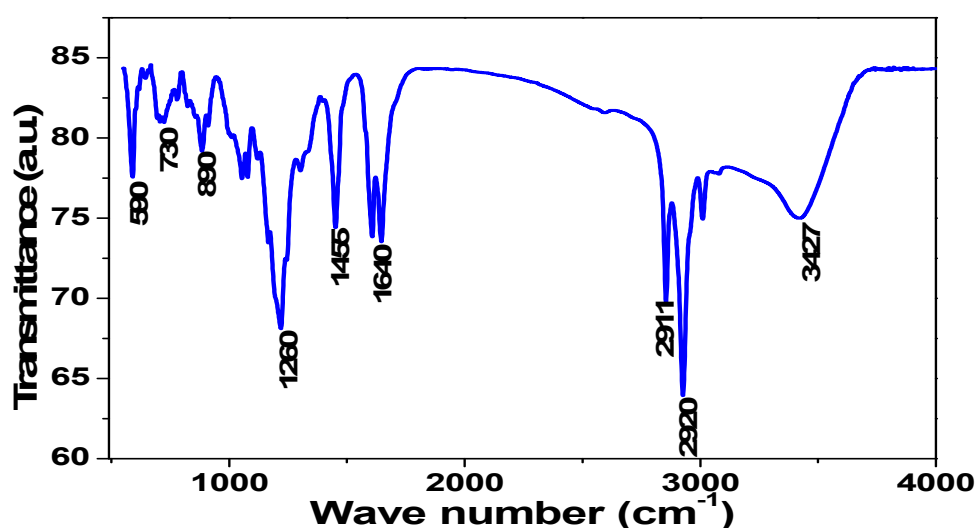


Figure 3. FTIR spectrum of e-CNSL.

Altogether, the FTIR spectral features corroborate the GC-MS results, confirming the presence of aromatic phenolic compounds with long alkyl side chains. These structural features portray the potential of antimicrobial performance of the extract, as they could enable both hydrophilic interactions (via OH groups) and lipophilic membrane

disruption (via alkyl chains). Based on previous studies on phenolic lipid-based preservation systems, such duality in molecular structure underlies the possibility for deployment as a natural antimicrobial preservative for beef meat [24].

3.2. Microbial Load and Bacterial Dynamics

In this study, microbiological assessments focused on three major spoilage and pathogenic organisms commonly associated with meat: *Pseudomonas* spp., *Clostridium* spp., and *Proteus* spp. The results of microbial counts across different storage conditions and time points are presented in Tables 2–4.

For *Pseudomonas* spp., the initial load on Day 0 ranged from 5.10 to 5.50 log₁₀ CFU/g across all samples (Table 2), which falls within the acceptable microbiological limits for fresh meat [25]. At ambient temperature (25 ± 2 °C), the untreated beef samples exhibited a steady increase in *Pseudomonas* load, reaching a peak value of 8.40 log₁₀ CFU/g by Day 14. However, the samples treated with 2.0% e-CNSL maintained significantly lower counts (7.75 log₁₀ CFU/g), showing a clear inhibitory effect. The antimicrobial action was concentration-dependent, with 1.0% e-CNSL showing optimal suppression by Day 7.

Under refrigeration (4 °C), the growth of *Pseudomonas* spp. was considerably slowed across all treatments. The 1.0% e-CNSL formulation offered the most consistent suppression (6.50 log₁₀ CFU/g on Day 14), whereas the 2.0% group showed slightly elevated counts, possibly due to cold-induced instability of the active phenolics in CNSL.

Table 2. Enumeration of *Pseudomonas* spp. (log₁₀ CFU/g) in beef during storage (mean ± SD). Values within the same row followed by different superscript letters (a–d) are significantly different ($p < 0.05$).

Storage	Day	0% e-CNSL	0.5% e-CNSL	1.0% e-CNSL	2.0% e-CNSL
Ambient	0	5.50 ± 0.30 ^a	5.40 ± 0.30 ^a	5.30 ± 0.20 ^a	5.10 ± 0.10 ^a
	3	6.60 ± 0.20 ^a	6.30 ± 0.20 ^b	6.00 ± 0.10 ^c	5.80 ± 0.30 ^c
	7	7.75 ± 0.30 ^a	7.10 ± 0.10 ^b	6.75 ± 0.30 ^c	6.60 ± 0.30 ^c
	14	8.40 ± 0.20 ^a	7.85 ± 0.40 ^b	7.15 ± 0.20 ^c	7.75 ± 0.40 ^b
Refrigerated	0	5.35 ± 0.35 ^a	5.25 ± 0.15 ^a	5.20 ± 0.25 ^a	5.00 ± 0.10 ^a
	3	6.10 ± 0.25 ^a	5.80 ± 0.35 ^b	5.50 ± 0.20 ^c	5.85 ± 0.15 ^b
	7	6.85 ± 0.20 ^a	6.45 ± 0.30 ^b	6.10 ± 0.15 ^c	6.50 ± 0.25 ^b
	14	7.50 ± 0.35 ^a	7.00 ± 0.25 ^b	6.50 ± 0.15 ^c	8.45 ± 0.30 ^d

For *Clostridium* spp., known to be an ±0.20 aerobic spore-formers, a different growth pattern was observed as shown in Table 3. At ambient temperature, untreated samples showed a gradual rise from 5.25 to 8.10 log₁₀ CFU/g by Day 14. The samples treated with 2.0% e-CNSL showed significantly suppressed growth until Day 7 but showed resurgence by Day 14 (7.60 log₁₀ CFU/g). This may likely due to compound degradation or microbial adaptation. However, the samples treated with 1.0% e-CNSL maintained more consistent suppression. Under refrigeration, *Clostridium* growth was delayed but not eliminated. The untreated sample reached 7.35 log₁₀ CFU/g by Day 14, while the 1.0% e-CNSL sample remained at 6.40 log₁₀ CFU/g, demonstrating the best inhibition under cold storage.

Table 3. Enumeration of *Clostridium* spp. (log₁₀ CFU/g) in beef during storage (mean ± SD). Values within the same row followed by different superscript letters (a–d) are significantly different ($p < 0.05$).

Storage	Day	0% e-CNSL	0.5% e-CNSL	1.0% e-CNSL	2.0% e-CNSL
Ambient	0	5.30 ± 0.10 ^a	5.20 ± 0.20 ^a	5.15 ± 0.25 ^a	5.00 ± 0.20 ^a
	3	6.45 ± 0.20 ^a	6.20 ± 0.15 ^b	5.90 ± 0.20 ^c	5.70 ± 0.10 ^c
	7	7.65 ± 0.25 ^a	7.05 ± 0.20 ^b	6.60 ± 0.15 ^c	6.40 ± 0.25 ^c
	14	8.10 ± 0.15 ^a	7.65 ± 0.15 ^b	7.00 ± 0.15 ^c	7.60 ± 0.25 ^b
Refrigerated	0	5.15 ± 0.15 ^a	5.10 ± 0.10 ^a	5.00 ± 0.10 ^a	4.95 ± 0.15 ^a
	3	5.95 ± 0.25 ^a	5.75 ± 0.15 ^b	5.40 ± 0.15 ^c	5.80 ± 0.20 ^b
	7	6.65 ± 0.20 ^a	6.35 ± 0.15 ^b	6.00 ± 0.10 ^c	6.30 ± 0.25 ^b
	14	7.35 ± 0.25 ^a	6.95 ± 0.10 ^b	6.40 ± 0.20 ^c	8.25 ± 0.15 ^d

For *Proteus* spp., known for their swarming motility and spoilage capability, results (Table 4) showed rapid proliferation under ambient conditions. Untreated meat had *Proteus* counts increasing from 5.40 to 8.20 log₁₀ CFU/g by Day 14. The 2.0% e-CNSL treatment suppressed counts to 7.55 log₁₀ CFU/g, with 1.0% showing a better inhibitory pattern up to Day 7. Refrigeration markedly delayed growth, with the 1.0% CNSL-treated sample maintaining the lowest

count ($6.30 \log_{10}$ CFU/g) by Day 14. However, the 2.0% treatment group under refrigeration showed late-stage increases, perhaps due to potential loss of compound activity or bacterial resistance.

Table 4. Enumeration of *Proteus* spp. ($\log_{10}$ CFU/g) in beef during storage (mean $\pm$ SD). Values within the same row followed by different superscript letters (a–d) are significantly different ($p < 0.05$).

Storage	Day	0% e-CNSL	0.5% e-CNSL	1.0% e-CNSL	2.0% e-CNSL
Ambient	0	5.45 ± 0.10^a	5.35 ± 0.20^a	5.25 ± 0.10^a	5.10 ± 0.25^a
	3	6.55 ± 0.25^a	6.25 ± 0.20^b	6.00 ± 0.20^c	5.85 ± 0.35^c
	7	7.65 ± 0.20^a	7.15 ± 0.15^b	6.80 ± 0.25^c	6.70 ± 0.30^c
	14	8.20 ± 0.30^a	7.75 ± 0.10^b	7.10 ± 0.20^c	7.55 ± 0.15^b
Refrigerated	0	5.30 ± 0.10^a	5.20 ± 0.20^a	5.10 ± 0.30^a	5.00 ± 0.20^a
	3	6.00 ± 0.15^a	5.70 ± 0.15^b	5.45 ± 0.35^c	5.75 ± 0.25^b
	7	6.70 ± 0.15^a	6.30 ± 0.25^b	6.00 ± 0.25^c	6.25 ± 0.15^b
	14	7.40 ± 0.25^a	6.85 ± 0.15^b	6.30 ± 0.25^c	8.10 ± 0.25^d

Across the three bacterial targets, the antimicrobial activity of e-CNSL was found to be dose-dependent. The 1.0% concentration was more consistent in balancing efficacy and stability under ambient and refrigerated conditions. It can be inferred from the results indicate that while higher concentrations (2.0%) may offer strong short-term inhibition, there may be a possibility of compound degradation or matrix instability over time. Overall, e-CNSL showed strong potential as a natural preservative for beef, particularly against spoilage bacteria such as *Pseudomonas*, facultative anaerobes like *Proteus*, and anaerobic spore-formers like *Clostridium*. Optimizing extract formulation and delivery may further enhance its preservation efficiency.

While e-CNSL generally reduced bacterial loads relative to the control, a few 2.0% treatments showed counts that were similar to, or marginally higher than, the corresponding controls at specific time points (Tables 2–4). These differences were not statistically significant (one-way ANOVA, $p > 0.05$). Non-monotonic responses to phenolic antimicrobials have been observed in complex food matrices, which may arise from matrix interactions and organism-specific susceptibility [26]. Phenolic lipids (e.g., anacardic acids, cresols) are typically more active against Gram-positive bacteria than Gram-negative organisms with robust outer membranes, which can partly explain treatment and taxa-dependent effects. Moreover, protein and lipid binding in meat can sequester phenolics and reduce their bioavailability at the microbial cell surface; higher nominal concentrations may not proportionally increase the effective antimicrobial dose at the target site [27]. In high fat-to-protein systems, such binding and phase partitioning can blunt activity or yield non-monotonic (biphasic) dose responses, which results in plateaus or small reversals that fall within experimental variability. Taken together, the few instances of “similar/higher” counts at 2.0% e-CNSL are consistent with matrix-limited bioavailability and taxon-specific tolerance, rather than a loss of intrinsic activity, and do not alter the overall trend of e-CNSL supporting microbial stability under the conditions tested.

3.3. pH Measurement

The pH of meat is an important physicochemical indicator of spoilage and biochemical changes. Generally, bacteria that cause spoilage act by generating metabolites that reduce the pH, while a preservative should exhibit more stable pH profiles. Results from treatment with e-CNSL at ambient temperature (Table 5) indicates that the pH of the control (untreated) dropped from 6.20 to 5.42 over 14 days. This behaviour shows increased acidification due to bacterial activities. However, the pH in the 2.0% e-CNSL treated samples remained around 5.76, showing significantly lower acidification. This trend was consistent with reduced microbial activity. Refrigerated samples showed more moderate changes, with the 1.0% CNSL group demonstrating optimal stability. This result demonstrates that treatment with e-CNSL effectively delayed acidification, particularly at higher concentrations, and hence preserved the biochemical integrity of meat over time.

Table 5. Measured pH of e-CNSL treated and untreated beef samples during storage (mean $\pm$ SD). Values within the same row followed by different superscript letters (a–d) are significantly different ($p < 0.05$).

Storage Condition	Duration (Days)	Concentration of e-CNSL (%)			
		0	0.5	1.0	2.0
Ambient temperature	0	6.20 $\pm$ 0.14 ^a	6.18 $\pm$ 0.15 ^a	6.19 $\pm$ 0.16 ^a	6.17 $\pm$ 0.15 ^a
	3	5.94 $\pm$ 0.17 ^a	6.00 $\pm$ 0.16 ^{ab}	6.05 $\pm$ 0.18 ^b	6.10 $\pm$ 0.19 ^c
	7	5.63 $\pm$ 0.16 ^a	5.79 $\pm$ 0.17 ^b	5.88 $\pm$ 0.18 ^c	5.90 $\pm$ 0.18 ^c
	14	5.42 $\pm$ 0.17 ^a	5.58 $\pm$ 0.18 ^b	5.66 $\pm$ 0.19 ^c	5.76 $\pm$ 0.20 ^d
Refrigeration	0	6.18 $\pm$ 0.14 ^a	6.16 $\pm$ 0.15 ^a	6.17 $\pm$ 0.15 ^a	6.15 $\pm$ 0.16 ^a
	3	6.08 $\pm$ 0.15 ^a	6.10 $\pm$ 0.15 ^a	6.13 $\pm$ 0.16 ^a	6.12 $\pm$ 0.16 ^a
	7	5.92 $\pm$ 0.16 ^a	5.95 $\pm$ 0.17 ^{ab}	6.00 $\pm$ 0.17 ^b	5.98 $\pm$ 0.18 ^{ab}
	14	5.81 $\pm$ 0.16 ^a	5.85 $\pm$ 0.16 ^a	5.89 $\pm$ 0.17 ^a	5.86 $\pm$ 0.17 ^a

3.4. Water Activity (a_w)

Water activity (a_w) is a parameter used to estimate microbial growth potential. It portrays a measure of free water available to support microbial growth. Microorganisms, particularly bacteria, require water activity above 0.90 to proliferate. As can be seen from results presented in Table 6, the control (untreated) group maintained higher water activity levels upto 0.96 throughout storage, especially under ambient conditions. On the other hand, e-CNSL-treated samples exhibited slightly reduced a_w values, notably at Day 14, where 2.0% CNSL reached 0.90 compared to 0.93 in the control. Similar results were obtained for the untreated refrigerated samples, but with subtler differences. However, the CNSL-treated groups consistently recorded lower a_w values, reflecting the possible moisture-binding or partial dehydration effects of eCNSL.

Overall, treatment with e-CNSL produced a modest but consistent reduction in water activity compared to the untreated control, which was very evident at Day 14 under ambient and refrigerated conditions. Although the absolute changes in a_w values were relatively small (generally within 0.02–0.05 units), statistical analysis (one-way ANOVA followed by Tukey's post hoc test) revealed that the reductions observed at 2.0% e-CNSL under both storage conditions were significant ($p < 0.05$) relative to the control. The reductions were not statistically significant at 0.5% and 1.0% concentrations ($p > 0.05$), but they displayed a consistent downward trend. This indicates that while water activity alone was not drastically altered, the small yet significant reductions at higher concentrations may still contribute synergistically with the antimicrobial and antifungal effects of e-CNSL. Thus, e-CNSL appears to act through a multifactorial mechanism, with water activity reduction complementing its more pronounced antimicrobial action.

Mechanistically, since CNSL itself does not possess water-binding properties, the mechanistic basis for the observed reductions in water activity with increasing e-CNSL concentration is likely to be indirect rather than a direct hygroscopic effect. A plausible explanation is that inhibition of microbial metabolism by e-CNSL may reduce the production of metabolic byproducts (e.g., organic acids, exopolysaccharides) that can influence free water availability in meat systems. In addition, phenolic lipids in CNSL may interact with meat proteins and lipids, subtly altering the water-binding capacity of the matrix. These interactions could lead to partial redistribution of bound and free water, thereby contributing to the modest but consistent decreases in a_w observed, especially at higher CNSL concentrations. Such indirect mechanisms are consistent with reports on other plant-derived antimicrobials, where antimicrobial action and minor physicochemical changes in the food matrix jointly influenced water activity and shelf stability [10,14].

Table 6. Measured water activity for the untreated and treated meat samples at day 0 and day 14 of storage under ambient and refrigerated conditions (mean $\pm$ SD). Values within the same row followed by different superscript letters (a–c) are significantly different ($p < 0.05$).

Storage Condition	Duration (Days)	Concentration of e-CNSL (%)			
		0	0.5	1.0	2.0
Ambient temperature	0	0.96 $\pm$ 0.02 ^a	0.95 $\pm$ 0.02 ^a	0.95 $\pm$ 0.02 ^a	0.94 $\pm$ 0.02 ^a
	14	0.93 $\pm$ 0.03 ^a	0.91 $\pm$ 0.03 ^b	0.91 $\pm$ 0.03 ^b	0.90 $\pm$ 0.04 ^c
Refrigeration	0	0.96 $\pm$ 0.02 ^a	0.95 $\pm$ 0.02 ^a	0.94 $\pm$ 0.02 ^a	0.94 $\pm$ 0.02 ^a
	14	0.91 $\pm$ 0.03 ^a	0.90 $\pm$ 0.03 ^{ab}	0.90 $\pm$ 0.03 ^{ab}	0.89 $\pm$ 0.03 ^b

3.5. Effect of e-CNSL on Fungal and Mold Growth

Fungal contamination is usually prominent under humid ambient conditions; hence, the influence of e-CNSL on fungal growth and mold growth was investigated by estimating the fungal counts at ambient conditions only. As can be observed from the results in Table 7, fungal counts increased progressively in untreated meat samples, especially under ambient storage conditions and reached 5.20 log₁₀ CFU/g by Day 14. Treatment with e-CNSL effectively suppressed fungal growth to less than 2.0 log₁₀ CFU/g at 2.0% concentration. Overall, all the e-CNSL-treated samples showed significant inhibition of fungal growth, with the 2.0% formulation affording a near-complete suppression. Morphologically, the e-CNSL-treated samples had less visible mold and discoloration, which could enhance the sensory attributes of the meat. These results align with the known antifungal action of cardol and anacardic acid components reported in literature [23]. Apart from complementing its antibacterial effect, the effective fungal and mold inhibition by e-CNSL also indicates its good potential for application in longer storage cycles.

Table 7. Measured fungal count (log₁₀ CFU/g) under ambient storage of beef under ambient storage treated with e-CNSL (mean ± SD). Values within the same row followed by different superscript letters (a–d) are significantly different ($p < 0.05$).

Day	Control	0.5% e-CNSL	1.0% e-CNSL	2.0% e-CNSL
0	1.05 ± 0.05 ^a	1.03 ± 0.05 ^a	1.02 ± 0.05 ^a	1.00 ± 0.02 ^a
7	3.85 ± 0.10 ^a	2.60 ± 0.04 ^b	2.10 ± 0.05 ^c	1.50 ± 0.05 ^d
14	5.20 ± 0.05 ^a	3.50 ± 0.11 ^b	2.50 ± 0.10 ^c	1.90 ± 0.10 ^d

3.6. Relevance of the Findings

In Tables 2–7, results are presented as mean ± SD. The inclusion of standard deviation values demonstrates that replicate measurements were consistent and that experimental error was minimal. Superscript letters (a, b, c, d) indicate significant differences across treatments at each time point ($p < 0.05$). For microbial counts (Tables 2–4) and fungal growth (Table 7), the progressive shift from ‘a’ in control samples to ‘c’ or ‘d’ in e-CNSL-treated samples clearly demonstrates a dose-dependent inhibitory effect. Similarly, the significant treatment-related differences can be observed in pH (Table 5) and water activity (Table 6), although modest in magnitude. This aligns with the microbial suppression trends and supports that the preservative effect of e-CNSL is statistically significant and biologically meaningful.

The results of e-CNSL demonstrated a broad-spectrum antimicrobial effect, and treatment with e-CNSL significantly reduced microbial load, slowed down acidification, reduced water activity, and suppressed key pathogens and spoilage fungi in treated meats. The samples treated with 1.0% e-CNSL concentration consistently exhibited optimum effective across the multiple indicators. In addition, the 1.0% ± 0.20 e-CNSL outperformed the 0.5% dose, which did not show the inconsistencies with the 2.0% concentration.

From a domestic and industrial perspective, e-CNSL offers a cost-effective, plant-derived alternative to synthetic preservatives. Its availability as a byproduct of the cashew industry aligns with circular economy goals, and its dual antibacterial and antifungal effects provide a multi-pronged defense against spoilage. However, the sensory impact of treatment with e-CNSL was not evaluated and is recommended for further studies. Also, the observation period for this study was limited to 14 days, which is expected to be the estimated longest range for domestic storage of beef by local processors and sellers. A long-term storage beyond 14 days may be considered in further studies. Lastly, the efficacy of e-CNSL in combination with packaging materials (e.g., vacuum sealing or edible films) remains another interesting investigation for the future to ensure proper validation for full-scale industry deployment.

3.7. Potential Toxicity of e-CNSL

To be considered for application in food systems, assessment of the potential toxicity of e-CNSL phenolic constituents is important. Cresols, which were identified as major components in the present study, are recognized as toxic at high levels and are regulated in occupational and environmental exposure contexts. While their antimicrobial activity contributes to preservative potential, their safety thresholds in food applications remain to be firmly established. Likewise, cardanol and anacardic acids, though naturally occurring in cashew shells, are bioactive compounds whose acceptable doses as food additives are yet to be set by some regulatory agencies. Although our experimental concentrations (1.0–2.0% w/w) demonstrated efficacy in meat preservation, these levels must be interpreted cautiously with respect to consumer safety. Further work is needed to quantify potential migration of CNSL components into edible portions, assess their toxicological profiles in vivo, and determine acceptable daily intakes. Until such data are

available, CNSL should be considered a promising candidate preservative whose practical application will require comprehensive safety and regulatory evaluation in parallel with efficacy testing.

4. Conclusions

The findings of this study confirm that ethanol-extracted cashew nut shell liquid (e-CNSL) possesses strong antimicrobial and antifungal properties that can effectively extend the shelf life of beef. The presence of phenolic compounds such as cresols and cardanol derivatives, as revealed by GC-MS and FTIR, underpins its preservative action. Application of e-CNSL, particularly at 1.0 and 2.0% concentrations suppressed microbial growth, maintained pH and water activity within acceptable limits, and reduced bacterial growth under refrigerated and short-term (14 days) ambient storage and fungal growth under ambient storage. These results highlight e-CNSL as a viable natural alternative to synthetic meat preservatives. Its effectiveness, coupled with its availability as an agro-industrial byproduct, makes it suitable for adoption in traditional meat preservation, especially in regions with limited refrigeration. Further studies are recommended to evaluate its sensory impact, consumer acceptance and potential in long-term storage or advanced packaging systems.

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Author Contributions

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Not applicable.

Informed Consent Statement

Not applicable.

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The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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The authors declare that there are no competing interests.

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