Bioinformatics-Guided Assessment of Luffa Cylindrica Seed Oil for Antimicrobial and Antioxidant Preservation of Ground Beef

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Abstract: This study evaluated *Luffa Cylindrica* seed oil (LSO) as a dual-action natural preservative for ground beef. The cold-pressed oil, obtained through mechanical extraction, was characterized by a high content of polyunsaturated fatty acids, mainly linoleic (45.77%) and oleic (30.58%) acids, which are known for their bioactive properties. Physicochemical and antioxidant analyses confirmed the strong radical scavenging potential of LSO. When incorporated into ground beef stored at 4 °C for 10 days, the treatment significantly reduced total viable counts by 0.73 log CFU/g compared to the untreated control, indicating effective microbial inhibition. Furthermore, LSO markedly suppressed lipid oxidation, maintaining the product's freshness and sensory quality throughout storage. Molecular docking studies supported these results by revealing that linoleic and oleic acids exhibit strong binding affinities (−6.8 kcal/mol and −6.4 kcal/mol, respectively) to the bacterial enzyme enoyl-acyl carrier protein reductase (FabI), suggesting a potential antimicrobial mechanism at the molecular level. These findings establish LSO as a promising, sustainable preservative with combined antimicrobial and antioxidant activities for perishable meat products.

1 INTRODUCTION

Your Ground beef is a highly perishable food product due to its high-water activity, protein and lipid content, and nutrient richness, which favor both microbial proliferation and lipid oxidation. Spoilage results from psychrotrophic bacteria such as *Pseudomonas spp.,* leading to off-odors, slime, and discoloration, as well as from oxidative rancidity that impairs sensory and nutritional quality [1]. While synthetic preservatives like BHA, BHT, and nitrites are widely used, consumer demand for natural alternatives has intensified concerns over their safety, encouraging research on plant-derived compounds with dual antimicrobial and antioxidant potential [2].

Luffa Cylindrica seeds, a byproduct of loofah sponge production, are a valuable source of oil abundant in linoleic and oleic acids, fatty acids recognized for their potent antimicrobial and antioxidant properties [3]. Valorizing this underutilized seed oil offers a sustainable route to natural food preservation while reducing agricultural waste. Although its composition has been described, the application of *L. Cylindrica* seed oil (LSO) in meat preservation remains unexplored. Beyond their direct antimicrobial activity, unsaturated fatty acids can interfere with bacterial enzymes critical for growth and survival, such as FabI, an essential enoyl-acyl carrier protein reductase in fatty acid biosynthesis [4]. Targeting FabI offers a promising strategy to inhibit foodborne pathogens. This study evaluates the preservative potential of *Luffa Cylindrica* seed oil (LSO) in ground beef by combining *in situ* assessments of microbial inhibition and oxidative stability within *silico* docking of its major fatty acids against FabI to elucidate possible enzyme-mediated antimicrobial mechanisms.

2 MATERIAL AND METHODS

2.1 Plant Material Collection and Seed Preparation

Mature fruits of *Luffa Cylindrica* were collected from the Essouassi region in Tunisia during April 2025. Fruits were manually dehisced to separate the fibrous matrix from the seeds. Seeds were carefully removed, cleaned of debris, washed with distilled water, blotted dry, and dried in a hot-air oven at 40 ± 2 °C for 48 h, to minimize the degradation of heat-sensitive bioactive compounds, until reaching a constant weight (<8% moisture). Dried seeds were stored in airtight polyethylene bags at 4 °C in a dark, dry environment until oil extraction.

2.2 Oil Extraction by Cold Pressing

Oil was extracted from 500 g of dried seeds using a mechanical screw-type cold press without external heating. Crude oil was collected in a sterile stainless-steel container, while the residual seed cake was collected separately. The crude oil was clarified by gravity settling for 24 h at room temperature, followed by filtration through Whatman No. 1 filter paper and the clarified oil was stored in amber bottles under nitrogen at 4 °C until use. The oil yield (%) was calculated as:
Yield (%) = (Initial seed weight (g)) / (Weight of extracted oil (g)) x 100.

2.3 Total Phenolic Compounds Content and Antioxidant Activity

Polyphenols were extracted from the oil following the method of Rezig et al., (2018)[5]. Briefly, 3 g of oil was dissolved in 6 mL of hexane and subjected to liquid-liquid extraction with 6 mL of 80:20 (v/v) methanol/water. The mixture was vortexed, sonicated for 10 minutes, and centrifuged at 6000 rpm for 20 minutes to separate the phases. This extraction was repeated three times, and the methanolic layers were pooled for analysis. Total polyphenolic content (TPC) was measured using the Folin-Ciocalteau method [6] on a Multiskan GO™ microplate spectrophotometer. Antioxidant activity was assessed by a DPPH assay, in which 20 µL of extract was added to 180 µL of 100 µM methanolic DPPH and incubated for 60 minutes. Absorbance at 515 nm was measured, and antioxidant capacity was expressed as Trolox equivalents (µM/g oil) using a standard curve.

2.4 Fatty Acid Analysis (GC–MS)

Approximately 10 mg of the oil residue was mixed with 4 mL of hexane and 5 mL of methanolic KOH (1 M). The mixture was vigorously shaken for about 10 s to allow transesterification. The upper hexane phase was collected, passed through a 0.45 µm membrane filter, and subjected to GC–MS analysis. The analyses were performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert triple-axis mass detector, equipped with an HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.20 µm). Helium served as the carrier gas at a constant flow rate of 1 mL/min, and the injection volume was 1 µL. The oven temperature program was as follows: initial hold at 120 °C for 4 min; ramp from 120°C to 200°C at 3 °C/min with a 10 min isothermal step; followed by an increase to 280 °C at 15 °C/min and a final hold for 15 min. Fatty acids were identified by comparing their retention times and mass spectra with those of authentic standards and reference data from the literature.

2.5 In Situ Antimicrobial Efficacy in Ground Beef

The preservative effect of LSO was tested in fresh ground beef. Samples (15 g each) were either treated with 5 mg of LSO (treatment) or left untreated (control). Each sample was stored at 4 °C for 10 days. Microbiological analysis was performed on days 1 and 7. For enumeration, 5 g of each sample was homogenized in 45 mL of sterile 0.9% saline, creating a 10^{-1} dilution. Ten-fold serial dilutions were prepared, and 0.1 mL of each dilution was spread onto Plate Count Agar plates. Plates were incubated at 30 °C for 72 h, and only plates with 15–300 colonies were used for calculating total viable counts, expressed as log CFU/g [7].

2.6 Lipid Oxidation (TBARS Assay)

Lipid oxidation inhibition capacity was assessed in control and LSO-treated ground beef and the oxidation was monitored on days 1, 6, and 10 [8]. 10 grams of each sample were homogenized with 50 mL of 7.5% TCA containing 0.1% EDTA and filtered through Whatman No. 1 paper. A 5 mL aliquot of the filtrate was mixed with 5 mL of freshly prepared 0.02 M TBA and incubated at 95 °C for 30 min. After cooling in an ice bath, absorbance was measured at 532 nm. The inhibition of lipid oxidation (%) was calculated relative to the control using:
Inhibition (%) = (Absorbance of control-Absorbance of sample) / (Absorbance of control) x 100.

2.7 Molecular Docking

To explore a potential mechanism of antibacterial activity, a molecular docking with staphylococcus aureus enoyl-acyl carrier protein reductase (fabI) (PDB: 3GR6) was performed. The protein structure was prepared using Maestro’s Protein Preparation Wizard, including bond order assignment, hydrogen addition, hydrogen-bond network optimization, and restrained minimization with the OPLS4 force field. Ligands, including the main fatty acids identified in LSO (linoleic and oleic acids), were processed using LigPrep to generate low-energy 3D structures and correct ionization states at pH 7.4 ± 0.5. Docking was performed in PyRx 0.8 using AutoDock Vina with a 25 Å × 25 Å × 25 Å cubic grid centered at coordinates X = 0.2929, Y = 30.7808, Z = -21.626. Exhaustiveness was set to 8. Top-scoring binding poses were analyzed in Maestro to visualize hydrogen bonds, hydrophobic interactions, and other contacts stabilizing the ligand-receptor complex.

3 RESULTS AND DISCUSSION

3.1 Oil Yield, Total Phenolic Content (TPC), and Antioxidant Activity

The oil was extracted from *Luffa Cylindrica* seeds via cold pressing, a method chosen to preserve thermosensitive bioactive compounds. The oil yield was 36.18% (w/w). This yield is slightly higher than yields obtained reported in previous studies (19.9–30.8%) [3].The total phenolic content (TPC) of the extracted oil was determined to be 1.02 mg GAE/g of oil. While modest compared to specialized phenolic-rich oils like extra virgin olive oil, this value is notable for a seed oil and suggests that these compounds may contribute, at least in part, to the oil's overall antioxidant capacity [9].

The antioxidant potential was further confirmed by the DPPH free radical scavenging assay, which showed an IC50 value of 1.26 mg/mL. This result indicates that the oil possesses intrinsic radical scavenging ability. Although polyphenols are primary contributors to this activity, the high concentration of unsaturated fatty acids in the oil likely plays a significant synergistic role, as discussed below.

3.2 Fatty Acid Profile of Luffa Cylindrica Seed Oil (LSO)

The composition of LSO was dominated by unsaturated fatty acids (UFAs), which constituted over 76% of the total fatty acids identified (Table 1).

The most abundant fatty acid was the polyunsaturated linoleic acid (C18:2) at 45.77%, followed by the monounsaturated oleic acid (C18:1) at 30.58%. The major saturated fatty acids were palmitic acid (C16:0) at 12.29% and stearic acid (C18:0) at 10.14%.

Table 1: Fatty acid composition of *Luffa Cylindrica* seed oil (LSO).

Fatty Acid (Cx:y)	Common Name	Percentage (%)
C16:0	Palmitic acid	12.29
C18:2	Linoleic acid	45.77
C18:1	Oleic acid	30.58
C18:0	Stearic acid	10.14

This fatty acid profile is consistent with previous reports on *L. Cylindrica* seed oil, confirming its identity as a rich source of essential omega-6 fatty acid. The composition is comparable to commonly used edible oils such as sunflower oil, which contains 48–74% linoleic acid and 14–39% oleic acid with total polyunsaturated fatty acids exceeding 80%, the same percentage is obtained in soybean oils [10], [11]. The prevalence of linoleic and oleic acids is critically important, as these specific UFAs are well-documented for possessing both antimicrobial and antioxidant properties [12]. Their proposed mechanism of antimicrobial action involves the disruption of the bacterial cytoplasmic membrane, leading to increased permeability and eventual cell death [13]. Their antioxidant activity is attributed to their ability to donate hydrogen atoms from the methylene groups between double bonds, thereby neutralizing free radicals [14]. This dual functionality of its major components positions LSO as an ideal candidate for a natural food preservative.

3.3 *In Situ* Preservative Efficacy in Ground Beef

3.3.1 Antimicrobial Activity

The effectiveness of LSO in controlling microbial growth in ground beef during refrigerated storage (4 °C) is shown in Table 2.

Table 2: Total viable counts (log CFU/g) in ground beef samples.

	Day 1 (log CFU/g)	Day 10 (Log CFU/g)
Control	5.98	8.71
Treated	5.93	7.98

The initial total viable count (TVC) in both control and treated samples was approximately 5.9 log CFU/g, indicating similar starting microbial loads. After 10 days, the TVC in the control group reached 8.71 log CFU/g, exceeding the typical spoilage threshold of 7 log CFU/g for fresh meat [15]. In contrast, the ground beef treated with LSO showed a significantly lower microbial count of 7.98 log CFU/g.

The addition of LSO resulted in a 0.73 log CFU/g reduction in the microbial population compared to the control after 10 days of storage. While this represents a bacteriostatic rather than bactericidal effect, a reduction of this magnitude is meaningful in extending the shelf-life and delaying the onset of spoilage caused by psychrotrophic bacteria like *Pseudomonas spp.*, which dominate the spoilage microflora of refrigerated meat [16]. This antimicrobial effect in a complex food matrix like ground beef can be directly attributed to the high concentration of linoleic and oleic acids in LSO.

3.3.2 Inhibition of Lipid Oxidation

Lipid oxidation is a major factor in meat quality deterioration, leading to rancidity and discoloration. The antioxidant effect of LSO in ground beef was assessed using the TBARS assay, which quantifies malondialdehyde (MDA) and other secondary oxidation products. LSO-treated samples showed significantly lower TBARS values than the control throughout storage, confirming its protective effect against lipid peroxidation. On the final day, the inhibition relative to the control corresponded to an IC_{50} of 0.88 mg/mL, indicating strong antioxidant capacity. Compared to existing natural preservatives, LSO provides a dual-action effect by combining antioxidant and antimicrobial properties, and its high content of unsaturated fatty acids may interfere with bacterial enzyme activity, offering enhanced preservative efficacy. Additionally, it is derived from an underutilized byproduct, making it a sustainable and cost-effective option for maintaining the sensory and nutritional quality of meat [17]. By limiting oxidative damage, LSO contributes to maintaining both the sensory and nutritional quality of ground beef.

3.4 *In Silico* Mechanistic Insight: Molecular Docking with Fabi

To explore a potential molecular mechanism for the observed antibacterial activity, *in silico* docking was performed with linoleic and oleic acids against the enoyl-acyl carrier protein reductase (FabI) from *Staphylococcus aureus*, a key enzyme in bacterial fatty acid synthesis.

The docking results revealed favorable binding affinities for both major fatty acids, with linoleic acid (-6.8 kcal/mol) showing a slightly stronger interaction than oleic acid (-6.4 kcal/mol). These negative binding energies indicate spontaneous and stable complex formation (Table 3). The interaction analysis (Fig. 1) showed that the carboxyl head group of both fatty acids forms crucial hydrogen bonds with polar residues in the active site (SER 93), anchoring the ligand. The long hydrophobic alkyl tail is stabilized within a deep pocket through extensive hydrophobic and van der Waals interactions with nonpolar residues such as ILE, ALA, VAL, and PHE. Fatty acids possess substantial conformational flexibility, and the unsaturated regions (C7–C10) of linoleic and oleic acids are prone to rotation and oxidation, which may influence their interactions with bacterial enzymes such as FabI [18], [19].

The inhibition of FabI disrupts the bacterial fatty acid synthesis (FAS-II) pathway, which is essential for membrane biogenesis and bacterial survival. By binding to the active site, linoleic and oleic acids could act as competitive inhibitors, preventing the natural substrate from binding and thereby halting bacterial proliferation [4], [20]. This provides a plausible molecular basis for the bacteriostatic effect observed in the *in situ* ground beef experiment.

Table 3: Docking results of fatty acids with FabI (PDB: 3GR6).

Ligand	Binding Affinity (kcal/mol)	Hydrogen Bonds with FabI Residues	Hydrophobic Interactions with FabI Residues
Linoleic Acid	-6.8	SER 93, ILE 207	HIS 92, ILE 94, PHE 95, THR 145, TYR 147, TYR 157, LYS 164, ALA 190, GLY 191, PRO 192, ILE 193, THR 195, SER 197, ALA 198, VAL 201, PHE 204
Oleic Acid	-6.4	GLY 13, ILE 20, SER 93	SER 19, ALA 21, THR 146, TYR 147, TYR 157, LYS 164, ALA 190, GLY 191, PRO 192, ILE 193, THR 195, ALA 198, VAL 201, PHE 204

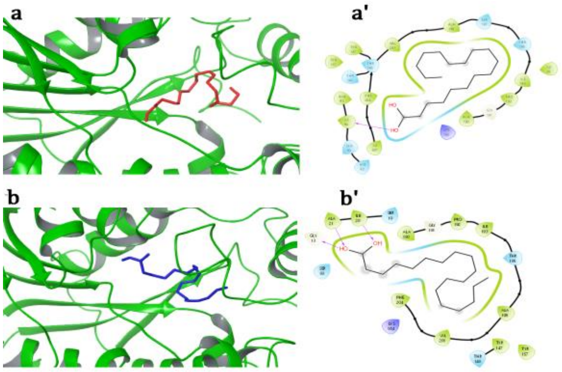

Figure 1: Docking of linolenic and oleic acids in the FabI active site. 3D poses (a, b) and 2D interaction maps (a', b') illustrate key binding interactions for (a, a') linolenic acid and (b, b') oleic acid

3 CONCLUSIONS

In summary, the present study provides a comprehensive evaluation of Luffa cylindrica seed oil (LSO) as a multifunctional natural preservative for ground beef systems. The results demonstrate that LSO, characterized by a high proportion of unsaturated fatty acids - predominantly linoleic and oleic acids - exhibits significant antioxidant and antimicrobial activities under refrigerated storage conditions. The incorporation of LSO effectively reduced microbial proliferation and delayed lipid oxidation, thereby contributing to the preservation of both microbiological safety and physicochemical quality of the meat matrix.

Importantly, the integration of in situ experimental data with in silico molecular docking analysis offers mechanistic insight into the observed bioactivity. The favorable binding affinities of the major fatty acids toward the bacterial FabI enzyme support a plausible inhibitory mechanism targeting fatty acid biosynthesis, which may underlie the bacteriostatic effect observed in treated samples.

Collectively, these findings highlight the potential of LSO as a sustainable, value-added alternative to synthetic preservatives. Its dual functionality, combined with its origin from an underutilized agro-industrial byproduct, underscores its relevance for clean-label food applications and circular bioeconomy strategies. Future studies should focus on optimizing formulation strategies, evaluating sensory acceptance at industrial scale, and validating efficacy against a broader spectrum of foodborne pathogens to facilitate its practical implementation in meat preservation systems.

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