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Modeling and optimization of essential oils and extracts for meat quality evaluation during storage, with high-performance liquid chromatography analysis of aflatoxin levels

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This research was focused on developing a suitable and optimizing model, for dried meat preservation (storability), through the application of natural essential oils and extracts. The dried meat (beef) samples were treated with onion peels extract (OPE), onion peels oil (OPO), plantain peels extract (PPE), and plantain peels oil (PPO), in accordance with concentrations and hybridization levels, provided by Central Composite Design (CCD) model. Two basic independent variables – treatment type and storage time – were evaluated, to establish their impact on six responses: total bacterial viable content (TBC), *Staphylococcus aureus*, *Salmonella* spp., total viable fungal content (TFC), aflatoxin B1 and aflatoxin B2. Based on the CCD optimization guidance, the meat samples were treated with 10 different natural additives, and coded T0 to T9, respectively. Nutritional and microbial evaluations of the samples were done by utilizing standard procedures. Specifically, the High-Performance Liquid Chromatography (HPLC) approach was used to explore the B vitamins and aflatoxins concentrations. The results specified that the plantain and onion waste products safeguarded the meat nutritional integrity and public health safety. Additionally, the optimization result depicted that the hybridized PPO and OPO treatment (T9), demonstrated the optimal efficacy in inhibiting microbial growth, and formation of aflatoxins. The maximum TBC and TFC populations of 3,130 and 2,180 cfu/g, were recorded in the control (T1) meat samples, respectively. The study found that the essential oils and extracts,

effectively reduced the aflatoxins B1/B2 levels, in preserved beef to safe limits, established by the World Health Organization for meat products suitable for human consumption. This study's results have highlighted the effectiveness of using oils and extracts derived from discarded onion and plantain peels, in improving meat quality, integrity and inhibiting pathogens survival.

KEYWORDS

aflatoxins, food security, meat products, microbial invasion, public health

Highlights

- Essential oils and plants extract influences on meat nutritional composition
- Essential oils and extracts play essential roles in inhibiting pathogens
- Plants materials are eco-friendly alternatives for meat preservation
- Employing the HPLC approach ensures accurate aflatoxins quantification

Introduction

Food is a vital requirement of the human body, as inadequate nutrition can lead to malnutrition-related issues, which are among the leading causes of death worldwide (Pawlak and Kołodziejczak, 2020). Protein and vitamins play crucial roles in maintaining health and supporting various bodily functions. Meat, as an animal-based protein unlike plant-based protein, is classified as complete protein since it naturally comprises of the nine essential amino acids in a proportions required for human health. However, by combining various plant foods, it is possible to achieve a complementary amino acid profile, which can result in the formation of a complete protein (Qin et al., 2022; Stadnik, 2024). Animal-based food items are perfect source of essential nutrients, some of which are challenging to obtain from plant-based sources alone. Meat and its products have been crucial in human nutrition, contributing to a balanced diet and supporting growth and performance of the body systems (Ruxton and Gordon, 2024; Sheffield et al., 2024). Despite its high cholesterol content, meat is one the most staple protein source in many human diets worldwide, resulting from its high concentration of essential amino acids, vital metals and vitamins (Stadnik, 2024).

Effective food storage is one of the major objectives of achieving food security, helping to reduce food wastage, maintains economic stability, and ensures food availability (Alirezalu et al., 2019; Pawlak and Kołodziejczak, 2020; Uguru et al., 2023; Ashraf et al., 2025). Food storage can be categorized into wet and dry methods, and the choice of method adopted is dependent on the type of the agricultural product, desired shelf life, and available resources (Sayed and Elsharkawy, 2021). The dry storage category is the widespread storage method used globally, due to its low level of complexity, availability of the materials, energy efficiency, and lower technical know-how (Uguru et al., 2023; Bradford et al., 2020; Lisboa et al., 2024). Fungal and bacteria growth is a major concern in dried meat storage, largely because this storage environment conditions (humidity, temperature and moisture content fluctuation conditions) often favors their survival (Ivane et al., 2024; Kunová et al., 2021; Kahraman and Tutun, 2021).

The key consequences in dried meat during storage are, decline in sensory quality, nutritional value, and microbial invasion (Sayed and Elsharkawy, 2021). *Salmonella* spp., *Pseudomonas*, *Staphylococcus* spp., *E. coli*, *Aspergillus*, *Penicillium*, and *Fusarium* have adverse effect on animal products quality, and posing significant public health issues (Kunová et al., 2021; Paswan and Park, 2020). Aflatoxins produced by some fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) are potent toxic compounds known for their carcinogenic and mutagenic effects; which are directly linked to severe health issues - organ (liver and kidney) failure, endocrine disruption and respiratory issues (Gruber-Dorninger et al., 2019; Ekwomadu et al., 2022; Uguru et al., 2023; Kahraman and Tutun, 2021).

Animal products are often treated with various natural additives (agro-based materials) during processing and storage operations, to improve their dietary integrity, shelf life, and consumer prevalence (Umaru et al., 2019; Beya et al., 2021; Djenane et al., 2024). Plant extracts and oils are rich in bioactive compounds, with antioxidants and antimicrobial properties (Gál et al., 2023; Enciso-Martínez et al., 2024), leading to improve food functional/nutritional properties (Kunová et al., 2021; Kačániová et al., 2024). Although natural extracts and oils offer many benefits in food preservation, their excessive applications can result in the emergence of resistant strains of microorganisms, thereby complicating food quality and safety (Zhou et al., 2023; Ashraf et al., 2025). Most meat processing industry uses synthetic preservatives; however, these inorganic chemicals toxicity (health challenges) includes - allergies, carcinogenic issues, and organs damage (Gál et al., 2023; Kačániová et al., 2024). This has spurred interest in the development of natural and environmental friendly alternatives, which include plant essential oils (EOs) and extracts having minimal health issues (Unar, 2022; Ivane et al., 2024). Additionally, the hybridization of EOs and extracts leverages antimicrobial and antioxidant properties of the treatments, which can combat spoilage-causing bacteria and delay oxidation (Al-Hijazeen, 2022).

Though numerous researches have been done on the use of organic preservatives in food preservation, most of them utilized plants edible parts as bio-preservatives (Turgut et al., 2017; Pateiro et al., 2021; Kačániová et al., 2024; Ashraf et al., 2025), inadvertently, contributing to food insecurity. Additionally, prior research primarily focused on the impact of green additives (bio-preservatives) on pathogenic infestation of preserved foods, and very little attention paid to the dietetic quality and human safety (Prakash et al., 2015; Yuan and Yuk, 2018; Kunová et al., 2021; Fu et al., 2022). This study tends to bridge these gaps by using agricultural waste materials (plantain finger peels and onion bulb wastes) as organic preservatives - essential oils (EOs) and extracts - for meat quality and integrity preservation, through mathematical modeling and optimization. Apart from microbial inhibition and nutrients stabilization, this

research appraised the aflatoxins toxicity status of the preserved meat. Specifically, this research uses a novel combination of High-Performance Liquid Chromatography (HPLC) for the aflatoxins evaluation, and predictive modeling techniques (Centre Composite Design “CCD” and Prediction Interval “PI” analysis), to create mathematical models capable of predicting meat quality alterations during storage; as well as, the optimal levels (concentrations) of these bio-preservatives, needed for maximize microbial defense and food safety. Eventually, this study’s outcomes will bridge essential knowledge gaps, connecting meat quality, food safety and computational optimization. This contributes to defensible and innovative methods in food preservation, which is in conformity with global sustainability trends like UN SDGs, European Union and Green Deal.

Materials and methods

Meat sampling

Ten kilogram of high-quality beef was collected from a local abattoir in Delta State, Nigeria on August 18th, 2023. The samples were placed in sterilized, ice-cooled plastic containers, and promptly transported to the microbiology laboratory for analysis at Delta State University of Science and Technology, Ozoro, Nigeria, and Docchy analytical laboratories and environmental services limited, Nigeria.

Quality control

The HPLC system, Plate Count Agar (PCA), Salmonella-Shigella (SS) agar, Baird-Parker Agar (BPA), Potato Dextrose Agar (PAD), methanol, helium (99% purity) and other solvents used for the analyses were manufactured by Thermo Fisher Scientific Inc. USA. All the materials and equipment used were sterilized to prevent cross-contamination. All measurements were conducted in triplicate, and the relative standard deviation was less than 4% across the measurements.

Extract and essential oil (EO) samples preparation

The onion bulb peels and plantain peels were sorted to eliminate contaminants, sun-dried and then ground into powder form using the laboratory mill (Ultra Centrifugal Mill ZM 300, produced by Retsch GmbH Ltd. Germany). Thereafter, the onion bulbs peels extract (OPE) and plantain peels extract (PPE) were prepared in accordance with standard procedures, through cold maceration approach using ethanol as the carrier solvent. 100 g of the pulverized onions and plantain peels wastes were soaked in 1 L of ethanol for four days, and filtered using a 0.22 μ m membrane. Thereafter, the filtrate was evaporated (concentrated) utilizing a rotary evaporator (model Rotavapor R-300, manufactured by Thermo Fisher Scientific Inc. America) at 45°C, to evaporate the excess methanol leaving the onion bulbs peels extract (OPE) and plantain peels extract (PPE) behind.

Additionally, the onion bulbs peels oil (OPO) and plantain peels oil (PPO), were extracted through the solvent extraction technique,

and using n-hexane as the solvent. 200 g of the sieved plants materials were poured into a Soxhlet extractor, containing 600 mL of n-hexane. The mixture was subjected to temperature of 67°C for 6 h, ensuring constant n-hexane cycling, and the high quality oil obtained was collected into a cylinder (Ashraf et al., 2025). Thereafter, the excess solvent in the EO was evaporated at 40°C, and dried with anhydrous sodium sulfate, and kept in cool dark environment.

Experiment design

The following treatment plans as presented in Table 1, was used to achieve the goals of this research. The experimental design was generated by Design Expert Software (Version 23.1), through CCD and considering these factors: storage duration, treatment concentration, treatment hybridization, and their interactions. Additionally, the PI analysis was employed to confirm (validate) the experiment results.

The optimization will involve all the treatments of T0–T9, and storage durations of 0–10 weeks. The extract and oil concentrations used as treatments in this research, were selected based on the range of concentrations previously applied by other authors (Kunová et al., 2021; Kačániová et al., 2024). This approach controls existing research to optimize their effectiveness and public safety in the stored beef quality control.

Meat samples preparation

Approximately 30 g of each meat sample was diced into 0.05 m x 0.05 m pieces. The cook-dried method was adopted for Treatments 1, 3, 5, 6, 7, and 8. For treatments involving plant extract application (Treatments 1 and 3), the meat was diced into 0.05 m x 0.05 m pieces, steamed with the appropriate extract concentration for 20 min, and then immediately cooled to room temperature. Also, for the treatments involving hybridized EO and extract application (Treatments 5, 6, 7, and 8), the meat pieces, already prepared and inoculated with the extract, were then sprayed with the appropriate volume of oil. The data indicated that, for treatments involving only oil application (Treatments 2, 4, and 9), the oil-dried method was

TABLE 1 The study’s experimental design.

Sampled code	Constituents
Control	100% Distilled water
T1	2% PPE
T2	1% PPO
T3	2% OPE
T4	1% OPO
T5	2% PPE + 1% PPO
T6	2% OPE + 1% OPO
T7	1%PPE + 1% OPE
T8	2% PPE + 0.5% OPO
T9	0.5%OPO + 0.5% PPO

Particularly, the concentrations of the essential oils and extracts used were in taken in percentage weight per weight (%w/w).

utilized: the meat cubes were blanched for 20 min, cooled and coated with the essential oils. Afterward, all the prepared meat samples were dried in a laboratory oven at 105°C to a moisture content of approximately 7%.

Storage condition

The dried meat samples were stored in a dry ambient environment ($30 \pm 5^\circ\text{C}$, $80 \pm 11\%$ RH), covered with netting to protect them from direct insect and pest exposure. This is similar to traditional storage practices commonly used in markets and households across many developing and underdeveloped nations. The nutritional quality of the meat and microbial analyses were conducted every 2 weeks over a 10-week period.

Proximate analyses

Essential oils and extracts phytochemicals composition

The composition of phytochemicals in the onion peels and plantain peels extracts and oils were analyzed using gas chromatography with flame ionization detection (GC-FID), manufactured by Chromatography Direct Ltd., United Kingdom. Firstly, the samples (extracts and oils) were prepared by mixing the oil/extract with n-hexane at a ratio of 1:10, trembled vigorously for 5 min and sieved through a 0.45 μm syringe filter. One μL of the prepared sample was injected into the system, which uses HP5 capillary column (30 m \times 0.25 mm \times 0.25 μm), and helium as the carrier medium with a flow rate of 1.0 mL/min. The injector temperature and detector temperature were 250°C and 280°C, respectively. The initial system's oven temperature was pre-set at 60°C for 2 min, before it was ramp at 10°C/min, and then a final temperature of 280°C. Additionally, the operational run time was 25 min, and standards were employed quantification and documentation of the phytochemicals.

Moisture content

The moisture content (MC) of the meat samples was determined using the gravimetric method, following the Association of Official Analytical Chemists (AOAC) guidelines.

Vitamins determination

The vitamin B profile and vitamin E level of the dried meat samples was measure through the HPLC method. To measure the vitamin E level in the samples, 2 g of the ground specimen was mixed with 202 mL of methanol, and strained using a 0.45 μm strainer. Basically, these factors/parameters were used to determine the vitamin E level of each specimen – injector volume of 20 μL , methanol mobile phase, C18 reversed-phase column, 25°C column temperature, flow speed of 0.06 L/h, UV detection of 295 nm, run duration of 15 min, retention time of 10 min, and recovery rate of 105%.

Likewise, to measure the B vitamins content of each sample, these were the preliminary preparations: 3 g of the meat the ground meat was normalized with acidified distill water, centrifuge at the speed of 10,000 rpm, sieved with a 0.45 μm filter. These were the major HPLC factors/parameters considered for the B vitamins evaluation: 20 μL

injector volume, methanol carrier, ≤ 30 min run time (depending on the B vitamin to be measured), of 0.06 L/h flow rate, C18 column, 30°C column temperature. Specifically, the UV detection wavelengths for Vitamins B1, B2, B6, B9 and B12 were 246, 270, 290, 290 and 361 nm, respectively; while the retention time for Vitamins B1, B2, B6, B9 and B12 were 7, 10, 12, 15, 17, and 24, respectively, (Albawarshi et al., 2022).

Protein determination

The Kjeldahl method was employed to assess the protein content in the meat samples. A 10 g portion of the meat was digested with sulfuric acid (H_2SO_4) at 90°C, utilizing copper as a catalyst. Following the digestion process, the resulting acidified solution was neutralized with sodium hydroxide (NaOH) to release ammonia gas, which was captured as ammonium. This ammonium solution was subsequently titrated with hydrochloric acid to measure the nitrogen content in the meat.

Microbial analyses

Preparation of sample

Twenty grams of each sample was blended in a laboratory mill, with the addition of 0.180 L of sterile saline solution, to produce a suspension having a 1:10 dilution factor. This dilution helps to homogenize the sample concentration, thereby enhancing the accuracy of microbial counts evaluation.

Bacterial count

One milliliter (1 mL) of the dilution was evenly spread onto sterile PCA, utilizing a disinfected glass spreader, incubated at 37°C for 48 h, and the bacterial clusters formed were counted. The total viable bacterial load (TBC) produced expressed as colony forming units per gram (cfu/g) (Ashraf et al., 2025).

Staphylococcus aureus determination

One (1) mL of the meat dilution, was spread across a BPA plate, and placed on incubation operation for 48 h at 37°C. *Staphylococcus aureus* colonies were identified through their black or dark gray coloration.

Salmonella spp. determination

The homogenized meat dilution (1 mL) was incubated (37°C for 24 h), before it was transferred to the Rappaport-Vassiliadis (RV) broth, and further incubated at 40°C for 24 h. This enriched sample was plated onto a SS agar plate, incubated at 37°C for 24 h, and the *Salmonella* spp. produced were identified and counted (Ashraf et al., 2025).

Fungal count

One mL of the diluted solution was uniformly spread across a sterile Potato Dextrose Agar (PDA) plate, and incubated at 25°C for 3 days. After incubation, the colony produced were counted and expressed as cfu/g.

Aflatoxins determination

The HPLC technique was used to determine aflatoxins (afB1 and afB2) count of the dried meat. Thirty gram of the meat sample was ground and mixed with 303 mL of methanol - Methanol: water

(80:20), and filtered with a Whatman No. 1 filter paper. 20 µL of the liquid (extract) specimen was introduced into the HPLC system, with a mobile phase that consist a mixture of water, methanol and acetonitrile, mixed at a ratio of 60:20:20. The system was operating at a flow speed of 1 mL/min, column temperature of 30°C, UV- diode array detector (DAD) detector, and typical emission wavelength of 460 nm. The retention period of AFB₁ and AFB₂ were 12 and 9 min, respectively. The retention times and peak areas were associated with individual aflatoxins, and the concentration of each aflatoxin was assessed by comparing the results to established aflatoxins standards (Algammal et al., 2021). The assay was conducted in triplicate, with recovery rates for aflatoxins B1 and B2 at 93 and 94%, respectively.

Statistical analysis

The laboratory data evaluated using Analysis of Variance (ANOVA), by utilizing the SPSS software (version 20.0) to establish the impact of the treatments on the meat quality and stability. Also the differences between the means were investigated using the Duncan's Multiple Range Test (DMRT), at 5% significance level ($p \leq 0.5$). Furthermore, descriptive statistic was conducted on the data and the findings presented in tables, to provide a clear and organized summary of the results. The experiments were conducted in triplicate to ensure consistency.

Results and discussion

Phytochemical properties of the bio-agents

The results of the oils and extracts phytochemical properties were presented in Table 2. The results indicated that in the OPE and OPO samples, the most prevalent phytochemicals were Naringenin, Quercetin, Artemetin, Baicalin, and Naringin. In contrast, the PPE and PPO samples contained Epicatechin, Naringenin, Robinetin, Ellagic acid, Baicalin, Lunamarin, and Tangeretin as their primary phytochemicals. The results illustrated that out of the parameters measured, the plants extracts generally had higher concentration of phytochemicals than the plants oils, which is in conformity with earlier reports on bioactive compounds availability in plants derivatives (Umaru et al., 2019). The high phytochemical concentrations in the extracts and oils contribute to their effectiveness in inhibiting microbial growth. This makes the OPE, PPE, OPO and PPO viable options for extending the shelf life of dried meat products. Apigenin, Ellagic acid, Naringenin, Cinnamic acid, and Resveratrol demonstrate antimicrobial properties against a wide range of bacteria and fungi, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Salmonella* spp., and several yeast species (Kačániová et al., 2024; Mandal and Domb, 2024).

Nutritional quality of the raw meat

The results of the vitamins and protein levels of the beef before its preservation are presented in Table 3. It was noted that the Vitamin B1, B2, B3, B5 B9, and B12 of the raw beef was 0.051, 0.018, 0.015,

TABLE 2 The phytochemicals properties of the plants extracts and oils (mg/kg).

Parameter	OPE	PPE	OPO	PPO
Catechin	NR	NR	0.929	1.532
Apigenin	NR	NR	45.066	33.171
Resveratrol	NR	NR	2.831	2.103
Genistein	4.432	2.773	2.129	1.163
Daidzin	0.622	0.315	0.405	0.318
Vinnilic acid	0.236	0.538	0.150	0.327
Butein	0.207	1.526	0.291	1.096
Naringenin	14.642	9.833	0.425	0.393
Luteolin	4.958	2.814	0.289	0.187
Kaempferol	1.083	0.682	0.312	0.395
Epicatechin	0.255	8.364	1.882	2.407
Epigallocatechin	3.081	2.950	0.147	0.125
Quercetin	11.665	6.931	4.266	4.104
Gallocatechin 3	1.457	3.558	NR	NR
Robinetin	24.901	17.523	NR	NR
Myricetin	2.200	1.063	19.250	15.091
Nobiletin	7.288	10.942	1.683	3.921
Artemetin	117.361	81.915	24.918	20.115
Ellagic acid	17.620	30.607	NR	NR
Tangeretin	3.306	18.795	NR	NR
Baicalin	43.195	76.082	0.577	NR
Naringin	28.154	22.073	1.682	0.973
Lunamarin	5.178	11.326	0.425	2.505
Cinnamic acid	5.307	3.116	0.605	0.394
Coumaric acid	NR	NR	0.408	1.306
Ferrulic acid	0.284	0.853	0.341	2.013
Flavone	NR	NR	6.815	4.225
Flavon-3-ol	NR	NR	0.429	1.851

NR, not read.

0.019, 0.052 and 0.029 mg/kg, respectively, while the total vitamin E and protein contents were 1.87 mg/kg and 52.73%, respectively. This is an indication that the raw meat flesh was rich in vitamins B and E; making beef a beneficial component of balanced diet. Vitamins and protein play crucial roles in energy production, nervous system functionality, and fetus development (Kačániová et al., 2024).

Meat quality after storage

Nutritional value

The results of the meat nutritional quality before and after the storage period are presented in Table 4. Following the processing of the meat samples, the levels of vitamin B, vitamin E, protein, and moisture content were observed to range from 0.177 to 0.336 mg/kg, 1.81 to 2.15 mg/kg, 47.16 to 47.26%, and 6.61 to 7.26%, respectively. It was observed that both the extract and oil treatments had an insignificant impact on the protein content of the meat after treatment. In contrast,

the plant extracts and oils enhanced the vitamin B complex and Vitamin E levels in the beef. This could be attributed to the rich antioxidant properties and vitamins levels of onions peels and plantain peels extracts and oils. Antioxidants and other essential compounds present in essential oils and plant extracts, tend to enhance the total vitamins concentration in the meat product during processing, and inhibit nutrients degradation during storage (Pateiro et al., 2018). These results are comparable to the findings reported by Baker (2023), for various meat samples treated with different natural additives.

Furthermore, at the end of the 10-week experimental period, each treatment had a distinct and significant impact on the proximate composition of the meat. The levels of vitamins Band E, protein, and moisture content of the preserved meat varied from 0.129 to 0.314 mg/kg, 1.61 to 2.05 mg/kg, 40.12 to 47.26%, 16.97 to 42.98%, respectively. The research data revealed that the control and T1–T9 meat samples showed total vitamin B reductions of 27.07, 8.86, 17.01, 10.19, 14.34, 7.23, 8.66, 6.64, 11.83, and 15.29%, respectively. Furthermore, the control and T1–T9 meat samples recorded vitamin E depreciation level 11.05, 5.80, 9.05, 6.90, 9.28, 8.87, 9.18, 4.65, 7.25 and 7.92%, respectively. Meanwhile, the protein content of the meat showed depreciation rates of 14.93, 9.49, 10.41, 8.89, 10.73, 10.22, 11.08, 5.12, 8.72, and 8.92% in the control and T1–T9 samples, respectively. Additionally, it was observed that the moisture level in the control sample was the highest at 84.62%. In contrast, the oil-based treatment samples T2, T4, and T9 exhibited the lowest moisture contents,

measuring 63.94, 64.09, and 57.21%, respectively. Meanwhile, the extract-based treatment samples T1, T3, and T7 had average moisture contents of 87.99, 69.43, and 68.59%, respectively.

Notably, the control sample showed the greatest decline in vitamins and protein levels during storage; whereas, the sample treated with hybridized extracts (T7) experienced the least depreciation in B vitamins (6.64% depreciation rate) and protein content (5.11%). Similar to Turgut et al. (2017) observation, where pomegranate (*Punica granatum*) peels extract hinders beef protein oxidation storage. These findings present perfect insight into plant extracts and EOs antioxidants attributes, in effectively retarding vitamins and proteins degradation during storage (Fu et al., 2022; Petcu et al., 2023). The higher B vitamins degradation rate, compared to vitamin E in the preserved meat samples, can be associated with the distinct chemical properties of these two vitamins. B vitamins been water-soluble are more vulnerable to degradation when exposed to heat and light (Hrubša et al., 2022); whereas, Vitamin E which is a fat-soluble vitamin had elevated stability under high temperatures and UV light (Napolitano et al., 2019).

Additionally, it was observed that the moisture level in the control sample was the highest at 84.62%. In contrast, the oil-based treatment samples - T2, T4, and T9 exhibited the lowest moisture contents, measuring 63.94, 64.09, and 57.21%, respectively. Meanwhile, the extract-based treatment samples - T1, T3, and T7 had average moisture contents of 87.99, 69.43, and 68.59%, respectively. These findings highlight the effectiveness of the oil, in reducing moisture absorption by the meat compared to the extracts. Oils act as impermeable barrier, which slow down water migration, microbial growth and food decay (Umaru et al., 2019). The reduced moisture content, especially in T9 (57.21%), points to improved storage stability, as lower moisture typically correlates with a lower risk of microbial contamination and oxidation (Uguru et al., 2023). By retarding nutrient breakdown, these preservatives have helped to extend the shelf life of the treated meat samples.

Microbial population evaluation

The microbiology results for the meat samples are presented in Tables 5, 6. The results highlight that both the storage duration and treatment plan, have significant effect on dried meat samples

TABLE 3 The raw meat quality.

Parameter		Concentration
Vitamin B (mg/kg)	B1	0.051 ± 0.006
	B2	0.018 ± 0.005
	B3	0.015 ± 0.001
	B6	0.019 ± 0.003
	B9	0.052 ± 0.006
	B12	0.029 ± 0.005
Vitamin E (mg/kg)		1.87 ± 0.21
Protein (%)		52.73 ± 0.97

Mean ± standard deviation ($n = 3$).

TABLE 4 Proximate compositions of the dried meat before and after storage.

Treat	Total B Vitamins (mg/kg)		Vitamin E (mg/kg)		Protein (%)		Moisture content (%)	
	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10	Week 0	Week 10
Cont.	0.177 ^a ± 0.009	0.129 ^a ± 0.002	1.81 ^a ± 0.02	1.61 ^a ± 0.02	47.16 ^a ± 0.06	40.12 ^a ± 0.09	6.61 ^b ± 0.04	42.98 ^b ± 0.15
T1	0.320 ^f ± 0.002	0.291 ^f ± 0.009	2.07 ^e ± 0.03	1.95 ^e ± 0.06	47.33 ^f ± 0.05	42.84 ^f ± 0.06	6.65 ^d ± 0.04	20.78 ^d ± 0.22
T2	0.241 ^b ± 0.004	0.200 ^b ± 0.006	1.99 ^{bc} ± 0.05	1.81 ^{bc} ± 0.04	47.16 ^c ± 0.02	42.25 ^c ± 0.04	7.05 ^{cd} ± 0.05	20.11 ^{cd} ± 0.12
T3	0.288 ^c ± 0.008	0.259 ^c ± 0.008	2.03 ^{cde} ± 0.04	1.89 ^{cde} ± 0.06	47.23 ^f ± 0.02	43.03 ^f ± 0.03	6.81 ^f ± 0.04	22.28 ^f ± 0.16
T4	0.256 ^c ± 0.004	0.219 ^c ± 0.004	1.94 ^b ± 0.04	1.76 ^c ± 0.05	47.15 ^c ± 0.04	42.09 ^c ± 0.08	7.12 ^c ± 0.02	19.83 ^c ± 0.09
T5	0.286 ^c ± 0.002	0.265 ^c ± 0.006	2.03 ^{cd} ± 0.04	1.85 ^{cd} ± 0.06	47.25 ^d ± 0.04	42.42 ^d ± 0.05	6.77 ^c ± 0.03	21.77 ^c ± 0.23
T6	0.269 ^d ± 0.002	0.246 ^d ± 0.004	1.96 ^b ± 0.05	1.78 ^b ± 0.06	47.21 ^b ± 0.02	41.98 ^b ± 0.06	6.78 ^c ± 0.03	21.75 ^c ± 0.09
T7	0.336 ^g ± 0.005	0.314 ^g ± 0.003	2.15 ^f ± 0.06	2.05 ^f ± 0.05	47.26 ^g ± 0.04	44.84 ^g ± 0.03	7.10 ^g ± 0.46	22.61 ^g ± 0.33
T8	0.262 ^c ± 0.002	0.231 ^c ± 0.003	2.07 ^{de} ± 0.07	1.92 ^{de} ± 0.06	47.16 ^f ± 0.02	43.05 ^f ± 0.04	6.62 ^b ± 0.06	19.90 ^b ± 0.22
T9	0.255 ^c ± 0.004	0.216 ^c ± 0.004	2.02 ^{cd} ± 0.06	1.86 ^{cd} ± 0.07	47.21 ^f ± 0.07	43.00 ^f ± 0.07	7.26 ^a ± 0.06	16.97 ^a ± 0.23

Mean ± standard deviation ($n = 3$); treat = treatment; Cont. = control; column with the same small alphabet for the sample nutriment represent significant difference ($p < 0.05$).

TABLE 5 The bacteria load of the meat samples.

Treatment	Storage (weeks)					
	0	2	4	6	8	10
TBVC (x10²cfu/g)						
Control	0.00 ^a	0.55 ^b ± 0.14	1.48 ^c ± 0.23	1.97 ^d ± 0.16	2.45 ^e ± 0.22	3.13 ^f ± 0.18
T1	0.00 ^a	0.40 ^b ± 0.12	0.60 ^c ± 0.09	0.88 ^d ± 0.03	1.34 ^e ± 0.12	1.67 ^f ± 0.11
T2	0.00 ^a	0.05 ^b ± 0.08	0.29 ^c ± 0.07	0.51 ^d ± 0.03	0.94 ^e ± 0.09	1.25 ^f ± 0.07
T3	0.00 ^a	0.16 ^b ± 0.07	0.49 ^c ± 0.07	0.73 ^d ± 0.11	0.98 ^e ± 0.11	1.42 ^f ± 0.11
T4	0.00 ^a	0.04 ^b ± 0.08	0.27 ^c ± 0.04	0.47 ^d ± 0.05	0.64 ^e ± 0.06	1.21 ^f ± 0.06
T5	0.00 ^a	0.00 ^b	0.37 ^c ± 0.04	0.57 ^d ± 0.07	0.63 ^e ± 0.03	0.86 ^f ± 0.07
T6	0.00 ^a	0.01 ^b ± 0.02	0.26 ^c ± 0.05	0.36 ^d ± 0.04	0.53 ^e ± 0.09	0.92 ± 0.07
T7	0.00 ^a	0.02 ^b ± 0.02	0.13 ^c ± 0.02	0.18 ^d ± 0.01	0.35 ^e ± 0.02	0.55 ^f ± 0.07
T8	0.00 ^a	0.00 ^b	0.01 ^c ± 0.02	0.13 ^d ± 0.04	0.22 ^e ± 0.04	0.43 ^f ± 0.03
T9	0.00 ^a	0.00 ^b	0.01 ^c ± 0.01	0.07 ^d ± 0.02	0.13 ^e ± 0.02	0.27 ^f ± 0.06
<i>Staphylococcus aureus</i> (cfu/g)						
Control	0.00 ^a	0.67 ^a ± 1.15	12.00 ^b ± 2.00	28.67 ^c ± 1.53	51.00 ^d ± 2.65	73.33 ^e ± 2.52
T1	0.00 ^a	0.00 ^a	3.33 ^b ± 2.52	10.00 ^c ± 2.00	14.67 ^d ± 2.08	25.67 ^e ± 2.08
T2	0.00 ^a	0.00 ^a	1.00 ^b ± 1.00	3.00 ^c ± 1.00	7.33 ^d ± 1.15	13.00 ^e ± 1.00
T3	0.00 ^a	0.67 ^a ± 1.15	3.00 ^b ± 1.00	8.67 ^c ± 0.58	12.00 ^d ± 2.00	21.67 ^e ± 2.52
T4	0.00 ^a	0.00 ^a	1.00 ^b ± 1.73	3.00 ^c ± 1.73	9.00 ^d ± 1.00	19.33 ^e ± 1.53
T5	0.00 ^a	0.00 ^a	0.00 ^b	1.00 ^c ± 1.00	6.67 ^d ± 0.58	12.00 ^e ± 2.00
T6	0.00 ^a	0.00 ^a	0.00 ^b	0.00 ^c	6.33 ^d ± 1.53	13.00 ^e ± 2.00
T7	0.00 ^a	0.00 ^a	0.00 ^b	2.00 ^c ± 1.00	5.67 ^d ± 1.15	7.67 ^e ± 1.53
T8	0.00 ^a	0.00 ^a	1.00 ^b ± 01.00	0.67 ^c ± 1.15	2.67 ^d ± 1.53	6.33 ^e ± 1.53
T9	0.00 ^a	0.00 ^a	0.00 ^b	0.00 ^c	0.00 ^d	3.33 ^e ± 2.08
<i>Salmonella</i> spp. (cfu/g)						
Control	0.00 ^a	0.00 ^a	0.00 ^a	13.33 ^b ± 2.08	30.33 ^c ± 3.21	52.67 ^d ± 4.73
T1	0.00 ^a	0.00 ^a	0.00 ^a	4.33 ^b ± 1.53	11.00 ^c ± 2.00	19.67 ^d ± 1.53
T2	0.00 ^a	0.00 ^a	1.3 ^a ± 30.58	4.67 ^b ± 1.53	8.67 ^c ± 2.08	14.00 ^d ± 2.00
T3	0.00 ^a	0.00 ^a	0.00 ^a	2.67 ^b ± 1.53	7.00 ^c ± 2.00	21.67 ^d ± 3.51
T4	0.00 ^a	0.00 ^a	0.00 ^a	2.00 ^b ± 1.00	8.00 ^c ± 2.00	16.33 ^d ± 1.53
T5	0.00 ^a	0.00 ^a	1.00 ^a ± 1.00	4.00 ^b ± 1.00	7.67 ^c ± 1.53	13.67 ^d ± 2.52
T6	0.00 ^a	0.00 ^a	0.00 ^a	2.33 ^b ± 0.58	7.67 ^c ± 2.08	17.00 ^d ± 1.00
T7	0.00 ^a	0.00 ^a	0.00 ^a	2.33 ^b ± 2.52	5.33 ^c ± 2.08	8.67 ^d ± 2.08
T8	0.00 ^a	0.00 ^a	1.00 ^a ± 1.73	1.00 ^b ± 1.73	3.33 ^c ± 1.53	6.00 ^d ± 1.00
T9	0.00 ^a	0.00 ^a	0.00 ^a	0.000 ^b ± .00	1.67 ^c ± 1.53	4.00 ^d ± 1.00

Mean ± standard deviation ($n = 3$); column with the same small alphabet for sample pathogen represent significant difference ($p \leq 0.05$).

microbial growth ($p \leq 0.05$). These findings confirmed earlier reports of these scholars (Gál et al., 2023; Ivane et al., 2024; Djenane et al., 2024), stating that EOs and extracts treatments, substantially retard microorganisms' growth and survival. Notably, at the end of the experimental duration (tenth week), the total viable bacterial counts (TVBC) for the T1 to T9 samples were 167, 125, 142, 121, 86, 92, 55, 43, and 27 cfu/g, respectively; the *Staphylococcus aureus* populations in the T1 to T9 treated meat specimens were 25.67, 13.00, 21.67, 19.33, 12.00, 13.00, 7.67, 6.33 and 3.33 cfu/g, respectively; the *Salmonella* spp. counts in the T1 to T9 meat samples were 19.67, 14.00, 21.67, 16.33, 13.67, 17.00, 8.67, 6.00 and

4.00 cfu/g, respectively. In terms of the fungal contamination, the TFC recorded in the T1 – T9 samples were 134, 115, 106, 93, 92, 88, 52, 60 and 56 cfu/g, respectively; the afB1 values among the treated samples ranged from 2.97 to 11.43 ppb, while the afB2 load varied from 2.60 to 10.17 ppb.

Generally, the mean samples treated with essential oils demonstrated better preservative attributes, than those treated with the extracts, similar to the findings of Ashraf et al. (2025). This study's outcomes depicted that meat samples preserved using hybridized treatments (T3–T9), exhibited a longer shelf life and stability, compared to those samples preserved with single

TABLE 6 Occurrence of fungal load and aflatoxins in analyzed samples.

Treatment	Storage (weeks)					
	0	2	4	6	8	10
TFC (x10²cfu/g)						
Control	0.00 ^a	0.01 ^a ± 0.01	0.53 ^b ± 0.26	1.20 ^c ± 0.14	1.45 ^d ± 0.38	2.18 ^e ± 0.14
T1	0.00 ^a	0.00 ^a	0.18 ^b ± 0.06	0.83 ^c ± 0.10	1.06 ^d ± 0.06	1.34 ^e ± 0.08
T2	0.00 ^a	0.00 ^a	0.03 ^b ± 0.04	0.12 ^c ± 0.03	1.02 ^d ± 0.09	1.15 ^e ± 0.18
T3	0.00 ^a	0.01 ^a ± 0.03	0.10 ^b ± 0.02	0.55 ^c ± 0.08	0.93 ^d ± 0.09	1.06 ^e ± 0.06
T4	0.00 ^a	0.01 ^a ± 0.03	0.11 ^b ± 0.03	0.47 ^c ± 0.09	0.73 ^d ± 0.09	0.93 ^e ± 0.08
T5	0.00 ^a	0.02 ^a ± 0.02	0.20 ^b ± 0.19	0.53 ^c ± 0.31	0.68 ^d ± 0.19	0.92 ^e ± 0.08
T6	0.00 ^a	0.02 ^a ± 0.01	0.17 ^b ± 0.17	0.57 ^c ± 0.18	0.59 ^d ± 0.25	0.88 ^e ± 0.07
T7	0.00 ^a	0.01 ^a ± 0.01	0.23 ^b ± 0.07	0.50 ^c ± 0.12	0.54 ^d ± 0.19	0.62 ^e ± 0.17
T8	0.00 ^a	0.00 ^a	0.10 ^b ± 0.06	0.18 ^c ± 0.04	0.27 ^d ± 0.08	0.60 ^e ± 0.13
T9	0.00 ^a	0.00 ^a	0.07 ^b ± 0.07	0.16 ^c ± 0.06	0.23 ^d ± 0.10	0.36 ^e ± 0.07
AFB1 (ppb)						
Control	0.00 ^a	0.23 ^a ± 0.40	3.67 ^b ± 1.15	7.00 ^c ± 2.00	13.00 ^d ± 2.00	20.67 ^e ± 1.53
T1	0.00 ^a	0.00 ^a	2.13 ^b ± 0.23	3.20 ^c ± 0.66	4.93 ^d ± 0.21	11.43 ^e ± 1.50
T2	0.00 ^a	0.00 ^a	2.00 ^b ± 1.00	3.67 ^c ± 0.58	5.00 ^d ± 2.00	8.33 ^e ± 1.53
T3	0.00 ^a	0.00 ^a	3.67 ^b ± 0.58	5.67 ^c ± 0.58	7.67 ^d ± 0.58	11.00 ^e ± 1.00
T4	0.00 ^a	0.00 ^a	2.33 ^b ± 1.15	4.00 ^c ± 1.00	8.00 ^d ± 1.00	8.67 ^e ± 1.53
T5	0.00 ^a	0.00 ^a	0.00 ^b	0.87 ^c ± 0.32	2.50 ^d ± 0.50	3.87 ^e ± 0.86
T6	0.00 ^a	0.00 ^a	0.20 ^b ± 0.35	1.17 ^c ± 0.40	2.67 ^d ± 1.53	4.33 ^e ± 1.15
T7	0.00 ^a	0.00 ^a	0.23 ^b ± 0.25	1.27 ^c ± 0.15	2.90 ^d ± 0.36	5.60 ^e ± 0.82
T8	0.00 ^a	0.00 ^a	0.00 ^b	0.33 ^c ± 0.15	2.23 ^d ± 0.23	4.83 ^e ± 0.25
T9	0.00 ^a	0.00 ^a	0.00 ^b	0.17 ^c ± 0.21	1.30 ^d ± 0.10	2.97 ^e ± 0.32
AFB2 (ppb)						
Control	0.00 ^a	0.20 ^a ± 0.20	3.30 ^b ± 0.75	6.27 ^c ± 1.86	12.10 ^d ± 1.87	17.87 ^e ± 0.96
T1	0.00 ^a	0.00 ^a	1.53 ^b ± 0.45	2.60 ^c ± 0.36	4.30 ^d ± 0.30	10.17 ^e ± 1.11
T2	0.00 ^a	0.00 ^a	1.50 ^b ± 1.05	3.60 ^c ± 0.95	4.00 ^d ± 1.65	6.43 ^e ± 0.49
T3	0.00 ^a	0.00 ^a	3.10 ^b ± 0.90	4.97 ^c ± 0.15	6.87 ^d ± 0.80	9.87 ^e ± 1.11
T4	0.00 ^a	0.00 ^a	1.90 ^b ± 0.95	3.67 ^c ± 1.05	6.97 ^d ± 0.78	7.43 ^e ± 1.42
T5	0.00 ^a	0.00 ^a	0.00 ^b	0.50 ^c ± 0.35	2.03 ^d ± 0.42	2.70 ^e ± 0.46
T6	0.00 ^a	0.00 ^a	0.20 ^b ± 0.35	0.73 ^c ± 0.21	2.00 ^d ± 1.31	4.67 ^e ± 0.55
T7	0.00 ^a	0.00 ^a	0.47 ^b ± 0.45	1.63 ^c ± 0.23	2.50 ^d ± 0.26	4.73 ^e ± 0.47
T8	0.00 ^a	0.00 ^a	0.00 ^b	0.20 ^c ± 0.10	1.90 ^d ± 0.20	3.97 ^e ± 0.81
T9	0.00 ^a	0.00 ^a	0.00 ^b	0.27 ^c ± 0.38	0.83 ^d ± 0.40	2.60 ^e ± 0.50

Mean ± standard deviation ($n = 3$); column with the same small alphabet for sample pathogen represent significant difference ($p < 0.05$).

treatments (T1 and T2). The hybridization of onion waste and plantain peel EOs at a concentration of 0.5% each (T9), demonstrated effective control over bacterial and fungal performance, particularly against afb1 and afb2. This could be attributed to the broad-spectrum antimicrobial properties of the oils (Alirezalu et al., 2019; Kačániová et al., 2024). The lower *Staphylococcus aureus*, *Salmonella* spp., and aflatoxins populations, observed in T9 meat sample can be attributed to the consistently low moisture content, this specimen maintained throughout the storage period. Lower moisture levels create an inhospitable environment, for microbial growth and toxin production (Uguru

et al., 2023; Kačániová et al., 2024). The statistical analysis revealed no significant difference in the populations of fungi, afb1, and afb2 between Weeks 0 and 2 ($p \leq 0.05$), an indication that the fungal growth and aflatoxins has very poor functionality during the initial storage period, which can be linked to the immediate effect of the organic additives (Beya et al., 2021).

The zero microbial count recorded at the first storage day, affirmed that the meat primarily picked up pathogenic microorganisms during storage, through contaminated environment and atmospheric depositions (Pleadin et al., 2021; Uguru et al., 2024; Djenane et al., 2024). Similarly, the rapid microbial growth observed after sixth week

TABLE 7 Optimal response surface experimental design result.

Run	Storage time (week)	Sample code	TBC	<i>Staphylococcus aureus</i>	<i>Salmonella</i> spp.	TFC	afB1	afB2
1	2	T9	0.000	0.000	0.000	0.000	0.000	0.000
2	2	T8	0.000	0.000	0.000	0.000	0.000	0.000
3	2	T1	5.000	0.000	0.000	0.000	0.000	0.000
4	10	T3	142.000	21.67	21.67	106.000	11.000	9.87
5	2	T9	0.000	0.000	0.000	0.000	0.000	0.000
6	4	T3	49.000	3	0.000	10.000	3.67	3.1
7	2	T4	4.000	0.000	0.000	10.000	0.000	0.000
8	8	T4	64.000	9.00	8.00	73.000	8.00	6.97
9	0	T3	0.000	0.000	0.000	0.000	0.000	0.000
10	2	T5	0.000	0.000	0.000	2.000	0.000	0.000
11	8	T0	245.000	51.00	30.33	145.000	13.00	12.10
12	8	T1	134.000	14.67	11.000	106.000	4.93	4.30
13	0	T2	0.000	0.000	0.000	0.000	0.000	0.000
14	8	T2	94.000	7.33	8.67	102.000	5.000	4.000
15	2	T2	5.000	0.000	0.000	0.000	0.000	0.000
16	0	T0	0.000	0.000	0.000	0.000	0.000	0.000
17	8	T7	35.000	5.670	5.330	54.000	2.90	2.500
18	2	T0	55.000	0 0.670	0.000	1.000	0.230	0.200
19	8	T6	53.000	6.330	7.670	59.000	2.230	2.000
20	8	T6	53.000	6.330	7.670	59.000	2.230	2.00
21	2	T7	2.000	0.000	0.000	1.000	0.000	0.000
22	2	T6	1.000	0.000	0.000	2.000	0.000	0.000
23	2	T3	16.000	0.000	0.000	1.000	0.000	0.000
24	4	T1	60.000	3.33	0.000	18.000	2.13	1.53
25	8	T9	13.000	0.000	0.000	23.000	1.30	0.83
26	8	T9	13.000	0.000	0.000	23.000	1.30	0.83
27	0	T1	0.000	0.000	0.000	0.000	0.000	0.000
28	8	T8	22.000	2.67	3.33	27.000	2.23	1.90
29	8	T4	64.000	9	8.000	73.000	8.000	6.87
30	8	T5	63.000	6.67	7.67	68.000	2.25	2.03
31	2	T4	4.000	0.000	0.000	1.000	0.000	0.000

Sample code is same as treatment code.

storage period, can be associated with the substantial decline in the meat’s antimicrobial and antioxidant properties over time; hence, creating favorable conditions for pathogen proliferation (Punia et al., 2021). Similar findings were documented by Mojaddar et al. (2018), when sumac (*Rhus coriaria*) extract was able to inhibit bacteria and fungi survivability. According to Lupia et al. (2024), due to the volatile nature of essential oils, they have greater ability of penetrating and disrupting microbial cellular structures, and metabolic functions, leading to enhance antimicrobial actions.

This experimental outcome aligned with the previous reports of these authors (Barbosa et al., 2009; Yuan and Yuk, 2018; Petcu et al., 2023; Kačániová et al., 2024; Ashraf et al., 2025), which stated that EOs and extracts derived from edible portions of peppermint (*Mentha piperita* L), lemongrass (*Cymbopogon citratus*), ginger

(*Zingiber officinale Roscoe*), clove (*Syzygium aromaticum*) and *Syzygium antisepticum*, successfully retarded *Salmonella* spp., *Staphylococcus aureus* and Coliform reproduction in chicken meat stored inside the refrigeration for 12 days. This affirms that EOs and extracts produced from agricultural waste materials, rather than the comestible plant parts, can be effectively utilized for meat preservation, thereby combating food insecurity problem, as well as waste valorization.

The fungal analysis depicted that Aflatoxins B1 (afB1) and Aflatoxins (afB2) presence were relatively small in T5, T8, and T9 samples, which can be linked to anti aflatoxins properties (bioactive compounds) of the extract and oil (Algammal et al., 2021; Baker, 2023). Specifically, the study revealed that the optimization and treatment combination provided by the CCD model revealed that,

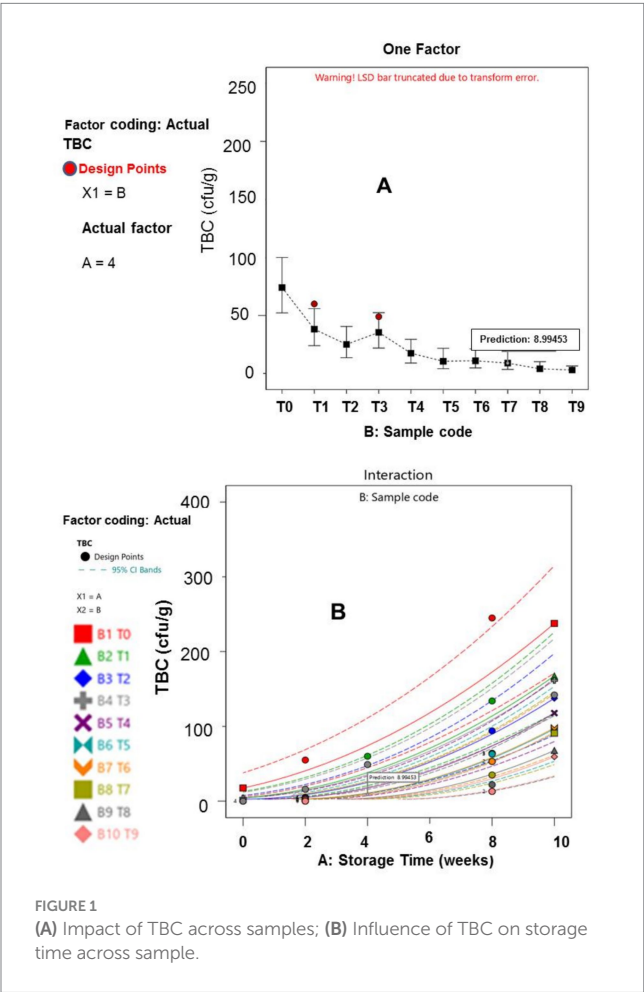
TABLE 8 ANOVA for response model and parameters.

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
TBC	Model	490.7	10	49.07	18.52	<0.0001	Significant
	Storage Time	407.63	1	407.63	153.89	<0.0001	Significant
	Sample code	153.12	9	17.01	6.42	0.0003	Significant
	Residual	52.98	20	2.65			
	Lack of Fit	52.98	15	3.53			
	Pure Error	0	5	0			
	Cor Total	543.68	30				
<i>Staphylococcus aureus</i>	Model	78.16	10	7.82	8.69	<0.0001	Significant
	Storage Time	64.44	1	64.44	71.62	<0.0001	Significant
	Sample code	22.41	9	2.49	2.77	0.0278	Significant
	Residual	18	20	0.8998			
	Lack of Fit	18	15	1.2			
	Pure Error	0	5	0			
	Cor Total	96.16	30				
<i>Salmonella</i> spp.	Model	65.92	10	6.59	7.58	<0.0001	Significant
	Storage Time	56.4	1	56.4	64.89	<0.0001	Significant
	Sample code	13.1	9	1.46	1.67	0.1611	Not significant
	Residual	17.38	20	0.8692			
	Lack of Fit	17.38	15	1.16			
	Pure Error	0	5	0			
	Cor Total	83.3	30				
TFC	Model	445.7	10	44.57	29.16	<0.0001	Significant
	Storage Time	412.91	1	412.91	270.1	<0.0001	Significant
	Sample code	41.31	9	4.59	3	0.0195	Not significant
	Residual	30.57	20	1.53			
	Lack of Fit	28.24	15	1.88	4.03	0.0658	Not significant
	Pure Error	2.34	5	0.4675			
	Cor Total	476.28	30				
afB1	Model	35.66	10	3.57	17.69	<0.0001	Significant
	Storage Time	32.95	1	32.95	163.47	<0.0001	Significant
	Sample code	5.85	9	0.6501	3.23	0.014	Significant
	Residual	4.03	20	0.2015			
	Lack of Fit	4.03	15	0.2687			
	Pure Error	0	5	0			
	Cor Total	39.69	30				
afB2	Model	31.14	10	3.11	16.34	<0.0001	Significant
	Storage Time	28.37	1	28.37	148.9	<0.0001	Significant
	Sample code	5.49	9	0.6102	3.2	0.0144	Significant
	Residual	3.81	20	0.1905			
	Lack of Fit	3.81	15	0.254	7031.12	<0.0001	Significant
	Pure Error	0.0002	5	0			
	Cor Total	34.95	30				

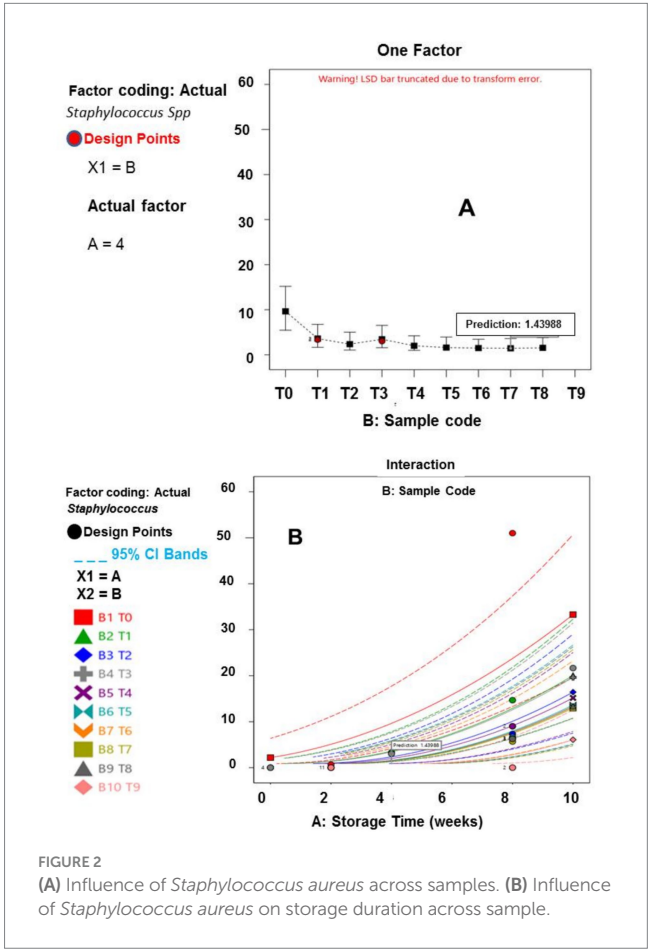
TABLE 9 Constrain applied for the optimization action.

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Storage Time	IIR	0	10	1	1	3
Sample code	IIR	T0	T9	1	1	3
TBC	IIR	0	245	1	1	3
<i>Staphylococcus aureus</i>	IIR	0	51	1	1	3
<i>Salmonella</i> spp.	IIR	0	33.33	1	1	3
TFC	IIR	0	145	1	1	3
Aflatoxins B1	IIR	0	13	1	1	3
Aflatoxins B2	IIR	0	12.1	1	1	3

IIR ~ is in range.

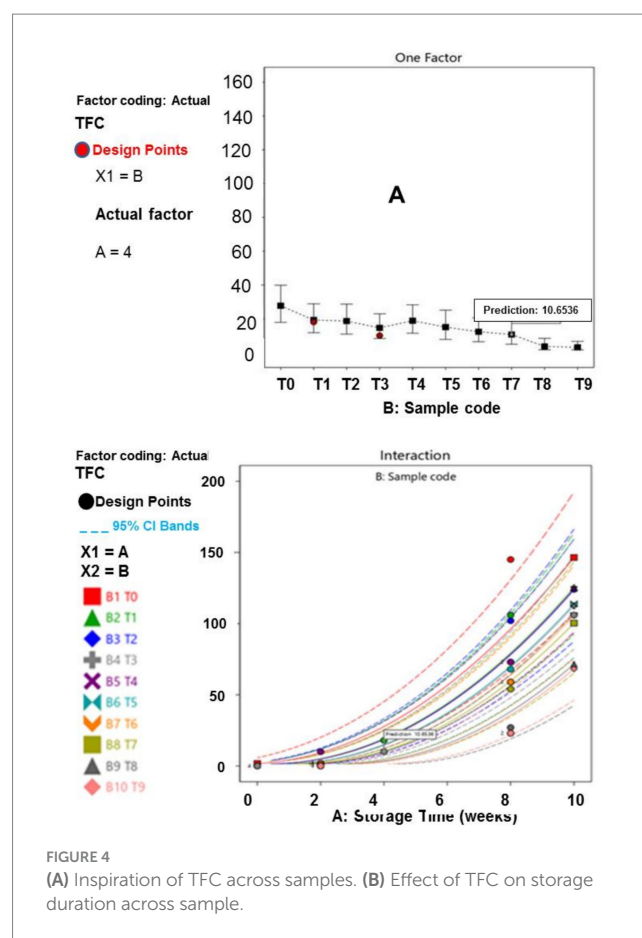
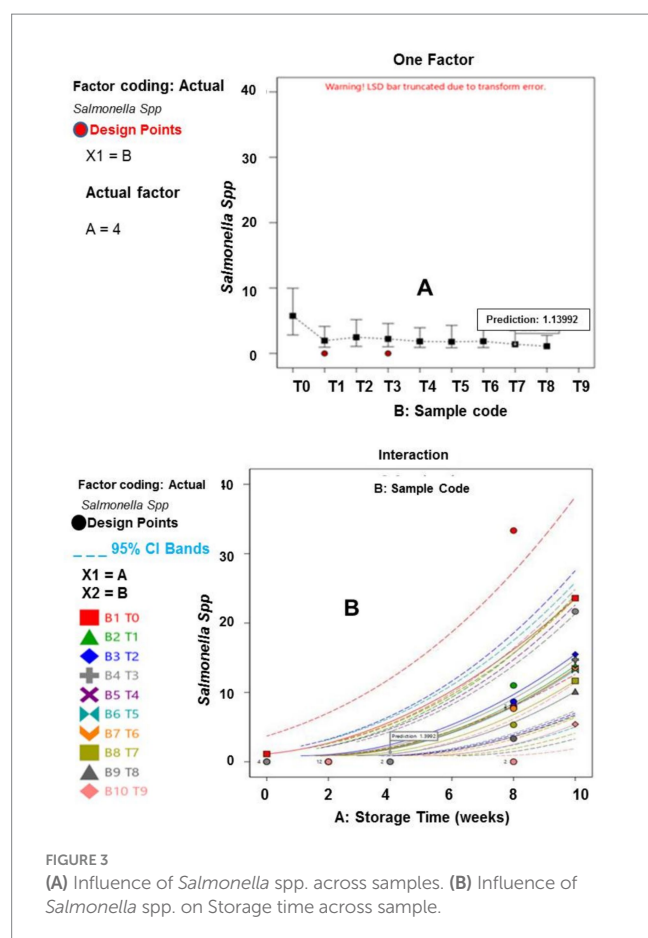


plants waste materials can be processed into EO and extract, with the capability of retarding aflatoxins formation within permissible limits for human safety, established by the European Council (EU) and World Health Organization (WHO). The WHO and European council approved maximum aflatoxins acceptable level of 10 ppb and 4 ppb, respectively, for food items (Bradford et al., 2020; Uguru et al., 2023). These outcomes underscore the importance of further research into pharmaceutical (antimicrobial) impact of agricultural waste materials, thereby ratifying the efficacy of the natural antioxidants as bio-preservation approach.



Modeling and optimization of the meat quality during storage

The results of the modeling and optimization of the meat storability, with respect to the treatment plans, were presented in Tables 7–9 and Supplementary Tables S1–S4. Table 7 displays the microbial results of the meat samples during storage for the 31 runs (based on Table 1), through the combination of these factors: treatment and storage duration. Table 8 shows the ANOVA values for response model and parameters. *Staphylococcus* spp., *Salmonella* spp. Aflatoxins B1 and B2 classified in the treated meat samples (Table 7)



were active pathogenic microbes, which have been linked to serious foodborne diseases (Bradford et al., 2020; Petcu et al., 2023; Kačanić et al., 2024). The data shown that in experimental runs 1, 2, 5, 9, 10, 13, 16, and 27, regardless of the response investigated, the contamination level was 0.000 (0%), which is an indication that the particular treatment successfully inhibited the pathogen persistence, at that exact storage period. Furthermore, the optimal response surface result (Table 7) depicts that, the untreated experimental batch (T0), had the worst microbial prevalence and toxins formation; while T8 and T9 units were very persuasive in microbial suppression, further buttressing the worth of the treatment agents in meat preservation (Alirezalu et al., 2019; Ashraf et al., 2025). Based on the optimization outcomes, T9 had the optimal results, yielding almost 0% microbial growth and toxin formation after several storage weeks; though T6–T8 recorded appreciable anti-pathogenic actions, and T1–T3 demonstrated moderate antimicrobial management behaviors. Fascinatingly, the reliability evaluation outcomes performed with runs 19, 10, 25, and 26, affirmed the optimization model constancy.

Specifically, Supplementary Tables S1–S4 displayed the following - optimization model selection for responses, response model equations used for optimization, response models statistic and optimization solutions, respectively. The information provided by Supplementary Table S4 (optimization solutions) revealed that, the minimal values solutions can be achieved in serial numbers 5, 14, 19, and 23. This sustained the fact that the distill water used for this research, was devoid of microbial pollution. Additionally, Supplementary Table S4 pointed out that, some of the natural agents used for the meat

treatment have traces of microbial contamination, which can be attributed to their mode of preparation and cross-contamination effects. Table 9 shows the constrain use for this study's optimization action. The values in Table 9 provide safe and suitable boundaries, for the parameters investigated in this work, primarily by identifying the combinations—treatments and storage period, which will yield minimal pathogenic contamination. Also, the importance level (3) signifies moderate primacy meant for each restraint. These conditions facilitate the attainment of reliable storage and treatment results.

Furthermore, the results of the interactions between the responses, storage time and treatment plan are presented in Figures 1–6. The models and optimization Figures revealed that Treatments 5, 8, and 9 had the best inhibiting power against TBC survival; while T7 and T8 exhibited the optimal *Staphylococcus* and *Salmonella* spp. survival inhibition. Similarly, the results indicated that T8 and T9 effectively retard fungal performance within the specimens; while T6, T8 and T9, exhibited high efficiency against aflB1 and aflB2 survival. Regarding the stability and storability of the meat products, the Figures 5, 6 revealed the reproduction tracks of the microbial within the preserved meat samples, as the storage period progresses from week 0 to week 10. Generally, there was sharp increment in pathogenic microorganism's performance, in the T0, T1, T2, T3, and T4 specimens, as storage progresses; which indicates that these treatments has poor preservation qualities. Also, the gentle slope curves, as reflected by T7, T8, and T9, are a sign of good microbial steadiness, irrespective of the microorganism examined in this research. Based on the treatments optimization outcomes, samples treated with T9 show the optimal microbial

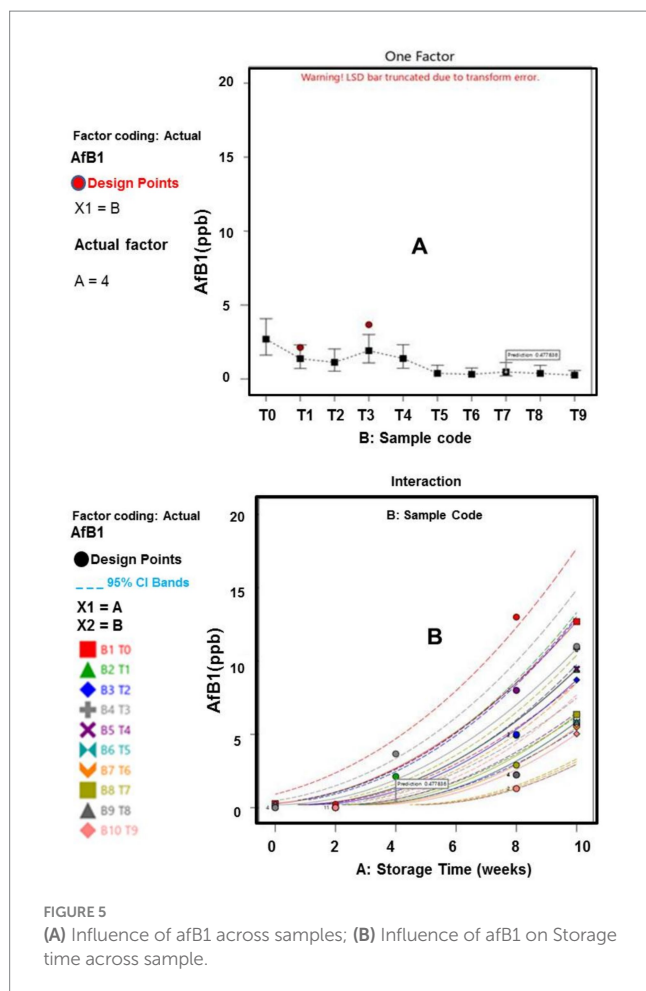


FIGURE 5
 (A) Influence of aflatoxin B1 across samples; (B) Influence of aflatoxin B1 on Storage time across sample.

inhibition potential; though samples treated with Treatments 5 to 8, display good effectiveness in hindering microbial growth. Specifically, samples T5 to T9 can be considered to be more shelf-stable, due to the higher resistivity to microbial invasion, which can lead to food spoilage. The prediction and optimization outcomes established that, the treatments have different antimicrobial potencies. Therefore, some treatments were able to retard microbial resilience significantly, when compared to others, signifying superior microbial control. Conversely, the rapid surge in microbial growth in T0, recorded across microorganisms evaluated, suggested that the untreated meat product is more vulnerable to microbial contamination during storage.

Confirmation of the model created and the optimization process

The result of the confirmation test preformed on the model is presented in Table 10. Validation test is necessary in optimization process, as it helps to authenticate the consistency and reproducibility of the outcomes documented from the experimental procedures (Teja et al., 2023); hence, boosting the assurance level of the optimized final results. Table 10 establishes that all the authentication experimental outcomes, were within the 95% prediction ranges, portraying substantial microbial growth inhibition, and high functionality of the model used. Categorically, it can be seen that the system has high

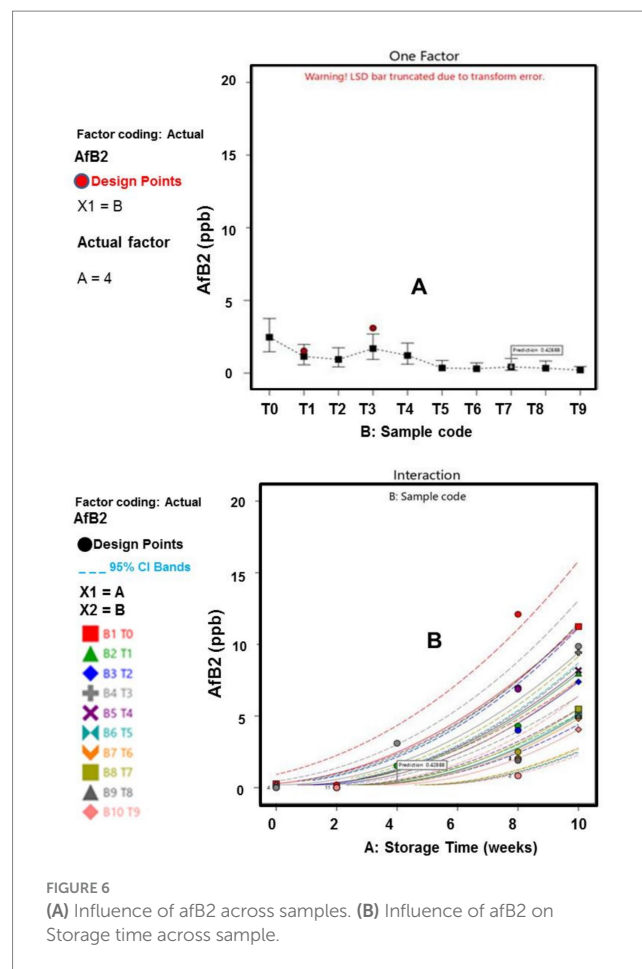


FIGURE 6
 (A) Influence of aflatoxin B2 across samples. (B) Influence of aflatoxin B2 on Storage time across sample.

predictive precision, and does not require further improvement, since the microbial burden documented by the validation exercise, did not significantly surpassed the model expectations.

Conclusion

Food insecurity resulting from nutrient degradation and microbial contamination during storage is indeed a significant challenge for the food industry. This study was conducted to optimize the utilization of natural additives, such as onion bulb peel oil (OPO), plantain peel oil (PPO), onion peel extract (OPE), and plantain peel extract (PPE), in enhancing meat quality and inhibit microbial growth in beef during storage. The hybridized effect of these process variables—treatment concentration and storage time, on the microbial reproductively and toxins production was comprehensively explored. The experimental outcomes revealed that the plant extracts (OPE and PPE), effectively slowed down nutrients degradation during storage; while the essential oils (OPO and PPO) demonstrated strong efficacy in inhibiting pathogens survival. The findings confirmed that moisture content significantly promotes microbial survival. The survival of aflatoxins, *Staphylococcus aureus*, and *Salmonella* spp. was negatively affected by OPO and PPO, when applied at a concentration of 1% (w/w). Precisely, the optimization program successfully pinpoints the treatments options, which are the most suitable for meat preservation, with minimum spoilage possibilities. It was noted

TABLE 10 Confirmation (Validation) experiment optimization model.

Factor	Predicted mean	Predicted median	SD	R	SE Pred	PI low (95%)	MER	PI high (95%)
TBC	94.80	92.16	31.47	3	N/A	32.11	109.21	183.14
Staph	12.55	11.65	6.59	3	N/A	1.26	10.83	32.55
Salm	7.81	6.943	5.06	3	N/A	0.15	5.99	23.89
TFC	40.84	39.31	15.65	3	N/A	10.77	50.83	85.71
afB1	3.83	3.63	1.73	3	N/A	0.67	5.02	8.939
afB2	3.48	3.29	1.61	3	N/A	0.57	4.78	8.22

Salm = *Salmonella* spp., Staph = *Staphylococcus aureus*, R = Replications, PI = predictive Interval, SD = Standard deviation, SE Pred = Standard Error of Prediction, N/A = Not Available, MER = Mean Experimental Result.

that T9 had the best microbial inhibition potential; while the T1 to T3 samples were vulnerable to microbial infestation, and aflatoxins production. Furthermore, the predictive modeling analysis established the optimal of EOs and extracts concentrations for maximal stabilizing efficiency. Notably, this study outcome emphasizes that EOs and extracts derived from agricultural by-products, instead of the edible parts can be effectively used to preserve meat integrity during storage, thereby conserving the public health safety and enhancing human performance.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Author contributions

SA: Methodology, Software, Writing – original draft. RS: Conceptualization, Methodology, Writing – review & editing. HU: Data curation, Methodology, Writing – review & editing. RB: Methodology, Supervision, Writing – review & editing. AMA: Investigation, Methodology, Writing – original draft. SuAA: Formal Analysis, Methodology, Writing – review & editing. RK: Methodology, Software, Writing – original draft. BA: Investigation, Methodology, Writing – original draft. EA: Methodology, Resources, Writing – review & editing. AAA: Data curation, Methodology, Writing – review & editing. SSA: Methodology, Supervision, Writing – original draft. SaAA: Investigation, Methodology, Writing – original draft. DA: Methodology, Project administration, Writing – review & editing. FA: Methodology, Software, Writing – original draft. AB: Investigation, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1626489/full#supplementary-material>

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