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Characterization and Evaluation of Tobacco Stem Waste Liquid Smoke as an Eco-Friendly Tofu Bio-preservation

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Abstract. Tofu preservation often uses unsafe chemicals, especially formaldehyde, that trigger health problems in humans. Therefore, it is necessary to develop preservation methods that are both effective and safe for human health. Liquid smoke derived from tobacco stem waste is well known to contain antioxidant and antimicrobial properties that may be suitable for use as a preservative compound for tofu. This study aims to evaluated the potential of tobacco stem liquid smoke as a natural preservative by analyzing its chemical profile, antimicrobial efficacy, and impact on tofu characteristics. Tofu samples were soaked in 0%, 5%, and 15% liquid smoke solutions and stored at room temperature for 24 hours. GC-MS analysis confirmed the presence of key compounds: phenolics (51.21%), fatty acids (30.82%), and ketones (17.96%). The treatment significantly improved preservation metrics, reducing the microbial load by up to 81% and lowering the pH to 5.1. The moisture content was reduced while protein levels were proportionally maintained. The 15% concentration provided the highest antimicrobial effect, enhanced aroma, and imparted a distinctive smoky flavor, yet the treatment was preferred by panelists for its better preservation of original color and soft texture. These findings highlight the potential of tobacco stem liquid smoke as a natural, low-cost preservative for tofu, promoting the valorization of biomass waste into value-added products.

Keywords: bio-preservative; liquid smoke; tobacco stem; tofu preservation

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INTRODUCTION

Tofu is soybean-based product that popular in Indonesia due to its affordability, nutritious, and health benefits (Faisal et al., 2016). However, its high protein and moisture content make it highly perishable, leading to rapid spoilage characterized by an ammonia-like odor, bitter taste, and a slimy texture, making it unpleasant for consumption (Cai et al., 2021). This spoilage reduces its marketability, as it significantly affects its quality and raises health concerns (Du et al., 2023). Therefore, it is important to apply preservation methods that can effectively extend the shelf life of tofu while maintaining its quality and nutritional value.Klik atau ketuk di sini untuk memasukkan teks.

In common practice, tofu preservation often uses chemical preservatives, including boric acid and formaldehyde, which are associated with serious health risks, such as an increased incidence of cancer (Al-Maqtari et al., 2023; Tuesta-Chavez et al., 2022; Lohita & Srijaya, 2024). Meanwhile, traditional tofu preservation is conducted using smoking techniques, which result in a black color, a hard texture, and a bitter taste due to exposure to soot from burning. Furthermore, the interaction between the proteins and fats in tofu and the carbon ash from burning can form carcinogenic compounds, such as nitrosamines, heterocyclic aromatic amines (HAAs) (Yang et al., 2019), and polycyclic aromatic hydrocarbons (PAHs) (Iko Afé et al., 2020). A modification of smoking-based preservation is conducted through the smoke condensation process to remove soot and minimize the formation of carcinogenic compounds. This process yields a smoke condensate, commonly known as liquid smoke, which has been widely applied as a safer alternative food preservative Klik atau ketuk di sini untuk memasukkan teks.(Xin et al., 2021; Desvita et al., 2023;Yulistiani et al., 2020).

Liquid smoke is commonly produced from various materials, such as coconut shells (Gani et al., 2024), corn cobs (Aladin et al., 2018), rice husks (Kim et al., 2011), and palm kernel shells (Faisal & Gani, 2018). Besides that, liquid smoke is primarily used for the preserving meat products such as sausages (Dedy et al., 2018), filleted ish (Saloko et al., 2014), and meatballs (Desvita et al., 2020). Application of liquid smoke as tofu preservative remains underexplored, particularly considering the optimal selection of biomass feedstock and processing conditions needed to produce a tofu-compatible condensate. Recent studies show that liquid smoke contains high antimicrobial phenolic and carbonyl compounds, supporting its application as a preservative for rapid perishable foods like tofu (Pisoschi et al., 2018). Moreover, another study also found that liquid smoke enhances sensory qualities, improving taste, imparting a vibrant color, and adding a smoky aroma, which increases consumer preference (Huang et al., 2023).

Liquid smoke is easily produced using an abundance of locally sourced biomass, such as tobacco stems. As we know, Indonesia is one of the world's largest tobacco producers, with production reaching 238,800 tons dry weight in 2023 (Badan Pusat Statistik, 2023). However, farmers and industries typically utilize only the leaves, leaving the stems unused and discarded as waste (Chen et al., 2024). The high content of lignin, cellulose, and hemicellulose in tobacco stems makes them suitable for use as a raw material in liquid smoke production through the pyrolysis process, which serves as a food preservative and helps address waste problems (Tian et al., 2023;Bai et al., 2022). Hence, this study aims to characterize the chemical profile of tobacco stem-derived liquid smoke, evaluate its antimicrobial activity, and assess its effects on the proximate composition and sensory properties of tofu. The findings are expected to contribute to advances in food biotechnology, offering practical benefits by reducing tobacco stem waste and increasing its economic value through the production of liquid smoke.

METHODS

Liquid Smoke Production

Five kilograms of tobacco stems were cut into small pieces and left to dry in the sunlight for approximately three days until fully dry. The dried tobacco stem was then placed into a pyrolysis chamber and ignited at 300°C in an oxygen-free environment, following the modified method of Faisal et al. (2020). The collected smoke condensate was then stored at room temperature for 24 hours to allow suspended solids to precipitate, resulting in a clarified solution. The liquid was then purified through sequential distillation at 130°C, followed by a second step distillation at 115°C. The resulting brown liquid smoke was then filtered through zeolite, activated charcoal coated with a mesh filter, and a paper filter before exiting the purification apparatus. The final liquid smoke product, characterized by a clear, vibrant brown color, a mild acidic taste, and a strong smoky aroma, was then stored in sterilized glass bottles.

Liquid Smoke and Tofu Preparation

Liquid smoke derived from tobacco stems was diluted to 5% and 15% concentrations using fresh, clean water to prepare the preservative solutions. Fresh tofu was cut into 0.5 g pieces and soaked in 0% (control), 5%, and 15% liquid smoke solutions for 15 minutes, then placed in sterile containers and stored at room temperature (±25 °C) for 24 hours.

Chemical Characterization of Liquid Smoke (GC-MS Analysis)

Liquid smoke samples were analyzed using a Shimadzu GCMS-QP2010 SE system. The GC was equipped with a capillary column, and the oven temperature was programmed to run from 50°C to 300°C. Compounds were identified by comparing retention times and mass spectra with those in the NIST spectral library, as described by Maskuri et al. (2025).

Proximate Analysis of Tofu

The analysis began with determining the moisture content using the gravimetric (oven-drying) method based on SNI 01-2891-1992. Approximately 0.5 grams of tofu sample were weighed into a porcelain crucible with a known constant weight. The crucible and its contents were then dried in an oven at 105°C for about 3 hours until a constant weight was achieved, confirmed by periodic weighing after cooling in a desiccator. The moisture content was calculated using the following formula:

$$Moisture\ Content(\%) = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight}\ x\ 100$$

The total protein content was then determined using the Kjeldahl method, as outlined in SNI 01-2891-1992. The procedure began with the digestion step, in which 0.5 grams of tofu sample were placed into a Kjeldahl flask. Then, 2 grams of selenium mixture were added as a catalyst, along with 25 ml of concentrated sulfuric acid (H_2SO_4). The mixture was heated at 420°C for about 2 hours. After cooling, the digested solution was diluted with distilled water and quantitatively transferred into a 100 ml volumetric flask, then filled up to the calibration mark. From this solution, 5 ml were taken for the distillation step using a distillation unit. Afterward, 5 ml of 30% NaOH solution and two drops of bromocresol green indicator were added. The resulting ammonia vapor was collected in an Erlenmeyer flask containing 10 ml of 2% boric acid (H_3BO_3) solution mixed with an indicator. The collected solution was then titrated with 0.01 N standard HCl. Protein content was determined from the total nitrogen obtained in the titration, using a conversion factor of 6.25, which represents the average nitrogen proportion in tofu protein, as shown in the formula below:

$$Protein\ Content\ \% = Total\ Nitrogen\ \%\ x\ 6.25$$

Microbial Analysis (Total Plate Count Test)

Ten grams of tofu sample were weighed, diluted, and homogenized in 90 mL of sterile distilled water. Serial dilutions up to 10^{-6} were plated onto nutrient agar and incubated at 37°C for 24 hours, following the procedure of Fathihatunnisa et al. (2025). Colony forming units (CFU) were counted and calculated as follows:

$$Total\ Plate\ Count = \frac{Number\ of\ Colonies\ x\ Dilution\ Factor}{Volume\ of\ Sample\ Plated\ (mL)}$$

Results were compared to the Indonesian National Standard for food safety (SNI 7388:2009).

pH Measurement

Ten milliliters of diluted tofu sample were mixed with 10 mL of distilled water and stirred for 30 seconds. The pH was then measured using a calibrated Mettler Toledo digital pH meter according to the manufacture's standard procedure.

Organoleptic Test

The organoleptic test was conducted to evaluate sensory aspects, including aroma, taste, texture, and color, based on the hedonic test, hedonic quality test, and favorability ranking following Hendrawati et al. (2021). This study involved 25 panelists (male and female students aged 20-25 years) who were briefed on the evaluation criteria and procedure. Panelists rated the sensory attributes (color, aroma, texture, and taste) using a five-point hedonic Likert scale (1 = least preferred; 5 = most preferred) to obtain both overall hedonic acceptance and attribute-specific hedonic scores (Table 1). Subsequently, each panelist ranked the tofu samples from most to least preferred based on their judgments of these attributes.

RESULTS AND DISCUSSION

Chemical Composition of Tobacco Stem Liquid Smoke

The chemical composition of liquid smoke from tobacco stems contains significant preserving compounds, including phenolic compounds, fatty acids, and ketones. All the compounds identified through GC-MS analysis are presented in Table 2.

Table 1. Combined scale for hedonic and hedonic quality tests of tofu attributes

Scale	Hedonic Test	Hedonic Quality Test			
		Aroma	Taste	Texture	Color
1	Strongly dislike	Very unpleasant smell	Very bland	Very soft	Pale white
2	Dislike	Unpleasant smell	Bland	Soft	Off-white
3	Neutral	Slight smoky smell	Slightly savory	Slightly soft	Bright yellowish
4	Like	Distinct smoky smell	Savory	Slightly chewy	Dark yellowish
5	Strongly like	Strong smoky aroma	Very savory	Very chewy	Brownish

Table 2. Chemical composition of tobacco stem liquid smoke identified by GC-MS

Group	Name	R. Time	Area	Percentage	Height
Phenolic Compounds	Phenol	6.158	3124016	34.92	200199
	Phenol, 3-methyl-	7.535	226966	2.54	24345
	Phenol, 2-methoxy-	7.734	623678	6.97	66874
	Phenol, 4-methyl-	8.043	304931	3.41	27024
	Phenol, 2-methoxy-4-methyl-	10.104	301789	3.37	33361
Total Percentage				**51.21**	
Fatty Acids and Esters	Hexadecanoic acid, methyl ester	27.668	343451	3.84	64968
	n-Hexadecanoic acid	29.125	601210	6.72	56543
	9-Octadecenoic acid (Z)-, methyl ester	31.189	544287	6.08	96681
	Octadecanoic acid, methyl ester	31.662	179235	2.00	33291
	Pentadecanoic acid	33.000	407404	4.55	37714
	9-Octadecenoic acid (Z)-, methyl ester	31.302	322076	3.60	55344
	9-Octadecenoic acid (Z)-, 9-hexadecenyl ester, (Z)-	37.150	185336	2.07	8263
	9-Octadecenal, (Z)-	37.922	175730	1.96	19446
Total Percentage				**30.82**	
Ketones	Cyclopentadecanone, 2-hydroxy-	32.696	1521841	17.01	78595
	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	6.876	84673	0.95	23326
Total Percentage				**17.96**	

Based on the GC-MS analysis, the abundance contents in liquid smoke are identified as phenolic compounds (51.21%), fatty acids (30.82%), and ketones (17.96%). The presence of these compounds is a direct result of the thermal degradation, or pyrolysis, of the biomass. These are the main degradation products derived from the lignocellulosic materials (cellulose, hemicellulose, and lignin), with the high phenolic content specifically originating from the decomposition of lignin. These findings are consistent with other research involving the production of liquid smoke from biomass materials such as coconut and palm kernel shells (Nithin et al., 2020).

Recent studies confirm that derivatives of phenolic compounds show strong antimicrobial activity against both Gram-positive and Gram-negative bacteria by denaturing essential structural proteins, disrupting cell wall formation, and increasing membrane permeability. This effect is particularly pronounced in Gram-positive bacteria because their cell structure lacks the outer membrane, making them highly susceptible to phenol compounds (Zhang et al., 2021). Conversely, Gram-negative bacteria are also affected, but to a lesser degree, as they possess a protective outer membrane, which makes them inherently less susceptible to antimicrobial agents (Stroppel et al., 2023; Wesseling & Martin, 2022) Furthermore, the strong antioxidant capacity of these phenolic compounds slow lipid oxidation, the primary cause of off-flavors and rancidity in lipid rich foods like tofu, thereby helping to extend food shelf life by maintaining sensory quality (Zhang et al., 2021). Fatty acids also contribute significantly to the overall preservation mechanism. Specifically, palmitic acid damages microbial membranes by inserting into the lipid bilayer, which disrupts the tight packing of membrane lipids and ultimately leads to cell leakage and death (Lima et al., 2019). Similarly, oleic acid inhibits pathogen growth by interfering with cell signaling and metabolic pathways essential for replication and survival (Casillas-Vargas et al., 2021; Todorov et al., 2019). Ketones, meanwhile, supplement this action by potentially hindering bacterial growth. This occurs primarily by altering membrane shape and inactivating essential enzymes (Lourenço et al., 2019; Lingbeck et al., 2014). These combined compounds ensure the broad-spectrum antimicrobial and antioxidant defense of the liquid smoke, supporting its effectiveness as a natural preservative in the food industry.

Effect of Tobacco Stem Liquid Smoke on Tofu's Moisture and Protein Content

Analysis results indicate that soaking tofu in tobacco stem liquid smoke for 24 hours was effective in both increasing the protein content and reducing the moisture level. Detailed data for the

0%, 5%, and 15% liquid smoke samples are presented in Table 3.

Soaking tofu in 5% and 15% liquid smoke resulted in a reduction of its moisture content by approximately 2% and 2.6%, respectively. This reduction is primarily caused by two factors: evaporation and the resulting concentration of soluble compounds in the liquid smoke. The high concentration of these compounds creates a hypertonic environment during soaking, effectively drawing water out of the tofu mass. Similar patterns were observed by Bora et al. (2022) and Wally et al. (2015), who found that higher smoke concentrations generally led to greater moisture loss. Andiana et al. (2019) also reported that increasing the amount of smoke components can limit water retention, making the soaking process more dehydrating. Thus, the data clearly shows that higher liquid smoke concentrations lead to a more substantial moisture reduction.

Table 3. Moisture and protein content of tofu soaked in tobacco stem liquid smoke

Liquid Smoke Concentrations (%)	Duration of Storage (hours)	Protein Content (%)	Moisture Content (%)
0		7.83	85
5	24	8.84	83
15		8.10	82.4

The proportion of protein appears higher with less water in the tofu, not because new protein forms, but due to a reduction in the overall water mass. This phenomenon is known as the concentration effect, which fundamentally explains the increase in protein measured compared to the control (0%). Specifically, the 5% concentration yielded the highest proportional increase (8.84%), while the protein at 15% (8.10%) was slightly lower, possibly due to minor protein degradation at the highest concentration, although both treated samples remained above the control value (7.83%). In this study, a 2.6% drop in moisture corresponded to roughly a 1.01% increase in measured protein. Budi et al. (2021) reported a similar trend in which protein levels seemed to rise as water content fell. Syafarina et al. (2014) stated that certain compounds in liquid smoke might also help bind water, which could influence protein measurements as moisture is lost. Taken together, these findings suggest that tobacco stem liquid smoke can lower moisture while maintaining protein levels, supporting its potential role as a natural additive for better nutritional retention, product quality, and shelf life.

Microbial Efficacy of Tobacco Stem Liquid Smoke

The treatment of tofu with tobacco stem liquid smoke demonstrated a clear antimicrobial effect after 24 hours of storage, significantly reducing the Total Plate Count (TPC) compared to the control. The specific TPC values for the 0%, 5%, and 15% treatments are detailed in Table 4.

Table 4. Total plate count (TPC) of tofu soaked in tobacco stem liquid smoke

Liquid Smoke Concentrations (%)	Duration of Storage (hours)	TPC (CFU)
0		5.8×10^2
5	24	3.8×10^2
15		1.1×10^2

All liquid smoke-treated tofu samples remained well below the maximum permissible microbial limit of 5×10^4 CFU/g, as established by the Indonesian National Standard (SNI 7388:2009), indicating that the treatment effectively controls microbial levels. Moreover, the reduction in microbial counts observed with increasing concentrations of tobacco stem liquid smoke indicates a dose-dependent antimicrobial effect. The 15% treatment led to an approximate 81% reduction in microbial load compared to the control, highlighting a clear increase in antimicrobial activity with higher concentrations. This increased efficacy is primarily attributed to the synergistic action of phenolic compounds, fatty acids, and ketones, which disrupt bacterial membranes and inhibit key metabolic processes. These results underscore the effectiveness of tobacco stem liquid smoke in suppressing microbial growth, contributing to the microbial stability of tofu during room-temperature storage, which consistent with previous findings by Desvita et al. (2020, 2023) and Faisal and Gani (2018), further emphasize the preservative role of liquid smoke in maintaining food quality and controlling microbial growth.

Tofu pH Test

The use of tobacco stem liquid smoke effectively lowered the pH value of tofu after 24 hours of storage at room temperature, indicating an improved preservation effect. The specific pH measurements for the 0% (control), 5%, and 15% treatments are presented in Table 5.

Table 5. pH levels of tofu soaked in tobacco stem liquid smoke

Liquid Smoke Concentrations (%)	Duration of Storage (hours)	pH Levels
0		5.5±0.03
5	24	5.2±0.07
15		5.1±0.03

Fresh tofu typically has a pH ranging from 6.0 to 7.0, an optimal range that supports microbial growth (Ali et al., 2021; Liu et al., 2013). This highlights the importance of pH as a crucial indicator of food freshness, since lower pH levels inhibit the growth of pathogenic microorganisms by disrupting bacterial enzyme activity, ionization of cellular compounds, and the transport of nutrients and toxins (Yudhistira et al., 2016). The acidity of the tofu samples decreased from 5.5 in the control to 5.2 with 5% liquid smoke treatment and further to 5.1 with 15% application, although the reduction between these two concentrations was not significantly distinct (Table 5). This acidification, attributed to the phenolic acids present in the liquid smoke, create an environment less favorable for microbial growth (Saloko et al., 2014) and indicates the potential of liquid smoke to naturally enhance the microbial safety of tofu through pH modulation.

Organoleptic Assessment of Tofu Preserved with Tobacco Stem Liquid Smoke

The organoleptic assessment indicated a strong overall consumer preference for the 5% liquid smoke treatment, highlighting a more balanced sensory profile compared to the 15% concentration. The specific hedonic and hedonic quality scores are detailed in Table 6, and overall favorability is presented in Figure 2.

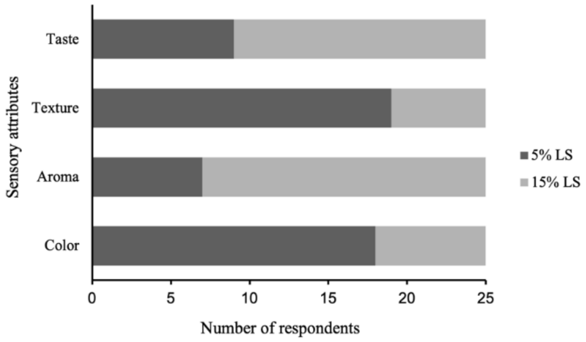

Figure 1. Favorability Ranking Test

Table 6. Organoleptic test of tofu soaked in tobacco stem liquid smoke

Sensory attributes	Liquid Smoke 5%	Liquid Smoke 15%
Hedonic Test		
Color	4.64±0.49	4.2±0.76
Aroma	3.92±0.99	4.4±0.91
Texture	4.32±1.07	3.92±0.72
Taste	4.48±0.51	4.28±0.54
Hedonic Quality Test		
Color	1.36±0.49	2.04±1.14
Aroma	3.32 ±0.69	3.8 ±1.25
Texture	3.64±0.71	3.12±1.09
Taste	2.24±0.66	2.44±0.82

1. Color

Color is critical, as visual appearance greatly influences consumer preference and often indicates the tofu's freshness and the chemical processes that have occurred (Faisal & Gani, 2018). The 5% liquid smoke sample scored highest in hedonic appeal (4.64), suggesting panellists preferred its visual appearance significantly more than the 15% sample (4.2). this preference carried over to the hedonic quality test: the 5% sample was rated closest to pale white (1.36), while the 15% sample was rated closer to off-white (2.04). The darker color observed at higher concentrations is directly linked to the naturally deep hue of concentrated liquid smoke, which contains high levels of phenols, ketones, and organic acids. During soaking, the diffusion of these compounds into the tofu produces a more noticeable yellow or brown tint. In the favorability ranking test, the 5% sample confirmed this preference, as its cleaner appearance more closely resembled fresh, untreated tofu.

2. Aroma

Aroma is an important quality attribute, as it relates directly to tofu's freshness and product quality (Ginayati et al., 2015; Aryanti et al., 2016). In the hedonic test, the 15% liquid smoke treatment obtained a higher aroma score (4.4) compared to the 5% treatment (3.92), clearly indicating a preference among panelists for a stronger smoky profile. This outcome was consistent in the hedonic quality test, where the 15% sample scored (3.8), surpassing the 5% sample score (3.32). The favorability ranking showed a similar pattern, which may be related to the higher concentrations of volatile compounds such as ketones and phenols in the 15% treatment. These compounds can diffuse more effectively into the tofu, producing sweet, smoky, and slightly sharp notes that are more noticeable at higher concentrations.

3. Texture

Texture played a major role in how panelists judged tofu quality. The 5% liquid smoke treatment achieved the highest score between the two concentrations tested. This tofu achieved a hedonic score of (4.32), while the 15% sample scored (3.92). Results from the hedonic quality test support these findings, as the 5% sample scored higher (3.64) than the 15% sample (3.12), indicating that the lower concentration produced a softer texture, while the higher concentration resulted in a denser, slightly chewier texture. The softer texture in the 5% sample may be due to less protein tightening during soaking, allowing more moisture retention. In contrast, the higher phenolic and acidic content in the 15% liquid smoke likely caused greater water loss and tighter protein binding, producing a denser, slightly tougher bite. The favorability ranking affirmed that panelists preferred the tofu preserved in 5% liquid smoke, likely because its softness aligns with the common perception of fresh tofu and offers less mouthfeel resistance during eating, resulting in a pleasant sensation.

4. Taste

Taste perception is crucial for consumer satisfaction, particularly in tofu, where taste differences are highly noticeable. In hedonic taste tests, the 5% was slightly preferred (4.48) over the 15% sample (4.28), indicating general acceptance for both concentrations. However, the hedonic quality evaluation revealed a bland taste characteristic at both liquid smoke concentration levels, with the 5% sample scored (2.24) and the 15% sample score (2.44). this outcome suggests that neither liquid smoke concentration significantly altered the fundamental taste profile of the tofu. Panelists typically described the 5% sample as having a cleaner, fresh-tofu flavor, while the 15% sample carried subtle smoky and slightly acidic notes from the higher concentration of liquid smoke compounds. Interestingly, despite the low scores in the hedonic test, the favorability rankings tended to favor the 15% liquid smoke concentration. This effect may be attributed to the stronger smoke aroma and enhanced taste perception resulting from the interaction between the olfactory and gustatory senses. Furthermore, the 15% tofu's higher carbonyl content may provide a mild umami flavor along with gentle acidity, ultimately leading to the 15% concertation being ranked higher due to its richer flavor complexity, despite the inherent blandness.

This research highlights significant practical implications for sustainable food preservation. The use of discarded tobacco stems demonstrates its potential as an accessible and low-cost raw material for liquid smoke production, offering a viable solution to agricultural waste management. Furthermore, the effectiveness demonstrated by the antimicrobial and preservative effects provides evidence for a safer, natural alternative to harmful chemical preservatives. The integration of chemical, microbial, and sensory data offers a comprehensive framework for the practical application of tobacco stem liquid smoke. This approach suggests a cost-effective preservation option that is particularly beneficial for small tofu producers or households without specialized refrigeration, enhancing food safety while utilizing agricultural by-products.

CONCLUSION

This research confirmed the efficacy of liquid smoke derived from tobacco stem waste as preservative. Chemical analysis revealed the presence of antimicrobial compounds, including phenols and fatty acids, which effectively reduced the microbial load, decreased pH and moisture content, and helped maintain protein content in tofu. Sensory evaluations indicated a panelists preference for the 5% concentration due to its balanced sensory profile, despite the 15% concentration yielding the highest antimicrobial effect. These findings affirm the potential of tobacco stem liquid smoke as a safe and eco-friendly preservative. Future research should explore the long-term storage effects and applications in other perishable food and optimize the pyrolysis and purification process to enhance overall efficacy and safety.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. Authors 1,2, and 3 conceived and designed the study, conducted the data analysis, and led the manuscript writing. Author 4 and 6 were responsible for data collection, contributed to data interpretation, and critically reviewed the manuscript for important intellectual content. Author 5 assisted in the literature review, supported data visualization and provided substantial feedback during the manuscript drafting process. Author 7 conducted a comprehensive critical revision of the entire manuscript to enhance clarity, coherence, and scientific rigor. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. Before participation, each subject was provided with a detailed explanation of the study's objectives, procedures, potential risks, and benefits. All participants voluntarily agreed to participate and signed a written informed consent form.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that artificial intelligence (AI) tools, including ChatGPT and Grammarly, were used solely for language editing and improvement. No AI-based technologies were employed in the generation of ideas, data collection, analysis, or interpretation of the research. All research activities and manuscript preparation were performed entirely by the authors.

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