AFSSAAE

Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering 2024, 7(2), 194-202
ISSN 2622-5921

ORIGINAL RESEARCH Open Access

Chemical composition and antibacterial activity of coconut-shell liquid smoke to maintain the texture of fresh meat

Susiana Purwantisari[1], Siti Nur Jannah[1], Dzakiyya Nabilla Fikri[1], Sukma Aulia Wulandari[1], Eflita Yohana[2], Budi Setiyana[2], Anindya Ardiansari[4], Daffa Ikhlasul Amal[3] and Hermawan Dwi Ariyanto[3*]

[1] Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia
[2] Department of Mechanical Engineering, Faculty of Engineering, Universitas Diponegoro, Semarang, Indonesia
[3] Department of Industrial Technology, Vocational College, Universitas Diponegoro, Semarang, Indonesia
[4] Department of Management, Faculty of Economics, Universitas Negeri Semarang, Semarang, Indonesia

KEYWORDS

Antibacterial
Chicken Meat
Food Preservative
Liquid Smoke
Texture

ABSTRACT

This research focused on the evaluation of raw broiler chicken meat texture and pathogenic antibacterial effect under the treatment of grade 2 coconut-shell liquid smoke (CSLS) as a food preservative. Compound identification of grade 2 CSLS was conducted using the GC-MS method. The main chemical compounds of CSLS were polyunsaturated fatty acid derivates, fatty acid and phenol. The evaluation of texture was conducted on raw broiler chicken meat under the treatment of grade 2 CSLS during storage at 25 ℃ for 7 days. The effect of antibacterial activity of grade 2 CSLS with different concentration (i.e., 5%, 25%, 50%, and 75%) were tested using Gram-positive bacteria on several pathogenic bacteria such as *L. monocytogenes*, *S. aureus*, *P. Aeruginosa*, and *E. coli*. The results indicated that grade 2 CSLS influenced the inhibited zone of *L. monocytogenes*, *S. aureus*, *P. Aeruginosa*, and *E. coli* respectively with linear correlation. The optimal concentration of grade 2 CSLS resulted in a concentration of 50%, which was the most optimal against *L. monocytogenes*, *S. aureus*, *P. Aeruginosa*, and *E. coli* as pathogenic bacteria strains.

Introduction

Broiler chicken meat is one of the most consumable meats in Indonesia. This meat, which has been consumed everyday as Indonesia's main dish, has expanded in consumption in line with the country's population expansion (Wahyono and Utami, 2018). However, raw broiler chicken meat is one of most perishable meat due to damage caused by the microbiological and chemical process. The damage caused by microbiological process can be originated from pathogenic bacteria contamination such as *E. coli*, *S. aureus*, *P. Aeruginosa*, and *L. Monocytogenes*, resulting in changes in physical properties and disease while consumed (Lopes et al., 2022; Silva et al., 2017). Furthermore, chemical processes such as oxidation, resulting in lipid peroxidation and protein oxidation, which can lead to meat texture degradation (Jiang and Xiong, 2016). The development of methods to maintain the raw broiler chicken meat's quality while being disseminated is necessary, given the increase in demand for it as a raw food material.

Liquid smoke as a food preservative has gained interest in recent years. Liquid smoke is a product of pyrolysis process of natural wood and biomass compounds. Pyrolysis at 200–500 ℃ may generate smoke to be distilled, produced liquid smoke containing phenol and carbonyl compounds (Ali and Al Fiqri, 2020). Phenol and carbonyl compounds in distilled liquid smoke are known to perform antibacterial effects when applied to food material to prevent microbiological damage. The mechanism is that to perform fluidal leak at cytoplasmic membranes of bacteria whether carbonyl compounds perform antibacterial effect by enzyme inactivation on cytoplasm and cytoplasmic membrane that caused growth inhibition of bacteria cell (Lingbeck et al., 2014). Carbonyl compounds in liquid smoke consist of fatty acids, aldehydes, ketones, and derivates, which are known can prevent chemical damage on raw meat. The mechanism of carbonyl compounds

*Corresponding author
E-mail address: hd.ariyanto@live.undip.ac.id

Received on 5 September 2023, Revised on 23 June 2024, Accepted on 30 June 2024

to prevent oxidation damage can be performed through an antioxidation mechanism between carbonyl and radical oxygen (Abustam et al., 2016; Jiang and Xiong, 2016). The antioxidation effect of the carbonyl compound in liquid smoke can imply to the texture improvement on raw meat. The mechanism of carbonyl compound in texture improvement on raw meat can be performed through myofibrils fiber loose on meat tissue caused by interaction between carbonyl compound and polypeptide that resulted in the increasing of meat tenderness (Abustam et al., 2016; Tiven et al., 2021).

According to the previous study on the application of liquid smoke as antibacterial and antioxidant agent, liquid smoke has potential to be used as food preservative (Desvita et al., 2023). Coconut shell liquid smoke (CSLS) is one of the applicable materials that can be used for food preservatives due to its abundance, economic-lower and give anti-toxicity properties that is suitable to be applied on food material (Keryanti et al., 2020; Surboyo et al., 2021). Coconut shell liquid based on previous research gives carbonyl compounds at 57.70% and phenol compounds at 24.55% from redistilled CSLS (Saloko et al., 2016). Effect of texture improvement on raw meat coated with CSLS has been known to give a lower shear force value than untreated, leading to improved on raw broiler chicken meat tenderness (Abustam et al., 2016; Sari et al., 2019). Inhibition properties of CSLS toward several microbes have been known against bacteria *S. aureus, E. Coli,* and *C. albicans* yeast, resulting in the antimicrobial effects being more effective to bacteria than yeast (Kailaku et al., 2019). According to a previous study, CSLS can be potentially used as a food preservative, thus providing antibacterial and antioxidant properties that lead to texture improvement in raw meat (Desvita et al., 2020).

Considering previous research, it is essential to investigate the impact of CSLS coating on the texture of raw meat and its antibacterial characteristics. The objective of this study was to evaluate the impact of CSLS coating on the texture of raw broiler chicken meat and its antibacterial qualities. Each component included in CSLS was assessed to determine its contribution to meat texture and antibacterial effects. Component identification towards CSLS can be conducted using the GC-MS to aim percentage of phenol and carbonyl compounds in the CSLS. Thus, the effect of CSLS coating on raw broiler chicken meat texture was evaluated using texture analyzer to aim texture improvement properties based on hardness value of the meat. Evaluation of antibacterial properties was conducted by using the Kirby-Bauer method to investigate the inhibition value of pathogenic bacteria (such as *L. monocytogenes, S. aureus, P. Aeruginosa,* and *E. Coli*) and the most optimal concentration for pathogenic bacteria inhibition.

Research Methods

Materials

Grade 2 CSLS, commercially produced using a high purified pyrolysis reactor, were obtained from CV. Ngudi Makmur, Temanggung, Indonesia. Ethanol 96% was purchased from Merck Indonesia. Fresh chicken meat was obtained from traditional market in Semarang, Indonesia. The bacterial suspense of *L. monocytogenes, S. aureus, P. aeruginosa,* and *E. coli* were provided by Biotechnology Laboratory, Universitas Diponegoro. Sodium chloride 0.9%, Crystal Violet, Safranin, Lugol and Chloramphenicol were purchased from Sigma-Aldrich.

Methods

Chemical compound characterization of CSLS

Chemical composition of CSLS was identified using the gas chromatography–mass spectrometry (GC-MS) (Shimadzu GC-14B; Shimadzu Corp., Kyoto, Japan), following the method reported by Kailaku et al. (2019). Injection and detector temperatures were set at 140 and 200 °C, respectively. The 1-MCP gas sample was separated using a packed column (2.1 m×3.2mm i.d.) with PEG–20M (20% on Chromosorb W 80/100 AW mesh; Shinwa Chemical Industries, Kyoto, Japan) equipped with a flame ionization detector. The column temperature was set at 130 °C. All measurements were done in triplicate.

Effect of grade 2 CSLS on chicken meat texture

Raw broiler chicken meat was coated using pure grade 2 CSLS immersion for 7 days at 25°C following the method reported by Ardilla et al. (2021). After 7 days of storage, the untreated and treated raw broiler chicken meat were analyzed using a texture analyzer to determine the hardness value of the meat with operating condition of the probe using a cylinder 35 mm length and diameter of 6 mm, trigger of 4.5 g, deformation of 10 mm and speed of 1 mm/s resulted in the hardness value on treated and untreated raw broiler chicken meat.

Gram staining of bacteria

Identification of bacteria Gram stain was conducted following the method reported by Su et

al. (2018). the sterile object glass plate was cleaned using 96% ethanol and fixed with Bunsen burner. Each isolated bacteria was suspended using an inoculating loop aseptically to the fixed object glass. Then, the bacteria isolation was diluted by a drop of sterile distilled water to spear the suspension and fixed the preparate on object glass using Bunsen burner fire. After fixation, a drop of crystal violet solution was added to the preparate, leave for 60 sec and washed with sterile distilled water, then leave until dry. A Lugol solution was dropped and leave for a minute, then washed with sterile distilled water and leave until dry. After that, decolorization was performed by washing the preparate using 96% ethanol and then leave until dry. The preparate was observed under binocular microscope with 100× magnification.

Bacteria inhibition test

The bacteria inhibition test was conducted using the Kirby-Bauer method following the method reported by Bhat and Vira (2018). Sterile NA medium was poured into petri dish and leave until the medium became solid. All bacteria suspensions of *L. monocytogenes*, *S. aureus*, *P. aeruginosa*, and *E. coli* were isolated through each petri dish using swab cotton, then leave for 15 min. Disc paper was attached to each petri dish, then add drops each concentration of CSLS (i.e., 5%, 25%, 50% and 75%), chloramphenicol, and distilled water to each disc paper inside petri dish. All samples were incubated at 37 °C for 24 hours until inhibition zone appears. Then, the inhibition zone was measured using a caliper and the inhibition zone diameter of each sample was determined.

Results and Discussion

Chemical composition of grade 2 CSLS

Figure 1 shows the chemical composition of grade 2 CSLS. This result was found by chemical analysis using the GC-MS method. Each component was detected using a mass detector and mass spectrum in way that determines each molecule of component by fragmentation analysis on mass detector during GC-MS analysis. The resulting compounds of CSLS was shown in Table 1. Grade 2 CSLS is dominated by fatty acid and its derivates, such as 10,13-eicosadienoic acid methyl ester, which is a derivate of polyunsaturated fatty acid with an area percentage of 14.05% as the most intense component on grade 2 CSLS. The remaining are derivates of saturated fatty acids such as tetradecyl oxirane with an area percentage of 13.85%, n-hexadecanoic acid or palmitic acid with an area percentage of 7.54% and oleic acid with an area percentage of 6.55%. The other components of grade 2 CSLS classified as non-fatty acid or derivation is phenol, which has an area percentage of 8.55%.

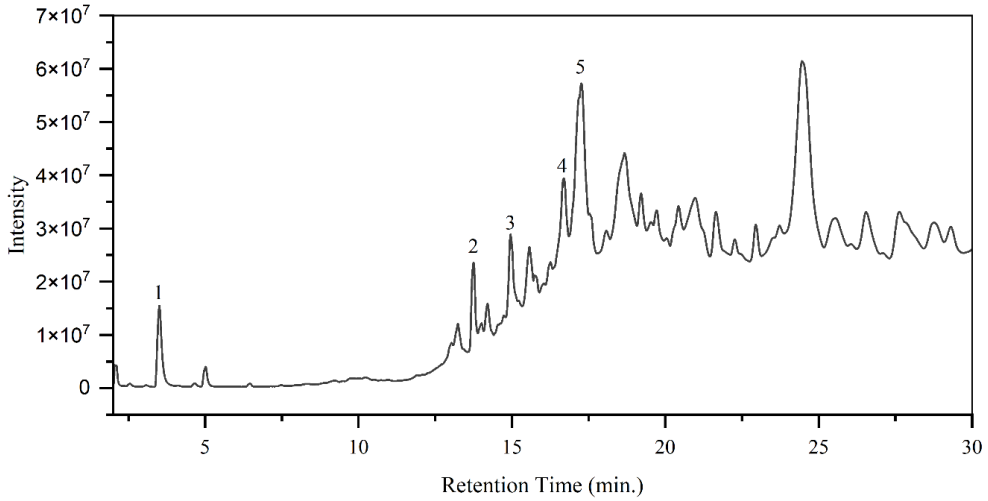

Figure 1. Chemical composition chromatogram of grade 2 CSLS from CG-MS identification

Table 1. Grade 2 CSLS compound list from CG-MS peak identification

Peak Number	Retention Time	Area	%Area	Compound
1	3.485	133888403	8.55	Phenol
2	13.734	118186970	7.54	n-hexadecanoic acid
3	14.968	102570507	6.55	Oleic Acid
4	17.273	220034440	14.05	10,13-eicosadienoic acid, methyl ester
5	17.300	216901452	13.85	tetradecyl oxirane

Table 2. Texture analysis result of grade 2 CSLS treated and untreated chicken meat

	Hardness (g)	
	Fish tail	**Fish body**
Untreated	1005.50±2.06	147.50±0.95
Treated with grade 2 CSLS	1887.00±1.42	285.75±0.77

The properties of CSLS as food preservatives depend on the activity of fatty acids and their derivates in CSLS. Liquid smoke with a higher concentration of fatty acid and its derivates has been proved in maintain raw meat properties with longer duration from 15 days until 90 days rather than phenolic based liquid smoke with a lower composition of fatty acid and its derivates (Guill et al., 2004; Sari et al., 2019). The mechanism of liquid smoke as a raw meat preservative is held by microbiological and chemical prevention. The microbiological mechanism of liquid smoke as a preservative of raw meat can be performed by preventing microbial growth, such as bacteria, that occurrs by the interaction between phenolic compound and bacteria cells. Several studies have proven antimicrobial properties towards bacteria (i.e., *E. coli* and *S. aureus*) and yeast (*Candida sp.*), but the antimicrobial effect is optimal only for bacteria in which phenolic compound concentration is proportionally linear along antimicrobial properties in CSLS (Kailaku et al., 2019). Meanwhile, the chemical reaction mechanism of liquid smoke improved meat texture and structure, which could be due to the carbonyl compounds. The compounds trigger the chemical interaction between liquid smoke, protein, and fat, hence improvingd texture and meat tissue (Guill et al., 2004; Tiven et al., 2021).

Texture analysis of chicken meat treated with grade 2 CSLS

Table 2 shows the texture analysis result of chicken meat. This result was found by a texture analyzer using a 35 mm × 6 mm cylinder probe with 4.5 g trigger force on body and tail part of CSLS-treated chicken meat and untreated chicken meat. The untreated chicken meat gives a hardness value of 1005.00±2.06 g for chicken tail part, dominated by skin tissue. A hardness value of 147.50±0.95 g for chicken body part, dominated by muscle tissue. The untreated data is the standard value used to determine hardness-increasing value in each part of grade 2 CSLS-treated chicken meat. The CSLS-treated chicken meat had a hardness value of 1887.00±1.42 g for chicken tail part (dominated by skin tissue) and 285.75±0.77 g for chicken body part (dominated by muscle tissue). The results indicate that treating chicken meat with CSLS improved hardness by 87.48% (for tail part) and 93.72% (for body part) respectively..

The findings confirmed that body part of raw broiler chicken meat had a higher increase in the hardness value after treated with CSLS. This could be due to the dominant component in CSLS, which is fatty acid (such as oleic acid and its derivates, and n-hexadecanoic and its derivates). The mechanism of fatty acid in giving texture improvement can be happened through a reaction between the carbonyl functional group in fatty acid with protein in muscle tissue of chicken meat, resulting in a firm increasing on muscle tissue that lead to increment of hardness value (Guill et al., 2004). According to Abustam et al. (2016), an increase in the tenderness of the breast part of broiler chicken meat was defined by Shear Force Value (SFV) reduction. CSLS-treated broiler chicken meat with CSLS immersion at a concentration of 10% (v/v) could decrease the SFV from 0.73 kg/cm^2 (untreated meat) to 0.63 kg/cm^2. A smaller SFV implied an increase in tenderness of the chicken broiler meat. Based on a previous research from Sasaki et al. (2017), the relation between hardness and tenderness is significantly related and defined by SFV reduction, which parallel with an increase in a hardness and tenderness simultaneously. This implies that this study aligned with the increase in hardness value. It also has a tenderness increase that needs further evaluation by defining SFV reduction in this study.

Unsaturated fatty acids are the main factor in meat texture improvement; recent research has been conducted on the relation between unsaturated fatty acid with meat texture. An Omega-3 and Omega-4 based polyunsaturated fatty acid (PUFA) existence has been known to cause the improvement of texture and sensory properties in raw chicken meat. A previous study reported that raw chicken meat injected with a PUFA ratio between omega-3 and omega-6 of 3.3:1.0 resulted in less-fishy meat, texture, tenderness, and juiciness are not significantly decreased even after storage for 25 days to40 days (Zelenka et al., 2008). The existence of PUFA in

CSLS was determined before by mass spectrum analysis on GC-MS. The results show PUFA components on grade 2 CSLS marked as peak number 4, implying the effect of hardness increase on treated raw broiler chicken meat in this study. The findings suggest that CSLS may improve the shelf-life of chicken meat. Yet, in-depth analysis is essential to evaluate the relation between texture change and meat life shelf-life.

Gram staining of bacterial suspension

Figure 2 shows the visualization result of Gram staining of bacterial suspension used in bacteria inhibition test. This result was found by the Gram staining method using crystal violet and safranin as bacterial colorizing agent followed by observation under binocular microscope with 100× magnification. The morphological characteristic of *P. aeruginosa* is shown in Figure 2A indicated by a basil-shaped cell with a red color from safranin colorizer. Therefore, *P. aeruginosa* is classified as a negative Gram strain, which has lipopolysaccharide as outer cell wall. This could imply that crystal violet discharged while decolorizing process with 96% ethanol, then binding with safranin to give a red color. The identification of *P. aeruginosa* was previously reported by Su et al. (2018) who reported similar shape and beahviour of *P. aeruginosa.* Their study found that, based on an indole test, *P. aeruginosa* could not perform sugar fermentation.

The results also demonstrate that *E. coli* also has similar shape and characteristics to P. aeruginosa, as shown in Figure 2D. A previous study by Singh and Prakash (2008), reported *E. coli* has a visualization of red basil cells, can perform lactose fermentation, and gives positive result on the indole test. The tendency of negative Gram bacteria strains to release crystal violet after decolorization is caused by bacterial cell walls that consist of lipopolysaccharide, which has higher permeability than peptidoglycan. Even some negative Gram strains bacteria have peptidoglycan, but it is limited to the inner cell walls adjoining the plasma membrane. However, positive Gram strain bacteria have higher peptidoglycan on the outer layer of cell walls, leading to cell resistance when decolorized. While negative Gram strain bacteria have higher cell wall permeability, therefore crystal violet is easily discharged during decolorization (Pasquina-Lemonche et al., 2020; Raja et al., 2006). Furthermore, due to the higher permeability of the outer cell wall layer, the activity of pathogenesis on negative Gram strain bacteria is higher than positive Gram strain bacteria. Also, negative Gram strain bacteria have higher resistance to antibacterial than positive Gram strain bacteria, implying a higher concentration of antibacterial is required to inhibit negative Gram strain bacteria (Godlewska et al., 2009; Jubeh et al., 2020).

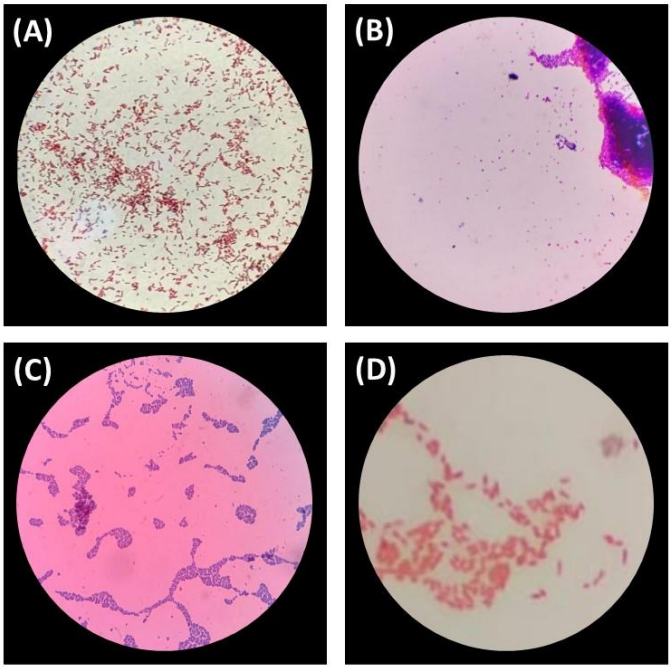

Figure 2. Gram staining result of *P. aeruginosa* (A), *L. monocytogenes* (B), *S. aureus* (C), and *E. coli* (D)

The morphological characteristic of *L. monocytogenes* is shown in Figure 2B, indicating a coccus-shaped cell with a violet color from a crystal violet colorizer. Therefore, *L. monocytogenes* is classified as a positive Gram strain bacteria with thick peptidoglycan in the outer cell wall. Such morphology enables the bacteria to maintain crystal violet even after decolorizing with 96% ethanol. Gohar et al. (2017) found that *L. monocytogenes* has a violet coccus bacteria cell visualization after a Gram staining test. Their study also reported that *L. monocytogenes* are not spore-forming bacteria and have peritrichous flagella on the cell. Another positive Gram strain bacteria identified in this study was *S. aureus* (Figure 2C), with similar morphology as found in *L. monocytogenes* Becerra et al. (2016) found that *S. aureus* isolated from biofilm tissue has an appearance of violet coccus bacteria cell. This positive Gram strain bacteria has a cell wall with a thick peptidoglycan layer, implying that no colour changes occurred during decolorization. Peptidoglycan consists of N-acetylglucosamine group that is built from disaccharides and N-acetylmuramic acid blocks bonded by cross-linking, lead to greater dependence on positive Gram strain cell wall than negative Gram strain (Pasquina-Lemonche et al., 2020). Positive or negative Gram strain bacteria have different morphological properties based on the outer cell wall layer. However, this layer could be adjusted to the environment, affecting their resistance towards several antibacterial (Rohde, 2019). Therefore, to evaluate the activity of antibacterial inhibition towards positive and negative Gram strain bacteria the Kirby-Bauer test of the inhibition zone should be carried out to determine their antibacterial performance.

Pathogenic bacteria inhibition characteristic of grade 2 CSLS

Figure 3 shows a visualization of the Kirby-Bauer test results from grade 2 CSLS with concentrations of 5%, 25%, 50%, and 75% towards *P. aeruginosa* (A), *L. monocytogenes* (C), *S. aureus* (E), *E. coli* (G), and positive-negative control (B, D, F, H). The visualization on Figure 3 shows a transparent zone around disc paper, indicating as inhibition zone on each variance of CSLS concentration and positive control of chloramphenicol. Each inhibition zone diameter of CSLS concentration variance on disc paper was measured and compared with each pathogenic bacteria in this study, as shown in Table 3. The inhibited zone diameter measurement of each pathogenic bacteria towards each concentration variance of CSLS gives a similar correlation. The inhibited zone diameter becomes higher as the concentration of CSLS gets higher. The lowest inhibited zone of grade 2 CSLS was in the concentration of 5% towards all pathogenic bacteria strains, particularly *L. monocytogenes* with an inhibited zone diameter of 0.40±0.02 mm. While, the highest inhibited zone of grade 2 CSLS was in the concentration of 75% toward all pathogenic bacteria strains, in particular . *S. aureus* with an inhibited zone diameter of 22.90±0.13 mm and a similarity percentage of 80.92% towards positive control of chloramphenicol. The relationship between the concentration of grade 2 CSLS towards inhibited zone on each bacteria strain can be evaluated from Figure 4.

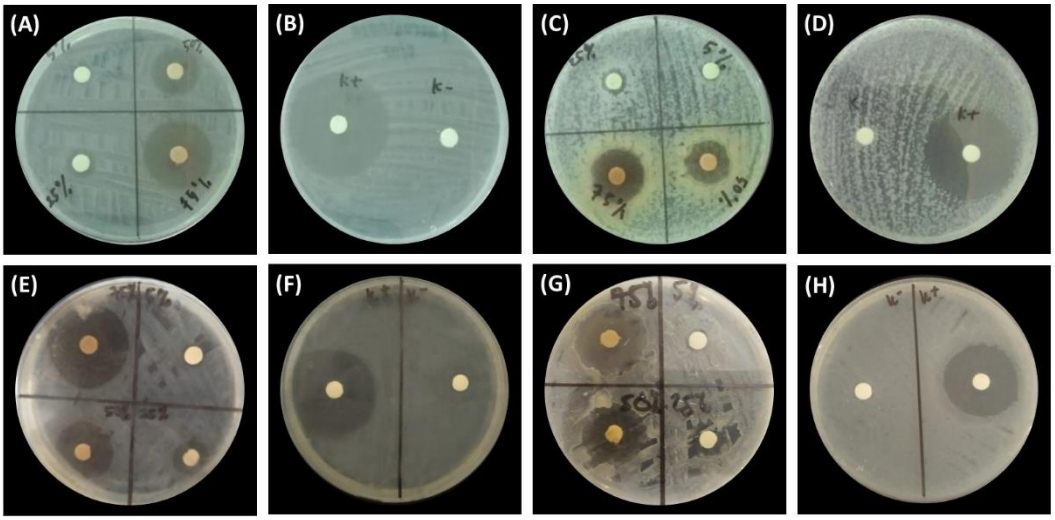

Figure 3. Kirby-Bauer inhibition test result of *P. aeruginosa* (A), *L. monocytogenes* (C), *S. aureus* (E), *E. coli* (G) and positive-negative control (B, D, F, H).

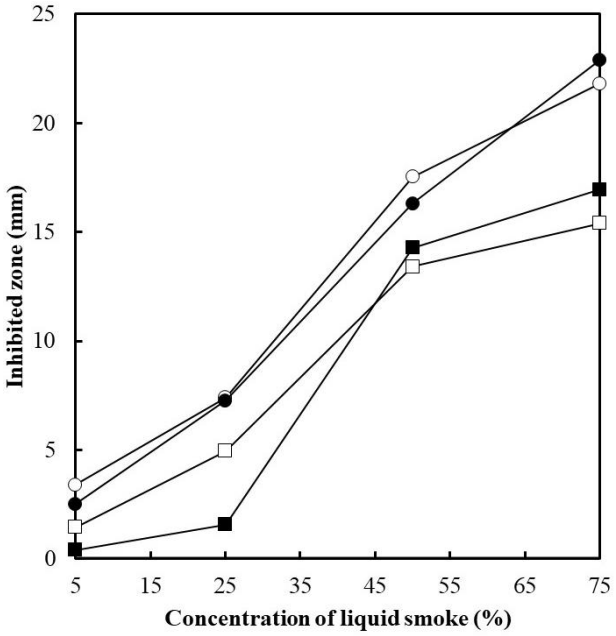

Figure 4. Correlation graph between concentration of grade 2 CSLS and inhibited zone of *S. aureus* (●), *P. aeruginosa* (○), *E. coli* (□), and *L. monocytogenes* (■).

Table 3. Kirby-Bauer inhibition zone test result of each grade 2 CSLS concentration towards each pathogenic bacteria strains

Bacteria strain	Gram-strain	Inhibited zone diameter (mm)					
		5% Liq. smoke	25% Liq. smoke	50% Liq. smoke	75% Liq. smoke	Control-positive	Control-negative
L. monocytogenes	(+)	0.40±0.02	1.57±0.41	14.27±0.11	16.95±0.03	26.80±0.19	-
S. aureus	(+)	2.52±0.31	7.27±0.04	16.30±0.23	22.90±0.13	28.30±0.32	-
P. aeruginosa	(-)	3.40±0.22	7.40±0.05	17.52±0.31	21.80±0.16	28.63±0.67	-
E. coli	(-)	1.45±0.16	4.94±0.04	13.40±0.03	15.40±0.37	24.00±0.01	-

Table 4. Slope interpretation from linear correlation between grade 2 CSLS towards each bacteria strain

Bacteria Strain	Gram Strain	Slope
L. monocytogenes	Gram-positive	26.723
S. aureus	Gram -positive	29.923
P. aeruginosa	Gram-negative	27.841
E. coli	Gram-negative	21.386

Figure 4 illustrates the linear correlation to obtain the slope value in determining the impact of increasing the CSLS concentration on the inhibited zone of all pathogenic bacteria strains. The results suggest that a significant increase in the inhibited zone was parallel to a higher slope value, indicating the resistance of bacteria strains. A lower slope gives a higher bacteria resistance to the variation of CSLS concentration. Table 4 shows that *S. aureus* has the highest slope value followed by *P. aeruginosa*, *L. monocytogenes*, and *E. coli*, respectively. However, when comparing negative and positive Gram strains, the results indicate that *L. monocytogenes has a lower slope than P. aeruginosa. This could be attributed to the a*daptive properties of *L. monocytogenes* towards antibacterial, depending on the highest motilities to another positive Gram stain bacteria (Meloni, 2015). Furthermore, other studies reported that *aeruginosa* is a negative Gram stain bacteria with lower motility than another negative Gram strain (de Sousa et al., 2021; Gilardi, 1971), leading to a bacteria strain resistance towards grade 2 CSLS. However, this study confirms that *E. coli* is the most resistant to grade 2 CSLS. *E. coli* resistance towards grade 2 CSLS relies on the morphological

properties of *E. coli* cell wall that consists of 3 layers of cell wall building from lipoprotein on the outer layer, lipopolysaccharide on the middle layer, and polypeptide R-layer on the inner layer (Lopes et al., 2022; Weidel et al., 1960). Such morphology indicates the complexity of *E. coli* cell walls, implying that *E. coli* is resistant to several antibacterial or disinfectants. Then, there should be an evaluation of the optimum concentration of antibacterial or disinfectant for *E. coli* inhibition. Figure 4 illustrates each resistance characteristic of pathogenic bacteria strains with the highest ability to inhibit *L. monocytogenes*, *S. aureus*, *P. aeruginosa,* and *E. coli* on a CSLS concentration of 75%. At the concentration of 5%, the inhibited zone towards *L. monocytogenes*, *S. aureus*, *P. aeruginosa,* and *E. coli* were still lower than that reported by Maniha et al. (2020). Their study reported that using natural preservatives of vinegar inhibits the zone towards *E. coli* at 10.02 mm, *S. aureus* at 12.02 mm, and *P. aeruginosa* at 24.95 mm. Therefore, following the Kirby-Bauer test and previous research as a reference, the optimal concentration of grade 2 CSLS to inhibit pathogenic bacteria strains (*L. monocytogenes*, *S. aureus*, *P. aeruginosa,* and *E. coli*) could be at 50%. However, further in-depth investigation is essential.

Conclusions

This study confirmed that grade 2 CSLS contains polyunsaturated fatty acid derivates, fatty acid, and phenol. Applying grade 2 CSLS on the tail and body of chicken meat, following storage of 7 days at 25°C, improved the hardness value by 87.48% and 93.72%, respectively. Different concentrations of grade 2 CSLS gave different and significant antibacterial properties towards positive Gram strain (i.e., *L. monocytogenes* and *S. aureus*) and negative Gram strain (i.e., *P. aeruginosa* and *E. coli*), as indicated with differed inhibited zone. The findings were found at a concentration of 50% in grade 2 CSLS to perform better in inhibiting pathogenic bacteria strains.

Declarations

Conflict of interests The authors declare no competing interests.

Open Access This Article is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License that allows others to use, share, adapt, distribute and reproduce the work in any medium or format with an acknowledgment to the original author(s) and the source. Publication and distribution of the work in the institutional repository or in a book are permessible as long as the author give an acknowledgment of its initial publication in this journal. To view a copy of this licence, visit https://creativecommons.org/licenses/by-sa/4.0/

References

Abustam, E., Yusuf, M., Ali, M., Said, M. I., and Yulianti, N. (2016) 'Quality of Bali beef and broiler after immersion in liquid smoke on different concentrations and storage times', *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, 10(2), pp. 69–73

Ali, F., and Al Fiqri, R. (2020) 'The simple design of pyrolysis tool for making liquid smoke from shells and rubber seeds as a food preservative', *Journal of Physics: Conference Series*, 1500(1), pp. 1-10

Ardilla, Y. N. N., Nuningtyas, Y. F., Djunaidi, I. H., Natsir, M. H., and Widodo, E. (2021) 'Encapsulation affects concentration of liquid smoke active substances and intestinal microflora of broiler', *EAS Journal of Veterinary Medical Science*, 3(3), pp. 17–20

Becerra, S. C., Roy, D. C., Sanchez, C. J., Christy, R. J., and Burmeister, D. M. (2016) 'An optimized staining technique for the detection of gram positive and gram negative bacteria within tissue', *BMC Research Notes*, 9(1), pp. 1–10

Bhat, V., and Vira, H. (2018) ' Quality Control issues in antibiotic susceptibility testing by disc diffusion technique', *Clinical Infectious Diseases*, 2(1), pp. 1-2

de Sousa, T., Hébraud, M., Dapkevicius, M. L. N. E., Maltez, L., Pereira, J. E., Capita, R., Alonso-Calleja, C., Igrejas, G., and Poeta, P. (2021) 'Genomic and metabolic characteristics of the pathogenicity in pseudomonas aeruginosa', *International Journal of Molecular Sciences*, 22, pp. 1-28

Desvita, H., Faisal, M., Mahidin, M., and Suhendrayatna, S. (2020) 'Edible coating for beef preservation from chitosan combined with liquid smoke', *International Journal of Technology*, 11(4), pp. 817–829

Desvita, H., Faisal, M., Mahidin, M., and Suhendrayatna, S. (2023) 'Natural antimicrobial properties of liquid smoke derived from cocoa pod shells in meatball preservation', *South African Journal of Chemical Engineering*, 46, pp. 106–111

Gilardi, G. L. (1971) 'Characterization of *Pseudomonas* species isolated from clinical specimens', *Applied Microbiology*, 21(3), pp. 414–419

Godlewska, R., Wiśniewska, K., Pietras, Z., and Jagusztyn-Krynicka, E. K. (2009) 'Peptidoglycan-associated lipoprotein (Pal) of gram-negative bacteria: Function, structure, role in pathogenesis and potential application in immunoprophylaxis', *FEMS Microbiology Letters*, *298*(1), pp. 1–11

Gohar, S., Abbas, G., Sajid, S., Sarfraz, M., Ali, S., Ashraf, M., Aslam, R., and Yaseen, K. (2017) 'Prevalence and antimicrobial resistance of Listeria monocytogenes isolated from raw milk and dairy products', *Matrix Science Medica*, 1(1), pp. 10–14

Guill, M. D., Martinez, O., Salmer, J., and Casas, C. (2004) 'Texture profile analysis of meat products treated with commercial liquid smoke flavourings', *Food Control*, 15, pp. 457–461

Jiang, J., and Xiong, Y. L. (2016) 'Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review', *Meat Science*, 120, pp. 107–117

Jubeh, B., Breijyeh, Z., and Karaman, R. (2020) 'Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches', *Molecules*, 25(12), pp. 1-22

Kailaku, S. I., Syakir, M., Mulyawanti, I., and Syah, A. N. A. (2019) 'Antimicrobial activity of coconut shell liquid smoke', *IOP Conference Series: Materials Science and Engineering*, 206, pp. 1-6

Keryanti, Permanasari, A. R., Yulistiani, F., Sihombing, R. P., and Wibisono, W. (2020) 'Applications of Liquid Smoke from Biomass on Food Products: A Review', *Proceedings of the International Seminar of Science and Applied Technology (ISSAT 2020)*, pp. 518–524

Lingbeck, J. M., Cordero, P., O'Bryan, C. A., Johnson, M. G., Ricke, S. C., and Crandall, P. G. (2014) 'Functionality of liquid smoke as an all-natural antimicrobial in food preservation', *Meat Science*, 97(2), pp. 197–206

Lopes, S. M., da Silva, D. C., and Tondo, E. C. (2022) 'Bactericidal effect of marinades on meats against different pathogens: a review', *Critical Reviews in Food Science and Nutrition*, 62(27), pp. 7650–7658

Maniha, S. M., Tabassum, T., Tabassum, T., Tabassum, N., and Noor, R. (2020) 'In vitro antibacterial traits of the commonly used food preservatives and spices in their crude forms', *Biomedical and Biotechnology Research Journal*, 4(1), pp. 26–30

Meloni, D. (2015) 'Presence of listeria monocytogenes in mediterranean-style dry fermented sausages', *Foods*, 4(1), pp. 34–50

Pasquina-Lemonche, L., Burns, J., Turner, R. D., Kumar, S., Tank, R., Mullin, N., Wilson, J. S., Chakrabarti, B., Bullough, P. A., Foster, S. J., and Hobbs, J. K. (2020) 'The architecture of the gram-positive bacterial cell wall', *Nature*, 582, pp. 294–297

Raja, C. E., Anbazhagan, K., and Selvam, G. S. (2006) 'Isolation and characterization of a metal-resistant *Pseudomonas Aeruginosa* strain', *World Journal of Microbiology and Biotechnology*, 22(6), pp. 577–585

Rohde, M. (2019) 'The gram-positive bacterial cell wall', *Microbiology Spectrum*, 7(3), pp. 1-9

Saloko, S., Darmadji, P., Setiaji, B., Pranoto, Y., and Widyastuti, S. (2016) 'Determination of principal volatile compounds of nanoencapsulated coconut shell-liquid smoke as a food biopreservative', *Original Research Article Journal of Advances in Food Science and Technology*, 3(3), pp. 114–118

Sari, E., Khatab, U., Burmawi, Rahman, E. D., Afriza, F., Maulidita, A., and Desti, V. (2019) 'Production of liquid smoke from the process of carbonization of durian skin biomass, coconut shell and palm shell for preservation of tilapia fish', *IOP Conference Series: Materials Science and Engineering*, 543(1), pp. 1-8

Sasaki, K., Motoyama, M., Tagawa, Y., Akama, K., Hayashi, T., Narita, T., and Chikuni, K. (2017) 'Qualitative and quantitative comparisons of texture characteristics between broiler and jidori-niku, Japanese indigenous chicken meat, assessed by a trained panel', *Journal of Poultry Science*, 54(1), pp. 87–96

Silva, F., Domingues, F. C., and Nerín, C. (2017) 'Trends in microbial control techniques for poultry products', *Critical Reviews in Food Science and Nutrition*, 58(4), pp. 591–609

Singh, P., and Prakash, A. (2008) 'Isolation of *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* from milk products sold under market conditions at agra region', *Acta Agriculture Slovenica*, *92*(1), pp. 83–88

Su, S. S., Lae, W. Z. K., and Ngwe, H. (2018) 'Isolation and identification of *Pseudomonas aeruginosa* from the clinical soil', *University of Yangon Research Journal*, 8, pp. 271–275

Surboyo, M. D. C., Mahdani, F. Y., Ayuningtas, N. F., Santosh, A. B. R., Ernawati, D. S., Mansur, D., Arundina, I., Nagoro, A. A. B., and Rahmadhany, I. P. (2021) 'The cytotoxicity, anti-inflammation, anti-nociceptive and oral ulcer healing properties of coconut shell liquid smoke', *Journal of HerbMed Pharmacology*, 10(4), pp. 459–467

Tiven, N. C., Hartati, L., and Simanjorang, T. M. (2021) 'Liquid smoke as fat protector and its effect on rumen fermentation characteristics and microbial activity', *Tropical Animal Science Journal*, 44(2), pp. 152–159

Wahyono, N. D., and Utami, M. M. D. (2018) 'A Review of the poultry meat production industry for food safety in Indonesia', *Journal of Physics: Conference Series*, 953(1), pp. 1–4

Weidel, W., Frank, H., and Martin, H. H. (1960) 'The rigid layer of the cell wall of *Escherichia coli* strain B', *Journal of General Microbiology*, 22(1), pp. 158–166

Zelenka, J., Jarošová, A., and Schneiderová, D. (2008) 'Influence of n-3 and n-6 polyunsaturated fatty acids on sensory characteristics of chicken meat', *Czech Journal of Animal Science*, 53(7), pp. 299–305