Chimica et Natura Acta
p-ISSN: 2355-0864 e-ISSN: 2541-2574
Homepage: http://jurnal.unpad.ac.id/jcena

Exploring the Synergistic Effect of Curcumin on Gelatin Films for Real-Time Pork Freshness Assessment

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DOI: https://doi.org/10.24198/cna.v13.n3.57995

Abstract: A smart film based on hydromethanol-curcumin has been developed as a freshness detector for local packaged pork. It was fabricated using solution casting method by varying the curcumin content to determine its effect on the chemical, physical, mechanical and functional characteristics of the film. Film characteristics were determined using colorimeter, TGA, FTIR, SEM and bioactivity assay. Film response was analyzed in different pH buffer solutions, different acid base media and pork samples during the storage period. Film characteristics such as antioxidant, antibacterial activity and surface color was improved by increasing curcumin concentration. On the other hand, mechanical dan physicochemical characteristics such as tensile strength, water content, swelling index and water solubility decreased along with increasing curcumin concentration. Curcumin concentration have no significant effect on the thermal stability and color brightness of the film. SEM images show that increasing curcumin concentration results in aggregation of the gelatin biopolymer matrix. Gelatin-curcumin film was sensitive to pH changes either in gas or liquid media. The properties of the proposed film have high potential to be used as a freshness detector for local packaged pork.

Keywords: film, gelatin, smoked pork, concentration, curcumin

Abstrak: *Film pintar berbasis hidrometanol-kurkumin telah dikembangkan sebagai detector kesegaran se'i babi dalam kemasan. Film difabrikasi menggunakan metode solution casting melalui variasi konsentrasi kurkumin untuk mengetahui pengaruhnya terhadap karakteristik kimia, fisik, mekanik dan karakteristik fungsional film. Karakteristik film ditentukan menggunakan Colorimeter, TGA, FTIR, SEM dan uji bioaktivitas. Respon film diuji dalam larutan buffer pH berbeda, media asam basa yang berbeda fasa dan sampel se'i babi selama masa penyimpanan. Peningkatan konsentrasi kurkumin meningkatkan karakteristik film seperti aktivitas antioksidan dan antibakteri serta visualisasi warna kuning yang semakin intens. Sebaliknya karakteristik seperti kuat tarik, kadar air, swelling indeks dan kelarutan air mengalami penurunan seiring dengan kenaikan konsentrasi kurkumin. Variasi konsentrasi film tidak berpengaruh nyata terhadap stabilitas termal dan kecerahan warna film. Hasil SEM menunjukkan bahwa peningkatan konsentrasi kurkumin mengakibatkan agregasi matriks biopolymer gelatin. Film gelatin-kurkumin yang dihasilkan sensitive terhadap perubahan pH baik dalam media gas maupun media cair. Film gelatin-kurkumin ini sangat berpotensi digunakan sebagai detector kesegaran se'i babi dalam kemasan.*

Kata kunci: *film, gelatin, se'i babi, konsentrasi, kurkumin*

INTRODUCTION

Smoked pork (se'i) is one of the traditional pork products from East Nusa Tenggara, processed by smoking the meat for a specific duration. Se'i contains water, fat, protein, minerals, carbohydrates, and vitamins (Pereira & Vicente 2013). Due to its composition, this product is prone to microbial contamination, making packaging an essential aspect of its marketing presentation. Packaging is generally done either by vacuum sealing or non-vacuum methods. Suluh (2019) stated that vacuum-sealed se'i has a longer shelf life compared to non-vacuum packaging. However, even under vacuum conditions, the physical state of se'i pork changes, becoming slimy, soft, and sour-smelling by the fourth week of storage. On the other hand, most packaging does not include expiration dates, leaving consumers unaware of the product's edibility. To ensure product freshness, consumers often have to open the packaging. Freshness assessment can then be

performed through sensory analysis, chemical experiments, and microbial population testing. Sensory analysis is considered less objective, while chemical methods (such as Total Volatile Basic Nitrogen tests) and microbial population testing are complex, time-consuming, and expensive (Biswas & Mandal 2019; Bonke *et al.* 2016).

A freshness indicator is an interactive tool embedded within smart packaging to monitor and inform consumers about the real-time condition of food by displaying specific visual signals on the packaging (Pereira *et al*. 2015). Freshness indicators can monitor changes in temperature, pH, oxygen concentration, or microbial activity in food during storage and distribution (Kuswandi 2017; Barska & Wyrwa 2017). A freshness indicator that detects pH as a parameter for assessing food freshness is known as a pH indicator. It uses color-based dyes that change in response to the pH conditions within the packaging environment (Moradi *et al*. 2019). The working principle of pH indicators is relatively simple: dye molecules react with metabolites produced by the food, leading to observable color changes (Priyadarshi *et al*. 2021).

pH indicators can be fabricated using synthetic or natural dyes. Several synthetic dyes sensitive to pH changes have been utilized in the development of freshness indicators, including methyl orange and bromocresol green (Musso *et al*. 2016) , methyl red (Kuswandi *et al*. 2014), bromophenol blue (Shukla *et al*. 2015) and bromocresol purple (Kim *et al*. 2017; Kuswandi & Nurfawaidi 2017). However, the use of synthetic dyes is unsuitable for food applications due to their toxic, carcinogenic, and non-eco-friendly properties (Zhang *et al*. 2016). Natural plant-based dyes such as curcumin are preferred because they are safer, easily prepared, and abundant. Curcumin is highly sensitive to pH changes and exhibits different color spectra under acidic and alkaline environments (Alizadeh-Sani *et al.* 2020; Balbinot-Alfaro *et al*. 2019; Rodrigues *et al*. 2021). Curcumin shows a visual color change from yellow to reddish-orange as pH increases (Liu *et al*. 2018; Priyadarshi *et al*. 2021), making it a promising candidate for freshness indicators. In addition to its ability to change color, curcumin also possesses functional characteristics as an antimicrobial and antioxidant, which can prolong product shelf life (Musso *et al*. 2017) , and it serves as a bioactive polymer that enhances the mechanical properties of films (Taghinia *et al*. 2021; Tyagi e*t al*. 2015).

Various biopolymers have been employed in the fabrication of curcumin-based indicators. The use of biopolymers such as carrageenan results in films with rough textures due to curcumin-carrageenan agglomeration, (Liu *et al*. 2018) while polysaccharides produce films with high swelling indices, leading to rapid degradation of the indicator's color (Taghinia *et al*. 2021). Gelatin is one of the most widely used biopolymers for indicator film formation due to its film-forming properties, biocompatibility, non-toxicity, availability, and biodegradability (Shahbazi 2017; Zhao *et al.* 2022). The physical, chemical, and mechanical characteristics of gelatin films are strongly influenced by the type of solvent used during the preparation of the film-forming solution. Previous researchers have used solvents such as distilled water (Abedi-Firoozjah *et al*. 2022; Rawdkuen *et al*. 2020; Sai-Ut 2021) and a mixture of distilled water and ethanol (Musso *et al*. 2019), to produce gelatin films, but the use of methanol as a solvent remains uncommon. Studies have shown that fabricating curcumin-gelatin films at different pH levels using distilled water and a 1:1 ethanol-water mixture results in films with intense curcumin color (Musso *et al*. 2017), however, the effect of curcumin concentration on film characteristics has not been thoroughly investigated. Other researchers have studied the effect of curcumin concentration ranging from 0.5-1.5% on the characteristics of gelatin-curcumin films using Sodium Dodecyl Sulfate (SDS) as an emulsifier (Roy & Rhim 2020) . The use of SDS is known to cause acute respiratory toxicity and skin irritation at concentrations of 5-10 mM (Welch *et al*. 2021), making it less safe for inclusion in the film-forming solution. Therefore, this study was conducted without the use of emulsifiers to investigate the effect of methanol-extracted curcumin concentration on the characteristics of gelatin-curcumin films and their application in detecting the freshness of packaged smoked pork.

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MATERIALS AND METHOD

Materials

The materials used include gelatin, distilled water, methanol (Merck), 37% hydrochloric acid (Merck), sodium hydroxide (Merck), glacial acetic acid (Merck), ammonia (Merck), glycerol, Whatman-41 filter paper, and pH buffers ranging from 2 to 10. The main equipment used includes a blender (Cosmos CB-282-AP), UV-Vis Spectroscopy (UV-Vis Analytic Jena, Specord 200 Plus), Fourier Transform Infrared Spectroscopy (Shimadzu Instrument Spectrum One 8400S), Scanning Electron Microscope (Zeiss EVO MA 10), thermogravimetric analysis device (Mettler Toledo, TGA/DSC1), Pyrex glassware, pH meter, petri dishes, desiccator, oven, analytical balance, hot plate, and micrometer.

Preparation and Fabrication of Gelatin-Curcumin Films

Methanol-extracted curcumin was prepared by blending 4 g of turmeric and dissolving it in 100 mL of methanol, labeled as MC (Methanol-Curcumin). The extract was filtered using a sieve followed by Whatman 41 filter paper. The absorbance of the curcumin extract was measured using a UV-Vis spectrophotometer. The curcumin stock solution was diluted to obtain concentrations of 0.4% (MC1),

0.8% (MC2), and 1.2% (MC3). The indicator films were prepared using the casting solution method (Musso *et al*. 2019). A 10% (w/v) gelatin solution (50 mL) was mixed with 1 mL of glycerol and 50 mL of each curcumin extract (MC1, MC2, MC3), and labeled as GMC1, GMC2, and GMC3. Each 10 mL of GMC solution was poured into a petri dish and dried at 60 °C for 24 hours. The dried films were stored for 48 hours at 20 °C in a desiccator and then characterized. The control film (GMC0) was prepared using the same procedure, with methanol added instead of curcumin.

Characterization of Gelatin-Curcumin Films

A Konica Minolta Chroma Meter CR-400 was used to measure the color of the films. The brightness (L*), redness (+a*) or greenness (−a*), and yellowness (+b*) or blueness (−b*) were determined using the CIE-Lab color scale. A standard white pellet with color coordinates L*standard=97.55, a*standard=0.03, and b*standard=1.73 was used to calibrate the instrument. The color of the film was evaluated on the surface of this standard pellet, and the total color difference (ΔE) was calculated using Equation 1.

Each GMC film was characterized using UV-Vis spectroscopy, SEM, FT-IR, and TGA. Other properties tested include moisture content (MC), water solubility (WS), and swelling index (SI). The moisture content (MC) was determined by weighing the sample before and after oven drying at 105°C, using the following Equation 2.

Water solubility (WS) was determined by cutting a square-shaped film (2 × 2 cm), drying it in an oven at 70°C for 24 hours to determine the initial dry weight (W_0), and then immersing the sample in 50 mL of distilled water at room temperature for 24 hours while stirring. The sample was then removed, blotted with filter paper, and weighed to determine the wet weight (W_1). The wet sample was further dried to obtain the final dry weight (W_2). The process was repeated three times, and WS and SI (%) were calculated using the following Equations 3 and 4.

The mechanical properties of the films tested include tensile strength (TS), elongation at break (EB), and elastic modulus (EM) using a Universal Testing Machine (Zwick, DO-FB0.5TS) following ASTM D 882-88. Rectangular Hstrips of film (3 × 9 cm) were cut and measured in tensile mode at a speed of 10 mm/min, a load of 500 N, and an initial grip separation of 50 mm. TS and EB were calculated using the following Equations 5 and 6.

Antibacterial and Antioxidant Activities

The antimicrobial activity was tested against E. coli and S. aureus using the broth dilution method. NaCl (1 µL) was added to test tubes followed by 1 loop of bacteria, adjusted to a turbidity corresponding to the McFarland standard of 0.1%. Nutrient broth (2.8 g dissolved in 100 mL of distilled water) was sterilized and added to test tubes containing GMC0, GMC1, GMC2, and GMC3 films. Then, 1 mL of bacterial suspension was added, and the absorbance was measured at a wavelength of 610 nm. The solution was incubated for 24 hours, and absorbance changes after incubation were recorded.

The antioxidant activity was determined using the DPPH assay. Film samples (20 × 20 mm) were placed in tubes containing 4 mL of methanol, stirred for 2 hours at 25°C. A mixture of 3 mL of the

$$\Delta E = \left[(L^*_{film} - L^*_{standar})^2 + (\alpha^*_{film} - \alpha^*_{standar})^2 + (b^*_{film} - b^*_{standar})^2 \right]^{0.5} \quad ...(1)$$

$$MC(\%) = \frac{100\ x\ (M_i - M_f)}{M_i} \quad ...(2)$$

Where: M_i = initial weight (g), M_f = final weight after drying (g)

$$WS(\%) = \frac{(W_0 - W_2)}{W_2}\ x\ 100 \quad ...(3)$$

$$SI(\%) = \frac{(W_1 - W_0)}{W_0}\ x\ 100 \quad ...(4)$$

$$TS(MPa) = \frac{F_{\max}}{A} \quad ...(5)$$

$$EB(\%) = \frac{\Delta L}{L_0}\ x\ 100 \quad ...(6)$$

Where: F_{max} = maximum force to break the film (N), A = cross-sectional area of the film (m^2), ΔL = elongation of the film at break, L_0 = initial film length (50 mm). The elastic modulus (YM) was determined by dividing the stress by the strain of the film samples.

supernatant, 1 mL of methanol, and 150 µM DPPH solution was prepared, and the absorbance was measured at 517 nm (A1). Another mixture of 3 mL of the supernatant and 1 mL of 150 µM methanol solution was measured for absorbance (A0). The DPPH radical scavenging rate was determined as Equation 7.

$$\text{LP DPPH}(\%) = \frac{A_0 - A_1}{A_0} \; x \; 100 \; ...(7)$$

Analysis of pH Indicator Color Response

Each film was exposed to liquid and gas media with varying pH levels:

i) Addition of 2 mol/L HCl or 2 mol/L NaOH solutions.

ii) Exposure of the films to acetic acid vapor ($C_2H_4O_2$, pKa ~4.8) and ammonia vapor (NH_3, pKa ~9.3).

Photographs of the films before and 30 minutes after exposure to the media were taken. Before testing the films for their response to meat freshness, the pH of the meat was analyzed during storage. A 10 g meat sample was vacuum-packed and stored at room temperature. The pH was monitored periodically (up to 3 days) by blending 10 g of meat with 90 mL of distilled water for 3 minutes and recording the pH using a pH meter. The freshness response test involved cutting the films into 2 × 2 cm pieces, placing them with 10 g of meat samples in vacuum-sealed packaging, and storing them for the test period (3 days). Changes in pH and film color were recorded and documented during testing.

RESULT AND DISSCUSION

Curcumin Extracts

The absorbance of the curcumin extract was measured using a UV-Vis spectrophotometer at wavelengths of 300–600 nm. The absorbance measurement results of the curcumin extract are shown in Figure 1. The methanol extract of curcumin exhibited a maximum absorbance at 419 nm, which is consistent with the maximum absorbance reported in previous studies (Wulandari *et al*. 2020). It has been stated that crude turmeric rhizome extract in alcohol typically absorbs at a wavelength of 425 nm (Ciuca & Racovita 2023).

Film Characteristics

Neat gelatin and gelatin-curcumin films prepared through hydroalcoholic dispersion exhibited homogeneous, thin, and flexible characteristics. The color characteristics of pure gelatin films and gelatin-curcumin films at various curcumin concentrations are shown in Table 1. Pure gelatin films appeared transparent and colorless, while films containing curcumin were also transparent but yellow.

The brightness (Hunter L value) was not significantly different between GMC0 and GMC1 films. The green component (Hunter α value) of pure gelatin films was not significantly different from GMC1 and GMC2 films, while increasing curcumin

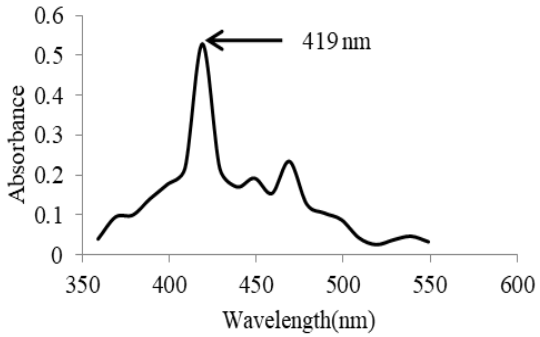


Figure 1. UV-Vis Spectra of curcumin extract

Table 1. Visual appearance of gelatin-curcumin films

Film	Visual Appearance	L	a	b	ΔE
GMC0		29.82 ± 0.91^{bc}	-1.62 ± 0.28^{a}	5.79±0.38^{c}	67.87±0.28
GMC1		28.70 ± 0.36^{c}	-1.72 ±0.09^{a}	14.84±0.25^{b}	70.10±0.11
GMC2		30.60 ± 0.23^{ab}	-1.61 ± 0.19^{a}	15.26±0.19^{b}	68.32±0.02
GMC3		31.50 ± 0.25^{a}	-2.34 ±0.17^{b}	16.04±0.2^{a}	67.43±0.03

The values are presented as mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

concentration significantly enhanced the yellow component of gelatin/curcumin films compared to pure gelatin films. The yellow color (Hunter b value) of the gelatin films was not significantly differ between GMC1 and GMC2, indicating that the yellow color depends on curcumin concentration but is not significantly affected within the concentration range used in this study. The yellow color is attributed to the yellow pigment, curcumin. The total color difference (ΔE) of the composite films was not significantly different from that of pure gelatin films (P value > 0.05), based on Bonferroni Test results.

Moisture content (MC), water solubility (WS), and swelling index (SI) are important factors influencing the stability and brittleness of the films. Low moisture and water solubility provide protection against physical activities and microbial growth (Thakur *et al*. 2016). A lower water solubility results in slower degradation of the films. High swelling indices cause faster fading of the film's color intensity. As curcumin concentration increased from 0% to 0.6%, MC, WS, and SI decreased significantly. This reduction can be attributed to the hydrophobic nature of curcumin (Rostami & Esfahani 2019). The addition of curcumin led to structural modifications that increased the hydrophobicity of the protein matrix, affecting the cross-linking of gelatin with water molecules. The interactions are illustrated in the following Figure 2.

This trend is consistent with previous findings (Pereira & Andrade 2017; Rostami & Esfahani 2019; Tyagi *et al*. 2015; Wu *et al*. 2019) which reported that curcumin addition influences the moisture of gum- and resin-based films. Conversely, Liu *et al.* (2016) found that curcumin addition was not significantly affect the MC of chitosan films. Generally, the tensile strength and other mechanical properties of composite films depend on the distribution of fillers and the interfacial interaction between polymer chains. Increased mechanical properties, such as tensile strength and elongation at break, can be attributed to interfacial interactions via hydrogen bonding between curcumin and the gelatin matrix. In this study, the mechanical properties of gelatin/curcumin composite films decreased with increasing curcumin concentration. This decrease in tensile strength and elongation at break may be due to aggregation caused by higher curcumin concentrations, as shown by SEM images. Another plausible explanation is the limited interaction between the biopolymer and filler due to phase separation caused by curcumin aggregation within the gelatin matrix. Proper filler distribution within the polymer matrix is key to productive stress transfer at

Table 2. Physicochemical and mechanical characteristics of the films

Film	Moisture Content (%)	Water Solubility (%)	Swelling Index (%)	Tensile Strength (MPa)	Elongation at Break (%)
GMC0	36.81 ± 3.11^{a}	44.61 ± 6.34^{a}	121.76 ± 16.69^{a}	12.04 ± 0.36^{a}	207.25 ± 1.77^{a}
GMC1	24.51± 3.36^{b}	28.28 ± 1.63^{b}	106.25 ± 11.43^{ab}	3.56 ± 0.30^{b}	175.26 ± 8.22^{b}
GMC2	15.74 ± 1.78^{c}	11.68 ± 1.45^{c}	83.51 ± 4.67^{bc}	1.44 ± 0.18^{c}	113.46 ± 10.91^{c}
GMC3	9.08 ± 1.40^{d}	4.21 ± 0.37^{c}	63.96 ± 6.28^{c}	0.89 ± 0.05^{c}	86.87 ± 3.40^{d}

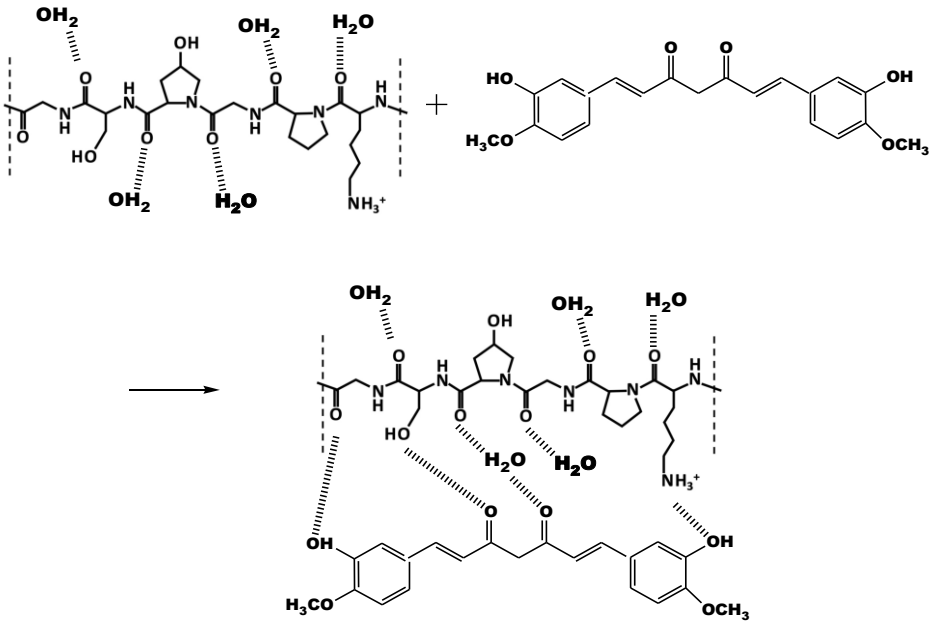


Figure 2. Interaction between gelatin, curcumin, and water molecules

the polymer-filler interface, which enhances the mechanical properties of composite films. Despite the decline in mechanical properties, the values are consistent with those reported by Musso *et al*. (2017) who used the same biopolymer and curcumin.

The effect of curcumin concentration on the chemical structure of gelatin and gelatin-curcumin films was evaluated using FT-IR spectroscopy. Changes in functional group frequencies were observed within the wavenumber range of 4000–500 cm^{-1}. The analysis results are shown in Figure 3.

Figure 3 indicated the incorporation of curcumin into the gelatin polymer, as evidenced by the absorption of specific functional groups from curcumin in GMC1, GMC2, and GMC3 films. Vibrational stretching of C–O–C was observed at 1057 cm^{-1}, C–H stretching at 2914 cm^{-1}, and aromatic C–O stretching at 1209 cm^{-1} (Chen *et al*. 2015; Cocean *et al*. 2021). Characteristic absorption peaks for gelatin films were observed at 3246–3248 cm^{-1} for N–H groups, 1685 cm^{-1} for C=O stretching (amide I), 1535–1539 cm^{-1} for N–H bending (amide II), and 1112 cm^{-1} for C–N stretching (amide III). Similar absorption peaks have been reported in previous studies (Bhowmik *et al*. 2017). Glycerol peaks were observed at 852 cm^{-1} (C–C of glycerol) and 923 cm^{-1} (C–O of glycerol) (Danish & Rashid 2016; Guimarães *et al*. 2016).

The thermal stability of gelatin-curcumin films at varying curcumin concentrations was analyzed using thermogravimetric analysis (TGA). The analysis was conducted at a heating rate of 10 °C/min. The results are shown in Figure 4.

TGA analysis indicated no significant differences in thermal stability between pure gelatin and gelatin-curcumin films. All films with different curcumin concentrations exhibited similar degradation rates. Overall, the films underwent two stages of thermal degradation: the first stage occurred at 50–280 °C, with an average mass loss of 78.12% from an initial average film mass of 6.38 mg. The higher mass loss in the first stage was due to the evaporation of free and bound water, glycerol, and other bound substituents (Wang *et al*. 2022). The second stage involved a mass loss of 2.71% between 280 and 450 °C, corresponding to the degradation of the gelatin polymer, with curcumin residues remaining as thermostable benzene rings (Roy & Rhim 2020). DTA data indicated that all films degraded at 250–315 °C, with residues amounting to 1.21 mg or 19.19% of the initial film mass at 500 °C.

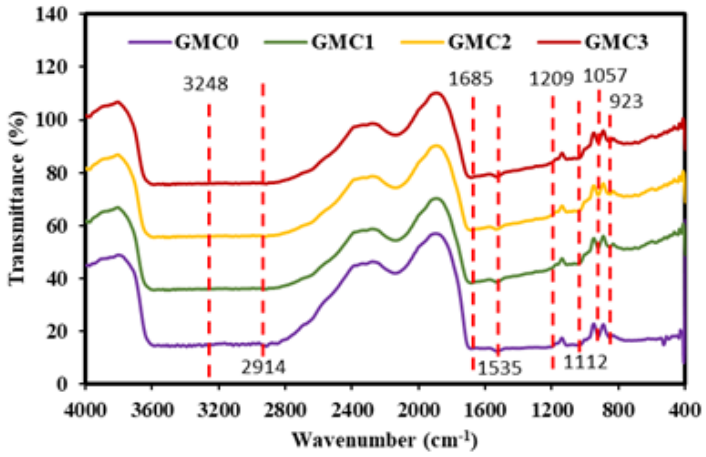


Figure 3. FTIR spectra of pure gelatin and gelatin-curcumin films

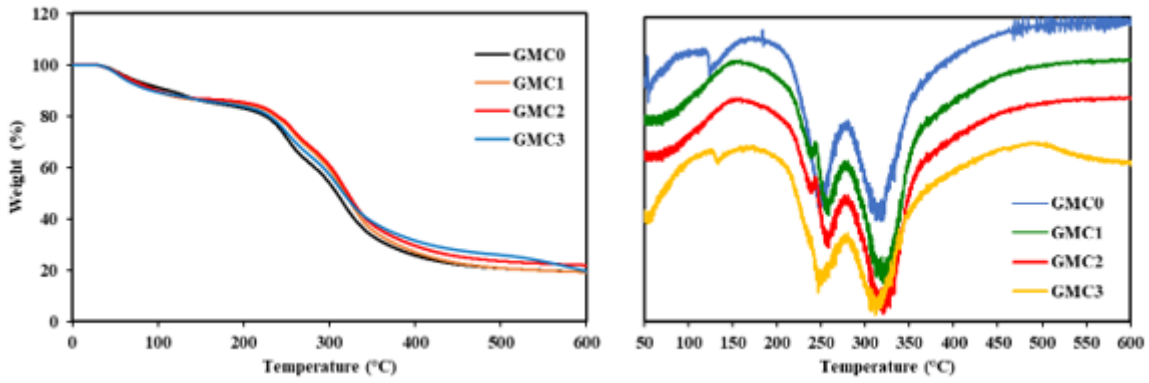


Figure 4. TGA/DTG curves of pure gelatin and gelatin-curcumin films

The surface morphology of pure gelatin and gelatin-curcumin films was observed using SEM. The effect of curcumin concentration on film surface morphology is shown in Figure 5. SEM images revealed that the surface of pure gelatin films and gelatin-curcumin films differed due to the addition of curcumin. Higher curcumin concentrations resulted in increasingly rough film surfaces due to curcumin particle aggregation. The hydrophobic nature of curcumin caused uneven particle dispersion within the gelatin matrix. Similar surface conditions were reported by Liu *et al*. (2018) and Roy & Rhim (2020).

The functional characteristics of pure gelatin and gelatin-curcumin films, determined by antioxidant and antibacterial activities, were evaluated. The films' ability to scavenge free radicals was tested using the DPPH method, with results shown in Figure 6. Increased curcumin concentration enhanced the films' antioxidant activity as expected. Pure gelatin films showed a free radical scavenging ability of 1.55%, attributed to hydrogen atom donation from gelatin peptides. Each 0.4% increase in curcumin concentration increased antioxidant activity by 28.3%. As previously reported, the ability of biopolymer films to scavenge free radicals correlates with the amount of added antioxidant compounds (Roy & Rhim 2020). The hydrogen atom donation from curcumin's phenolic groups enables it to stabilize DPPH radicals through bonding with nitrogen atoms (Priyadarsini *et al*. 2003; Vlocskó *et al*. 2025). This characteristic allows the gelatin-curcumin film to be used in the development of active packaging because curcumin is capable of inhibiting the oxidation reactions of packaged food products.

Curcumin, an essential compound found in turmeric, also possesses antibacterial properties. The antibacterial activity of gelatin-curcumin films was tested against pathogenic bacteria E. coli and S. aureus using the broth dilution method. The minimum inhibitory concentration (MIC) values of pure gelatin and gelatin-curcumin films are presented

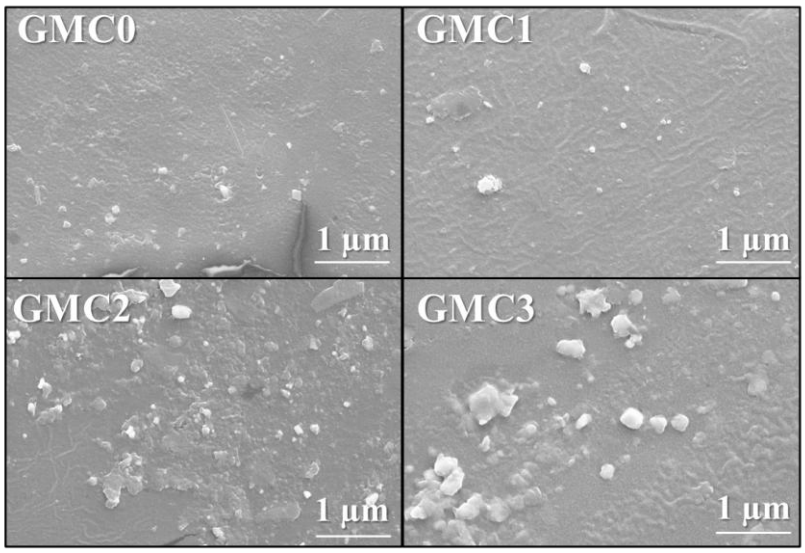


Figure 5. Surface morphology of pure gelatin and gelatin-curcumin films

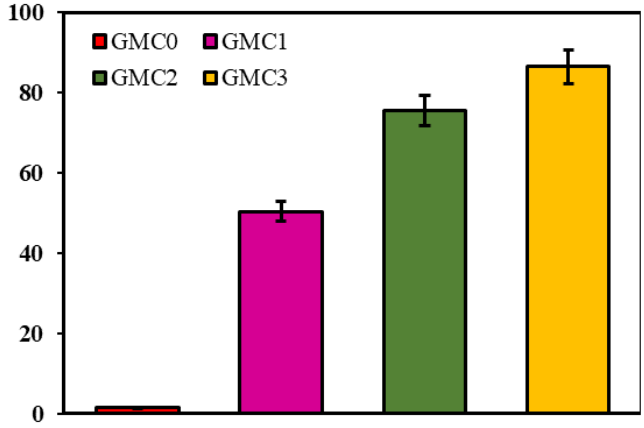


Figure 6. Antioxidant activity of gelatin-curcumin films

in Table 3. The MIC was determined based on the difference between the mean absorbance values before and after incubation, as measured by a spectrophotometer. Table 3 shows that all films, including pure gelatin films and positive controls, exhibited negative mean absorbance values, indicating their inhibitory effect on bacterial growth. The MIC was observed at a 0.4% concentration of curcumin. Curcumin effectively kills both Gram-positive and Gram-negative bacterial pathogens, such as S. aureus and E. coli, due to its phenolic compounds that disrupt FtsZ function, a bacterial cell division and survival protein, thereby inhibiting bacterial proliferation (Luo *et al*. 2012).

Film Response

The color change of curcumin extract in response to pH variations was observed using buffer solutions ranging from pH 2 to 12, and the absorbance was measured with a UV-Vis spectrophotometer. Figure 7 illustrates that the curcumin extract exhibited strong yellow-orange-red color transitions under different pH conditions. At pH levels between 2 and 4, the curcumin appeared yellow, while at neutral pH, the extract turned orange. Above pH 8, the curcumin turned reddish-orange.

The color change is attributed to the dominant structure of curcumin under different pH conditions (Priyadarshi *et al*. 2021). Curcumin's crystalline structure comprises seven carbon chains, including α,β-unsaturated β-diketone sections bound to two aromatic rings with ortho-methoxy phenolic hydroxyl groups. At neutral and basic pH, bond cleavage occurs in the α,β-unsaturated β-diketone section, which acts as a hydrogen donor site, leading to hydrolysis and curcumin degradation. Intramolecular hydrogen atom transfer in the β-diketone chain results in the keto-enol tautomeric forms of curcumin, depending on the solvent's nature. The bis-keto form is predominant under acidic or neutral conditions, displaying a yellow color with low water solubility. Under neutral or basic conditions, curcumin rapidly degrades due to proton loss from phenolic groups, resulting in the enolate form dominating. As the pH increases, curcumin degradation leads to the formation of trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal as the main degradation product, while feruloyl methane, ferulic acid, and vanillin are minor

Table 3. Antibacterial activity of gelatin and gelatin-curcumin films

Film	N	Mean	
		S. aureus	***E. coli***
GMC0	3	-0.218	-0.001
GMC1	3	-0.236	-0.086
GMC2	3	-0.133	-0.016
GMC3	3	-0.008	0.000
Positive control	3	-0.28	-0.28

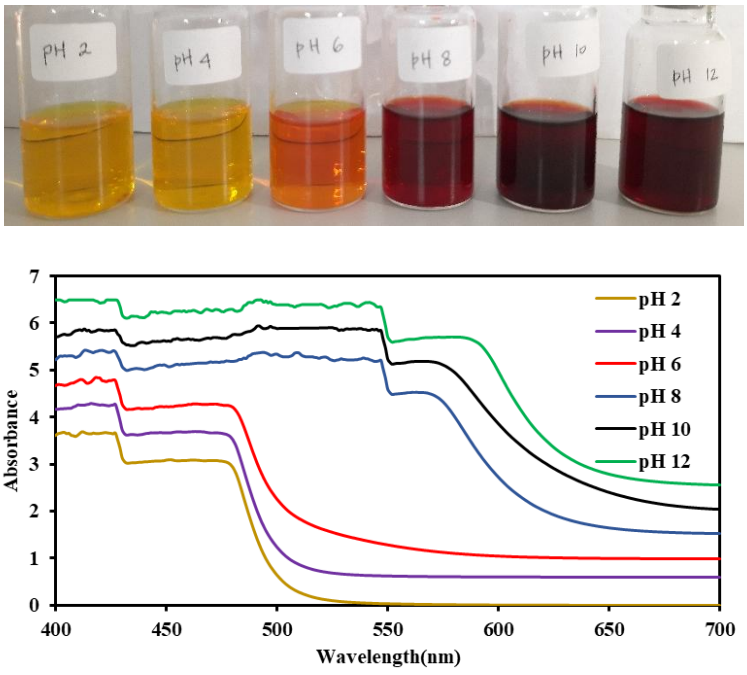


Figure 7. Color response of curcumin extract due to pH changes

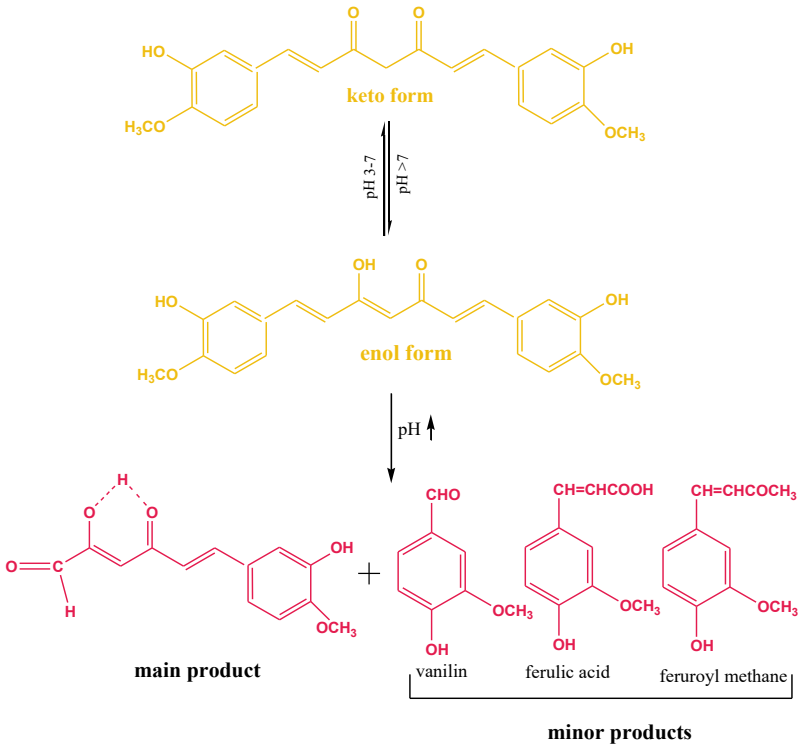


Figure 8. Curcumin degradation products due to pH changes

Table 4. Film response in liquid and gaseous media

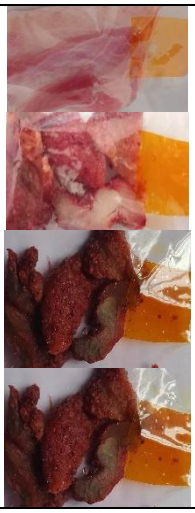
Film	Media			
	HCl 2M	NaOH 2M	NH_3	CH_3COOH
GMC0				
GMC1				
GMC2				
GMC3				

degradation products that cause the color change to red. The degradation mechanism is illustrated as shown in Figure 8.

The color change due to pH variations of curcumin extracts was observed through the extract's absorbance at UV-Vis wavelengths. At pH levels 2, 4, and 6, the extract exhibited maximum absorbance at 433 and 477 nm. Peaks at 433 and 477 nm correspond to π-π* transitions in curcumin, while the peak at 552 nm indicates deprotonated curcumin compounds under basic conditions. Increasing the pH to basic conditions caused a bathochromic shift from 470 to 552 nm due to curcumin degradation into its enolic form, which appears red (Musso *et al*. 2017). As a weak Bronsted acid with three unstable protons, curcumin exists as H4A+ in solutions with pH < 1. As the pH increases, H4A+ sequentially loses protons, shifting from carrying one positive charge to three negative charges (Liu *et al*. 2018).

The film response in strong acidic (HCl) and strong basic (NaOH) liquid media, as well as in weak acidic (CH_3COOH) and weak basic (NH_3) gaseous media, is shown in Table 4. Pure gelatin films did not exhibit any color changes in any media due to the absence of pigments. Gelatin-curcumin films (GMC1, GMC2, and GMC3) displayed color changes from yellow to reddish-orange in basic liquid and gas media, while no color changes were observed in acidic media. All gelatin-curcumin films demonstrated sensitivity to pH changes toward basic conditions. This phenomenon simulates pH changes resulting from food degradation in packaging, producing liquid and gaseous metabolites that interact with the film and visually indicate changes in color.

Table 5. Film response to freshness changes in se'i

Storage time (day)	pH	Visual condition of meat and film color
1	5.2	
2	6.5	
3	8.3	
4	9.2	

The gelatin-curcumin films exhibited slightly different color responses due to freshness changes in vacuum-sealed se'i. The film displayed a yellow color when the fresh meat had a pH of 5.2. As the meat's pH increased to 8.3 on the third day of storage, the film color shifted to reddish-orange and remained consistent through the fourth day. The increase in meat pH resulted from the degradation of meat proteins into volatile basic compounds, such as amines and ammonia, which raised the surrounding pH. The basic environment induced the color change in the film due to alterations in the curcumin structure within the film.

CONCLUSION

Adding curcumin content have distinct effects on the characteristics of gelatin-curcumin-based smart films. Increasing curcumin concentration enhances film properties such as antioxidant activity by 28.3%, antibacterial activity (MIC = 0.4%), and more intense yellow color visualization. Mechanical and physical characteristics, such as tensile strength, moisture content, and water solubility, decreased as curcumin concentration increased. Curcumin concentration variations did not significantly affect the thermal stability (≈50–280 °C) or brightness of the films. SEM results showed that higher curcumin concentrations caused aggregation of the gelatin biopolymer matrix on the film surface morphology. Response tests indicated that gelatin-curcumin films were sensitive to pH changes in both gaseous and liquid media. The resulting gelatin-curcumin films have significant potential for use as freshness detectors in vacuum-sealed smoked pork packaging.

ACKNOWLEDGMENT

The authors express their gratitude to all parties who contributed to this research, especially to LPPM Universitas Timor for supporting this research through funding under the Basic Research Program Contract Number: 65/UN60.6/PP/2023.

REFERENCES

Abedi-Firoozjah, R., Yousefi, S., Heydari, M., Seyedfatehi, F., Jafarzadeh, S., Mohammadi, R., Rouhi, M. & Garavand, F. (2022). Application of red cabbage anthocyanins as pH-sensitive pigments in smart food packaging and sensors. *Polymers*. **14**: 1-21.

Alizadeh-Sani, M., Mohammadian, E., Rhim, J.W. & Jafari, S.M. (2020). pH-sensitive (halochromic) smart packaging films based on natural food colorants for the monitoring of food quality and safety. *Trends in Food Science & Technology*. **105**: 93-144.

Balbinot-Alfaro, E., Craveiro, D.V., Lima, K.O., Costa, H.L.G., Lopes, D.R. & Prentice, C. (2019). Intelligent packaging with pH indicator potential. *Food Engineering Reviews*. **11(4)**: 235-244.

Barska, A. & Wyrwa, J. (2017). Innovations in the Food Packaging Market - Intelligent Packaging - a Review. *Czech Journal of Food Sciences*. **35(1)**: 1-6.

Bhowmik, S., Islam, J.M., Debnath, T., Miah, M.Y., Bhattacharjee, S. & Khan, M.A. (2017). Reinforcement of gelatin-based nanofilled polymer biocomposite by crystalline cellulose from cotton for advanced wound dressing applications. *Polymers*. **9(6)**: 1-13.

Biswas, A.K. & Mandal, P. (2019). *Meat quality analysis: advanced evaluation methods, techniques, and technologies*. Academic Press. London.

Bonke, R., Drees, N. & Gareis, M. (2016). Detection of psychrophilic and psychrotolerant *Clostridium spp.* in chilled fresh vacuum-packed meat using different PCR methods. *FEMS Microbiology Letters*. **363(1)**: 1-7.

Chen, X., Zou, L.Q., Niu, J., Liu, W. Peng, S.F. & Liu, C.M. (2015). The stability, sustained release and cellular antioxidant activity of curcumin nanoliposomes. *Molecules*. **20(8)**: 14293-14311.

Ciuca, M.D., & Racovita, R. C. (2023). Curcumin: Overview of extraction methods, health benefits, and encapsulation and delivery using microemulsions and nanoemulsions. *International Journal of Molecular Sciences*. **24(10)**: 1-29.

Cocean, A., Cocean, I., Cimpoesu, N., Cocean, G., Cimpoesu, R., Postolachi, C., Popescu, V. & Gurlui, S. (2021). Laser induced method to produce Curcuminoid-Silanol thin films for transdermal patches using irradiation of turmeric target. *Applied Sciences*. **11(9)**: 1-12.

Danish, M., Mumtaz, M.W., Fakhar, M. & Rashid, U. (2017). Response surface methodology based optimized purification of the residual glycerol from biodiesel production process. *Chiang Mai Journal of Science*. **44(4)**: 1570-1582.

Guimarães, J.L., Cursino, A.C.T., Saul, C.K, Sierrakowski, M.R., Ramos, L.P. & Satyanarayana, K.G. (2016). Evaluation of castor oil cake starch and recovered glycerol and development of "Green" composites based on those with plant fibers. *Materials*. **9(2)**: 1-18.

Kim, D., Lee, S., Lee, K., Baek, S. & Seo, J. (2017). Development of a pH indicator composed of high moisture-absorbing materials for real-time monitoring of chicken breast freshness. Food Science and Biotechnology. **26(1)**: 37-42.

Kuswandi, B. & Nurfawaidi, A. (2017). On-package dual sensors label based on pH indicators for real-time monitoring of beef freshness. *Food Control*. **82**: 91-100.

Kuswandi, B. (2017). *Freshness sensors for food packaging*. Reference Module in Food Science. Elsevier. Amsterdam.

Kuswandi, B., Jayus, Oktaviana, R., Abdullah, A. & Heng, L.Y. (2014). A novel on-package sticker sensor based on methyl red for real-time monitoring of broiler chicken cut freshness. Packaging Technology and Science. **27(1)**: 69-81.

Liu, J., Wang, H., Wang, P., Guo, M., Jiang, S., Li, X. & Jiang, S. (2018). Films based on κ-carrageenan incorporated with curcumin for freshness monitoring. *Food Hydrocolloids*. **83**: 134-142.

Liu, Y., Cai, Y., Jiang, X., Wu, J. & Le, X. (2016). Molecular interactions, characterization and antimicrobial activity of curcumin–chitosan blend films. *Food Hydrocolloids*. **52**: 564-572.

Luo, N., Varaprasad, K., Reddy, G.V.S., Rajulu, A. V. & Zhang, J. (2012). Preparation and characterization of cellulose/curcumin composite films. *Rsc Advances*. **2(22)**: 8483-8488.

Moradi, M., Tajik, H., Almasi, H., Forough, M. & Ezati, P. (2019). A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness. *Carbohydrate Polymers*. **222**: 1-10.

Musso, Y.S., Salgado, P.R. & Mauri, A.N. (2016). Gelatin based films capable of modifying its color against environmental pH changes. *Food Hydrocolloids*. **61**: 523-530.

Musso, Y.S., Salgado, P.R. & Mauri, A.N. (2017). Smart edible films based on gelatin and curcumin. *Food Hydrocolloids*. **66**: 8-15.

Musso, Y.S., Salgado, P.R. & Mauri, A.N. (2019). Smart gelatin films prepared using red cabbage (*Brassica oleracea* L.) extracts as solvent. *Food Hydrocolloids*. **89**: 674-681.

Pereira Jr, V.A., de Arruda, I.N.Q. & Stefani, R. (2015). Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as Time–Temperature Indicators for application in intelligent food packaging. Food Hydrocolloids. **43**: 180-188.

Pereira, P.F. & Andrade, C.T. (2017). Optimized pH-responsive film based on a eutectic mixture-plasticized chitosan. *Carbohydrate Polymers*. **165**: 238-246.

Pereira, P.M. de C.C.& Vicente, A.F. dos R.B. (2013). Meat nutritional composition and nutritive role in the human diet. *Meat Science* . **93**: 586–592

Priyadarshi, R., Ezati, P. & Rhim, J.W. (2021). Recent advances in intelligent food packaging applications using natural food colorants. *ACS Food Science & Technology*. **1(2)**: 124-138.

Priyadarsini, K.I., Maity, D.K., Naik, G.H., Kumar, M.S., Unnikrishnan, M.K., Satav, J.G. & Mohan, H. (2003). Role of phenolic OH and methylene hydrogen on the free radical reactions and antioxidant activity of curcumin. *Free Radical Biology and Medicine*. **35(5)**: 475-484.

Rawdkuen, S., Faseha, A., Benjakul, S. & Kaewprachu, P. (2020). Application of anthocyanin as a color indicator in gelatin films. *Food Bioscience*. **36**: 100603.

Rodrigues, C., Souza, V.G.L., Coelhoso, I. & Fernando, A.L. (2021). Bio-based sensors for smart food packaging—current applications and future trends. *Sensors*. **21(6)**: 2148.

Rostami, H. & Esfahani, A.A. (2019). Development a smart edible nanocomposite based on mucilage of Melissa officinalis seed/montmorillonite (MMT)/curcumin. *International Journal of Biological Macromolecules*. **141**: 171-177.

Roy, S. & Rhim, J. W. (2020). Preparation of antimicrobial and antioxidant gelatin/curcumin composite films for active food packaging application. *Colloids and Surfaces B: Biointerfaces*. **188**: 110761.

Sai-Ut, S., Suthiluk, P., Tongdeesoontorn, W., Rawdkuen, S., Kaewprachu, P., Karbowiak, T. Debeaufort & Degraeve, P. (2021). Using anthocyanin extracts from butterfly pea as pH indicator for intelligent gelatin film and methylcellulose film. *Current Applied Science and Technology*. **21(4)**: 652-661.

Shahbazi, Y. (2017). The properties of chitosan and gelatin films incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil as biodegradable materials for active food packaging. *International Journal of Biological Macromolecules.* **99**: 746-753.

Shukla, V., Kandeepan, G. & Vishnuraj, M.R. (2015). Development of on package indicator sensor for real-time monitoring of meat quality. *Veterinary World*. **8(3)**: 393-397.

Suluh, D.G. (2019). Studi perkembangan kuman pada daging se'i yang dikemas divakum dan dikemas tanpa divakum. *Oehònis.* **3(1)**: 167-174.

Taghinia, P., Abdolshahi, A., Sedaghati, S. & Shokrollahi, B. (2021). Smart edible films based on mucilage of *Lallemantia iberica* seed incorporated with curcumin for freshness monitoring. *Food Science & Nutrition*. **9(2)**: 1222-1231.

Thakur, G., Singh, A. & Singh, I. (2016). Formulation and evaluation of transdermal composite films of chitosan-montmorillonite for the delivery of curcumin. *International Journal of Pharmaceutical Investigation*. **6(1)**: 23-31.

Tyagi, P., Singh, M., Kumari, H., Kumari, A. & Mukhopadhyay, K. (2015). Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. *PloS One*. **10(3)**: e0121313.

Vlocskó, R. B., Mastyugin, M., Török, B., & Török, M. (2025). Correlation of physicochemical properties with antioxidant activity in phenol and thiophenol analogues. *Scientific Reports*. **15(1)**: 73.

Wang, F., Wang, R., Pan, Y., Du, M., Zhao, Y. & Liu, H. (2022). Gelatin/chitosan films incorporated with curcumin based on photodynamic inactivation technology for antibacterial food packaging. *Polymers*. **14(8)**: 1-16.

Welch, J., Wallace, J., Lansley, A.B. & Roper, C. (2021). Evaluation of the toxicity of sodium dodecyl sulphate (SDS) in the MucilAir™ human airway model in vitro. *Regulatory Toxicology and Pharmacology*. **125**: 105022.

Wu, C., Sun, J., Chen, M., Ge, Y., Ma, J., Hu, Y., Pang, J. & Yan, Z. (2019). Effect of oxidized chitin nanocrystals and curcumin into chitosan films for seafood freshness monitoring. Food Hydrocolloids. **95**: 308-317.

Wulandari, A., Sunarti, T.C. & Fahma, F. (2020). Potentiality of ethanol curcumin extract as biosensor for detection of sodium tetraborate. *In IOP Conference Series: Earth and Environmental Science.* **472(1)**: 012018.

Zhang, S.L., Peng, D.E.N.G. & LÜ, S.W. (2016). Quantification and analysis of anthocyanin and flavonoids compositions, and antioxidant activities in onions with three different colors. *Journal of Integrative Agriculture*. **15(9)**: 2175-2181.

Zhao, L., Liu, Y., Zhao, L. & Wang, Y. (2022). Anthocyanin-based pH-sensitive smart packaging films for monitoring food freshness. *Journal of Agriculture and Food Research*. **9**: 100340.

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