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Preparation and Characterisation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) Thin Film for Food Coating Application

Nur Najmina Rafiae[a], Muhammad Bisyrul Hafi Othman[a,b*], Muhamad Shirwan Abdullah Sani[c], Mohamad Nasir Mohamad Ibrahim[b] and Najwa Najihah Mohamad Daud[a,b]

[a]School of Chemical Sciences, Universiti Sains Malaysia (USM), 11800 Minden, Penang, Malaysia.
[b]Materials Technology Research Group (MaTRec), School of Chemical Sciences, Universiti Sains Malaysia (USM), 11800 Minden, Penang, Malaysia.
[c]International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia (IIUM), 53100 Kuala Lumpur, Malaysia.
*Corresponding author: E-mail address: bisyrul@usm.my

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Abstract

Recognising the importance of preventing rapid food deterioration and prolonging the shelf life of fruits and vegetables from oxidation, we successfully created thin films composed of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) through a traditional blending technique. Fourier Transform Infrared Spectroscopy (FTIR) confirmed the successful preparation of nanolignin structures, with characteristic peaks observed at 513 cm-1 (C-C stretching in aromatic), 1222 cm-1 (phenolic OH), and 1107 cm-1 (Ar-H and syringyl group). Ultraviolet-visible spectroscopy (UV-Vis) revealed an absorption capacity within the 280 to 300 nm range. Film opacity increased with a greater nanolignin composition in the thin film, attributed to the presence of chromophore structures. Thermogravimetric Analysis (TGA) demonstrated a thermal degradation temperature exceeding 300°C. Differential Scanning Calorimetry (DSC) analysis unveiled two distinct glass transition temperatures (Tg) at approximately 60°C and 80°C, indicating microphase separation and immiscibility between gelatine and nanolignin particles. The nanolignin content significantly influenced solubility and water uptake, with higher nanolignin content leading to reduced solubility and water absorption. The application of NLGGCs film coatings on banana surfaces extended their shelf life compared to control samples after 10 days. Furthermore, NLGGCs underscore the pivotal role in enhancing the performance as a promising bio-based food coating alternative for future applications.

1. Introduction

Harmful microorganisms can pose a threat to fruits and vegetables, both in terms of reduced shelf-life and potential health risks. Additionally, food products can undergo degradation due to oxidation and exposure to artificial light. Wu *et al.* (2020) report that oxidation is a significant factor in the deterioration of harvested goods, causing decreased shelf life, unwanted sensory changes, and reduced food quality. To combat this problem, food coatings that exhibit resistance to UV radiation and antimicrobial properties have emerged as a viable solution for preserving food quality. Protective films can be applied to the surface of food items to extend their shelf life and maintain their integrity.

Moreover, there has been a notable surge in worldwide interest towards the halal food industry, owing to its potential to cater to a significant population of over 1.8 billion Muslim consumers. This industry's current value stands at $2.2 trillion and is poised to reach $4.7 trillion, primarily driven by the rapid expansion of the global Muslim population (Hafiz *et al.*, 2022). Several non-Islamic countries, such as China, Thailand, Brazil, New Zealand, Singapore, the Philippines, and Korea, have recognised its immense potential and are actively exploring opportunities within this industry (Najihah *et al.*, 2023). Consequently, the need to prioritise innovation in halal-based product manufacturing to stimulate national economies is rising.

The source of gelatine has sparked a contentious debate within the halal community due to its pivotal role in determining the halal status of products. This debate stems from the religious significance of adhering to Islamic dietary law, which is the fundamental basis of Islamic principles. Gelatine derived from non-halal sources, such as non-slaughtered animals in accordance with Islamic principles or, worse yet, from pigs, directly conflicts with these dietary regulations, rendering products containing such gelatine as haram. Approximately 326,000 tons of gelatine is produced annually, with pig skin as the highest contributor of gelatine source, making it 46 percent of the total production (Pradini D. *et al.*, 2018). In Europe, 80 percent of edible gelatine comes from porcine sources, making it harder for Muslim consumers to navigate the market confidently and select products aligned with Islamic dietary principles (Demirhan D. *et al.*, 2012). The significance of clarifying the halal status of gelatine and the innovation of dietary products from halal sources to replace non-halal source products will uphold the trust and confidence of Muslim

consumers toward the halal industry and ease them to make informed choices in line with their religious beliefs.

Besides that, significant headway has been made in developing food coatings with antioxidant properties for fruits and vegetables. However, using synthetic antioxidants has raised concerns due to potential health hazards. Synthetic phenolic antioxidants (SPAs), including butyl hydroxyanisole (BHA), dibutyl hydroxytoluene 2 (BHT), and tert-butyl hydroquinone (TBHQ), have been associated with adverse impacts on aquatic life and mammals, such as reproductive and developmental toxicity, as well as carcinogenic effects and disruptions to endocrine systems (Wang *et al.*, 2021). Since the requirements of manufacturing halal products entail the usage of halal and *toyyib* (safe, clean and nutritious) ingredients (Mohamad Asri *et al.*, 2022), it is necessary to encourage substantial innovation in creating halal-based products that could fulfil these requirements while stimulating economic growth.

Aadil *et al.* (2016) reported that lignin-gelatine film exhibits remarkable barrier properties, including effective UV light, high water uptake and thermal stability. El-Nemr *et al.* (2020) reported that the gelatine-lignin blend had shown high effectiveness against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* due to the presence of phenolic hydroxyl. However, a research gap exists regarding the impact of nanolignin composition in these films, with no prior studies on this specific aspect. Consequently, the factors influencing the physical properties of Nanolignin-Gelatine-Glycerol composites (NLGGCs), such as thermal stability, glass transition temperature, UV shielding capacity, solubility, and water uptake, remain unknown. According to Low *et al.* (2021), lignin nanoparticles exhibit superior antioxidant, antibacterial, and UV protectant applications compared to macro-sized lignin particles. The increased specific surface area enhances phenolic hydroxyl group distribution, contributing to superior UV protectant capabilities (Qian *et al.*, 2017). However, the performance of nanolignin in nanolignin-gelatine film is still unknown due to no previous research conducted. Comprehending these elements is critical in innovating outstanding food coating with exceptional features.

The current study has effectively acknowledged the importance of addressing specific bio-based thin film development issues. The study successfully produced a range of NLGGCs thin films using a conventional blending-casting method. The impact of nanolignin composition on NLGGCs thin film properties was evaluated through various analytical techniques, including FTIR Spectroscopy, UV-Vis Spectrophotometry, DSC, TGA, solubility assessments, and examinations of swelling properties. The results of this study are expected to help develop new and improved bio-based thin films with enhanced properties that can be utilised in various industries.

2. Methodology

2.1 Raw materials

Gelatine powder from a Bovine source was obtained from Take It Global Sdn. Bhd certified as a halal supplier, Refine Glycerol 99 %, sodium hydroxide pallet (NaOH), sulfuric acid (H_2SO_4, 95 - 97 %) and *Elais Guineensis* empty fruit bunch (EFB). The lignin Nanoparticles were extracted from *Elais Guineensis* EFB from the work reported by Sekeri *et al.* (2020) and Yaqoob *et al.* (2021).

2.2 Preparation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

NLGGCs thin film series was prepared by dissolving 0.5% w/v of nanolignin in 0.0125 N NaOH respectively in 100 mL of deionised water. After the nanolignin colour changed to black and a sign of homogeneity was shown, gelatine powder was dissolved into the solution according to Table 1. Next, 2% v/v of refined glycerol was added to plasticise the thin film solution and stirred for 1 hour at 60°C. A series of prepared NLGGCs thin films is summarised in Table 1.

Table 1: Ratio of Nanolignin: Gelatine: Glycerol in NLGGCs thin film series

Sample Designation	Nanolignin (g)	Gelatine in g (ratio)	Refined Glycerol in g (ratio)	Nanolignin (%)
NLGGCs 59	0.5	1.6 (0.2)	1.26 (0.5)	58.82
NLGGCs 50	0.5	4 (0.5)	1.26 (0.5)	50.00
NLGGCs 43	0.5	6.4 (0.8)	1.26 (0.5)	43.48

Next, 26.5 mL of composite thin film solution was put into an 11 cm petri dish sprayed with mould release and dried for 16 hours at 60°C in the oven. The film was peeled and kept in desiccators with P_2O_5 at 25°C with 0% relative humidity. In this formulation, glycerol composition was set as constant to study the effect of nanolignin and gelatine composition in NLGGCs thin film.

2.3 Characterisation of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed using a Perkin Elmer model system 2000 FT-IR (United Kingdom) to determine the functional group in a series of NLGGCs thin film according to *ASTM E168-06: Standard Practices for General Techniques of Infrared Quantitative Analysis* at a resolution of 4 cm^{-1} from 400 to 4000 cm^{-1} of wavenumber. UV-Vis spectroscopy analysis was performed using a Perkin Elmer Lambda 365 UV-Vis Spectrophotometer to determine the UV light absorbance according to ASTM E169-16: *Standard Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis* in the wavelength range of 200-800 nm. A wavelength of 600 nm was used for the determination of film transparency.

Thermogravimetric analysis (TGA) was performed using a Perkin Elmer Diamond STA-6000 analyser to determine the thermal stability according to ASTM E1131-08: *Standard Test Method for Compositional Analysis by Thermogravimetry* under a nitrogen atmosphere at a flow rate of 200 mL/min. About 5 mg of the film will be heated at room temperature to 700°C at 10°C/min. Differential Scanning Calorimetry (DSC) analysis was performed using Mettler Toledo DSC 822e to determine the glass transition temperature (Tg). About 5 mg samples were encapsulated tightly in aluminium pans and scanned under 50 mL/min dry nitrogen from 0°C to 300°C at 10°C/min.

The solubility was performed to determine the chemical stability of NLGGCs thin film using ASTM D5226-21: *Standard practice for Dissolving Polymer Materials*. About 10 mm x 10 mm were cut and soaked into 5 mL of sulfuric acid, acetic acid,

sodium hydroxide, ammonia solution, distilled water, dimethylformamide, and hexane. Water uptake analysis was conducted according to ASTM-D2765: *Standard Test Methods for Determination of Gel Content and Swell Ratio of Crosslinked Ethylene Plastics*. About 20mm x 20mm were cut, and initial mass was recorded.

Finally, the pre-coated banana shelf-life was investigated at room temperatures with moderate lighting and dry environments for 10 days. The appearance banana was selected, pre-cleaned and coated with a series of NLGGCs solutions for every banana. The control banana was prepared as a benchmark for banana ripening.

3. Results and discussion

3.1 Preparation of NLGGCs thin film series consideration

The choice of *Elais Guineensis sp*. (Empty Fruit Bunch) as a primary material is strategic. This resource was favoured due to its abundant and sustainable presence in Malaysia. Previous research findings have underscored its exceptional qualities, including its antioxidative attributes, antibacterial properties, UV resistance, and suitability for nano-enhancement applications (Aadil *et al*., 2016; Low *et al*., 2021; Yun *et al*., 2021). The properties displayed aligned well with the objective of crafting biobased food coatings.

In the realm of polymer matrices, the selection of bovine gelatine is a well-considered choice. This material boasts proven attributes that have been thoroughly documented in prior research. Notably, gelatine can be sourced from the halal livestock industry, effectively utilising resources that might otherwise be discarded. Furthermore, this choice carries significant potential within the halal industry, especially considering the projected growth of the global Muslim population to 29.7% by 2050 (Mohd *et al*., 2023). Gelatine is recognised for its strong resistance to oxygen permeation, although there is room for enhancement in its mechanical properties (Tyuftin & Kerry, 2021). Incorporating nanolignin and glycerol is justified to improve the properties of gelatine.

Glycerol plays a pivotal role as a plasticiser to counteract the inherent brittleness of dry gelatine. This addition effectively enhances the film's flexibility, elasticity, and extensibility, a phenomenon supported by Sanyang *et al.* (2015). It is important to emphasise that all materials employed in this study originated from natural sources, are renewable, and are deemed safe for use in food coatings. This ethical consideration aligned with the overarching goal of ensuring the safety of these materials when they are applied in food-related contexts.

3.2 Spectroscopy analysis

FTIR analysis was carried out to delve into the chemical composition of the NLGGCs thin films. It sought to reveal the unique characteristics of these films, with a particular focus on the lignin nanoparticles sourced from *Elaeis Guineensis* sp. EFB through the High Shear Homogenization method. Figure 1 provides visual evidence of the successful preparation of NLGGCs thin films, showcasing the absorption peak of nanolignin.

Within Figure 1, a broad absorption peak at 3334 cm^{-1} signified the presence of aliphatic and aromatic O-H groups in the nanolignin. Additionally, moderate-intensity peaks at 1513 cm^{-1} indicated C-C stretching within the aromatic structures of

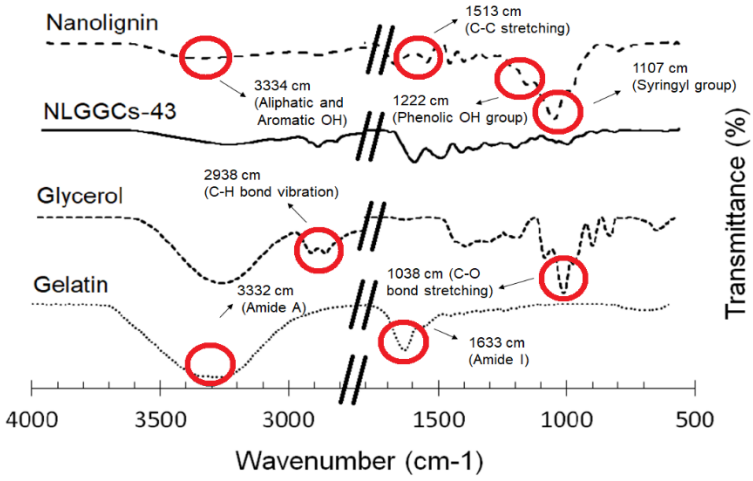

Figure 1: FTIR spectra of Nanolignin, Bovine Gelatine, Refined Glycerol and NLGGCs-43.

lignin. Furthermore, 1222 and 1107 cm^{-1} peaks corresponded to phenolic OH and Ar-H and syringyl groups, respectively. These findings aligned with the report by Sekeri *et al.* (2020). The FTIR analysis of gelatine uncovered a broad peak at 3332 cm^{-1} and another at 1633 cm^{-1}, suggesting the presence of Amide A and Amide I, respectively. These findings were consistent with prior research by Aadil *et al.* (2016). Notably, in the FTIR peak of NLGGCs-59, several distinctive peaks emerged. The most prominent band was a broad band at 3281 cm^{-1}, indicating the presence of O-H groups derived from nanolignin, glycerol, and gelatine's aromatic and aliphatic compounds. This observation was further supported by peaks at 2938 cm^{-1}, which imply the stretching vibration of C-H bonds within the aliphatic chain of glycerol, a correlation documented by Danish *et al.* (2016) in the context of refined glycerol. The thin film's absorption peaks at 3281 cm^{-1} and 1633 cm^{-1} were consistent with FTIR peaks identified in the gelatine raw materials. Furthermore, the 1038 cm^{-1} band was attributed to the C-O stretch of glycerol. Notably, the peaks were associated with the Ar-H syringyl and phenolic OH groups in nanolignin that remained unchanged after the interaction. Figure 2 offers insight into the FTIR peaks across all NLGGCs thin film series, revealing a consistent absorption peak similar to that observed in NLGGCs-59.

However, intensity varied due to differing compound compositions. The O-H group at 3600-3200 cm^{-1} exhibited variable intensities due to variations in glycerol composition. This phenomenon illustrated the plasticising effect of glycerol, with increasing glycerol content leading to a reduction in hydrogen bonding intensity. This outcome can be primarily attributed to glycerol's role in forming new hydrogen bonds within the gelatine network while disrupting internal hydrogen bonding within the gelatine protein network. These activities decrease internal forces and increase intermolecular spacing, as Chen *et al.* (2018) and Chen *et al.* (2018) explained.

The introduction of nanolignin plays a crucial role in shaping the physical and mechanical properties of the NLGGCs thin film series. According to Núñez-Flores *et al.* (2013), the inclusion of nanolignin leads to a reduction in the intensity of Amide I, Amide II, and Amide III in gelatine due to a "dilution effect," as nanolignin takes the place of gelatine. The mostnotable alterations occur in the 953-1633 cm-1 range, signifying substantial interference caused by nanolignin in the hydrogen bonding between water and imide residues. The addition of nanolignin has caused a reduction in the intensity

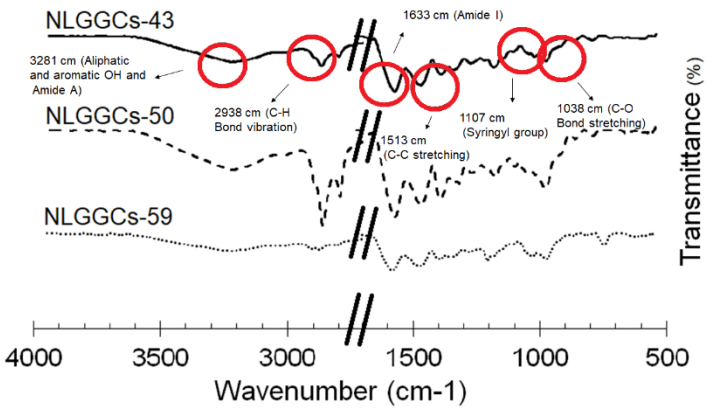

Figure 2: FTIR spectra of NLGGCs thin film series.

of Amide I, Amide II and Amide III of gelatine. The intensity reduction is due to the 'dilution effect' of gelatine replacement by nanolignin. The most notable changes in the thin film series range from 953-1633 $cm^{-1,}$ indicating strong intrusion caused by nanolignin in the hydrogen bonding between water and imide residues (Núñez-Flores *et al.*, 2013). This interaction arose from the hydrophobic group of polyphenols interacting with the hydrophobic region of the protein through hydrophobic interactions and hydrogen bonding between the phenolic hydroxy group of polyphenols and the polar group of the protein.

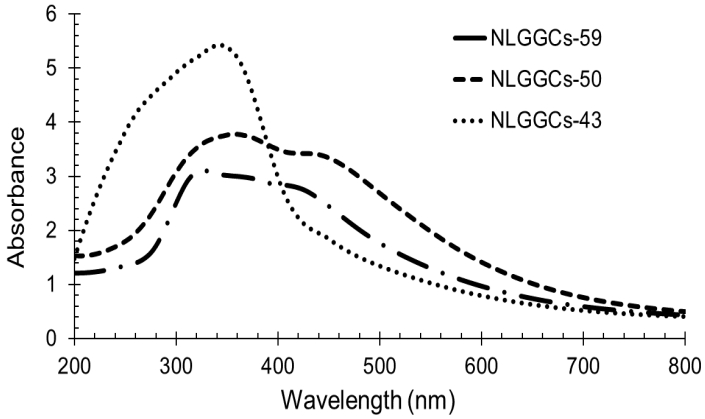

Figure 3: The effect of gelatine content on UV light absorption of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film.

UV-Vis was employed to assess the light barrier properties and film transparency, particularly within the 200-800 nm range, with a specific focus on transparency at 600 nm. Film transparency was inversely correlated with light barrier properties; as transparency decreased, the light barrier properties also increased. Figure 3 visually presents the opacity and light absorption of the NLGGCs thin film series across the 200-800 nm wavelength range.

Figure 3 illustrates that all NLGGCs series exhibit substantial UV absorption capacity within the 280-350 nm range. This phenomenon has been primarily attributed to chromophores within nanolignin, including phenolic and ketone groups (Qian *et al.*, 2015). These chromophores responsible for UV absorption have encompassed double bonds (CH=CH) conjugated with aromatic rings, quinone methide and quinones, chalcone structures, free radicals, and metal complexes with catechol structures (Zhang & Naebe, 2021). Additionally, the gelatine content has also contributed to high absorption within the 200-300 nm wavelength range, with absorption in the 200-250 nm range linked to peptide bonds in gelatine and absorption in the 250-300 nm range attributed to chromophore structures within gelatine, such as the phenylalanine and tyrosine amino acid aromatic groups (Etxabide *et al.*, 2015).

Table 2: The opacity of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film

Sample Designation	Film Thickness (cm)	A_{600}	Opacity
NLGGCs 59	0.05	0.9667	19.34
NLGGCs 50	0.31	1.4123	4.56
NLGGCs 43	0.22	0.7958	3.62

Specifically, NLGGCs-59, with the highest nanolignin composition, exhibited the highest opacity (19.34), with opacity values decreasing as nanolignin composition decreases. This observation aligned with the findings of Yang *et al.* (2020), who noted that an increasing content of lignin nanoparticles in poly (lactic acid)-poly (ε-caprolactone)-nanolignin composite films have progressively reduced optical transparency. The opacity arises primarily due to the absorption of light in the visible range by chromophore structures within the 400-800 nm wavelength range, resulting in the dark brown colour of lignin that enhances UV shielding properties while potentially compromising the film's visual appeal in food coating applications (Aadil *et al.*, 2016). These properties hold significant importance in the food coating industry for addressing or mitigating lipid oxidation, a prominent concern in the food industry (Sá *et al.*, 2020).

3.3 Thermal properties

The study delved into the thermal behaviour of the NLGGCs thin films through TGA in a nitrogen environment, as shown in Figure 4.

The TGA curves unveiled four distinct decomposition stages common to all NLGGCs thin films. The initial stage witnessed a weight loss between 50°C and 180°C, attributed to the evaporation of water content absorbed within the films. These findings corroborated prior research by Benbettaïeb *et al.* (2016) and Inamura *et al.* (2013), which reported similar initial degradation occurring within the temperature ranges of 52°C to 220°C and 46°C to 140°C, respectively. The second stage of decomposition, occurring between 200°C and 280°C, corresponded to the volatilisation of glycerol and bound water within the films. This observation concurred with findings reported by Phreecha & Chinpa (2019), where glycerol decomposition occurred at approximately 250°C, proximate to its boiling point of 289°C. The most substantial degradation was noted between 280°C and 480°C, indicating the decomposition of gelatine and lignin structures, resulting in the highest weight loss percentage (35% to 55%). This decomposition was linked to the breakage of helical protein chain structures, rupturing of peptide bonds, and the breakdown of lignin C-C linkages. Remarkably, gelatine's primary and secondary structures remained unaffected by thermal degradation, aligning with the findings of Benbettaïeb *et al.* (2016). In the final stages, a minor weight loss occurred at temperatures ranging from 460°C to 550°C, attributed to the

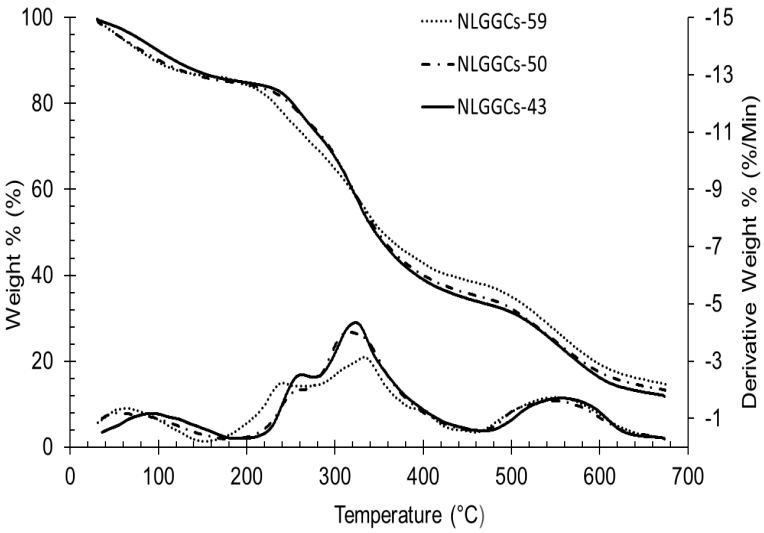

Figure 4: TG and DTG trace for thermal decomposition of a series of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) thin films in nitrogen gas atmosphere.

decomposition of more thermally stable compounds within the films.

Incorporating nanolignin played a pivotal role in elevating the thermal stability of the NLGGCs thin film series. As outlined in Table 3, all NLGGCs thin films exhibited maximum degradation temperatures surpassing 300°C, indicating robust thermal stability. This enhancement can be ascribed to the robust interaction between lignin nanoparticles and the gelatine polymer network in the NLGGCs thin film through hydrogen bonding, as elucidated by Huang *et al.* (2020). Similar results were achieved in the study conducted by Bian *et al.* (2018). Additionally, an escalation in nanolignin composition led to a substantial increase in the percentage of char yield at 800°C. This phenomenon of char formation might be linked to the condensation of lignin aromatic rings at temperatures exceeding 550°C, serving as a protective mechanism against polymer matrix degradation, as Monteiro *et al.* 2021 suggested.

DSC analysis was employed to scrutinise the impact of temperature on the properties of the NLGGCs thin film series, specifically focusing on determining the glass transition temperature (Tg), Figure 5.

Table 3: Results of TG/DTG traces of the Nanolignin-Gelatine-Glycerol Composites thin films in nitrogen gas atmosphere

Sample Designation	$T_{10\%}$ (°C)	T_{onset} (°C)	T_{endset} (°C)	ΔT (°C)	T_{max} (°C)	Char Yield at 800°C (%)
NLGGCs 59	88.03	154	461	307	334	14.509
NLGGCs 50	92.67	179	463	284	315	13.146
NLGGCs 43	110.33	194	470	276	324	11.85

Figure 5 visually encapsulates the heat flow within the NLGGCs series, revealing Tg values clustered around 80°C for all NLGGCs films. In comparison, bulk lignin showcased a Tg at 139.7°C, while mechanically sheared nanolignin exhibited a Tg at 156.6°C, as documented by Juikar & Vigneshwaran (2017). Nair corroborated these findings *et al.* (2014), where diverse lignin nanoparticles displayed Tg values ranging from 120°C to 150°C. However, including nanolignin within the thin film series reduced the Tg compared to the composite materials.

Furthermore, it was observed that certain series displayed an additional Tg at approximately 60°C. As per Núñez-Flores *et al.* (2013) report, the presence of two distinct Tg values might have stemmed from the immiscibility between lignin's hydrophobic structure and gelatine's hydrophilic nature, resulting in microphase separation that has enhanced gelatine mobility. As highlighted by Aadil *et al.* (2016), an escalation in nanolignin composition has led to a decrease in the Tg of the gelatine/glycerol thin film. This decline in Tg within the NLGGCs films can be attributed to interactions between nanolignin and gelatine, facilitated by hydrogen bonding and hydrophobic interactions, subsequently diminishing crystallinity and inducing changes in the helical structures of gelatine. In summary, this section provides an in-depth exploration of the thermal properties of the NLGGCs thin films, shedding light on their decomposition patterns, improved thermal stability due to nanolignin integration, and shifts in Tg.

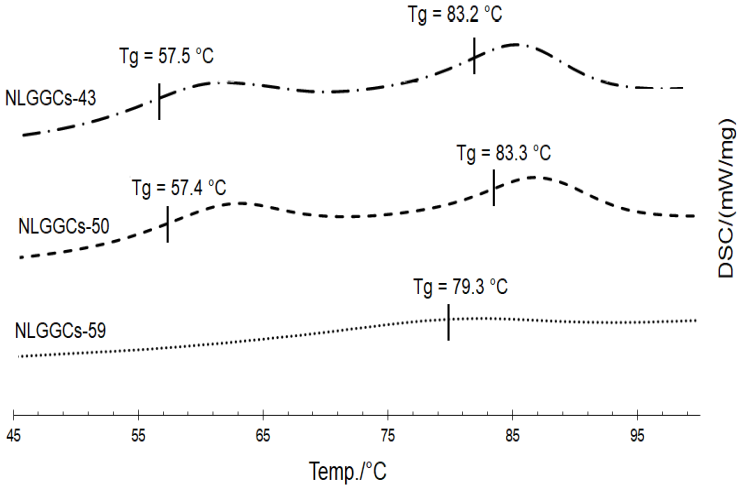

Figure 5: Heat flow of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film series.

3.4 Chemical stability

Table 4 elucidates the solubility of the NLGGCs thin film series across diverse solvents at 25°C to explore the chemical durability of NLGGCs thin films. It offered insights into their solubility across diverse solvents and swelling behaviour. The solubility of these thin films is profoundly influenced by the functional groups inherent in each constituent material within the film.

As outlined, all NLGGCs thin film series exhibited limited solubility in polar protic, polar aprotic, and non-polar solvents. The restricted solubility has arisen from differing chemical affinities between the solvents and the materials composing the film and the strength of interactions encompassing London dispersion forces, electrostatic bonds, and hydrogen bonding

(Zhao *et al.*, 2021). Remarkably, sulfuric acid, a potent degrading agent, has entirely dissolved all NLGGCs series.

Integrating nanolignin into the NLGGCs thin films is a pivotal factor influencing their solubility. Table 4 underscores that most NLGGCs thin films dissolved in strong (NaOH) bases and weak (ammonia solution), except for NLGGCs-59. This phenomenon stemmed from the substantial nanolignin content present in the thin film series. A plausible explanation was the interference of hydrophobic interactions between lignin nanoparticles and the gelatine network, hindering the dissolution of gelatine polymer chains in water (Aadil *et al.*, 2016). Núñez-Flores *et al.* (2012) have noted analogous observations in a study where the addition of lignin induced alterations in gelatine's helical structures, thereby influencing water solubility.

To investigate the swelling propensity of the NLGGCs thin film, a swelling analysis was conducted following immersion in distilled water for 30 minutes. The results, graphically represented in Figure 6, manifest a significant upsurge in swelling percentages during the initial phase, eventually reaching a saturation point where swelling degrees were stabilised and subsequently diminished, signifying an equilibrium in swelling. All NLGGCs thin films exhibited notable swelling, with percentages ranging from 241% to 249% after just 1 minute of immersion. The remarkable swelling properties are primarily attributed to the inherent hydrophilic properties of gelatine and glycerol, which facilitate water molecule binding via hydrogen bonding (Ciannamea *et al.*, 2018). Additionally, the porous structure within the gelatine network has contributed significantly to the high swelling ratios, allowing for enhanced water absorption (Kavoosi *et al.*, 2014). These augmented swelling properties hold promise for fruit coating applications, facilitating the complete removal of the thin film layer during fruit washing.

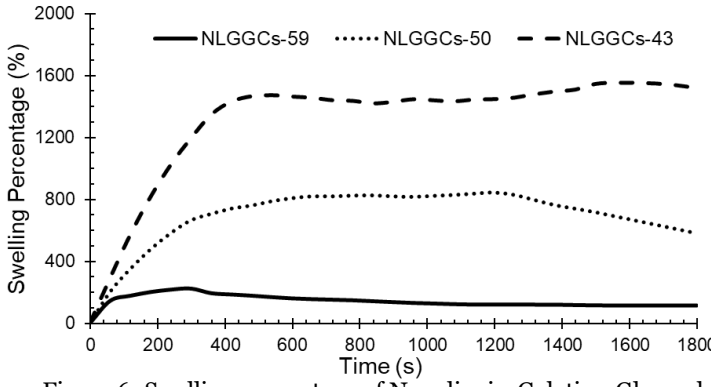

Figure 6: Swelling percentage of Nanolignin-Gelatine-Glycerol Composites in distilled water.

The influences of nanolignin composition on swelling properties proved noteworthy. The swelling graph depicted a declining water uptake trend with escalating nanolignin composition. NLGGCs-59 exhibited the lowest swelling percentage due to its elevated nanolignin content, while NLGGCs-43, characterised by the lowest nanolignin composition, registered the highest swelling percentage. This decrease in swelling propensity can be attributed to plausible interactions between the phenolic components of nanolignin and the amino groups within gelatine. These interactions have shielded the polar side chains of gelatine from water exposure, thus limiting interaction with water molecules (Bhat *et al.*, 2013). This section comprehensively explored the chemical durability of NLGGCs thin films, offering insights into their solubility across diverse solvents and swelling behaviour. These findings enhance our understanding of the films' chemical attributes and potential applications.

3.5 Pre-coated banana analysis

In this segment, the evaluation of pre-coating fruit shelf-life critically examined the NLGGCs thin film's potential utility in food coating applications. The results stemmed from a 10-day observation of bananas treated with the NLGGCs thin film solution, illustrated in Figure 7.

The outcome of this assessment demonstrated a noteworthy contrast between bananas coated with NLGGCs thin film series and the control sample. Following a 10-day storage period under room temperature conditions, the NLGGCs-coated fruit retained a fresh appearance devoid of any discernible juice leakage. In stark contrast, the control sample adhered to the standard banana ripening process expected in a non-coated scenario. This uncoated sample exhibited conspicuous mould growth, characterised by numerous black mould spots and a pronounced putrid odour

To summarise, this section provided compelling evidence of the potential advantages of utilising NLGGCs thin film in food coating applications, particularly in extending the shelf-life of coated fruits. The juxtaposition with a control sample enhanced the credibility of the observed effects. However, supplementing the qualitative observations with quantitative data, such as measurements of ripening rates, would further corroborate the findings and fortify the conclusions. Furthermore, these findings elucidated the practical implications and prospective applications within the food industry that would augment their overall impact.

To summarise, this section provided compelling evidence of the potential advantages of utilising NLGGCs thin film in food coating applications, particularly in extending the shelf-life of

Table 4: Solubility of Nanolignin-Gelatine-Glycerol Composites (NLGGCs) thin film in various solvent mediums at 25 °C

Sample Designation	Solvent	1.0 M H_2SO_4		1.0 M NaOH		Distilled Water	Hexane	DMF
	Medium Type	Strong acid	Acetic acid Weak acid	Strong base	Ammonia solution Weak base	Polar protic	Non-polar	Polar aprotic
NLGGCs-59	88.03	+	-	-	-	-	-	-
NLGGCs-50	92.67	+	-	+	+	-	-	-
NLGGCs-43	110.33	+	-	+	+	-	-	-

(+): Soluble, (-): Insoluble

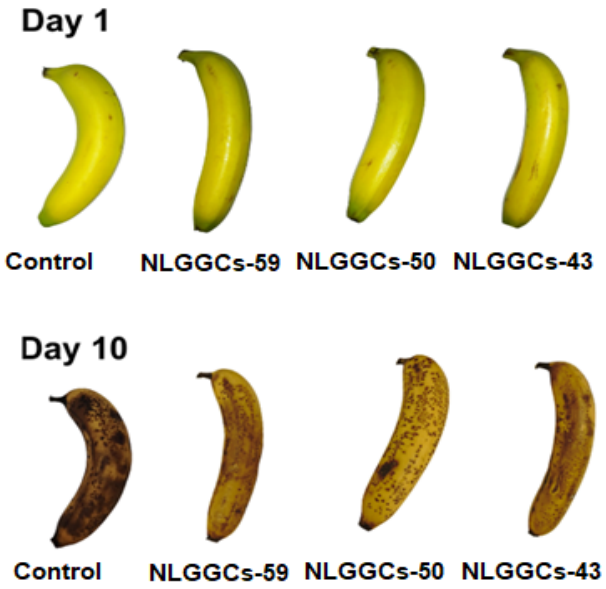

Figure 7: Pre-coated banana shelf-life analysis of Nanolignin-Gelatine-Glycerol Composite (NLGGCs) thin film after day 10.

coated fruits. The juxtaposition with a control sample enhanced the credibility of the observed effects. However, supplementing the qualitative observations with quantitative data, such as measurements of ripening rates, would further corroborate the findings and fortify the conclusions. Furthermore, these findings elucidated the practical implications and prospective applications within the food industry that would augment their overall impact.

4. Conclusion

In conclusion, this study successfully achieved its initial objective: creating thin films composed of the Nanolignin-Gelatine-Glycerol Composite (NLGGCs). Throughout this investigation, we thoroughly explored the impact of incorporating nanolignin into NLGGCs thin films using diverse analytical methods.

One notable discovery was the dark colouration exhibited by NLGGCs thin films, a direct result of nanolignin's presence. This characteristic was coupled with exceptional flexibility and elasticity within the film's textures. Furthermore, our FTIR characterisation unveiled the existence of crucial functional groups inherent to lignin nanoparticles. The peaks at 1222 and 1107 cm^{-1} are particularly noteworthy, which confirm the presence of phenolic OH groups and Ar-H syringyl groups, respectively.

The UV-Vis analysis underscored nanolignin's remarkable UV shielding properties, which are attributed to the presence of chromophore structures and contribute to the films' high opacity. The films also exhibited impressive thermal stability, as evidenced by TGA, with all NLGGCs series showcasing maximum degradation temperatures surpassing 300°C.

DSC unveiled intriguing findings, manifesting as dual Tg at 80°C and 60°C in certain NLGGCs series. This phenomenon signifies microphase separation and immiscibility between gelatine and nanolignin particles, shedding light on their intricate interactions.

Our investigations into chemical stability highlighted nanolignin's role in rendering the thin films' poor solubility in weak acids, polar protic, polar aprotic, and non-polar solvents. Additionally, the reduction in water uptake with increasing nanolignin composition in NLGGCs can be attributed to its inherent hydrophobic nature.

Finally, the analysis of banana ripening following pre-coating with NLGGCs thin film demonstrated substantial enhancements in the shelf-life of the treated bananas compared to control samples over 10 days. This outcome underscored the promising potential of NLGGCs thin films as safe, bio-based food coatings with exceptional properties, paving the way for future advancements in the food industry.

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7. Author contributions

Muhammad Bisyrul Hafi Othman, Muhamad Shirwan Abdullah Sani, Mohamad Nasir Mohamad Ibrahim and Nur Najmina Rafiae: Conceptualisation and resources.
Nur Najmina Rafiae and Najwa Najihah Mohamad Daud: Formal analysis and investigation.
Muhammad Bisyrul Hafi Othman and Nur Najmina Rafiae: Data curation, writing, and original draft preparation.
Muhammad Bisyrul Hafi Othman, Mohamad Nasir Mohamad Ibrahim and Muhamad Shirwan Abdullah Sani: Validation, editing, visualisation, and supervision.
Muhammad Bisyrul Hafi Othman: Project administration.
All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

8. Conflict of interest

We declare no conflict of interest.

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