
| RESEARCH ARTICLE**Sesame Seed Protein Bioplastic Films Modified with Maleic Acid: Structural, Morphological and Functional-Group Characterizations****Jaji Saheed Olatunde¹ ✉ Yusuf Shukurat Opeyemi², Lateef AbdulMujeeb³, Damazio Olanrewaju⁴, Oluwole Folorunsho⁵, Adedokun Aderinsola Omotayo⁶ and Jaji-Ibrahim Hawaw Kikelomo⁷**¹²³⁴⁵⁶*Chemical Sciences Department, Lagos State University of Science and Technology, Lagos State, Nigeria*⁷*National Open University of Nigeria (NOUN)***Corresponding Author:** Jaji Saheed Olatunde, **E-mail:** jaji101us@gmail.com, jaji.so@lasustech.edu.ng

| ABSTRACT

The development of protein-based bioplastics from renewable agricultural resources provides a promising route towards alternative polymeric materials derived from non-petroleum feedstocks. This study investigated the effect of maleic acid concentration on the structural, morphological, and spectroscopic characteristics of sesame (*Sesamum indicum* L.) protein bioplastic films. Sesame protein isolate was prepared through alkaline extraction followed by acid precipitation and subsequently modified with 4% and 5% maleic acid to obtain 4MX and 5MX formulations, respectively. The modified protein materials were plasticised with glycerol and processed into films before characterisation by X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier-transform infrared (FTIR) spectroscopy. XRD patterns exhibited broad diffraction features characteristic of predominantly disordered protein-based structures. The principal reflection shifted from $2\theta = 16.800^\circ$ for 4MX to 17.800° for 5MX, corresponding to calculated interplanar spacings of 5.2778 and 4.9835 Å, respectively. Thus, increasing maleic acid concentration produced a 1.000° shift in the principal reflection and an approximately 5.57% reduction in calculated d-spacing. SEM examination revealed concentration-dependent differences in surface organisation, including variations in surface texture, interconnected regions and voids. FTIR spectra retained characteristic protein amide absorptions, while the broad O–H/N–H stretching band shifted from 3261.46 cm^{-1} for 4MX to 3248.38 cm^{-1} for 5MX, representing a 13.08 cm^{-1} shift. The combined XRD, SEM and FTIR findings demonstrate that increasing maleic acid concentration from 4% to 5% alters the structural organisation, surface morphology and molecular environment of the sesame-protein matrix. However, the available data do not independently establish covalent esterification, increased crosslink density, or improved functional performance. Further mechanical, barrier, thermal and controlled chemical analyses are required to establish the structure–property relationships of the resulting films.

| KEYWORDS

Sesame protein isolate; maleic acid; bioplastic; X-ray diffraction; FTIR; scanning electron microscopy; biopolymer films.

| ARTICLE INFORMATION**ACCEPTED:** September 10, 2026 **PUBLISHED:** October 19, 2026 **DOI:** <https://doi.org/10.61424/ijans.v4i4.1096>

1. Introduction

The development of bio-based polymeric materials from renewable agricultural resources has become an important research direction because conventional petroleum-derived plastics are persistent in the environment and remain

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difficult to manage at end of life. Plant proteins are particularly attractive as biomaterial precursors because they contain chemically active groups and can form cohesive networks through hydrogen bonding, electrostatic interactions, hydrophobic associations and, where appropriate, covalent modification. Recent reviews identify plant proteins as promising feedstocks for biodegradable films and packaging because of their renewability, film-forming ability and capacity for functionalisation, although moisture sensitivity and brittleness remain important limitations (Chen et al., 2019; Hadidi et al., 2022; Pawde et al., 2025).

Sesame (*Sesamum indicum* L.) is an oilseed crop of considerable nutritional and industrial importance, and its protein-rich seed and meal fractions provide a potentially valuable renewable feedstock for biomaterial development. Sesame protein isolate has been shown to possess useful functional properties and a defined protein secondary-structure profile, with extraction behaviour influenced strongly by pH and ionic conditions (Achouri et al., 2012; Ghorbani et al., 2025). The utilisation of sesame-derived protein for bioplastic or film production is therefore attractive not only because it exploits a plant-derived polymeric resource, but also because it provides an avenue for adding value to an agricultural material that is primarily associated with oil production. Recent work on sesame protein films demonstrated that chemical modification with carboxylic acids can substantially alter film structure and performance, establishing sesame protein as a relevant matrix for acid-mediated modification (Sharma et al., 2018).

Protein-based films, however, are not inherently optimal materials. Strong protein–protein interactions can produce cohesive but relatively brittle matrices, whereas the hydrophilic character of many proteins promotes water uptake and poor water-vapour resistance. Plasticisers such as glycerol are consequently used to increase chain mobility and flexibility, although excessive plasticisation may increase hydrophilicity and free volume (Chen et al., 2019; Hadidi et al., 2022). Film properties are also strongly dependent on the processing route, protein concentration, pH, thermal treatment, plasticiser content and the nature and concentration of modifying agents (Zhang, 2010; Kaewprachu et al., 2016). These structure–processing relationships make controlled chemical modification important when developing protein-based bioplastics with reproducible properties.

Dicarboxylic acids are of particular interest as protein-modifying agents because their carboxyl groups can participate in hydrogen bonding and ionic interactions and, under suitable reaction conditions, may form covalent linkages with nucleophilic functional groups in biopolymer matrices. Evidence from carboxylic-acid-crosslinked biopolymers shows that such modification can change molecular interactions, swelling, mechanical behaviour and thermal characteristics (Awadhiya et al., 2016; Shen et al., 2015). Importantly, the presence of a new infrared absorption band should not be interpreted in isolation as definitive evidence of a particular chemical reaction; functional-group assignments must be considered together with the complete spectrum and appropriate controls (Barth & Zscherp, 2002).

Maleic acid is a dicarboxylic acid containing two carboxyl groups and an unsaturated C=C bond. Its use in the present system is therefore relevant to the broader strategy of modifying plant-protein matrices through multifunctional organic acids. Nevertheless, the chemical evidence for covalent crosslinking must be distinguished from evidence of simple incorporation, hydrogen bonding, or ionic association. This distinction is especially important for FTIR interpretation because protein amide bands, O–H/N–H stretching, and C–O/C–O–C vibrations can all respond to changes in molecular environment without uniquely identifying a reaction pathway (Barth & Zscherp, 2002).

Previous work by Sharma et al. (2018) is particularly relevant because it examined sesame-protein films crosslinked with malic, citric, and succinic acids at concentrations up to 5% and evaluated their barrier, mechanical, thermal, crystalline, and morphological properties. Their study showed that carboxylic-acid modification could produce measurable changes in the structure and performance of sesame-protein films, while the films remained predominantly amorphous. The present study extends this research direction by examining maleic-acid-modified sesame protein at 4% and 5% and focusing on the structural information obtainable from X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR).

The objective of this study was therefore to characterise sesame-protein bioplastic films modified with 4% and 5% maleic acid, designated 4MX and 5MX, respectively, and to determine how the increase in maleic-acid concentration affected their diffraction behaviour, surface morphology and infrared spectral characteristics. Particular emphasis was placed on distinguishing observations directly supported by the measured data from mechanistic interpretations that require further confirmation by complementary chemical, mechanical, barrier or thermal analyses.

2. Materials and Methods

2.1 Materials

Sesame seeds (*Sesamum indicum* L.) of Nigerian origin were obtained from a local market in Lagos, Nigeria. The materials and reagents reported in the original experimental record included n-hexane, sodium hydroxide, hydrochloric acid, maleic acid, glycerol, and distilled water. Analytical-grade reagents were used.

2.2 Preparation of Sesame Seed Flour

The sesame seeds were processed to obtain flour according to the procedure recorded in the experimental work. The resulting material was used for protein isolation.

2.3 Preparation of Sesame Protein Isolate

Defatting was performed using n-hexane at a flour-to-solvent ratio of 1:5 (w/v) with stirring for 6 h. For protein extraction, 360 g of defatted sesame flour was dispersed in 3600 mL of distilled water. The suspension was adjusted to pH 10.5 with sodium hydroxide and stirred for 30 min. The suspension was centrifuged at 5000 rpm for 15 min, and the supernatant was collected. The supernatant was adjusted to pH 4.5 with hydrochloric acid to precipitate the protein fraction and centrifuged. The recovered precipitate was washed three times with distilled water, adjusted to approximately pH 7 with sodium hydroxide, and dried at 40 °C.

2.4 Preparation of Maleic-Acid-Modified Sesame Protein

Maleic-acid solutions corresponding to 0–5% (w/v) were prepared and adjusted to pH 9 using 2 M sodium hydroxide. For the modified formulations reported here, 20 g of sesame protein was combined with 20 mL of the maleic-acid solution. The mixtures were heated at 90 °C with stirring at 500 rpm for 1 h 10 min. The treated materials were dried at 50 °C and subsequently annealed at 100 °C for 1 h. The formulations designated 4MX and 5MX corresponded to 4% and 5% maleic acid, respectively.

2.5 Film Preparation

For film formation, 10 g of the modified sesame-protein material was combined with water at 40% (v/w) and glycerol at 30% (v/w), based on the formulation described in the experimental record. The material was compression-moulded at 3.0 MPa and 130 °C for 2 min using a 9 × 9 × 2 mm mould. The moulded films were dried at 30 °C and conditioned in a desiccator for 48 h before characterisation.

2.6 X-ray Diffraction

XRD measurements were performed using a Rigaku D/Max-III C diffractometer (MiniFlex) with Cu K α radiation (λ = 1.542 Å), operated at 40 kV and 20 mA. The stated scan range was 2θ = 14–47°, with a step size of 0.020° and a counting time of 0.250 s per step. Savitzky–Golay smoothing was applied to the diffraction data.

Interplanar spacings were recalculated from the reported 2θ values using first-order Bragg diffraction, $n\lambda = 2d \sin \theta$, where $n = 1$ and $\theta = 2\theta/2$. The calculated values are reported to four decimal places.

2.7 Scanning Electron Microscopy

Surface morphology was examined using scanning electron microscopy at an accelerating voltage of 15 kV. Film specimens were mounted on aluminium stubs, sputter-coated with gold and examined at magnifications of approximately ×8000–×10,000, with working distances of 12–15 mm.

2.8 FTIR Spectroscopy

FTIR spectra were collected over 500–4000 cm^{-1} . For 4MX, the recorded instrument settings were sensitivity 62 and threshold 95.7; for 5MX, sensitivity 26 and threshold 103. Peak positions and transmittance values were recorded for functional-group interpretation.

2.9 Biodegradability

The evaluation of the biodegradability of the films used the soil burial degradation method as described by Martucci and Ruseckaite (2009). The films were cut with scissor (40 x 20 mm^2), in triplicate for each treatment, weighed, and placed on a mesh. Each set was dehydrated in the air oven at 60 $^{\circ}\text{C}$ for 6 h. Subsequently, the samples were placed in plastic containers containing soil at room temperature and then covered with more soil so that the films were 4 cm from the surface and exposed to the natural microbiota. Water (20 ml) was added every two days to maintain the soil moisture.

3. Results and Discussion

3.1 XRD characteristics

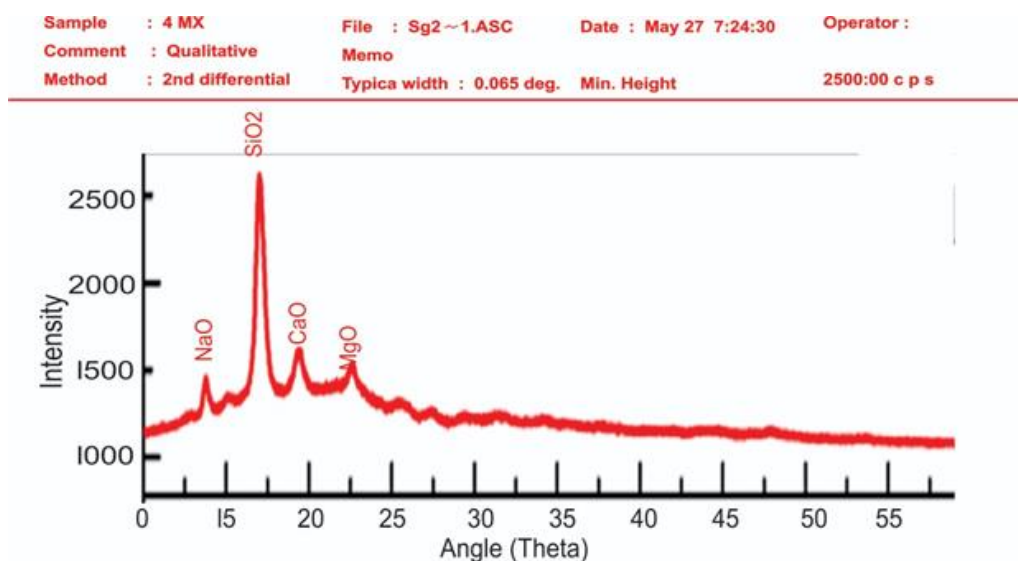


Figure1: XRD diffractogram of 4 MX

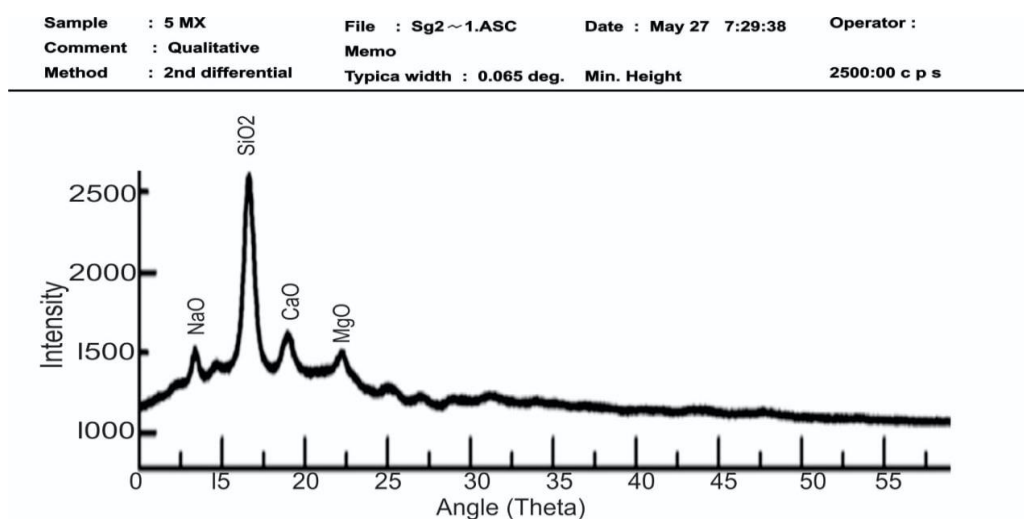


Figure2: XRD diffractogram of 5 MX

The reported XRD data for the two formulations are presented below. Because the original d-values were not consistent with Bragg's equation using the stated wavelength, the d-spacings have been recalculated from the measured 2θ values without changing the reported peak positions or intensities.

Both formulations exhibited broad diffraction features rather than a pattern dominated by numerous sharp reflections. The strongest reported reflection occurred at $2\theta = 16.800^\circ$ for 4MX and $2\theta = 17.800^\circ$ for 5MX, with both peaks reaching 2500 cps. Using the stated Cu K α wavelength of 1.542 Å and first-order Bragg diffraction, the corresponding d-spacings are 5.278 Å and 4.984 Å, respectively. The 1.000° shift towards higher 2θ therefore corresponds to a 0.294 Å decrease in the calculated d-spacing, or approximately 5.57%. This change is best interpreted as a modification of the average intermolecular packing or short-range structural organisation of the protein matrix rather than as definitive evidence of crystalline lattice compression.

The predominantly broad character of the patterns is consistent with the structural behaviour reported for sesame-protein films. Sharma et al. (2018) observed a broad principal reflection near 20° in carboxylic-acid-crosslinked sesame protein films and reported that the films remained predominantly amorphous. Protein-based materials commonly contain substantial disordered regions because protein chains adopt heterogeneous conformations and processing can disrupt native organisation (Barth & Zscherp, 2002). Consequently, the present XRD data support a change in molecular organisation following the increase from 4% to 5% maleic acid, but they do not by themselves establish a quantitative change in crystallinity or crosslink density.

The shift in the principal reflection is nevertheless structurally meaningful. A movement to higher 2θ is accompanied by a reduction in the calculated average interplanar spacing, indicating that the structural environment responsible for the broad diffraction maximum became somewhat more compact at the higher maleic-acid concentration. Similar structure–property changes are recognised when biopolymer networks are chemically modified because crosslinking or stronger intermolecular association can restrict chain mobility and alter packing (Awadhiya et al., 2016; Shen et al., 2015). In the present case, however, the XRD result should be described as evidence of altered organisation rather than proof that maleic acid produced a specific covalent crosslinking mechanism.

A further reflection was reported for 5MX at $2\theta = 47.980^\circ$. This value lies slightly outside the stated acquisition range of $14\text{--}47^\circ$. It is therefore inappropriate to assign this reflection to a specific phase or structural feature without checking the original raw instrument file or diffractogram. The discrepancy has been retained explicitly to preserve data integrity rather than silently deleting or altering the reported observation.

The original I/I₀ values were not retained in the corrected interpretation because their numerical values are not consistent with the reported cps intensities and the calculation basis was not specified. Relative intensity should normally be defined explicitly, for example as $I/I_{\text{max}} \times 100$, and calculated from the raw intensity values. Omitting an internally inconsistent derived column is preferable to presenting a numerical parameter whose calculation cannot be reproduced.

3.3 SEM Results and Discussion

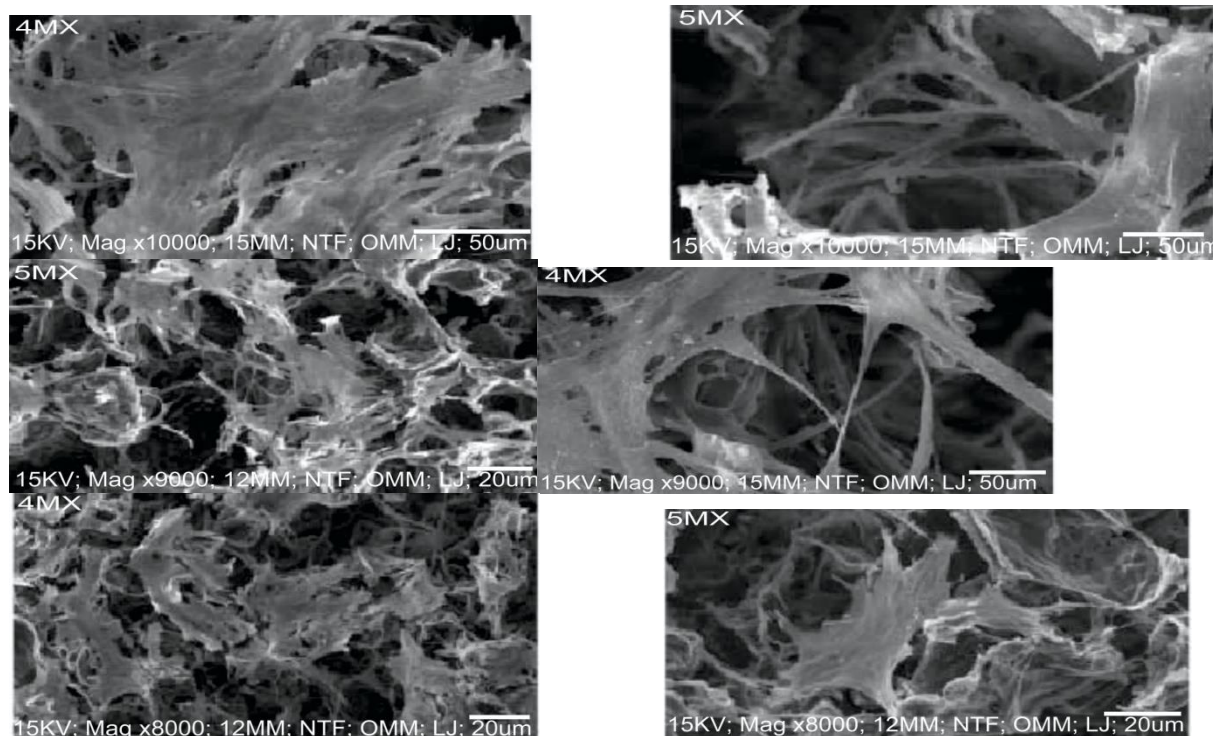


Figure 3: SEM micrographs of 4 MX and 5 MX

The SEM micrographs demonstrated differences in surface organisation between the maleic-acid-modified formulations. Both materials showed a heterogeneous protein-based morphology containing interconnected regions and voids, while the relative compactness and surface texture differed between 4MX and 5MX. Such differences are plausible consequences of changes in protein–protein and protein–modifier interactions during heating, moulding and drying. Protein–film morphology is known to depend strongly on formulation, plasticiser content, processing conditions and the degree of molecular association established during film formation (Kaewprachu et al., 2016; Hadidi et al., 2022).

The observed voids and irregular regions can arise from several mechanisms, including evaporation of entrapped water, local differences in component concentration, incomplete redistribution of the plasticiser, and rearrangement of the protein-rich phase during thermal processing. Thus, the morphological contrast between 4MX and 5MX is consistent with a concentration-dependent change in matrix organisation, but SEM alone cannot identify which molecular mechanism dominates. This is important because a visually denser surface does not necessarily translate directly into higher tensile strength, lower water-vapour permeability or greater thermal stability.

The role of glycerol should also be considered when interpreting the micrographs. Glycerol is widely used as a plasticiser in protein-based films because it increases chain mobility and flexibility, but its hydrophilic nature can also increase moisture sensitivity (Zhang, 2010; Chen et al., 2019). Consequently, the final morphology reflects not only maleic-acid treatment but also the combined effects of protein composition, glycerol, water content, and thermal compression. The present SEM observations therefore provide useful qualitative evidence of morphological differences, while quantitative structure–property claims require direct mechanical, water-vapour and thermal measurements. Overall, the SEM results support the conclusion that increasing maleic-acid concentration from 4% to 5% altered the physical organisation of the sesame-protein matrix. This interpretation is compatible with previous work on modified sesame-protein films, in which carboxylic-acid treatment produced changes in film morphology (Sharma et al., 2018). However, the present images should not be used alone to claim increased crosslink density or improved end-use performance; those conclusions require independent quantitative measurements.

3.4 FTIR Results

3.4.1 FTIR Peak Data for 4%M (4% Maleic Acid Sesame Seed Bioplastic)

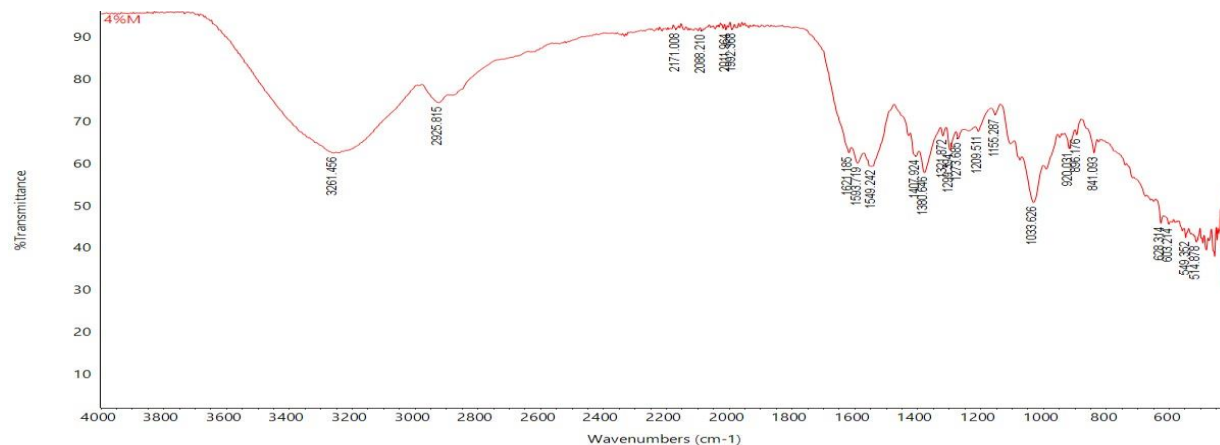


Figure4: FTIR spectrum of 4 %

3.4.2 FTIR Peak Data for 5%M (5% Maleic Acid Sesame Seed Bioplastic)

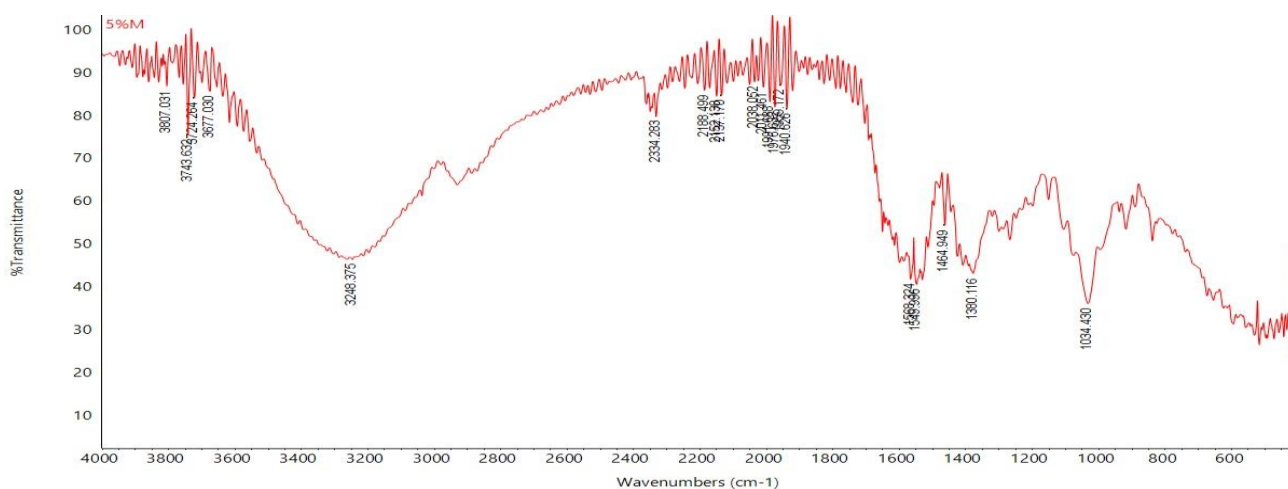


Figure4: FTIR spectrum of 5 %

4. Discussion

The FTIR spectra retained characteristic protein absorptions, particularly in the amide I and amide II regions. For 4MX, the principal amide I-region band occurred at 1621.19 cm^{-1} , while amide II features were observed at 1549.24 and 1593.72 cm^{-1} . In 5MX, amide II features occurred at 1550.00 and 1568.32 cm^{-1} . Amide I arises mainly from vibrations of the peptide backbone and is particularly sensitive to protein conformation and secondary structure, whereas amide II is associated principally with N–H bending and C–N stretching contributions (Barth & Zscherp, 2002). Retention of these bands indicates that the protein backbone remained spectroscopically evident after processing and maleic-acid treatment.

The broad O–H/N–H stretching band shifted from 3261.46 cm^{-1} in 4MX to 3248.38 cm^{-1} in 5MX, an absolute shift of 13.08 cm^{-1} , equivalent to approximately 0.40% relative to the 4MX position. A shift towards lower wavenumber can be consistent with a change in hydrogen-bonding environment because hydrogen bonding influences the vibrational frequency of O–H and N–H groups. Similar sensitivity of protein infrared bands to molecular interactions and conformational environment is well established (Barth & Zscherp, 2002). The present shift therefore supports altered intermolecular interactions at the higher maleic-acid concentration, but its magnitude cannot be used alone to quantify crosslink density.

The absorptions near 1033.63 cm^{-1} in 4MX and 1034.43 cm^{-1} in 5MX occur in the C–O/C–O–C vibrational region. These bands confirm the presence of oxygen-containing functionalities within the matrix, but a band near 1034 cm^{-1} is not, by itself, diagnostic of ester formation. This distinction is important because proteins and other matrix components can contribute C–O-containing vibrations, and infrared bands can overlap. In a closely related sesame-protein study, Sharma et al. (2018) used a band near 1700 cm^{-1} as supporting evidence for carboxylic-acid/protein crosslinking. Because the present dataset does not report a distinct new carbonyl band in the 1700–1750 cm^{-1} region, the current FTIR evidence should be interpreted conservatively as demonstrating changes in the chemical environment and incorporation/interaction of the modifier rather than unequivocally proving ester-bond formation.

The high-wavenumber features observed only in 5MX at 3677.03, 3724.26, 3743.63 and 3807.03 cm^{-1} require particular caution. They should not be assigned to 'maleic acid hydroxyl groups', because maleic acid is a dicarboxylic acid containing carboxyl O–H groups rather than alcohol hydroxyl groups. Free or weakly hydrogen-bonded O–H contributions may originate from residual moisture or other oxygen-containing components, but the available spectrum does not permit their source to be established with certainty. This restrained interpretation is preferable to assigning a specific unreacted species without an appropriate control spectrum.

The amide-region changes between 4MX and 5MX are also compatible with modification of the protein molecular environment. However, FTIR spectra of complex protein films contain overlapping contributions, and small shifts can arise from changes in hydrogen bonding, hydration, conformation, and local chemical environment rather than from a single reaction pathway (Barth & Zscherp, 2002). Accordingly, the FTIR results provide molecular-level evidence that the 5% maleic-acid treatment produced a different chemical environment from the 4% treatment, while the exact balance between covalent reaction, ionic association and hydrogen bonding remains to be established.

Taken together, the FTIR findings are consistent with concentration-dependent modification of the sesame-protein matrix. This conclusion agrees with the broader literature showing that chemical and physical treatments can alter the infrared response and functional behaviour of plant-protein films (Hadidi et al., 2022; Chen et al., 2019). Nevertheless, stronger evidence for a specific crosslinking reaction would require comparison with an untreated protein control, maleic acid alone, and preferably complementary analytical evidence capable of distinguishing covalent ester formation from non-covalent association.

4.1 Integrated Interpretation

Taken together, the XRD, SEM, and FTIR results indicate that increasing maleic-acid concentration from 4% to 5% changed the structural and molecular environment of the sesame-protein matrix. XRD showed a 1.000° shift in the principal diffraction maximum and a corresponding 5.57% decrease in calculated d-spacing; SEM showed concentration-dependent differences in surface organisation; and FTIR showed a 13.08 cm^{-1} shift in the broad O–H/N–H stretching region together with changes in the amide-region features. These independent observations are mutually compatible with altered molecular association and matrix organisation following the increase in maleic-acid concentration.

The findings also agree with published evidence that modification of protein-based films can change their morphology, molecular interactions and physical performance. In sesame-protein films, Sharma et al. (2018) demonstrated that carboxylic-acid treatment altered crystalline and morphological characteristics, while broader studies show that plasticisation and chemical modification are important strategies for balancing the strength, flexibility and moisture sensitivity of protein-based materials (Kaewprachu et al., 2016; Hadidi et al., 2022; Pawde et al., 2025).

Importantly, the present dataset does not justify a quantitative claim of increased crosslink density, improved mechanical strength, reduced water-vapour permeability or enhanced thermal stability. Such conclusions require direct measurements. The strongest conclusion supported by the available evidence is that 5% maleic-acid

treatment produced measurable changes in diffraction, morphology and infrared spectral characteristics relative to 4% treatment, indicating a changed structural environment within the sesame-protein bioplastic matrix.

4.2 Biodegradability

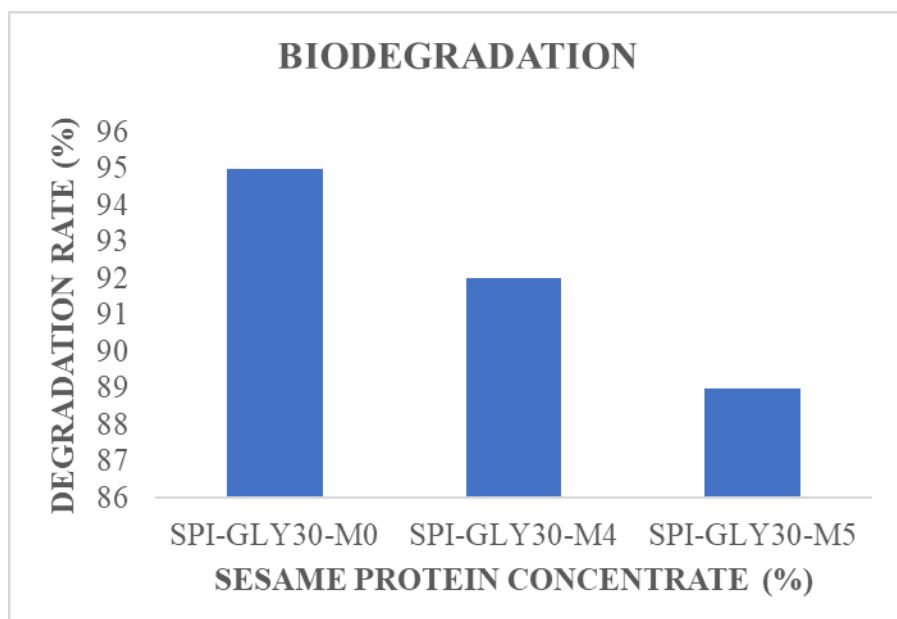


Figure 6: The biodegradability of the SPI Films

Materials that can disintegrate or decompose naturally into biogases and biomass (mainly carbon dioxide and water) when exposed to a microbiological environment and humidity are healthy for our environment. Biodegradable bioplastics are completely broken down by microorganisms, without leaving hazardous residues that can be seen. (Jain et al. 2010). The biodegradation test (Figure 6) showed that SPIF with either castor oil or glycerol degraded within 2 weeks. By the 12th day of soil burial, both glycerol- and castor oil plasticized films were 100 % biodegraded. This further confirmed the potentiality of castor oil plasticized SPIF as good packaging films.

5. Conclusion

Sesame-protein bioplastic films modified with 4% and 5% maleic acid were prepared and characterised using XRD, SEM and FTIR. The XRD data showed broad diffraction features consistent with a predominantly disordered/amorphous protein-based matrix. Recalculation using Bragg's law and the reported Cu K α wavelength gave d-spacings of 5.278 Å for the 4MX principal peak at 16.800° and 4.984 Å for the 5MX principal peak at 17.800°. FTIR analysis showed characteristic protein amide bands and concentration-dependent changes in the broad O–H/N–H stretching region, while SEM revealed differences in surface morphology between the formulations.

The results support the conclusion that maleic acid modifies the structural and molecular environment of the sesame-protein matrix. However, esterification, increased cross-link density, and improved functional performance should not be claimed as unequivocally established by the present XRD, SEM, and FTIR data alone. Additional analyses, particularly mechanical testing, water-vapour permeability, thermal analysis, controlled FTIR comparison with an untreated film, and confirmation of the reported 47.980° XRD reflection from the raw instrument data, would strengthen the material-performance assessment.

6. Recommendations

Future work should include an untreated sesame-protein control and a broader maleic-acid concentration range to establish concentration–structure relationships. Tensile strength, elongation at break, water absorption, water-vapour permeability and thermal stability should be measured directly. The chemical nature of the proposed

crosslinking should be investigated using appropriate controls and, where possible, complementary spectroscopic or chemical analyses. The 47.980° XRD reflection reported for 5MX should be verified against the original raw diffractogram because it lies outside the stated 14–47° acquisition range.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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Artificial Intelligence (AI) Use Disclosure: The authors declare that no artificial intelligence tools were used in the preparation of this manuscript.

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