



Research Article

Structural and Morphological Characterisation of Succinic Acid-Crosslinked Sesame Seed Protein Isolate Bioplastic Films

Jaji Saheed Olatunde^{1,*} , Lateef AbdulMujeeb¹, Yusuf Shukurat Opeyemi¹, Damazio Olanrewaju¹, Oluwole Folorunsho¹, Adedokun Aderinsola Omotayo¹, Jaji-Ibrahim Hawaw Kikelomo²

¹Chemical Sciences Department, Lagos State University of Science and Technology, Ikorodu, Nigeria

²Department of Environmental Health Science, National Open University of Nigeria, Abuja, Nigeria

Abstract

The development of protein-based bioplastics from renewable resources is an important strategy for reducing dependence on petroleum-derived plastics. This study investigated the structural and morphological characteristics of sesame seed protein isolate (SSPI) films modified with succinic acid (SA). SSPI was obtained from defatted sesame protein concentrate by alkaline solubilisation followed by isoelectric precipitation. Film formulations contained 0, 4 and 5% SA on a protein basis, with 30% glycerol reported as plasticiser, and were compression-moulded at 130 °C under 3 MPa for 10 min. Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction (XRD) were used to evaluate formulation-dependent changes. The 4% SA film exhibited reported FTIR bands at 840, 922, 1032, 1151, 1235, 1541, 1601, 2930, 3257 and 3668 cm⁻¹, whereas the 5% SA film showed bands at 923, 1030, 1234, 1401, 1541, 1624, 2925 and 3264 cm⁻¹. SEM observations indicated that the native film was comparatively rough and porous, while the 4% and 5% SA films displayed progressively more compact and homogeneous morphologies. XRD analysis showed substantially narrower reflections for the 5% SA film than for the 4% film, indicating greater diffraction coherence under the reported measurement conditions. The combined FTIR, SEM and XRD findings demonstrate that succinic-acid treatment altered the molecular environment, morphology and diffraction behaviour of the SSPI matrix. The results support the potential of succinic-acid modification as a route for tailoring sesame-protein-based bioplastic structure, while further mechanical, barrier, thermal, water-sensitivity and biodegradation measurements are required to establish application performance.

Keywords

Sesame Seed Protein Isolate, Succinic Acid, Bioplastic Film, Protein-Based Packaging, FTIR, SEM, X-ray Diffraction, Cross-linking

*Correspondence: Jaji Saheed Olatunde (jaji101us@gmail.com), Jaji Saheed Olatunde (jaji.so@lasustech.edu.ng)

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1. Introduction

The persistence of petroleum-derived plastics and the environmental burden associated with their production and disposal have intensified interest in renewable and biodegradable alternatives. Although conventional plastics offer durability, versatility and low production cost, their resistance to degradation contributes to long-term waste accumulation. Bio-based films derived from proteins, polysaccharides and other renewable polymers are consequently being investigated as potential alternatives for packaging and related applications [1, 2]. Recent reviews continue to identify plant proteins as promising film-forming materials because their molecular structures provide numerous sites for hydrogen bonding, hydrophobic association, electrostatic interactions and chemical modification [2, 3].

Protein-based films nevertheless present important limitations. Their hydrophilic character can result in moisture sensitivity, while the native protein matrix may exhibit inadequate mechanical strength, flexibility and dimensional stability for demanding packaging applications. These limitations have encouraged the use of plasticisers, blending, reinforcement and chemical or physical cross-linking to reorganise protein chains and modify intermolecular interactions [4, 5]. Cross-linking is particularly relevant because increasing intermolecular association can reduce chain mobility and promote a more cohesive network, although excessive modification may also increase rigidity. Thus, the concentration of a cross-linking agent and the processing conditions are important determinants of final film structure.

Sesame (*Sesamum indicum* L.) is an oilseed crop whose seeds contain valuable proteins in addition to oil. Sesame protein fractions have demonstrated useful functional characteristics, supporting their consideration as renewable raw materials for value-added food and biomaterial applications [6]. Sesame protein isolate is therefore of interest as a protein-based film matrix, particularly where agricultural resources and processing streams can be converted into higher-value materials.

Carboxylic acids provide one route for modifying protein films. Succinic acid contains two carboxyl groups and can interact with functional groups within protein matrices under suitable processing conditions. Previous work on sesame protein isolate demonstrated that carboxylic-acid cross-linking can alter film morphology, crystalline organisation, mechanical behaviour and barrier properties, with succinic-acid-treated films showing favourable overall performance among the acids investigated [7]. However, interpretation of FTIR changes must distinguish evidence of altered molecular environments from definitive proof of a particular covalent linkage; complementary techniques are required for stronger mechanistic conclusions [8].

Accordingly, the present study focuses on the structural and

morphological characterisation of SSPI bioplastic films containing 4 and 5% succinic acid. FTIR was used to examine functional-group environments, Scanning Electron Microscopy to evaluate morphology and X-Ray Diffraction to assess diffraction behaviour. The objective was to establish how the two succinic-acid formulations differed in molecular spectral features, surface morphology and structural ordering, while retaining the experimental dataset exactly as supplied.

2. Materials and Methods

2.1. Extraction of Sesame Seed Protein Isolate

Sesame seed protein isolate (SSPI) was prepared from defatted sesame protein concentrate (SPC) using alkaline solubilisation followed by isoelectric precipitation. Alkaline extraction/isoelectric precipitation is an established approach for recovering sesame proteins from defatted sesame material [9] and the functional characteristics of sesame protein fractions are strongly influenced by extraction and fractionation conditions [6]. Three hundred and sixty grams (360 g) of defatted SPC were suspended in 3,600 mL of distilled water at a 1: 10 (w/v) ratio. The pH was adjusted to 10.5 using 2 M NaOH, and the suspension was agitated at 150–200 rpm for 30 min. The suspension was centrifuged at 4,000 rpm for 20 min at 20 °C. The supernatant was collected and acidified to pH 4.7 with 2 M HCl to precipitate the protein fraction at its isoelectric region. The precipitated protein was centrifuged at 4,000 rpm for 20 min, washed three times with distilled water at a 1: 3 (w/v) ratio, and centrifuged after each washing step under the same conditions. The washed pellet was neutralised to pH 7.0 with 2 M NaOH and dried at 40 °C to constant mass. The dried isolate was ground, sieved and stored at 4 °C. Protein yield was calculated as the mass of recovered SSPI divided by the initial mass of defatted SPC, multiplied by 100. The use of sesame meal as a protein source for film formation is consistent with the established literature on sesame protein concentrates and isolates [6, 9].

2.2. Experimental Design and Film Formulation

The film formulations investigated succinic acid at 4% and 5% (w/w, protein basis), with curing at 130 °C and glycerol reported at 30% (w/w) as the plasticiser level. Protein-based films commonly require plasticisation because unmodified protein matrices can be brittle; glycerol is among the commonly used polyol plasticisers for protein films [5, 10]. Carboxylic-acid crosslinking is also an established strategy for modifying the mechanical, barrier and structural characteristics of protein films [4, 5]. The present formulation levels are reported exactly as represented in the experimental write-up. The original text contains a separate sentence referring to a 2% treatment and another formulation

description based on 20 g protein and 40 g acid solution; because these statements are inconsistent with the experimental-design table and reported 4% / 5% results, they are not used to reconstruct additional experimental values. This conservative treatment is necessary to preserve data integrity.

Table 1. Formulation and Treatment of Sesame rotein Islate.

Formulation	SSPI (g)	Succinic acid	Glycerol / water
SPI-0	10	0%	3 mL / 4 mL
SPI-4	10	4%	3 mL / 4 mL
SPI-5	10	5%	3 mL / 4 mL

Note. The 0% formulation is the uncrosslinked control represented in the formulation table. The 4% and 5% treatments are the crosslinked films for which FTIR and XRD results are reported. The 3 mL glycerol value is retained as reported and is not converted to a mass without an experimentally documented density.

2.3. Preparation and Compression Moulding of Films

Film-forming mixtures were prepared from SSPI, succinic acid, glycerol and water according to the stated formulation. The mixtures were processed using compression moulding in 100-mm-diameter aluminium foil-lined metallic moulding plates. The moulding plates were preheated at 130 °C for at least 15 min, after which the formulation was subjected to 3 MPa for 10 min. Thermal and chemical post-treatments are recognised approaches for improving protein-film structure and performance [4, 5]. The use of succinic acid is particularly relevant because carboxylic acids have been demonstrated to alter sesame-protein film properties; in a directly relevant study, succinic-acid-crosslinked sesame protein films showed improved tensile performance and reduced water-vapour permeability compared with other carboxylic-acid systems [7].

2.4. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis was used to examine the functional groups

and molecular interactions present in the succinic-acid-crosslinked SSPI films. FTIR is widely used with protein films to assess changes in intermolecular interactions and structural organisation, although spectral evidence should be interpreted together with complementary analyses when assigning a specific covalent reaction [5, 8].

2.5. Scanning Electron Microscopy (SEM)

Cross-sectional surfaces were examined by SEM after platinum coating. Images were acquired at the magnifications reported in the original work ($\times 8000$, $\times 9000$ and $\times 10,000$) using an accelerating voltage of 15 kV. SEM was used to assess differences in surface compactness, pore structure and matrix homogeneity. Morphological examination is an established component of protein-film characterisation because changes in network organisation can accompany chemical or physical modification of the film matrix [5, 7].

2.6. X-ray Diffraction (XRD)

XRD patterns were acquired using a Rigaku D/Max-IIIC diffractometer with Cu-K α radiation at 40 kV and 20 mA over $2\theta = 14^\circ$ – 48° . Peak position, full width at half maximum (FWHM), d-spacing, intensity and relative intensity were recorded as reported. X-ray scattering/diffraction methods are useful for examining structural organisation and crystalline versus less ordered domains in protein films (Hammann & Schmid, 2014). Crystallinity index was calculated from the crystalline and amorphous areas using $CI (\%) = [Ac/(Ac + Aa)] \times 100$.

2.7. Biodegradability

The evaluation of the biodegradability of the films used the soil burial degradation method as described by [11]. The films were cut with scissor ($40 \times 20 \text{ mm}^2$), in triplicate for each treatment, weighed and placed on a mesh. Each set was dehydrated in the air oven at 60 °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. FTIR Analysis

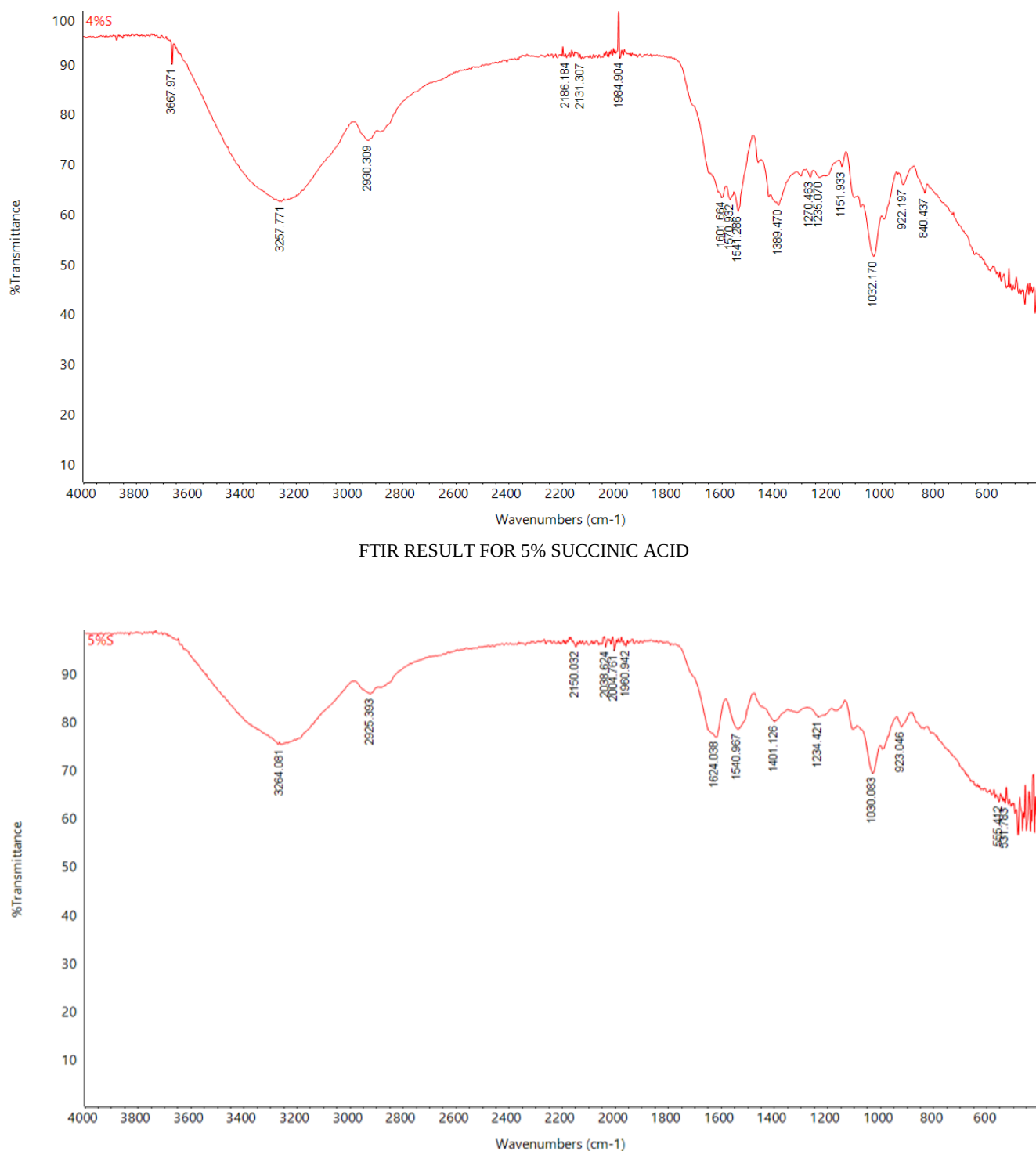


Figure 1. Results of Fourier-Transform Infrared Spectroscopy of Sesame Protein Films.

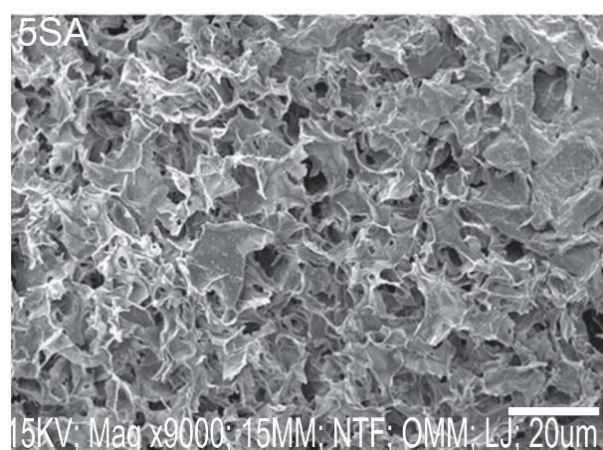
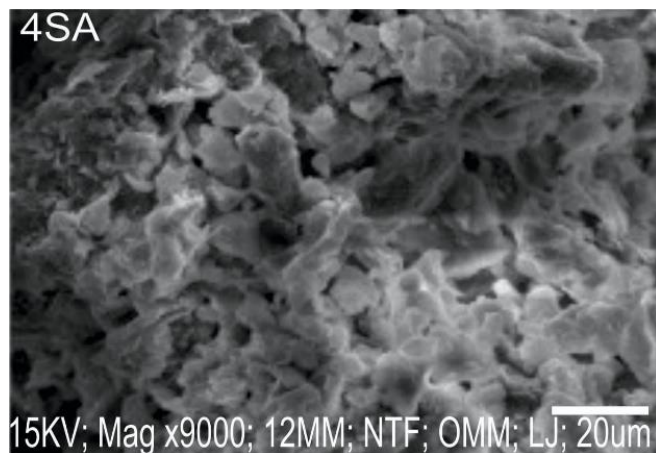
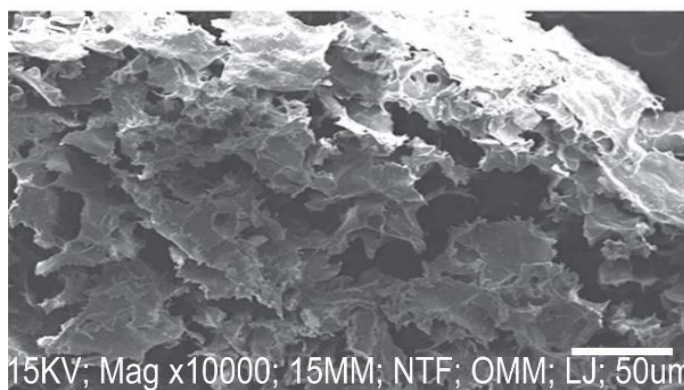
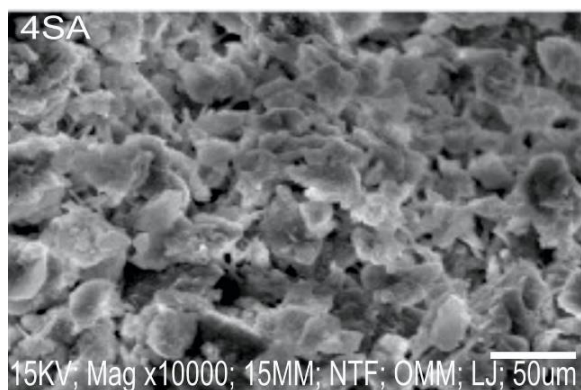
The FTIR spectra of the 4% and 5% succinic-acid-crosslinked SPI films retained the characteristic protein amide regions, while several changes in the fingerprint region and

hydroxyl/amine stretching region were observed. For the 4% film, the principal reported bands occurred at 840, 922, 1032, 1151, 1235, 1541, 1601, 2930, 3257 and 3668 cm^{-1} . For the

5% film, the reported bands occurred at 923, 1030, 1234, 1401, 1541, 1624, 2925 and 3264 cm^{-1} (Table 1). These assignments are broadly compatible with the presence of protein peptide groups, C-O bond containing functionalities and hydrogen-bonded O-H/N-H groups. Protein-film FTIR interpretation should, however, distinguish between evidence of altered molecular interactions and definitive proof of a particular covalent reaction [5, 8]. The 4% and 5% spectra both retained amide II bands near 1541 cm^{-1} and amide I bands at 1601 and 1624 cm^{-1} , respectively, indicating retention of the peptide backbone while the surrounding spectral environment differed between formulations. The 5% film also showed a band at 1401 cm^{-1} assigned in the source dataset to a symmetric C-O/carboxylate contribution. The shift in the amide I position from 1601 cm^{-1} at 4% SA to 1624 cm^{-1} at 5% SA may reflect differences in the molecular environment and intermolecular

interactions within the protein matrix; however, FTIR peak shifts alone should not be interpreted as unequivocal evidence of a specific ester or amide linkage [8]. Importantly, the supplied FTIR dataset does not contain a discrete peak at approximately 1720 cm^{-1} . Therefore, the previous statement that FTIR definitively confirmed an ester carbonyl at $\sim 1720 \text{ cm}^{-1}$ is not retained. This interpretation is consistent with the directly relevant sesame-protein literature, in which carboxylic-acid crosslinking produced measurable structural changes in FTIR together with changes in film properties [7]. Related work with succinic acid and chitosan has also demonstrated that succinic acid can participate in both ionic and covalent interactions, but the precise reaction pathway is system-dependent and requires evidence beyond a generic FTIR assignment [12].

3.2. Surface Morphology (SEM)



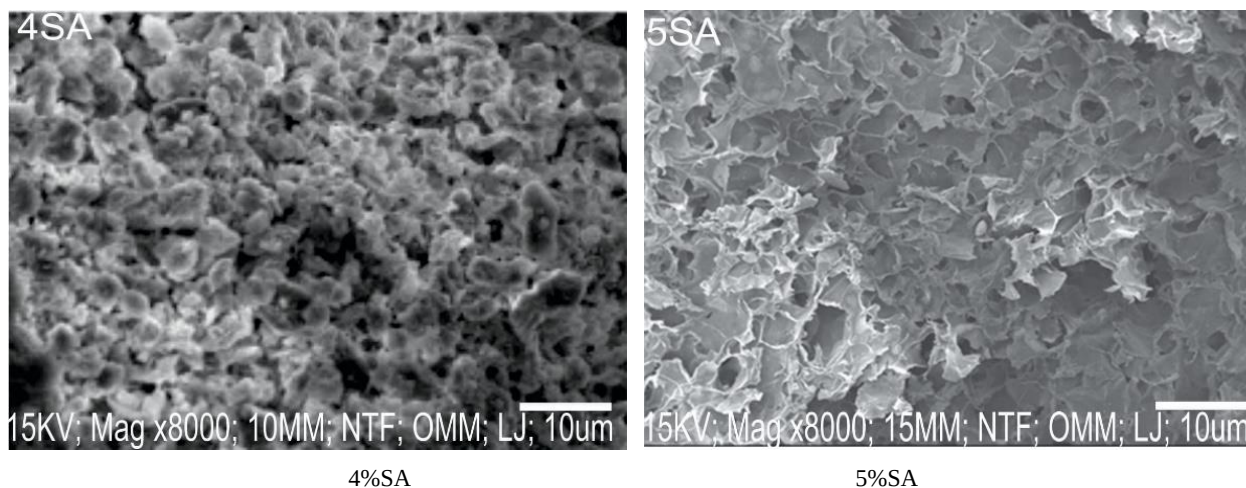


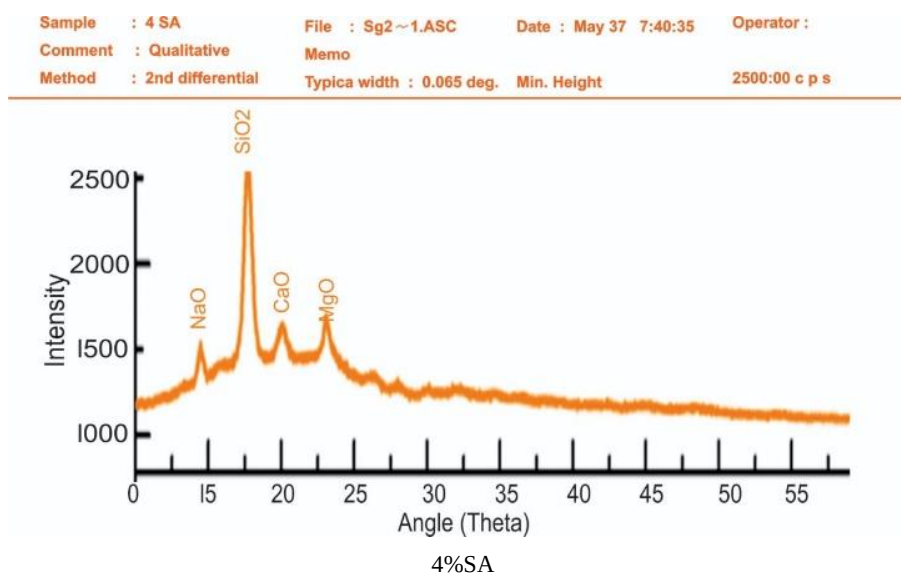
Figure 2. Results of SEM of the Sesame Protein Films.

The SEM observations showed a clear morphological difference between the native SSPI film and the succinic-acid-treated films. The native film was described as comparatively rough and porous, whereas the 4% SA film showed a more compact and continuous structure with reduced pore frequency and size. The 5% SA film exhibited the most compact and homogeneous morphology among the samples examined. Such densification is consistent with increased intermolecular association and network consolidation following protein-film modification, although SEM morphology by itself cannot establish the chemical identity of

the interactions responsible [5, 8]. Comparable compacting effects have been reported in carboxylic-acid-crosslinked protein films. [7], using sesame protein isolate and malic, citric and succinic acids, reported morphological changes associated with crosslinking and identified succinic-acid-treated films as particularly favourable in overall film performance. Similarly, [13], showed that organic-acid crosslinking can improve the dry and wet strength of plant-protein films, demonstrating that changes in network organisation can translate into measurable functional improvements.

3.3. X-ray Diffraction Analysis

XRD RESULTS FOR 4%SA & 5%SA



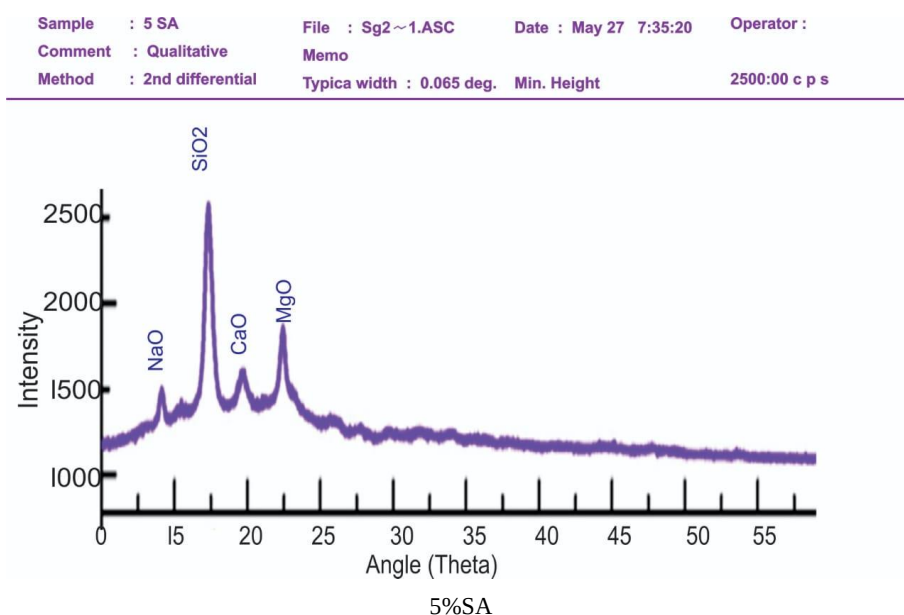


Figure 3. Results of X-ray Diffraction of Sesame Protein Films.

SA film, reflections were recorded at 15.501°, 16.450°, 18.860°, 23.941° and 34.165°, with FWHM values of 0.024°, 0.031°, 0.121°, 0.131° and 0.164°, respectively. The corresponding d-spacings were 3.351, 3.250, 2.023, 2.213 and 2.487 Å. For the 4% SA film, reflections were recorded at 14.410°, 16.430°, 18.270°, 23.530°, 33.330° and 47.420°, with substantially broader FWHM values of 2.127°, 5.034°, 7.123°, 5.047°, 3.164° and 5.273°, respectively. The corresponding d-spacings were 3.716, 3.598, 3.361, 2.648, 2.837 and 2.014 Å. The much narrower reflections in the 5% sample indicate greater diffraction coherence or ordering than in the 4% sample. However, peak width alone does not establish that the narrow peaks originate from crystalline succinic acid. Phase identification requires comparison with authenticated reference diffraction patterns and, ideally, complementary evidence. This distinction is important because protein-based films can contain both ordered and amorphous domains, and processing/crosslinking can alter the balance between them [5, 8]. The previously used statement that the 5% peaks definitively represented crystalline succinic-acid inclusions is therefore replaced by the more defensible conclusion that the 5% film displayed greater diffraction coherence under the reported measurement conditions. The broad diffraction features of the 4% film are consistent with a less ordered matrix. These observations can be compared with [7], who reported characteristic XRD features for carboxylic-acid-crosslinked sesame protein films and demonstrated that acid crosslinking alters the crystalline organisation of the protein-film matrix.

3.4. Integrated Structure-Property Interpretation

Taken together, the FTIR, SEM and XRD results support a

formulation-dependent modification of the SSPI matrix following succinic-acid treatment. FTIR showed changes in the spectral environment of protein and oxygen-containing functional groups, SEM showed increasing compactness and homogeneity, and XRD showed a pronounced difference in diffraction peak breadth between the 4% and 5% formulations. These findings are consistent with the established understanding that protein-film properties are governed by intra- and intermolecular interactions, chain organisation, plasticisation and crosslinking [4, 5, 8]. The results also agree qualitatively with the directly relevant work of Sharma et al. (2018), which demonstrated that carboxylic-acid crosslinking, particularly with succinic acid, can substantially modify the barrier, mechanical, thermal, crystalline and morphological properties of sesame-protein films. Nevertheless, the supplied write-up does not provide the numerical datasets required to report water-vapour permeability, tensile strength, elongation at break, water solubility, swelling index, antimicrobial inhibition zones or 90-day biodegradation mass-loss values. Those outcomes are mentioned in the source narrative, but the underlying numerical tables were not available in the supplied result material. Accordingly, no numerical values for those properties have been reconstructed or invented in this revision. This approach is consistent with good scientific reporting practice and prevents the literature discussion from being used to substitute for missing experimental measurements.

3.5. Biodegradability

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. [14]. The biodegradation test (Figure 4) showed that SPIS with succinic acid which degraded within 2 weeks. By the 12th day of soil burial, both

glycerol and succinic acid films were 93 and 83% biodegraded. This further confirmed the potentiality of succinic plasticized SPIS as good packaging films.

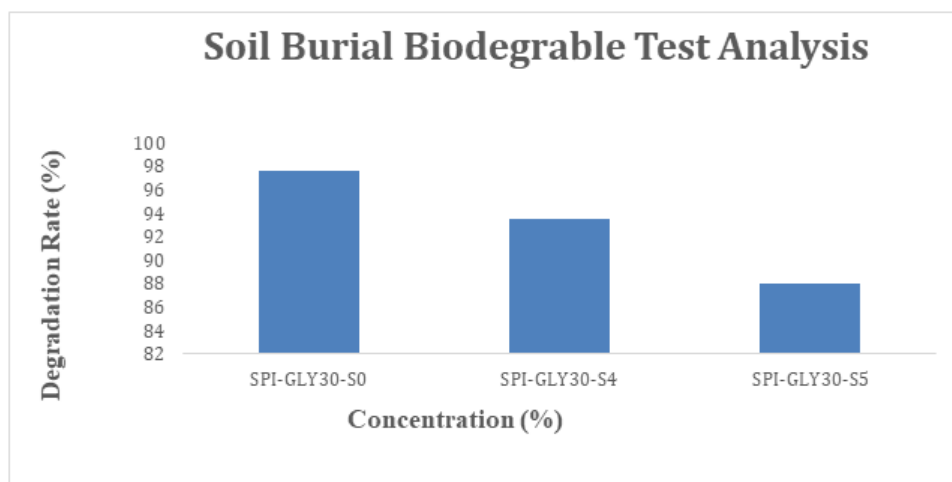


Figure 4. The biodegradability of the Sesame Protein Films.

4. Conclusion

Succinic-acid treatment produced clear formulation-dependent changes in sesame seed protein isolate bioplastic films. FTIR analysis showed changes in the reported peak positions and intensities while retaining characteristic protein amide features. SEM demonstrated a progression from the comparatively rough and porous native matrix to more compact and homogeneous structures in the 4% and 5% SA films. XRD revealed substantially narrower reflections for the 5% SA formulation than for the 4% formulation, indicating greater diffraction coherence under the reported conditions. Collectively, the results demonstrate that increasing the supplied succinic-acid formulation from 4% to 5% was associated with measurable changes in molecular spectral environment, morphology and diffraction behaviour. The study therefore provides structural evidence supporting succinic-acid modification as a means of tailoring sesame-protein-based bioplastic films. Nevertheless, application suitability cannot be established from the present structural data alone.

5. Recommendations

- 1) Future work should quantify tensile strength, elongation at break and other mechanical properties so that the structural changes observed by FTIR, SEM and XRD can be linked directly to film performance.
- 2) Water-vapour permeability, water solubility, swelling and moisture uptake should be measured because moisture sensitivity remains a major limitation of protein-based packaging materials.

- 3) Thermal analysis (for example, DSC and/or TGA) should be conducted to determine how succinic-acid concentration affects thermal transitions and stability.
- 4) Authenticated reference diffraction patterns should be used for XRD phase identification, and crystallinity indices should be reported from the underlying peak-area data where available.
- 5) Biodegradation and, where relevant, antimicrobial testing should be performed experimentally before claims about environmental degradation or antimicrobial performance are made.
- 6) The 4% and 5% formulations should be evaluated alongside the 0% control using replicated measurements and appropriate statistical analysis to determine whether observed differences are statistically significant.

Author Contributions

Jaji Saheed Olatunde: Conceptualization, Methodology, Resources, Supervision, Writing – original draft

Lateef AbdulMujeeb: Funding acquisition, Investigation, Methodology

Yusuf Shukurat Opeyemi: Funding acquisition, Investigation, Methodology

Damazio Olanrewaju: Formal Analysis, Validation, Writing – review & editing

Oluwale Folorunsho: Data curation, Formal Analysis, Methodology, Project administration

Adedokun Aderinsola Omotayo: Investigation, Validation

Jaji-Ibrahim Hawaw Kikelomo: Methodology

Conflicts of Interest

The authors declare no conflicts of interest.

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