FRI-LCR-P-1-CT(R)-07

OPTIMIZATION OF PROPERTIES OF GELATIN-BASED BIOFILMS WITH VARYING GLYCEROL CONTENT

Assoc. Prof. Dimitrina Kiryakova, PhD
Senior Assist. Ganka Kolchakova, PhD
Department of Material Science,
Burgas State University
E-mail: dskiryakova@abv.bg
E-mail: gkolchakova@gmail.com

Senior Assist. Antoniya Ilieva, PhD
Departament of Chemical Technologies,
Burgas State University
E-mail: a.s.ilieva@abv.bg

Student Krasimir Petrov, Engineering Materials
Department of Material Science,
Burgas State University
E-mail: krasi187@mail.bg

Abstract: *In the present study, an approach was applied to evaluate and optimize the physicomechanical properties of pre-prepared gelatin biofilms with different glycerol contents (0–75%). Experimental data on tensile strength, elongation at break, Young's modulus, Shore hardness, and film thickness were used to build correlation and regression relationships, which allowed visualizing the relationships between the individual indicators. The analysis confirmed the strong negative relationship between strength and elongation, as well as a linear relationship between strength and Young's modulus, which turned out to be the most reliable predictor of the tensile strength of the films. The optimal glycerol content to achieve a balance between mechanical strength (>10 MPa) and sufficient flexibility (>70% elongation) of the biofilms is in the range of 25–40%. The obtained results demonstrate that the applied approach is an effective tool for rational design of biodegradable gelatin-based materials, which allows optimization of properties depending on the application.*

Keywords: *Gelatin, Glycerol, Biofilms, Properties, Optimization.*

INTRODUCTION

In response to the growing environmental challenges associated with conventional plastics, especially in the packaging industry, significant research efforts are being directed towards developing sustainable alternatives based on biodegradable materials from renewable sources. (Islam, M., Xayachak, T., Haque, N., Lau, D., Bhuiyan, M., & Pramanik, B., 2024) (Harrazi, N., Özbek, H. N., Yanık, D. K., Zaghbib, I., & Göğüş, F., 2024). Gelatin stands out as a remarkable biopolymer due to its numerous advantages (Alipal, J., Mohd Pu'ad, N., Lee, T., Nayan, N., Sahari, N., Basri, H., Idris, M., & Abdullah, H., 2021). It is widely available, biodegradable, biocompatible, and has the ability to form films (Abdullah, J., Jiménez-Rosado, M., Guerrero, A., & Romero, A., 2022). Despite the above-mentioned benefits, gelatin-based film has some significant disadvantages, such as high water olubility, hygroscopicity, and low mechanical properties, which limit its application in food packaging. (Mousazadeh, S., Ehsani, A., Moghaddas Kia, E., & Ghasempour, Z., 2021). To overcome these limitations, various strategies are used, with one of the most effective and widely applied approaches being the incorporation of plasticizers. Glycerol ranks among the most common and studied plasticizers due to its effectiveness, safety, and compatibility with the gelatin matrix (Liu, F., Chiou, B. S., Avena-Bustillos, R. J., Zhang, Y., Li, Y., McHugh, T. H., & Zhong, F., 2017). It acts as a coupling agent that reduces intermolecular forces in polymer chains, thereby increasing the flexibility of the film and reducing its brittleness (Suderman, N., Isa, M., & Sarbon, N., 2018). Established that glycerol improves elasticity, but its influence on other critical

properties such as mechanical strength, barrier properties, and water solubility is complex and not always linear (Abdullah, J., Jiménez-Rosado, M., Guerrero, A., & Romero, A., 2022).

Despite the numerous studies devoted to the influence of glycerol on the individual properties of gelatin biofilms (Pinto, E., Tavares, W., Matos, R., Ferreira, A., Menezes, R., Costa, M., Souza, T., Ferreira, I., Sousa, F., & Zamora, R., 2018) (Mitra, B., Ziyadi, H., Ramakrishna, S., Chernova, A., & Di Martino, A., 2023) there is still a lack of a comprehensive analysis of the interrelationships between the different characteristics when changing its content. Most current studies present fragmented information that does not allow for reliable prediction of the overall behavior of the material (Janse, R. J., Hoekstra, T., Jager, K. J., Zoccali, C., Tripepi, G., Dekker, F. W., & van Diepen, M., 2021). In this context, it is necessary to apply more comprehensive research approaches that provide a better understanding of the interdependencies and support the targeted optimization of properties (Gowtham, G., Somashekarappa, H., Bharath, K., & Somashekar, R., 2021). In this way, models are created that can serve as a reliable tool for improving compositions and developing materials with improved performance and higher applied value.

The aim of this study was to investigate the effect of glycerol content on the physicomechanical properties of gelatin-based biofilms and, by applying statistical approaches (correlation, regression, and PCA analysis) for modeling and optimization of their characteristics for potential applications.

EXPERIMENTAL

Materials and Methods

Preparation of plasticized gelatin films and determination of their physicomechanical properties

Gelatin films with varying glycerol content (0–75%) were previously prepared by solution casting, according to the methodology described in detail in our earlier publication (Kiryakova, D., Ilieva, A., Kolchakova, G., 2025). The same work also provides a detailed description of the procedures used to determine the physicomechanical properties: tensile strength, elongation at break, Young’s modulus, Shore hardness, and film thickness. In the present study, these experimental data were employed for statistical analysis, modeling, and optimization.

Statistical analysis

Statistical methods were applied to process the experimental data and to establish the relationships between glycerol content and the physicomechanical properties of gelatin films.

Correlation analysis

The relationships between the individual parameters (tensile strength, elongation at break, Young's modulus, thickness, and Shore hardness) were assessed by calculating Pearson correlation coefficients. The results were visualized as a heatmap, enabling straightforward identification of positive and negative dependencies.

Regression Analysis

To model the effects of glycerol content on film properties, linear and polynomial regression models were applied. Regression relationships between tensile strength and selected properties (elongation, Young's modulus, thickness, and hardness) were plotted, with the experimental data shown as dots and regression lines as red curves. The statistical significance of the models was checked by analysis of variance (ANOVA).

Principal Component Analysis (PCA)

To synthesize the multivariate dependencies and visualize the grouping of samples according to the glycerol content, PCA was performed. Prior to the analysis, all data were standardized (mean = 0, standard deviation = 1) to eliminate the influence of different dimensions of the indicators. The following film properties were included in the analysis: tensile strength, elongation at break, Young’s modulus, thickness, Shore hardness. The results are presented as a biplot including the samples (from 0 to 75% glycerol) and variable vectors (loadings), reflecting their relative contribution to the formation of the principal components.

All statistical analyses were performed using Python with the NumPy, pandas, matplotlib, scikit-learn, seaborn libraries.

RESULTS AND DISCUSSION

This study focuses on the statistical processing and interpretation of experimental data on the physicomechanical properties of gelatin films plasticized with different amounts of glycerol. The obtained values of tensile strength, elongation at break, Young's modulus, Shore hardness, and thickness were employed to construct correlation and regression models, as well as to perform Principal Component Analysis (PCA). This approach not only enables the visualization of the main relationships between parameters but also helps to identify the optimal glycerol content range at which a balance between film strength and flexibility can be achieved.

Correlation dependencies between the physicomechanical properties of the investigated films

The correlation analysis (Fig. 1) revealed clearly pronounced relationships between the key physicomechanical properties of the gelatin films. Tensile strength showed a strong positive correlation with Young's modulus, confirming that stiffer and more rigid structures demonstrate higher resistance to rupture. At the same time, a distinct negative correlation was observed between tensile strength and elongation at break, indicating that more flexible samples are characterized by lower strength (Bergo, P., Sobral, P., 2007; Rivero, S., García, M., Pnotti, A., 2010) and higher extensibility.

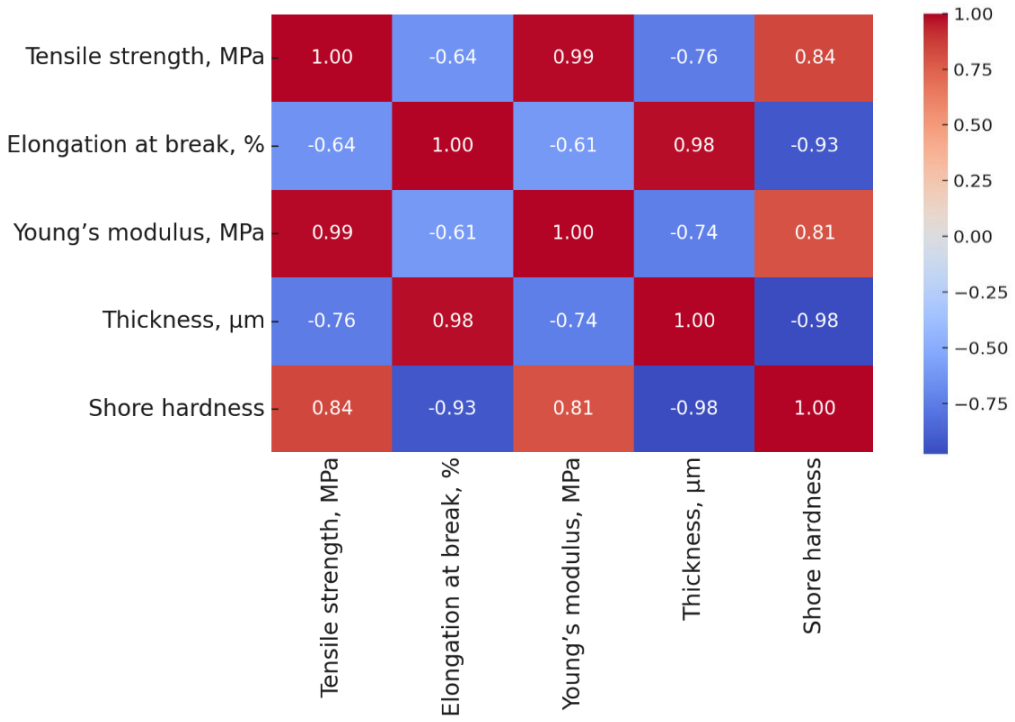

Fig. 1. Heatmap of the correlations between the main properties of gelatin films with different glycerol content. The color scale indicates the strength and direction of the relationships (blue – negative, red – positive)

Film thickness was positively associated with elongation, suggesting that higher glycerol content leads to the formation of thicker (Cuq, B., Gontard, N., Cuq, J., Guilbert, S., 1997; Rezaei, M., Motamedzadegan, A., 2015) and more elastic materials (Rivero, S., García, M., Pnotti, A., 2010; Said, N., Sarbon, N., 2022), but with lower hardness (Pulla-Huillca, P., Gomes, A., Bittante, A. et al., 2021; Tarique, J., Sapuan, S., Khalina, A., 2021) according to Shore measurements. These dependencies are consistent with the trends typically observed when glycerol is added as a plasticizer.

Regression dependencies between the physicomechanical properties of the investigated films

Figure 2 presents the regression relationships between tensile strength and selected physicomechanical properties of gelatin films with varying glycerol content. The dots represent the experimental data, while the red line indicates the linear regression.

A clear negative relationship was observed between tensile strength and elongation at break (Fig. 2a). With increasing glycerol concentration, elongation rose sharply (up to ~400% at 75% glycerol), while tensile strength decreased from 47.5 MPa (0% glycerol) to only 4.5 MPa. This trend illustrates the classical effect of a plasticizer: enhancing the mobility of macromolecular chains at the expense of reduced strength (Rivero, S., García, M., Pnotti, A., 2010; Said, N., Sarbon, N., 2022).

The correlation between tensile strength and Young's modulus was strongly positive, with both parameters decreasing in a similar fashion as glycerol content increased (Fig. 2b). The rigid and brittle film without plasticizer exhibited a modulus above 2000 MPa, whereas at 75% glycerol the modulus dropped to 1.1 MPa. This finding confirms the weakening of intermolecular interactions in the gelatin matrix and the transition toward a more elastic but less structured state.

Figure 2c shows the relationship between tensile strength and Shore hardness. As evident, hardness also correlated positively with tensile strength. Films without glycerol exhibited a hardness of ~80 Shore A, while at 75% glycerol it decreased to ~67.5. This confirms that glycerol acts as a softening agent, reducing hardness and consequently mechanical strength.

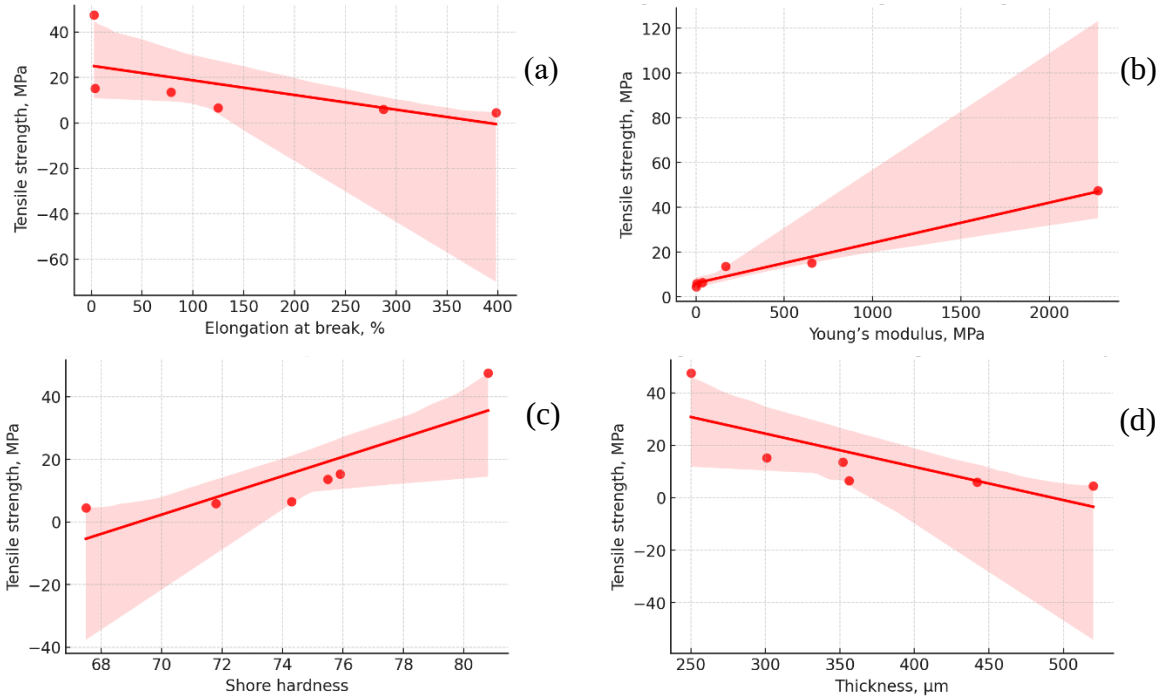

Fig. 2. Regression relationships between: (a) tensile strength vs. elongation at break; (b) tensile strength vs. Young's modulus; (c) tensile strength vs. Shore hardness; and (d) tensile strength vs. film thickness

With increasing glycerol concentration, film thickness increased from 250 to 520 µm, while an inverse relationship with tensile strength was observed (Fig. 2d). Thicker films were mechanically weaker, likely due to higher water content and a less compact protein network in the presence of elevated contents of plasticizer (Bergo, P., Sobral, P., 2007; Sahari, J., Sapuan, S., Zainudin, E., Maleque, M., 2012; Tarique, J., Sapuan, S., Khalina, A., 2021).

The regression models shown in Fig. 2 confirm that Young's modulus is the most reliable predictor of tensile strength, with a linear relationship between these two parameters. The relationships with thickness and elongation were less pronounced, suggesting more complex mechanisms of influence that are not strictly linear. These results are consistent with published findings for other protein- and polysaccharide-based films, where the addition of hydrophilic plasticizers such as glycerol leads to increased elasticity but reduced tensile strength and modulus of elasticity (Bergo, P., Sobral, P., 2007; Tarique, J., Sapuan, S., Khalina, A., 2021). Despite the significant reduction in strength, the addition of moderate amounts of glycerol (12.5–25%) enables achieving a compromise between sufficient strength (>10 MPa) and enhanced flexibility (>70%), which is particularly important for packaging applications requiring film pliability.

PCA analysis of the physicomechanical properties of the investigated films

To visualize the interrelationships between the different parameters and to identify groups of samples with similar characteristics according to glycerol content, Principal Component Analysis (PCA) was performed (Janse, R. J., Hoekstra, T., Jager, K. J., Zoccali, C., Tripepi, G., Dekker, F. W., & van Diepen, M., 2021).

In Fig. 3, the samples are projected into the space of the first two principal components, PC1 and PC2, which explain 86.3% and 12.9% of the total variance, respectively. PC1 clearly distinguishes samples with low glycerol content (up to 12.5%) from those with medium and high levels (50–75%). Tensile strength, Young's modulus, and Shore hardness are located on the positive side of PC1, while elongation and thickness dominate on the negative side, reflecting the increased elasticity and thickness associated with higher glycerol addition (Bergo, P., Sobral, P.,2007; Rivero, S., García, M., Pnotti, A., 2010; Liu, F., Chiou, B. S., Avena-Bustillos, R. J., Zhang, Y., Li, Y., McHugh, T. H., & Zhong, F., 2017).

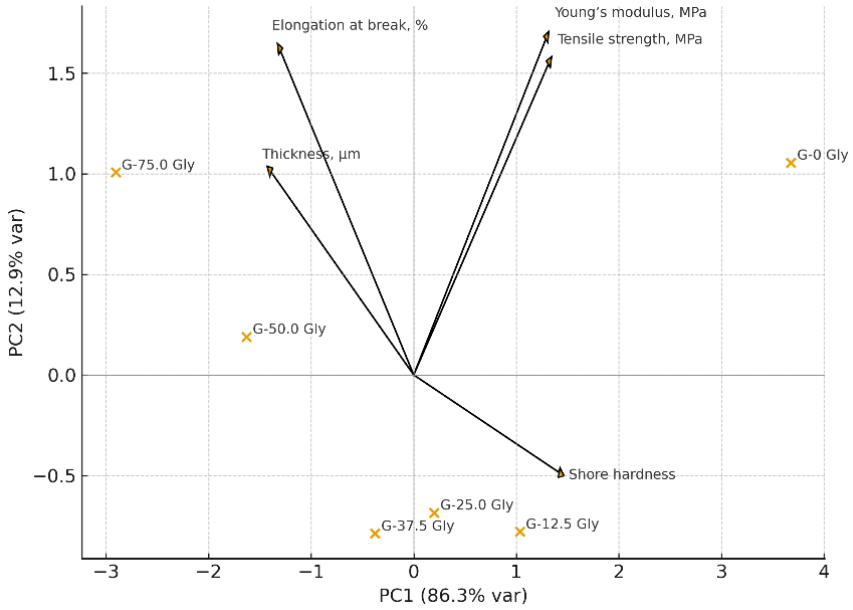

Fig. 3. Principal Component Analysis (PCA) plot of gelatin films with different glycerol contents. PC1 and PC2 explain the main variation in the dataset, separating groups of samples according to composition and plasticizer content

PC2 captures additional variation, mainly related to Shore hardness, and differentiates samples containing 12.5%, 25%, and 37.5% glycerol (Mitra Baghali, Ziyadi, H., Ramakrishna, S., Chernova, A., & Di Martino, A., 2023).

The biofilms without glycerol and those containing 12.5% glycerol formed a group characterized by high hardness and mechanical strength, while films with 50% and 75% plasticizer clustered separately, exhibiting high elongation at break and greater thickness (Cuq, B., Gontard, N., Cuq, J., Guilbert, S., 1997; Pulla-Huillca, P., Gomes, A., Bittante, A. et al., 2021). Samples containing 25% and 37.5% glycerol occupied an intermediate position, confirming that this concentration range represents a balance between the two extremes (Tarique, J., Sapuan, S., Khalina, A., 2021). The observed grouping demonstrates that with increasing plasticizer content, the materials transition from rigid and brittle to soft and elastic (Said, N., Sarbon, N., 2022; Suderman, N., Isa, M., & Sarbon, N., 2018).

In summary, the PCA analysis provides valuable insights for data systematization and confirms the key role of glycerol content as the primary factor determining the properties of gelatin films. These relationships facilitate targeted optimization and enable the selection of film composition depending on the intended application: for uses where mechanical strength is a priority (e.g., rigid packaging), lower glycerol concentrations are more suitable, whereas for applications requiring flexibility (e.g., wraps and edible films), higher plasticizer levels are optimal (Sahari, J., Sapuan, S., Zainudin, E., Maleque, M., 2012; Islam, M., Xayachak, T., Haque, N., Lau, D., Bhuiyan, M., & Pramanik, B. K., 2024).

CONCLUSION

With increasing glycerol content, tensile strength and Young's modulus decreased, while elongation at break and film thickness increased, confirming the role of glycerol as an effective plasticizer. Correlation and PCA analyses revealed a clear separation of the samples. The optimal glycerol content range for achieving a balance between mechanical strength (>10 MPa) and sufficient flexibility (>70% elongation) in the biofilms was identified as 25–40%. This makes them suitable for application as biodegradable packaging materials.

REFERENCES

Abdullah, J. A. A., Jiménez-Rosado, M., Guerrero, A., & Romero, A. (2022). Gelatin-Based Biofilms with FexOy-NPs Incorporated for Antioxidant and Antimicrobial Applications. *Materials*, *15*(5), 1966.

Alipal, J., Mohd Pu'ad, N., Lee, T., Nayan, N., Sahari, N., Basri, H., Idris, M., & Abdullah, H. (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, 42, 240–250.

Bergo, P., Sobral, P. (2007). Effects of plasticizer on physical properties of pigskin gelatin films. *Food Hydrocoll*, 21 (8), 1285.

Cuq, B., Gontard, N., Cuq, J., Guilbert, S. (1997). Selected functional properties of fish myofibrilar protein-based films as affected by hydrophilic plasticizers. *J Agric Food Chem,* 45 (3), 622.

Gowtham, G. K., Somashekarappa, H., Bharath, K., & Somashekar, R. (2021). Measure of polymer performance based on correlated physical parameters. *Journal of Applied Polymer Science*, 138(29).

Harrazi, N., Özbek, H. N., Yanık, D. K., Zaghbib, I., & Göğüş, F. (2024). Development and characterization of gelatin-based biodegradable films incorporated with pistachio shell hemicellulose. *Journal of Food Science and Technology*, 61(10), 1919–1929.

Islam, M., Xayachak, T., Haque, N., Lau, D., Bhuiyan, M., & Pramanik, B. K. (2024). Impact of bioplastics on environment from its production to end-of-life. *Process Safety and Environmental Protection*, 188, 151–166.

Janse, R. J., Hoekstra, T., Jager, K. J., Zoccali, C., Tripepi, G., Dekker, F. W., & van Diepen, M. (2021). Conducting correlation analysis: important limitations and pitfalls. *Clinical Kidney Journal*, 14(11), 2332–2337.

Kiryakova, D., Ilieva, A., Kolchakova, G. (2025). Evaluation of the biodegradability and tensile properties of gelatin-based biofilms with varying glycerol plasticizer content. *Journal of Environmental Protection and Ecology*, 26 (5), 1702–1710.

Liu, F., Chiou, B. S., Avena-Bustillos, R. J., Zhang, Y., Li, Y., McHugh, T. H., & Zhong, F. (2017). Study of combined effects of glycerol and transglutaminase on properties of gelatin films. *Food Hydrocolloids*, 65, 1–9.

Mitra Baghali, Ziyadi, H., Ramakrishna, S., Chernova, A., & Di Martino, A. (2023). Effect of Glycerol Content in Gelatin/Glycerol Composite Films Loaded with Erythromycin. *Polymer Science, Series A*, 65(6), 714–724.

Mousazadeh, S., Ehsani, A., Moghaddas Kia, E., & Ghasempour, Z. (2021). Zinc oxide nanoparticles and periodate oxidation in developing pH-sensitive packaging film based on modified gelatin. *Food Packaging and Shelf Life*, 28, 100654.

Pinto, E., Tavares, W., Matos, R., Ferreira, A., Menezes, R., Costa, M., Souza, T., Ferreira, I., Sousa, F., & Zamora, R. (2018). Influence of low and high glycerol concentrations on wettability and flexibility of chitosan biofilms. *Química Nova*, 41(10), 1109-1116.

Pulla-Huillca, P., Gomes, A., Bittante, A. et al. (2021). Wettability of gelatinbased films: the effects of hydrophilic or hydrophobic plasticisers and nanoparticle loads. *J Food Eng*, 297, 110480.

Rivero, S., García, M., Pnotti, A. (2010). Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films. *Innov Food Sci Emerg Technol*, 11 (2), 369.

Rezaei, M., Motamedzadegan, A. (2015). The effect of plasticisers on mechanical properties and water vapor permeability of gelatin-based edible films containing clay nanoparticles. *World J Nano Sci Eng*, 5, 178.

Sahari, J., Sapuan, S., Zainudin, E., Maleque, M. (2012). A new approach to use arenga pinnata as sustainable biopolymer: effects of plasticisers on physical properties. *Proc Chem*, 4, 254.

Said, N., Sarbon, N. (2022). Physical and mechanical characteristics of gelatin-based films as a potential food packaging material: a review. *Membranes*, 12 (5), 442.

Suderman, N., Isa, M., & Sarbon, N. (2018). The effect of plasticizers on the functional properties of biodegradable gelatin-based film: A review. *Food Bioscience*, 24, 111–119.

Tarique, J., Sapuan, S., Khalina, A. (2021). Effect of glycerol plasticiser loading on the physical, mechanical, thermal, and barrier properties of arrowroot (Maranta arundinacea) starch biopolymers. *Sci Rep*, 11, 13900.