Does the Hydrogel:Oleogel Ratio Affect the Physicochemical Properties of Gelatin–Beeswax Bioplastic Films?

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Abstract. Plastic waste from packaging poses a serious environmental problem due to its persistence and contribution to microplastic formation. Bigels, composed of hydrogel and oleogel, have emerged as an innovative approach to eco-friendly food packaging. This study aimed to analyze the effect of varying hydrogel–oleogel ratios on the physicochemical properties of bigel-based bioplastic films for food packaging applications. An experimental design was employed using a Completely Randomized Design (CRD) with four treatments and five replications. Data were analyzed using ANOVA, and when significant ($p<0.05$) or highly significant ($p<0.01$) differences were detected, further testing was conducted using Duncan's Multiple Range Test (DMRT). The treatments were Hydrogel: Oleogel ratios, consisted of T1 (90 :10), T2 (80 :20), T3 (70 :30) and T4 (60 :40) respectively. The findings indicated that the optimal ratio was achieved in T1 (90:10). Detailed results included solubility (21.133%), syneresis (81.186%), water-holding capacity (77.174%), moisture content (7.549%), thermal properties (TGA/DTG) more stable and optical microscopy which shows small and relatively homogeneous droplets. Overall, the results demonstrated that T1 (90:10) provided the most favorable physicochemical characteristics of bigels bioplastic films for food packaging applications. These findings highlight formulation-dependent structural mechanisms that advance the use of gelatin–beeswax gels for food packaging.
Keywords: bigel, bioplastic, films, hydrogel-to-oleogel ratios, packaging.

1 Introduction

The increasing accumulation of plastic waste, particularly from non-biodegradable food packaging, poses serious environmental concerns due to its persistence and contribution to microplastic contamination in the food chain. In Indonesia, total waste generation reached 37.8 million tons in 2023 [1]. To address this issue, environmentally friendly packaging alternatives are urgently needed. Bigel-based packaging a biphasic system composed of

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hydrogel and oleogel phases, has emerged as a promising solution due to its biodegradability, enhanced mechanical stability and tunable physicochemical properties through formulation optimization [2].

Hydrogels are typically derived from collagen or gelatin. Gelatin-based hydrogels offer advantages such as elasticity, transparency, and biodegradability. Oleogels, on the other hand, are commonly structured using vegetable oils, including corn oil [3]. Corn oil is rich in unsaturated fatty acids that are beneficial for health and can be structured by adding oil-structuring and gelling agents such as plant waxes, monoglycerides, lecithin, and cellulose derivatives. Beeswax is among the most widely used structuring agents, as it plays a crucial role in enhancing mechanical properties, improving water resistance, and preventing oxidation in oleogels [4].

Bigel formulations based on gelatin, corn oil, and beeswax hold great potential as sustainable food packaging materials. The hydrogel-to-oleogel ratio in bigel systems significantly influences their physicochemical properties, including solubility and system stability. Therefore, research on the effect of hydrogel-to-oleogel ratios in bigel formulations is essential to optimize the characteristics of the resulting bioplastics as an environmentally friendly alternative to conventional food packaging.

2 Materials and methods

2.1 Material and Equipment

This study was conducted at the Laboratory of Animal Product Technology, Faculty of Animal Science, Universitas Brawijaya, for the preparation of hydrogel, oleogel, bigel, and bioplastic, as well as for the analysis of solubility, water-holding capacity (WHC), syneresis, moisture content, and optical microscopy. Thermal property analysis was performed at the Integrated Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya. The materials used in this study included corn oil (Tropicana Slim), beeswax, glyceryl monostearate (GMS), bone gelatin (beef gelatin), and distilled water (Hydrobatt). The equipment utilized comprised an analytical balance (BC Series OHAUS Centrogram Balance), 500 mL beaker glass (Iwaki), hot plate (Duta Nusantara), spatula, thermometer, 100 mL and 10 mL graduated cylinders (Iwaki), magnetic stirrer, refrigerator, object glass, cover glass, filter paper, 15 mL centrifuge tubes, centrifuge, oven (Sharp), desiccator, Petri dishes, scissors, plastic clips, ruler, pen, silicone pad, Teflon plate, label paper, Thermogravimetric Analyzer (TGA) (STA 449F3), optical microscope (Olympus CX 21 FS 1) and a stopwatch.

2.2 Research Methods

The research method used an experimental method. Statistical calculations were performed using analysis of variance (ANOVA) with a Completely Randomized Design (CRD) consisting 4 treatments and 5 replications. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was applied for mean comparison on the variables of solubility, moisture content, water-holding capacity (WHC), and syneresis. A descriptive analysis was performed for the variables of optical microscopy and thermal properties.The treatments were Hydrogel: Oleogel ratios, consisted of T1 (90 :10), T2 (80 :20), T3 (70 :30) and T4 (60 :40) respectively. These ratios were selected based on preliminary experiments to minimize excessive moisture or oil exudation and to ensure balanced phase distribution and structural integrity.

2.3 Hydrogel Preparation

The procedure for hydrogel preparation was prepared by weighing 20 g of gelatin powder and dissolving it in 180 mL of distilled water using a beaker glass. The solution was then heated and homogenized using a hot plate magnetic stirrer at 40°C for 15 minutes. Once homogenized, the solution was transferred into a container for further processing.

2.4 Oleogel Preparation

The procedure for oleogel preparation was prepared by dissolving 1% beeswax and 3% glycerol monostearate as oleogelators in 90 mL of corn oil using a beaker glass. The mixture was heated and homogenized with a hot plate magnetic stirrer at 90°C for 5 minutes. After homogenization, the mixture was cooled at room temperature for 30 minutes, then stored in a freezer at −21°C for 1 hour to allow the oleogel to fully set.

2.5 Bigel Preparation

The bigel samples were prepared by combining hydrogel and oleogel phases according to the designated hydrogel:oleogel ratios, namely T1 (90:10), T2 (80:20), T3 (70:30) and T4 (60:40). Each phase was prepared separately and subsequently homogenized at 500 rpm for 5 min using hot plate stirrer at 70 °C.

2.6 Bioplastic Casting

The bioplastic casting was prepared by a total of 10 mL of the homogenized bigel mixture was poured into a teflon mold lined with a silicone base and allowed to dry at room temperature for 24 hours. Once dried, the bigel-based bioplastic films were carefully peeled off, cut into 5 × 5 cm pieces, and subjected to further analyses.

2.7 Solubillity

The solubility properties of the films were measured following by bigel-based bioplastic films (5 × 5 cm) were weighed together with filter paper using an analytical balance. An empty Petri dish was dried at 105°C for 30 minutes, cooled in a desiccator for 10 minutes, and weighed to a constant weight. The bioplastic was placed on the filter paper within the Petri dish and heated at 105°C for 30 minutes, then cooled in the desiccator for 10 minutes and weighed as the initial weight (w_0). The samples were then immersed in 20 mL of distilled water at room temperature for 5 minutes. After immersion, the samples were filtered through filter paper and dried in an oven at 105°C for 10 minutes. The Petri dish was removed using tongs, cooled in the desiccator for 10 minutes, and weighed as the final weight (w_1). Solubility was calculated using the following formula:

$$Solubillity\ (\%) = \frac{(w0-w1)}{w0} x\ 100 \quad (1)$$

2.8 Water Holding Capacity (WHC)

The WHC properties of the films were measured following by a total of 10 mL of bigel bioplastic solution was placed into a 15 mL centrifuge tube and weighed as the initial weight (m_1). The samples were centrifuged at 4.500 rpm for 10 minutes, and the separated water was

discarded. The remaining gel was weighed as the final weight (m_2). WHC was calculated using the following equation:

$$WHC\ (\%) = \left(1\ \frac{m2}{m1}\right) x\ 100 \quad (2)$$

2.9 Syneresis

The syneresis properties of the films were measured following by a total of 10 mL of bigel bioplastic solution was placed into a 15 mL centrifuge tube and weighed as the initial weight (m_1). The samples were centrifuged at 4.000 rpm for 15 minutes, and the separated water was discarded. The remaining gel was weighed as the final weight (m_2). Syneresis was calculated using the following equation:

$$Syneresis\ (\%) = \frac{m1-m2}{m1} x\ 100 \quad (3)$$

2.10 Moisture Content

The moisture content properties of the films were measured following by bioplastic samples measuring 5 × 5 cm were prepared and weighed using an analytical balance (m_1). An empty Petri dish was dried in an oven at 105 °C for 30 minutes, cooled in a desiccator for 10 minutes, and weighed. The bioplastic sample was then placed in the Petri dish and dried in the oven at 105°C for 24 hours until a constant weight was reached. After drying, the sample was cooled in a desiccator for 10 minutes and reweighed (m_2). The moisture content was calculated using the following equation:

$$Moisture\ Content(\%) = \frac{m1-m2}{m1} x\ 100 \quad (4)$$

2.11 Optical Microscopy

Optical microscopy properties of the films were measured following by a bioplastic sample was placed on an object glass and covered with a cover glass. The sample was observed under an optical microscope at 40× magnification, and images were captured using a camera. Observations were then repeated at 100× magnification, and additional images were taken for documentation and structural analysis.

2.12 Thermal Properties (DTA/TGA)

Thermal properties of the films were measured following by bioplastic samples measuring 5 × 5 cm were placed in a stainless-steel crucible equipped with a torque-sealed lid. The samples were heated from 0 °C to 300 °C at a heating rate of 5 °C per minute. Enthalpy changes and phase transition temperatures (onset and endset of melting) were recorded using a Differential Scanning Calorimeter (DSC). The melting temperature (Tm) and enthalpy (ΔH) were determined to evaluate the thermal stability and melting behavior of the bigel-based bioplastics.

3 Results and Discussion

The results of the gelatin–beeswax bigel bioplastic analysis are presented in Table 1.

Table 1. Average value of gelatin-beeswax based bioplastic

Hydrogel : Oleogel ratios	Solubillity (%)	Syneresis (%)	WHC (%)	Moisture Content (%)
T1 (90 :10)	21.133 ± 3.283	81.186^{a} ± 4.289	77.174^{a} ± 7.632	7.549 ± 2.170
T2 (80 :20)	21.523 ± 1.363	75.196^{a} ± 4.032	65.662^{b} ± 7.510	8.144 ± 1.362
T3 (70 :30)	22.544 ± 2.221	56.032^{b} ± 3.631	62.812^{b} ± 4.220	8.622 ± 1.802
T4 (60 :40)	23.706 ± 7.545	41.904^{c} ± 5.711	45.258^{c} ± 2.707	9.103 ± 0.989

Description: a, b, c Different superscripts in the same column indicate highly significant differences ($P<0.01$).

3.1 Solubility

Based on the results shown in Table 1, different hydrogel-to-oleogel ratios in bigel bioplastics did not produce a statistically significant difference ($P > 0.05$) in solubility values among treatments. The solubility values for T1, T2, T3, and T4 were 21.133 ± 3.283%, 21.523 ± 1.363%, 22.544 ± 2.221%, and 23.706 ± 7.545%, respectively. The lowest mean value was observed in T1 (90:10) at 21.133%, while the highest was recorded in T4 (60:40) at 23.706%. These results indicate that different hydrogel-to-oleogel ratios produce varying solubility levels, showing that even with lower hydrogel and higher oleogel concentrations, the resulting bioplastics remain biodegradable.

The increase in solubility values corresponded to increasing oleogel concentrations. This can be attributed to the instability of the ratio, as indicated by the presence of larger droplets leading to aggregation and heterogeneity in the matrix. This heterogeneity also interferes with the crystallization process of beeswax, resulting in rapid cooling and the formation of cracks in the film structure. Theoretically, higher oleogel content should lead to reduced solubility however, in this study, the opposite trend was observed. This may be due to the inability of the oleogel to form a continuous wax layer, creating pore spaces within the structure. These pores allow water to penetrate more easily, dissolving the hydrogel fraction. This phenomenon occurs because the oleogel phase failed to form a continuous barrier [5]. In this study, lower solubility values were considered advantageous, as they enhance the protective function of the bioplastic during storage. Low solubility also contributes to better water barrier properties, thereby improving the structural integrity and functionality of the film.

3.2 Water Holding Capacity

Based on the results presented in Table 1, different hydrogel-to-oleogel ratios in bigel bioplastics showed a highly significant effect ($P < 0.01$) on water-holding capacity (WHC). The WHC values for T1, T2, T3, and T4 were 77.174 ± 7.632%, 65.662 ± 7.510%, 62.812 ± 4.220%, and 45.258 ± 2.707%, respectively. The lowest WHC was observed in T4 (60:40) with a value of 45.258%, whereas the highest WHC was recorded in T1 (90:10) at 77.174%. These results indicate that different hydrogel-to-oleogel ratios lead to variations in WHC values, showing that higher hydrogel concentrations provide better water retention capacity, while increased oleogel concentrations result in a gradual decline in WHC.

This phenomenon can be attributed to the instability of the hydrogel-to-oleogel ratio, where the hydrophilic phase is partially substituted by the hydrophobic phase. This substitution reduces the availability of polar groups capable of forming hydrogen bonds with

water molecules. The decrease in WHC is also influenced by the crystalline network formed by beeswax, which limits water retention within the hydrogel matrix. The dense crystalline structure of beeswax acts as a barrier, reducing water diffusion [6]. In addition, the type of oil used affects the morphology of the beeswax crystals. Corn oil, which is rich in unsaturated fatty acids, tends to produce a more fragile crystalline structure, thereby lowering WHC. The decline in WHC values is therefore associated with increasing oleogel content in the bigel system [7]. High WHC values indicate a well-developed gel network with a strong capacity to bind water, leading to reduced free water in the bioplastic matrix.

3.3 Syneresis

Based on the results presented in Table 1, different hydrogel-to-oleogel ratios in bigel bioplastics showed a highly significant effect ($P < 0.01$) on syneresis values. The syneresis values for T1, T2, T3, and T4 were 81.186 ± 4.289%, 75.196 ± 4.032%, 56.032 ± 3.631%, and 41.904 ± 5.711%, respectively. The lowest syneresis was observed in T4 (60H:40O) with a value of 41.904%, while the highest was found in T1 (90:10) at 81.186%. These results indicate that different hydrogel-to-oleogel ratios lead to variations in syneresis values, with syneresis decreasing as the oleogel concentration increases. This behavior is primarily attributed to the beeswax content in oleogel. Beeswax reduces syneresis through the formation of a crystalline network.

The crystalline network formed by beeswax effectively retains oil within the gel matrix, thereby reducing the mobility of free water and oil molecules. Beeswax can form a dense three-dimensional structure that strongly binds oil, preventing its release [8]. Additionally, the presence of corn oil further contributes to reducing syneresis. Corn oil, which is rich in unsaturated fatty acids, enhances beeswax distribution and facilitates the formation of a more homogeneous system. This effect is also supported by hydrophobic interactions between beeswax and corn oil, which strengthen the oleogel network and prevent fluid loss from the gel structure [9]. As a result, the oleogel phase exhibits improved physical stability, extended shelf life, and optimal performance in food applications.

3.4 Water Content

Based on the results presented in Table 1, different hydrogel-to-oleogel ratios in bigel bioplastics showed no statistically significant effect ($P > 0.05$) on moisture content values. The moisture content values for T1, T2, T3, and T4 were 7.549 ± 2.170%, 8.144 ± 1.362%, 8.622 ± 1.802%, and 9.103 ± 0.989%, respectively. The lowest mean moisture content was observed in T1 (90:10) at 7.549%, while the highest was recorded in T4 (60:40) at 9.103%. These results indicate that different hydrogel-to-oleogel ratios yield varying moisture content values, with increasing oleogel concentration contributing to higher moisture levels. Moisture content is a key parameter in bioplastic characterization, as it strongly influences structural stability and potential food packaging applications.

The observed increase in moisture content with increasing oleogel concentration is associated with the hydrophobic properties of beeswax. Beeswax tends to form a barrier layer, which limits water loss and creates an interfacial network. This network produces a compact structure that hinders water release while maintaining the water bound to the gelatin phase, thereby increasing moisture retention [10]. The hydrogel-to-oleogel ratio in bigel systems plays a crucial role in regulating water dynamics and vapor barrier properties. This indicates that gelatin–beeswax–corn oil bigel formulations offer advantages in controlling moisture, providing flexible films with good structural stability. Moreover, the combination of hydrophilic polymers with hydrophobic lipids such as beeswax can produce films with excellent moisture barrier properties. The moisture content values obtained in this study

remain below the acceptable range for packaging applications, specifically below 13% and 16% [11], indicating the suitability of the resulting bioplastics for practical use.

3.5 Optical Microscopy

Optical microscopy analysis was conducted on gelatin–beeswax bigel-based bioplastic samples with different hydrogel-to-oleogel ratios using optical microscopy at 40× and 100× magnifications (Figure 1). A single bioplastic sample was placed on an object glass for each treatment. The purpose of this test was to identify the presence of crystalline structures and analyze the microstructural patterns, which can indicate whether the bigel bioplastic matrix is homogeneous. The results revealed that the hydrogel-to-oleogel ratio plays a key role in determining droplet size and homogeneity within the bioplastic structure.

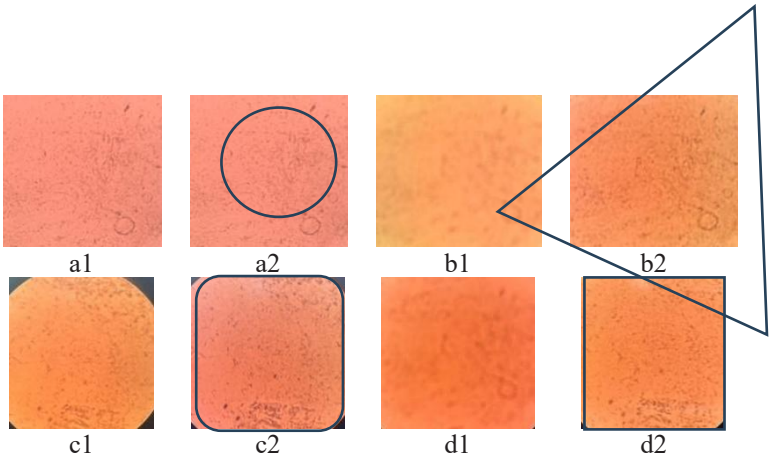

Fig 1. Optical microscopy display of all samples each treatment
Description: The indicator 1 for magnification 40× and 2 for magnification 100×.
a for T1, b for T2, c for T3 and d for T4.

A higher oleogel ratio resulted in larger droplet sizes with broader bright areas in the micrographs. In contrast, lower beeswax concentrations in the oleogel reduced light intensity during observation. This phenomenon was clearly observed in T4, characterized by large, bright droplets. This condition can be attributed to the nature of corn oil, which leads to the formation of a more fragile and porous beeswax crystal network, producing a softer light pattern. Increasing the oleogel fraction in the bigel system decreased structural homogeneity due to the reduced strength of the hydrogel network to hold droplets, making the large droplets more prone to coalescence and lowering long-term stability [12].

In contrast, T1 exhibited smaller droplet sizes. The dominant hydrogel phase effectively stabilized the droplets, enhancing the structural stability of the system. Smaller droplets indicate a more homogeneous matrix, which contributes to consistent texture, as well as improved thermal and mechanical stability. Rapid cooling also produced finer beeswax crystals, which reinforced the network structure. The limited number of beeswax crystals within the droplets resulted in lower light intensity during microscopic observation. Uniform droplet distribution allowed for smoother light scattering, creating micrographs with finer bright speckles [13]. Therefore, smaller droplet sizes enhance microstructural homogeneity even though they reduce the dominance of bright areas in the microscopic images.

3.6 Thermal Properties (DTA/TGA)

Based on the results presented in Figure 2, different hydrogel-to-oleogel ratios in bigel bioplastics exhibited distinct thermal behaviors as reflected in their DTA/TGA profiles. The TGA curves of all treatments revealed three major stages of weight loss (Stage I–III), corresponding to different thermal events in the biopolymer system.

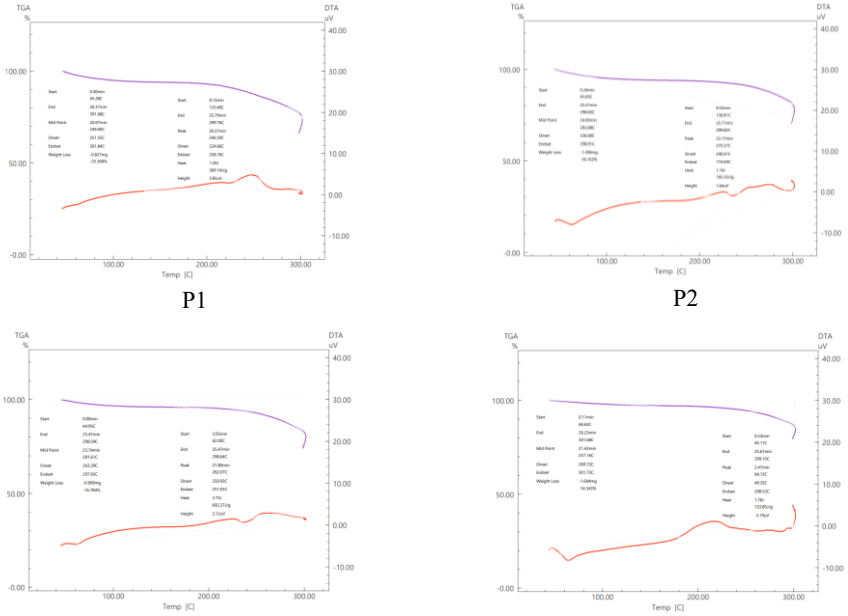

Fig 2. DTA and TGA pada bioplastik tiap perlakuan
Description: DTA
TGA

The TGA/DTA analysis indicated a stepwise degradation pattern of the protein–lipid biopolymer matrix. Stage I, which occurred below 100 °C, was associated with moisture evaporation. T1 exhibited the highest mass loss in this stage due to the dominance of the hydrophilic gelatin phase. This observation is consistent with [34], who reported that hydrogels are three-dimensional hydrophilic networks capable of retaining large volumes of hydro-fluids. A higher gelatin content accelerates natural degradation. Increasing the oleogel ratio from T2 to T4 gradually reduced the initial mass loss, indicating the formation of a hydrophobic barrier effect from beeswax and corn oil, which restricts moisture diffusion. Stage II (100–200 °C) corresponded to additional weight loss related to water evaporation, cleavage of gelatin polymer chains, and the onset of beeswax degradation. A shift of onset temperature to higher values in T3 and T4 was observed, indicating enhanced thermal stability due to the formation of a dense crystalline wax network. This is in line with [35], which states that beeswax contributes to forming a hydrophobic layer that acts as an effective barrier to both water and heat diffusion. This phase reduces early mass loss and increases onset degradation temperature, reflecting improved thermal stability.

Stage III (above 200 °C) was associated with oxidation, thermal decomposition, and the formation of carbonate residues. Treatments with higher oleogel ratios (T3 and T4) left more residual mass compared to T1 and T2. Overall, the order of thermal stability was T4 > T3 > T2 > T1. This aligns with [36], who reported that gelatin-dominant systems (T1) produce a homogeneous and flexible bigel structure with finely dispersed oleogel droplets within the hydrogel matrix. The oleogel content in T1 was sufficient to provide a hydrophobic effect,

improving water resistance without compromising transparency and elasticity. Although T1 showed higher weight loss at lower temperatures, this formulation remains the most optimal as it maintains a balance between thermal resistance, structural homogeneity, and environmental compatibility, making it a strong candidate for bigel-based bioplastic applications.

Overall, the performance of gelatin-beeswax bigel based bioplastic films is governed by the combined effects of microstructural organization, water-oil phase distribution and thermal stability. A hydrogel dominant formulation in T1 (90:10) produced a more homogeneous microstructure with finely dispersed oleogel droplets, which restricted water mobility and resulted in higher water holding capacity, controlled solubility and improved moisture stability. In contrast, increasing oleogel content reduced structural homogeneity, leading to lower water holding capacity and compromised matrix integrity. These structural differences were reflected in the thermal behavior, where formulations with balanced phase distribution exhibited more stable degradation profiles. Collectively, solubility, water holding capacity, syneresis, moisture content, microstructure and thermal properties interact synergistically to define the functional performance of bigel-based films for food packaging applications.

4 Conclusion

The hydrogel-to-oleogel ratio was found to significantly influence the physicochemical properties of gelatin–beeswax bigel bioplastics. The ratio of hydrogel: oleogel at 90:10 formulation (T1) exhibited the most optimal performance, characterized by low solubility, high water-holding capacity, stable thermal behavior, and a homogeneous microstructure. In contrast, higher oleogel ratios decreased gel stability and increased moisture content. Therefore, a hydrogel-dominant ratio is recommended for the development of eco-friendly bioplastics with optimal functional characteristics.

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