***Original Research Article***

**Development and Characterization of Bigel Based on Corn Oil and Beeswax Oleogel with Gelatin Hydrogel as a Fat Replacer**

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**ABSTRACT**

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| The aim of this study was to determine the optimal ratio of bigel based on beeswax-corn oil oleogel and gelatin hydrogel as a fat replacer. The study employed a completely randomized design (CRD). Data obtained were processed using Microsoft Excel and analyzed using analysis of variance (ANOVA). If significant or highly significant differences were found, Duncan's Multiple Range Test (DMRT) was applied. The study was conducted at the Laboratory of Animal Product Technology, Faculty of Animal Science, and the Food Technology Laboratory, Brawijaya University, from February 3 to March 25, 2025. The research investigated bigel formulations made from beeswax-corn oil oleogel and gelatin hydrogel at various ratios as a fat substitute. The variables observed included moisture content, fat content, texture, color, oil binding capacity (OBC), weight loss, antioxidant activity, FTIR, and microstructure. The results showed highly significant differences (P<0.01) in moisture content (18.51%-42.53%), fat content (81.87%-50.53%), texture (1.96-2.80 N), weight loss (0.81%-1.92%), and antioxidant activity (26.08 µg/ml - 61.48 µg/ml). However, OBC and color did not show significant differences (P>0.05), with OBC ranging from 97.98%-98.22% and color values of L\* (87.13-88.98), a\* (0.25-0.22), and b\* (15.29-15.03). The study concluded that bigel based on beeswax-corn oil oleogel and gelatin hydrogel had a significant effect on moisture, fat content, texture, weight loss, and antioxidant activity, but did not significantly affect OBC and color. The best oleogel-to-hydrogel ratio was found in treatment P1 with a ratio of 80:20. This ratio not only improved the texture and nutritional quality of the product but also demonstrated potential for practical application in the development of functional foods. |

*Keywords: Bigel; Oleogel; Hydrogel; Beeswax; Corn Oil; Gelatine.*

**1. INTRODUCTION**

Bigel is an innovative semi-solid system consisting of a combination of hydrogel and oleogel that combines the two phases to produce a material with superior stability, encapsulation ability and mechanical properties (Shakeel et al., 2022). Bigel is a gel form of vegetable fat and gelatin that can be used to partially replace saturated fat as a healthier alternative technique with the potential to decrease saturated fat content and increase unsaturated fatty acid profile (Jing et al., 2015). Beeswax is able to form a dense network that can maintain the physical integrity of the bigel, slow the migration of active substances and provide rheological characteristics suitable for topical and food applications (Meng et al., 2018). Oleogels are generally rich in unsaturated fatty acids that acquire a solid texture when trapped in a three-dimensional crystalline network formed by an organizing agent (oleogelator) at a certain concentration. The characteristics of each gel such as viscosity, hardness and melting point can vary depending on the regulating agent thus allowing the development of different products (Floter et al., 2021). The use of oleogels is not only to replace fat but can act as carriers of water-insoluble bioactive substances, stabilizers for products that do not use emulsifiers, oil binders or provide heat resistance in food products (Puscas et al., 2020).

Corn oil is used as the oil phase in the development of bigel due to the content of polyunsaturated fatty acids, especially linoleic acid and vitamin E which functions as a natural antioxidant (Dwiputra et al., 2015). The combination of beeswax and corn oil in the formation of oleogel is able to produce a matrix that is stable and flexible in the regulation of its textural and functional properties. Hydrogels derived from natural polymers such as gelatin have a good ability to absorb water and form stable hydrophilic networks through hydrogen bond interactions and three-dimensional structures. (Chen et al., 2011). The combination of oleogel made from beeswax and corn oil with gelatin hydrogel in a bigel system is expected to form a stable multiphase structure. The main focus in this formulation is to evaluate the role of beeswax in forming and reinforcing the oleogel network, which has a direct impact on the overall physicochemical characteristics of the bigel. Thus, the development of bigels based on beeswax, corn oil and gelatin is expected to contribute to producing food or pharmaceutical products that are healthier, stable and have good sensory quality.

**2. materials and methods**

**2.1 Sampling of Raw Materials**

The materials used in this study were *beeswax* oleogel-based bigel with corn oil (*Zea mays L*.) and gelatin hydrogel. The materials used in this study include beeswax (kembang joyo), corn oil (Tropicana Slim), GMS (*Glycerol monostearate*), bovine gelatin (Haqiqi) and distilled water. The tools used include *beaker glass* (Pyrex), glass *stirrer*, SH-2 *magnetic stirrer hotplate*, electric stove, handblender (Bosch) knife, 200ml plastic container.

**2.2 Preparation of Oleogel, Hydrogel and Bigel**

The hydrogel manufacturing procedure refers to the method developed by Noman and Singh (2024) with some modifications. The preparation of hydrogels was carried out through several stages, namely preparing 20 grams of gelatin powder, then dissolving it in 180 ml of distilled water in a glass beaker. The solution was heated and homogenized until the temperature reached 40˚C for 15 minutes. Put in a container for 2.5 hours to harden.

The oleogel preparation procedure refers to the method proposed by Zulfiqar et al. (2024) with some modifications. The stages of making oleogel begin with heating beeswax as much as 6% of the amount of 500 ml corn oil. Next, add 5.1 grams of GMS (*Glycerol monostearate*) and corn oil. The mixture was then heated on an electric stove under the supervision of a thermometer until it reached a temperature of 70˚C, while being homogenized until the beeswax dissolved completely. Afterwards, solution was poured into a storage container and left at room temperature of 27.5 ˚C for 1.5 hours to solidify.

The bigel generation procedure refers to the method of Prasad et al. (2023) which has been modified. Corn oil (*Zea mays L*.) oleogel and gelatin hydrogel were prepared in the ratio of 80:20, 70:30, 60:40, and 50:50. The mixture was then homogenized using a handblender (Bosch). After mixing evenly, the bigel was stored in a container and ready for testing.

**2.3 Moisture Content**

The procedure for testing moisture content uses the gravimetric method of Sakul et al. (2019) with modifications. The cup was put into the oven for 24 hours at 105˚C, then placed in a desiccator for 30 minutes and left to cool and then weighed (A). 3 grams of sample was put into a weighing bottle and then weighed (B), the cup was put into the oven at 105˚C for 24 hours. After completion, the sample was put into a desiccator and left to cool and then weighed (C).

Moisture content =$\frac{B-C}{B-A} x$ 100

**2.4 Fat Content**

The test procedure for fat content used the Soxhlet method of AOAC (2005) with modifications: filter paper and cotton were baked at 105°C for 12 hours. Put in a desiccator for 15 minutes then weighed as (BS). Weighed 1 gram of sample and wrapped with filter paper and cotton to form a cylinder then weighed as (BSK). Petroleum Ether (PE) solution was added as much as 60 ml above the cylinder and 150 ml below the cylinder. The sample was extracted for 6 hours. Samples wrapped in filter paper were taken. Cooled and placed in an oven at 105°C (24 hours). Put in a desiccator for 15 minutes and then weighed as (BK). Here is the formula:

Fat Content (%) =$\frac{BS – (BSK – BK)}{BK}$ X 100%

Dry Fat Content % = $\frac{\left(100 –\%moisture content\right) \%fat content}{100}$

**2.5 Texture**

The texture testing procedure uses Texture AnalyzerType NCT 2150 MSAT Lite software. Kaimal and Shinghal (2023) as follows: prepared a sample of 50 grams in the cup to be measured and placed under the probe, installed and set the speed of 50 mm/min, then positioned the sample properly. Turn on the device and make sure that the value displayed on the monitor is at zero. Select the "*start test*" menu and pull the handle down so that the probe moves through the sample. The test is considered complete when the probe returns to the starting position. The test result will be displayed in Newton (N) on the monitor.

**2.6 Color**

The L\*a\*b\* Color testing procedure using a colorimeter refers to Phatarhe et al. (2013) is as follows: *The color reader* is turned on by pressing the power button. Color measurement using the color *reader color* scale L\* (white), a\* (red), and b\* (yellow). Samples as much as 10 grams were put and flattened into a plastic clip measuring 6x10 cm, then placed on the lens on the tool. The first time chromameter with white color as standard. The measurement results are in the form of L\*, a\*, and b\* values.

**2.7 Weight Loss**

The weight loss testing procedure of Calligaris et al*.* (2021) which has been modified is as follows: 2 grams of bigel was weighed and placed in a Petri dish, then the bigel was left for 2 weeks at room temperature 25. The final weight of the bigel was obtained by weighing the bigel. After that, calculate the weight loss with the following formula:

weight loss (%) =$\frac{early weight-final weight)}{early weight}$ x 100%

**2.8 *Oil Binding Capacity* (OBC)**

*The oil binding capacity* test procedure refers to Li et al. (2024) with some modifications as follows: Weighed an empty centrifuge tube as (m1), then prepared a sample of 6 grams and placed in a 15 ml centrifuge tube and weighed as (m2), centrifuged at 4500 rpm for 15 minutes. Inverted the tube for 5 minutes until all the oil comes out and weighed as (m3). The OBC formula is as follows:

OBC (%) = 100% - $\frac{m2-m3}{m2-m1}$

**2.9 Microstructure**

Microstructure testing using *Confocal Laser Scanning Microscopy* (LEICA) as follows: 6 grams of bigel was stained with 40 μL of Rhodamine B dye (0.2%, b/v), then the sample was diluted with 100 μL of distilled water. After that, the sample was taken and 10 μL of type G emersion oil was dripped on the slide and covered with *cover glass*. The sample was observed with *Confocal Laser Scanning Microscopy*. Images were taken using a 40x objective.

**2.10 Antioxidant Activity**

The antioxidant activity testing procedure is based on AOAC (2005). The principle of antioxidant activity testing using DPPH method and continued with the use of IC₅₀ as follows: diluted 0.1 gram sample with methanol as much as 10 ml in a centrifuge tube, then allowed to stand for 24 hours. Then three concentrations were made, namely 100%, 50% and 25% and 1 ml of DPPH was added to the concentration. Homogenized and titrated for 30 minutes. Next, put into a cuvette and measured the absorbance with a wavelength of 517 nm with a methanol blank. The absorbance value was measured using a 517 nm wavelength spectrophotometer. The IC₅₀ value was determined by entering the sample concentration and % inhibition into a linear regression equation. The x value is for the sample concentration and the y value is for the y axis. The determination of the IC₅₀ value was entered into the equation Y = ax + b, y is 50 and x is the expected IC₅₀ value.

**2.11 *Fourier Transform Infrared* (FTIR)**

The FTIR testing procedure refers to Martins et al. (2023) using Shimadzhu IRSprint as follows: Turn on the Shimadzu IRSpirit FTIR instrument and wait until the system is ready. Heated the tool for about 15-30 minutes. Samples were dropped and measurements were taken in a vacuum atmosphere, in the wave number range of 400 to 4000 cm⁻ ¹.

**2.12 Statistical Analysis**

The data obtained were collected and processed using Microsoft Excel. Data were analyzed using analysis of variance (ANOVA). If there are significant or highly significant differences in the results of the analysis, Duncan's Multiple Range Test (UJBD) will be conducted. FTIR testing was obtained in the wavelength range of 400-4000 cm-1. Absorption bands were observed and compared between samples. Confocal Laser Scanning Microscopy (CLSM) testing was analyzed using Rhodamine dye to see the protein phase. The data was visually analyzed using a Leica device to evaluate the dispersion of components

**3. results and discussion**

The results of this study are presented in tables summarizing the proximate analysis and physical quality of with varying ratios of oleogel to hydrogel. Table 1 shows data on moisture content, fat content, and antioxidant activity. Table 2 presents sensory and physical quality parameters of the bigel, including color values (L, a\*, b\*), oil binding capacity (OBC), weight loss, and texture.

**Table 1. Proximate Analysis**

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| --- | --- | --- | --- |
| Parameters | Moisture content (%) | Fat Content (%) | Antioxidant (µg/ml) |
| P1 (80:20) | 18,33 ± 2,04ª | 81,87 ± 4,69ª | 26,08 ± 8,08ª |
| P2 (70:30) | 24,90 ± 1,05ᵇ | 71,68 ± 0,72ᵇ | 32.38 ± 0.93 ab |
| P3 (60:40) | 35,05 ± 0,90ᶜ | 60,65 ± 1,93ᶜ | 51,28 ± 4,59ᵇ |
| P4 (50:50) | 42,53 ± 2,42ᵈ | 50,53 ± 2,27ᵈ | 61,48 ± 12,70c |

*Values are presented as mean ± Standard Deviation from four replications. Means in the same column with different superscripts are significantly different at p<0.05.*

**Table 2. Physical Analysis**

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| --- | --- |
| Treatment | Bigel Physical Quality Variables |
| L | a\* | b\* | OBC (%) | Weight Loss(%) | Texture (N) |
| P1 (80:20) | 18,33 ± 2,04ª | 81,87 ± 4,69ª | 1,96 ± 0,49ª | 97,98 ± 0,12 | 0,81 ± 0,11ª | 1,96 ± 0,49ª |
| P2 (70:30) | 24,90 ± 1,05ᵇ | 71,68 ± 0,72ᵇ | 2,67 ± 0,67ª | 97,99 ± 0,06 | 0.86 ± 0.07ªᵇ | 2,67 ± 0,67ª |
| P3 (60:40) | 35,05 ± 0,90ᶜ | 60,65 ± 1,93ᶜ | 2.80 ± 0.70ªᵇ | 98,00 ± 0,05 | 1,64 ± 0,73ᵇ | 2.80 ± 0.70ªᵇ |
| P4 (50:50) | 42,53 ± 2,42ᵈ | 50,53 ± 2,27ᵈ | 2,74 ± 0,91ᵇ | 98,22 ± 0,44 | 2,18 ± 0,95ᵇ | 2,74 ± 0,91ᵇ |

*Values are presented as mean ± Standard Deviation from four replications. Means in the same column with different superscripts are significantly different at p<0.05*

* 1. **Moisture content**

The results of the analysis of variance showed that the ratio of oleogel and hydrogel based on beeswax and gelatin had a very significant effect (*P < 0*.01) on the average value of moisture content in bigel. The lowest moisture content value was obtained in sample P1 at 18.33% with an oleogel and hydrogel ratio of 80:20, followed by sample P2 at 24.90%, sample P3 at 35.05% and the highest value was in sample P4 at 42.53% with a ratio of 50:50. The increase in moisture content along with the increase in the proportion of hydrogel shows that the higher the composition of hydrogel in bigel, the greater the absorbed moisture content.

Based on Table 1, moisture content gives a very significant difference to bigel characteristics, with a range of values between 18.33% and 42.53%. This result is not much different from the research conducted by Gulsac, et al*.* (2024), who reported that the moisture content in vegetable-based bigel from chickpea protein hydrogel and glyceryl monostearate oleogel ranged from 49.81% to 56.54%. This difference can be explained through the nature of each component forming the bigel. The use of gelatin as a hydrogel material allows the formation of a three-dimensional network of hydrophilic polymer structures capable of absorbing and retaining large amounts of water. (In addition, based on research conducted by Kaimal and Shinghal (2023), bigel with hydrogel to oleogel ratios of 75:25, 50.55 and 25:75 had moisture content values of 73.84%, 49.14% and 23.41%, respectively. This shows that increasing the hydrogel fraction significantly contributes to the increase in moisture content in the bigel system.

* 1. **Fat Content**

The results of the analysis of variance showed that the ratio of oleogel and hydrogel in beeswax and gelatin-based bigel made a very significant difference to the fat content. The highest average value was found in sample P1 at 81.87%, while the lowest average value was found in sample P4 at 50.53%. The average value of P1-P4 decreased because the proportion of oleogel decreased. Oleogel is the fat phase in bigel which is composed of corn oil and beeswax, so an increase in the proportion of oleogel contributes to the high fat content in bigel. In contrast, increasing the proportion of gelatin-based hydrogels that contain a large amount of water tends to significantly reduce the fat content. Overall, the fat content of bigel in this study was in the range of 81.87%-50.53%. This is not in accordance with the research of Gulsac, et al (2024), where the fat content of bigel based on chickpea protein hydrogel and glyceryl monostereat oleogel was 12.10%-28.65%. This difference is due to the type of base material used because beeswax and corn oil have a much higher fat content than glyceryl monostearate.

* 1. **Antioxidant Activity**

The results of analysis of variance showed that the ratio of oleogel and hydrogel gave a highly significant difference (*P<0*.01) to the antioxidant activity of bigel. The mean IC₅₀ of antioxidant activity of sample P1 was 26.08 µg/ml, sample P2 was 32.38 µg/ml, sample P3 was 51.28 µg/ml and the sample with the highest value in sample P4 was 61.48 µg/ml. The highest average value was found in sample P1 of 26.08 µg/ml with the proportion of oleogel and hydrogel 80:20. This is not much different from the research conducted by Firdaus and Sunita (2024), the IC₅₀ value of antioxidant activity of beeswax oleogel-based bigel and coconut oil is 26.13 µg/ml. sample P1 has a high enough antioxidant activity value because the proportion of oleogel containing more corn oil. The research is not much different from Khelifi, *et al* (2019), 5,8-dihydroxy-1,4-naphthoquinone-based bigel with DPPH methofe has a value of 19 ×$10^{-3}$ mg/mL and 35 ×$10^{-3}$ mg/ml. According to Kholifah, et al. (2023), IC₅₀ (µg/ml) values between <50 indicate very strong antioxidant activity, values of 50-100 indicating strong antioxidant activity and values above 100 indicating weak antioxidant activity. Based on Table 1, it can be observed that the beeswax and corn oil based bigel showed strong antioxidant activity as corn oil is a good source of antioxidants.

* 1. **Color**

The results of the analysis of variance showed that the ratio of oleogel and hydrogel in bigel did not give a significant difference (*P>0*.05*)* to the L\*, a\* or b\* values. The mean value of the L\* (brightness) parameter ranged from 87.13-88.98. The mean value of parameter a\* (redness) ranged from 1.00-0.86, while for parameter b\* (yellowness) had a value of 15.29-15.03. This can be explained by the fact that oleogel based on beeswax and corn oil has a natural brownish-yellow color, so that a decrease in the amount of oleogel causes a reduction in the intensity of redness and yellowness in bigel but is not statistically significant. This study is similar to Lee, et al. (2024), the color value of bigel based onadelic wax oleogel with *guar gum* hydrogel has an L\* value of 32.59-35.57, an a\* value of 14.35-11.59 and a b\* value of 30.50-28.76 indicating that bigel also has yellowish color characteristics that are quite dominant. In addition, research conducted by Shaikh, et al (2022), bigel based on candelilla wax oleogel and sesame oil with guar gum hydrogel, the L\* color value is 97.76-98.81, the a\* parameter is -5.74 to -7.05, while the b\* parameter is 19.06-23.92.

* 1. ***Oil Binding Capacity* (OBC)**

The result of analysis of variance showed that the ratio of oleogel and hydrogel in bigel did not give significant difference (*P*> 0.05) to OBC. The average value of OBC increased with each sample. Sample P1 had the lowest value of 97.98%, sample P2 of 97.99%, sample P3 of 98.00% and sample P4 had the highest value of 98.22% ranging from 97%-98.22% as the proportion of hydrogel increased. The increase in the proportion of hydrogel in the bigel formulation contributed significantly to the increase in OBC. The ability of hydrogel to form a stable and organized hydrophilic network. The three-dimensional structure produced by the hydrogel is supported by the high water absorption capacity so as to maintain elasticity. This is in accordance with Shakouri, et al (2025), the increase in hydrogel phase restricts the movement of oil in the bigel and increases OBC so that it acts as a surfactant to effectively reduce interfacial tension and inhibit phase separation. The highest average value of OBC was found in sample P4 at 98.22%. This is in accordance with Liu, et al (2025), the highest OBC value in soybean oil and insect wax based bigel was 99.8%. Meanwhile, research conducted by Samui, et al (2021), bigel with homogenization time treatment of 1, 3, 5 and 7 minutes was 85%, 99%, 99% and 91%.

* 1. **Weight Loss**

The results of the analysis of variance showed that the ratio of oleogel and hydrogel in bigel gave a very significant difference (*P<0*.01) to the weight loss. The average value of weight loss of sample P1 was 0.81%, sample P2 was 0.86%, sample P3 was 1.64% and sample P4 had a value of 2.18%. The highest value is found in sample P4 which is 2.18% and the lowest value is 0.81%. Weight shrinkage increased along with the increase in the proportion of hydrogel in the bigel formulation. This is because the hydrogel has a high moisture content so that during the process of evaporation or storage there is mass loss due to evaporation of water from the system. The greater the proportion of hydrogel, the amount of water available to evaporate increases and causes the weight loss value to be higher. The average value of weight loss in bigel samples during the two-week observation ranged from 0.81%-2.18%. This weight loss value is relatively low when compared to the results of Silva, et al (2022), who reported that the weight loss in their bigels ranged from 13.20% to 23.90% after a 28-day observation period.

* 1. **Texture**

The results of the analysis of variance showed that the ratio of oleogel and hydrogel based on beeswax and gelatin gave very significant differences (*P < 0*.01) to the texture of the bigel. The lowest average value of texture was found in sample P1, which had an average value of 1.96 N, followed by sample P2 at 2.67 N, sample P3 with the highest value of 2.80 N and sample P4 at 2.74 N. Based on the measurement results, the average value of bigel texture ranged from 1.96 N to 2.80 N. There was an increase in hardness as the proportion of hydrogel in the bigel formulation increased. This is in line with Martins, et al (2023), the ratio of oleogel and hydrogel 50:50 to 99:1 decreased the hardness value of bigel when the proportion of oleogel was increased. This is due to the ability of hydrogels, especially gelatin, to form a three-dimensional polymer network that is hydrophilic. This network is able to retain water well and provide a stable mechanical structure, thus strengthening the overall physical integrity of the bigel. The results of this study are in line with the findings of Shakouri, et al. (2024), the texture value of bigel decreased as the proportion of gelatin decreased and the proportion of CMC increased with a range of 2.62 N to 1.72 N. Bigel samples with higher gelatin content showed a firmer texture, indicating that gelatin plays an important role in forming a strong and dense gel network compared to CMC which produces a softer gel structure. According to Samui, et al (2021), bigel research with different homogenization durations of 1,3,5 and 7 minutes was 0.35 N, 0.35 N, 0.30 N and 0.20 N.

* 1. **FTIR**

FTIR analysis was performed to identify the functional groups and chemical interactions between the bigel components. The graph shows the absorption spectra of each treatment indicating a change in structure or interaction. The graph can be seen in Figure 1.

Figure 1. FTIR testing graph of bigel

The FTIR analysis in Figure 1 shows several absorption bands that illustrate changes in the chemical composition of the sample. The absorption band in the 3100-3500 cm-1 region corresponding to the O-H (stretching*)* of alcohol or hydroxyl groups, increased in intensity in sample P4. This indicates high moisture content or gelatin hydrogel in accordance with the findings of Mallamace et al. (2015), the broad bands in this range are closely related to O-H and N-H vibrations, both in free and bound forms commonly found in hydrogel-based systems. In contrast, the absorption bands in the 2800-3000 cm-1 region indicating aliphatic C-H stretches of the lipid or oleogel components of beeswax and corn oil, decreased in intensity from P1 to P4. This is in accordance with Sagiri et al. (2015), the C-H peaks at 2920 and 2850 cm⁻ ¹ decreased with the reduction of lipid fraction in the bigel system. The absorption band of 1600-1750 cm⁻ ¹ region, which originates from the C=O stretch of ester or carbonyl groups (from triglycerides or beeswax esters), was most intense at P1, indicating the dominance of oleogel. This is in agreement with Martins et al. (2023), the presence of absorption bands around 1745 and 1160 cm⁻ ¹ indicates an increase in oleogel fraction associated with C=O and C-O stretch vibrations of triglycerides. In sample P4, the intensity of this band decreases due to the decrease in oleogel proportion. The band around 1630 cm⁻ ¹ originates from the C=O stretch of amide I (gelatin protein) or -OH of structured water and is more prominent in samples with high hydrogel ratio (P4) indicating an increased hydrogel content of gelatin or structured water. This is in agreement with Hashemi et al, (2017), the band around 1620 cm⁻ ¹ attributes to the characteristics of gelatin protein in protein-polyphenol gel system. The absorption band in the 1000-1200 cm region⁻ ¹ associated with ether or polysaccharide groups in the hydrogel material showed a decrease in intensity as the gelatin hydrogel ratio increased in the sample (P4). This is suggested by Cakman and Arslan (2022), the reduced fraction of polysaccharides sourced from oleogel or interactions between components affecting their presence and corroborates the idea that structural changes in bigel are physical in nature.

* 1. **Microstructure**

Observation using Confocal Laser Scanning Microscopy (CLSM) shows that the ratio of oleogel and hydrogel affects the protein distribution in the bigel system. Figure a (80:20) shows uneven protein distribution with a few large visible as bright red patches. Figure b (70:30) shows a more even distribution of proteins compared to 80:20. Although there are still some aggregates, the fluorescence intensity becomes more dispersed indicating interactions are starting to form on the proteins in the hydrogel system. Figure c (60:40) shows a smooth and homogeneous protein distribution without any large aggregates. This indicates that this ratio provides an ideal balance between the oil and water phases, resulting in an optimal interaction between the proteins and the hydrogel network that is stable and uniform. Figure d (50:50) shows that an excess of hydrogel phase in the system can lead to an overabundance of aqueous phase, resulting in protein phase separation or precipitation. This finding is in line with the results of Luo et al. (2021), a bigel-based emulsion system with a balanced oil and water phase ratio results in better protein dispersion and high structural stability. In addition, Wang et al. (2022) also showed that the presence of gelatin in the hydrogel phase can strengthen the gel network and facilitate the even distribution of protein in semi-solid food systems. *Confocal Laser Scanning Microscopy* (CLSM) observation results to see the distribution of protein phases in bigel. The observation results can be seen in Figure 2.

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Figure 2. *Confocal Laser Scanning Microscopy* (CLSM) image of beeswax-based bigel and gelatin with rhodamine dye. The top row shows the protein distribution (red) and the bottom row shows the *gray scale* image. a.) 80:20, b.) 70:30, c.) 60:40 and d.) 50:50. Scale bar = 25 µm.

**4. Conclusion**

The results showed that beeswax and corn oil oleogel-based bigel with gelatin hydrogel gave a very significant effect on moisture content, fat content, texture, weight loss and antioxidant activity, but did not give significant differences on OBC and color. The best ratio of oleogel and hydrogel was found in treatment P1 with a ratio of 80:20 which produced vegetable fat with good physical and functional characteristics.

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**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**References**

AOAC. (2005). Official Methods of Analysis of AOAC International 18th Edition. Maryland: AOAC International. *Science*. 1(2), 68-74.

Cakmak-Arslan, G. (2022). *Characterization of Triglyceride-Based Oleogels Using Spectroscopic and Thermal Analysis Techniques*. *Journal of Food Science and Technology*, 59(5), 2134-2142. [https://doi.](https://doi.org/10.1007/s13197-021-05233-7)org/10.1007/s13197-021-05233-7

Calligaris, S., Valoppi, F., Barba, L., & Martini, S. (2021). Structuring Edible Oils with Rice Starch and Hydrocolloids: Effects of Composition and Processing Conditions. *Journal of Food Engineering*, 291, 110263.

Chen, J.P., Leu, Y.L., Fang, C.L., Chen, C.H. & Fang, J.Y. (2011). Thermosensitive Hydrogels Composed of Hyaluronic Acid and Gelatin as Carriers for the Intravesical Administration of Cisplatin. J. Pharm. *Sci*, 100(2), 655-666.

Dwiputra, D., Jagat, A. N., Wulandari, F. K., Prakarsa, A. S., Puspaningrum, D. A. & Islamiyah, F. (2015). Corn Oil as a Healthy Alternative Oil Substitute. *Journal of Food Technology Applications*, 4(2).

Firdaus, M.A. & Sunita, M. (2024). characterization of Coconut Oil-Beeswax/Gelatin Based Food-Grade Bigel and Its Application in Muffin as Trans-Fat Replacement. *Journal of Chemical Health Risks*, 14(1), 512-521. [https://doi.](https://doi.org/10.22034/jchr.2024.1988999.1234)org/10.22034/jchr.2024.1988999.1234

Flöter, E., Wettlaufer, T., Conty, V., & Scharfe, M. (2021). Oleogels Their Applicability and Methods of Characterization. *Molecules*, 26(6), 1673. [https://doi.](https://doi.org/10.3390/molecules26061673)org/10.3390/molecules26061673

Glusac, J., Moguiliansky, S., Fishman, A., & Davidovich-Pinhas, M. (2024). Plant-Based Bigel Based on Chickpea-Potato Protein Hydrogel and Glycerol Monostearate Oleogel. *Food Structure*, *41*,100378. [https://doi.](https://doi.org/10.1016/j.foostr.2024.100378)org/10.1016/j.foostr.2024.100378

Hashemi, S. M. B., Khaneghah, A. M., & Barba, F. J. (2017). *Interaction of Proteins and Polyphenols in Food Systems: Characterization and Applications Using FTIR Spectroscopy*. *Critical Reviews in Food Science and Nutrition,* 57(16), 3443-3451. [https://doi.](https://doi.org/10.1080/10408398.2015.1121911)org/10.1080/10408398.2015.1121911

Jing, X., Chen, Z., Tang, Z., Tao, Y., Huang, Q., Wu, Y. & Sun, Y. (2022). Preparation of Camellia Oil Oleogel and its Application in an Ice Cream System. *Lwt*, 169, 113985.

Kaimal, A. M., & Singhal, R. S. (2023). Bigels for Controlled Gastric Release of Ascorbic Acid: Impact on Rheology, Texture, Thermal Stability and Antioxidant Activity. *Food Hydrocolloids for Health*, *4*, 100171. [https://doi.](https://doi.org/10.1016/j.fhfh.2023.100171)org/10.1016/j.fhfh.2023.100171

Kavya, N. P., & Pal, K. (2024). Pectin Emulsions and Emulgels: Bridging the Correlation Between Rheology and Microstructure. *Food Hydrocolloids*, 137, 107345.

Khelifi, I., Saada, M., Hayouni, E. A., Tourette, A., Bouajila, J. & Ksouri, R. (2020). Development and Characterization of Novel Bigel-Based 1, 4-Naphthoquinones for Topical Application with Antioxidant Potential. *Arabian Journal for Science and Engineering*, 45(1), 53-61.

Kholifah, E., Nurazizah, D., & Noviyanto, F. (2023). Antioxidant Activity and Vitamin C Concentration Analysis of Gandaria (*Bouae macrophylla griff*) Ethanol Extract Using UV Vis Spectrophotometry. *Journal of Fundamental and Applied Pharmaceutical Science*, *3*(2), 54-63.

Lee, G. R., Baek, Y., Jeong, E., & Lee, H. G. (2024). Development and Characterization of a Novel Bigel System Based on Candelilla Wax Oleogel and Guar Gum Hydrogel for Heat-Triggered Release Properties. *Food Hydrocolloids*, *152*, 109892. [https://doi.](https://doi.org/10.1016/j.foodhyd.2024.109892)org/10.1016/j.foodhyd.2024.109892

Li, X., Wang, Y., & Liu, Y. (2024). Novel Bigels Based on Walnut Oil Oleogel and Chitosan Hydrogel: Preparation, Characterization, and Application as Food Spread. *International Journal of Biological Macromolecules*, 242, 124567.

Liu, W., Liu, K., McClements, D. J., Jin, Z., & Chen, L. (2025). Fabrication and Characterization of Starch-Based Bigels Under Phase Control: Structural, Physicochemical and 3D Printing Properties. *Food Hydrocolloids*, *159*, 110623. [https://doi.](https://doi.org/10.1016/j.foodhyd.2025.110623)org/10.1016/j.foodhyd.2025.110623

Mallamace, F., Vasi, S., Corsaro, C., et al. (2015). *The Role of Hydrogen Bonding in the FTIR Spectra of Water. The Journal of Chemical Physics*, 142(21), 212502. [https://doi.](https://doi.org/10.1063/1.4919366)org/10.1063/1.4919366

Martins, A. J., Guimarães, A., Fuciños, P., Sousa, P., Venâncio, A., Pastrana, L. M. & Cerqueira, M. A. (2023). Food-Grade Bigels: Evaluation of hydrogels: Oleogel Ratio and Gelator Concentration on Their Physicochemical Properties. *Food Hydrocolloids*, *143*, 108893. [https://doi.](https://doi.org/10.1016/j.foodhyd.2023.108497)org/10.1016/j.foodhyd.2023.108497

Meng, Z., Qi, K., Guo, Y., Wang, Y. and Liu, Y. (2018). Edible Oleogels Prepared from Beeswax and Vegetable Oils: Gel Properties and Crystallization Behavior. *Food Research International*, 105, 36-45. h[ttps://doi.org/10.1016/j.foodres.2017.11.057](https://doi.org/10.1016/j.foodres.2017.11.057)

Noman, S., & Singh, N. (2024). Efficacy of Bigel in Improving the Viability of Probiotic: Experimental Study. *Sustainability, Agri, Food and Environmental Research-DISCONTINUED*, *12*(2). [https://doi.](https://doi.org/10.7770/safer-V12N2-art3209)org/10.7770/safer-V12N2-art3209

Pathare, P. B., Opara, U. L. & Al-Said, F. A. J. (2013). Color Measurement and Analysis in Fresh and Processed Foods: A Review. *Food and Bioprocess Technology*, *6*, 36-60. [https://doi.](https://doi.org/10.1007/s11947-012-0867-9)org/10.1007/s11947-012-0867-9

Prasad, K., & Kumar, A. (2023). Physiochemical, Thermal, Textural, Functional, And Microstructural Characterization of The Developed Shelf Stable Bigel Using Hydrogel and Oleogel. *In Journal of Physics: Conference Series*, 2663(1), 012029. h[ttps://doi.org/10.1088/1742-6596/2663/1/012029](https://doi.org/10.1088/1742-6596/2663/1/012029)

Pușcaș, A., Mureșan, V., Socaciu, C., and Muste, S. (2020). Oleogels in Food: A Review of Current and Potential Applications. *Foods*, 9(1). [https://doi.](https://doi.org/10.3390/foods9010070)org/10.3390/foods9010070

Sagiri, S. S., Singh, V. K., Kulanthaivel, S., Banerjee, I., Basak, P., Battacharya, M. K., & Pal, K. (2015). *Physical, Mechanical, and Biological Characterization of Oleogel-Based Bigels for Topical Delivery of Diclofenac*. *Journal of Materials Science: Materials in Medicine*, 26(1), 5346. [https://doi.](https://doi.org/10.1007/s10856-014-5346-5)org/10.1007/s10856-014-5346-5

Sakul, S. E., Rosyidi, D., & Radiati, L. E. (2019). Effect of White Oyster Mushroom (*Pleurotus ostreatus*) Juice Addition on Fat Content, Moisture Content, Ash Content, Water Binding Capacity, and pH Value of Cow's Milk Yogurt. *Journal of Animal Science*, 7(1), 41-46.

Samui, T., Goldenisky, D., Rosen-Kligvasser, J., & Davidovich-Pinhas, M. (2021). The Development and Characterization of Novel in-situ Bigel Formulation. *Food Hydrocolloids*, *113*, 106416. [https://doi.](https://doi.org/10.1016/j.foodhyd.2020.106416)org/10.1016/j.foodhyd.2020.106416

Shaikh, H. M., Anis, A., Poulose, A. M., Madhar, N. A. & Al-Zahrani, S. M. (2022). Development of Bigels Based on Date Palm-Derived Cellulose Nanocrystal-Reinforced Guar Gum Hydrogel and Sesame Oil/Candelilla Wax Oleogel as Delivery Vehicles for Moxifloxacin. *Gels,* 8(6), 330.

Shakouri, S., Arabshahi, S., Madanchi, H., Mohammadifar, M. A., and Abdolshahi, A. (2024). Effect of Carboxymethyl Cellulose Incorporation to Gelatin-Sunflower Oil Bigel on the Physicochemical and Structural Properties. *Polymers for Advanced Technologies*, *35*(9), e6567.

Silva, R., Ferdaus, M. J., Foguel, A., & da Silva, T. L. T. (2023). Oleogels as a Fat Substitute in Food: A Current Review. *Gels*, 9, 180. [https://doi.](https://doi.org/10.3390/gels9030180)org/10.3390/gels9030180

Silva, S. S., Rodrigues, L. C., Fernandes, E. M., Lobo, F. C., Gomes, J. M., & Reis, R. L. (2022). Tailoring Natural-Based Oleogels Combining Ethylcellulose and Virgin Coconut Oil. *Polymers*, *14*(12), 2473. [https://doi.](https://doi.org/10.3390/polym14122473)org/10.3390/polym14122473

Zhu, Q., Gao, J., Han, L., & Zhang, M. (2021)**.** Development and Characterization of Novel Bigels Based on Monoglyceride-Beeswax Oleogel and High Acyl Gellan Gum Hydrogel for Lycopene Delivery. *Food Chemistry*, 344, 128624.

Zulfiqar, A., Shabbir, M. A., Tahir, F., Khan, M. R., Ahmed, W., Yıkmış, S., & Aadil, R. M. (2024). Development of Oleogel by Structuring the Blend of Corn Oil and Sunflower Oil with Beeswax to Replace Margarine in Cookies. *Food Chemistry:* 10, 23, 101676.