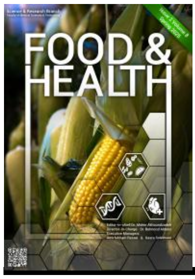

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Development and Characterization of Food-Grade Bigels Based on HPMC and Beeswax with Saffron Petal Powder as a Natural Antioxidant

Nafise Javidi[1], Khadije Abdolmaleki[2], Leyla Alizadeh[*1]

[1] Department of Food Quality Control and Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran.

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A B S T R A C T

Bigels are semi-solid, two-phase systems composed of an organic and an aqueous phase. This study aims to produce bigels with hydrogel of HPMC at concentrations of 1% and 2% (0.4% and 0.8% in bigel) and oleogel of beeswax at concentrations of 5% and 10% (3% and 6% in bigel). Additionally, to evaluate antioxidant properties, Saffron petal powder was added to bigel samples at two levels of 0% and 2%. The steady state measurement revealed that all samples exhibited pseudoplastic behavior (n<1). An increase in the concentrations of HPMC and beeswax resulted in significant increase of viscosity (pv< 0.05). The highest values of consistency index (K) and yield stress ($\tau 0$), (238.74Pa and 92.16Pa.sn) were related to sample 8 (bigel with 0.8% HPMC and 6% beeswax). Furthermore, in frequency Sweeps tests, the storage modulus was greater than the loss modulus in all samples, confirming their viscoelastic solid characteristics. When comparing the rheological properties of the bigel samples to those of mayonnaise, samples 5 (bigel with 0.8% HPMC and 3% beeswax) and 6 (bigel with 0.8% HPMC, 3% beeswax, and 2% saffron petal powder) were identified as the best treatments. The antioxidant evaluation demonstrated that saffron petal powder significantly reduced peroxide and anisidine levels, and increased oil stability (pv< 0.05). The saffron petal powder addition resulted in 34.33% and 11.45% reduction in peroxide and anisidine values, respectively, in sample 6 compared to sample 5, after 90 days of storage. Sensory evaluations indicated that sample 5 received the highest scores for texture and color.

Introduction

Fats and oils are important for people's nutrition because they not only provide energy but also help supply vitamins and essential fatty acids. They enhance the texture, aroma, and flavor of food products (1). In recent years, modern lifestyles have led to excessive consumption of saturated and trans fats through high-calorie fast foods and ready-to-eat meals (2). This has led to more cases of obesity, type 2 diabetes, heart disease, and cancer around the world (3). To maintain a healthy diet, the World Health Organization recommends limiting total fat intake to less than 30% and saturated fats to less than 10% of total energy (4). Consumer awareness has driven demand for healthier food products with reduced saturated and trans fats, prompting the food industry to reformulate fats to maintain competitive sensory and functional properties (5). Replacing solid fats with liquid oils often reduces product quality and acceptance, making the elimination of saturated and trans fats challenging (6). New methods, including carbohydrate, protein, emulsifier, and fat-based approaches, are being explored to replace unhealthy fats, but each has its limitations. For example,

* Corresponding Author: Leyla Alizadeh
Email: Lm.alizadeh2014@gmail.com

using unsaturated oils instead of saturated ones can result in undesirable texture and mouthfeel. Although hydrogenated oils are derived from healthy vegetable oils, they contain harmful trans fats. Interesterification does not produce trans fats and does not alter the fatty acid composition of the raw material, but it changes the distribution of fatty acids in triglycerides, affecting properties like melting point and crystallization(7, 8) . Recently, oleogels, hydrogels, and emulsion gels have been introduced as fat substitutes to answer consumer demand for healthier products (9). Oleogels are formed by trapping oil droplets in a three-dimensional network created by high and low molecular weight oleogelators through direct and indirect methods, converting liquid oil into semi-solid fat (10). Hydrogels are three-dimensional gel networks capable of trapping large amounts of water. Both gel systems exhibit viscoelastic properties, influenced by the type and concentration of gelators and the liquid phase (water or oil)(11). Bigels are semi-solid, structured biphasic gel systems prepared by combining hydrogel and organogel at high speed and specific temperatures (1, 12). The combination of these gels gives bigels an advantage over hydrogels and oleogels (13). Bigel properties depend on factors such as the structure and preparation of hydrogel and oleogel, the ratio of the two phases, and the type and concentration of gelling agents (14, 15). Since both phases are immiscible and structured, biphasic gels can be considered the next step for emulsions. The first study on biphasic gels was published in 2008, combining the cooling effect of hydrogels and the moisturizing effect of oleogels to create a better moisturizing agent for pharmaceutical applications (16). In 2008, Almeida et al, achieved a semi-solid, physically stable formulation by combining hydrogel and oleogel. The hydrogel, as an aqueous system, temporarily hydrates the skin before water evaporation, while the oleogel effectively moisturizes by creating a lipid barrier between the skin and the environment. However, the tactile feel of oleogel may be unpleasant due to its greasiness, prompting researchers to combine hydrogel and oleogel to create a non-greasy yet moisturizing skin cream. Although the formulation by Almeida et al. in 2008 was not suitable for food applications, the idea of merging two immiscible systems to achieve a new system with combined properties can be applied to food products. Biphasic gels are superior to other gels due to their stability and ability to carry both hydrophobic and hydrophilic compounds, due to their organic and aqueous solvents (17). Bigels have the capability to encapsulate lipophilic and hydrophilic bioactive compounds, serve as fat substitutes, and modify aqueous and oil phases to enhance gel properties and physical stability (18). They have been extensively used in pharmaceutical and cosmetic industries, and research on their application in food products is ongoing (19). These biphasic gel systems, due to their distinct rheological properties, have potential as solid fat substitutes to achieve desirable textures. Saffron, scientifically known as Crocus sativus, is used as a flavor and color agent in foods. During saffron production, the stigma and style are used commercially, while other parts like petals are discarded as waste, highlighting the importance of finding ways to recycle them (20, 21). Saffron petals are a rich plant source of polyphenolic compounds, possessing significant phenolic content and high antioxidant capacity. A concentration of 500 ppm of petal extract is comparable to 100 ppm of TBHQ, a strong synthetic antioxidant (21). Natural antioxidants play a role in reducing the incidence of diseases such as cancer, heart, and brain diseases without the adverse effects of synthetic antioxidants, emphasizing the need for natural antioxidant compounds from plants which are not only potent but also have fewer side effects (20). The existing literature has extensively explored the application of bigels in pharmaceutical and cosmetic fields; however, there is a significant gap in research regarding the development of bigels specifically for food products, particularly as fat substitutes with integrated antioxidant properties. This area remains underexplored, with limited studies addressing the formulation and characterization of food-grade bigels. Consequently, there is a need for investigation into the sensory, physicochemical, and rheological properties of bigels derived from beeswax oleogel and hydroxypropyl methylcellulose (HPMC) hydrogel. Furthermore, the potential antioxidant effects of natural additives, such as saffron petal powder, on these bigels have not been adequately examined. This study aims to address these gaps by producing and characterizing bigels for food applications, compaing it with mayonnaise properties and evaluating the impact of saffron petal powder on their antioxidant properties.

2. Materials and methods

2.1. Materials

Refined sunflower oil (Tabiat Co., saveh, Iran), mayonnaise (Mahram Co.,Qazvin) and saffron petal were purchased from a local market. Hydroxypropyl meyhyl cellulose (viscosity 2600–5600 cP, 2% in distilled water at 20 °C, MW ~86 kDa, methoxyl content of 28–30% and a hydroxypropoxyl content of 7–12%) and Beeswax (yellow flakes, odorless, melting range of 60–65 °C) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Sodium Azide.(for microbial growth inhibition) and All other chemicals and reagents were obtained from Merck Company (Darmstadt, Germany)

2.2. Sample preparation

The hydrogel (aqueous phase of bigel) was prepared by dissolving hydroxypropyl methylcellulose and sodium azide in distilled water at 1% and 2% concentrations. Saffron petal Powder was added to hydrogel phase in treatments 2,4,6 and 8. The oleogel (oil phase of bigel) was prepared by dissolving Beeswax and lecithin in oil phase (sunflower oil) under agitation at 85 °C until complete solubilization. Hydrogels were poured into oleogels in a 40:60 ratio and the prepared Bigel samples (8 samples) composition are presented in table 1. The mayonnaise is addressed as sample 9 in this study. The resulting mixtures were homogenized for 2 minutes at a speed of 15,000 rpm and a temperature of 85 °C. After homogenization, the samples were placed in ice water and maintained there until complete solidification occurred. Subsequently, the samples were transferred to a refrigerator and stored prior to analysis.

Table 1 . Composition (%) of Bigel samples prepared from beeswax and hydroxypropyl methylcellulose

Sample names	HPMC	Beeswax	oil	Saffron petal powder	Eemulsifier	Water%
1	0.4	3	56.7	0	0.3	39.6
2	0.4	3	56.7	2	0.3	37.6
3	0.4	6	53.7	0	0.3	39.6
4	0.4	6	53.7	2	0.3	37.6
5	0.8	3	56.7	0	0.3	39.2
6	0.8	3	56.7	2	0.3	37.2
7	0.8	6	53.7	0	0.3	39.2
8	0.8	6	53.7	2	0.3	37.2

2.3. Rheological Tests

All rheological tests were conducted using an Anton Paar Physica rheometer with a parallel plate probe. The gap between the plates was set to 1 mm, and excess sample outside the plate was removed. Initially, a strain Sweeps test was performed to identify the linear viscoelastic region (LVR). Subsequently, a frequency Sweeps test was conducted in the LVR at a constant shear strain to determine changes in the elastic modulus (G') and viscous modulus (G"). Data collection and fitting to models were done using RheoPlus software version 3.21 (Anton Paar).

2.3.1. Strain Sweeps Test

The elastic modulus (G') and viscous modulus (G") were determined in the strain range of 0.01% to 600% at a frequency of 1 Hz. From the data, the corresponding strain for the LVR (γ LVR), structural strength (G' LVR), loss tangent ($\tan \delta$), and yield stress (τy) in the linear region were determined. Based on the strain test results, a strain of 0.1% was used for the frequency Sweeps test. The loss tangent is calculated as $\tan \delta$ = G'/G".

2.3.2. Frequency Sweeps Test

Based on the LVR obtained from the strain Sweeps test, the frequency Sweeps was conducted in the range of 0.1 to 100 Hz at room temperature and within the LVR strain to obtain the elastic and viscous modulus as a function of frequency and reveal frequency dependence of the samples. The storage modulus (G´) was modeled as a power function of angular frequency (ω, rad/s) to determine intercept (A), slope (b) and R2 using the Bohlin equation (Eq.(1)) $G' = A \times (\omega) b$

2.3.3. Steady state measurement

The Herschel-Bulkley and Power Law models were fitted to data, and the following parameters were determined for the samples (Eq.(2) and (3)):

Herschel-Bulkley model: y=kx^n+τ0 (2)
K= consistency index n= flow behavior index x=shear rate τ0=yield stress

Power Law model: y = a·x b (3)
b=flow behavior index a=consistency index

2.4. Antioxidant Effect of Saffron Petals

Oxidation parameters of the model food system were evaluated over three months in four periods (immediately after production, end of the first month, end of the second month, and end of the third month) at refrigerator temperature. The indices are as follows:

2.4.1. Peroxide Value

Measured in milliequivalents of oxygen per kilogram using the AOSC method (cd 8-53) with acetic acid/chloroform and potassium iodide solution (22).

2.4.2. Anisidine Value

Since the peroxide number only indicates the presence of primary oxidation products, determining the anisidine number, which indicates secondary products, is important. Anisidine value measurement was done according to AOCS method (Cd 18–90) (23).

2.4.3. Rancimat Test

To determine the oxidative stability index (OSI), samples were subjected to accelerated conditions in a Rancimat device. For this, 2.5 grams of sample were tested at 120°C with an airflow rate of 20 liters per hour, and the volume of water in the conductivity measurement vessel was 60 mL(24).

2.5. Sensory Evaluation

Sensory evaluation of samples treated with different concentrations of saffron petal powder was conducted using a 5-point hedonic scale, ranging from very desirable (5) to very undesirable (1). During this test, attributes such as aroma, texture, and color were evaluated by seven assessors(25).

2.6. Statistical Analysis of Data

All analyses were performed using SPSS software version 21. The one-way ANOVA was applied to compare the parametric features of different samples and the significance of means differences was determined (at the level of $p < 0.05$) by Duncan’s multiple-range post-hoc test. Repeated measures were used to compare the antioxidant properties of saffron petal powder in each sample, over the storage period. Experiments were conducted in triplicate, and results were reported as mean ± standard deviation.

3. Results and Discussion

3.1. Rheological Properties

3.1.1. Strain Sweeps Test

Dynamic oscillatory tests provide valuable information about the flow and deformation behavior of viscoelastic materials. The strain Sweeps test was conducted to determine the linear viscoelastic region (LVR) for other tests. Parameters from the strain Sweeps test include the strain corresponding to the end of the LVR (γ LVR), elastic modulus (G'), viscous modulus (G"), and loss tangent (tan δ = G"/G'), as shown in the Table 2. In the LVR, the elastic modulus was higher than the viscous modulus for all samples, resulting in a loss tangent less than one. Among samples 1, 4, and 5, sample 5 had the lowest values for these factors. Additionally, with an increase in wax percentage, the elastic modulus significantly increased ($p \leq 0.05$). Comparing samples 1 and 4 with the same HPMC content, an increase in wax percentage in sample 4 showed a significant increase in both elastic and viscous moduli. For all samples in the LVR, the storage or elastic modulus was greater than the loss or viscous modulus (G' > G"), indicating a predominantly elastic characteristic. The gap between the G' and G" curves represents the loss tangent (tan δ = G"/G'). In the LVR, the material maintains its structure, and beyond this point, it breaks down, indicating the yield stress. This point is seen on the graph as the intersection of the elastic and viscous moduli (G" = G'). Beyond this point, if G" becomes greater than G', it means the material behaves more like a liquid and begins to flow (flow point). A longer LVR indicates greater resistance to deformation and higher stability(26). Sample 5 had a longer LVR compared to other samples, indicating that it maintained its structure under applied strain for a longer period before breaking, suggesting greater stability.

Table 2. parameters obtained from the strain Sweeps test

sample	γ_{LVR}	tan δ_{LVE}	G''_{LVE} (Pa)	G'_{LVE} (Pa)
1	0.41 ± 0.001^{a}	0.317 ± 0.001^{a}	691.6 ± 1.5^{a}	2181.7 ± 1.52^{a}
2	0.26 ± 0.007^{a}	0.408 ± 0.005^{b}	6912.3±۲/۰۸^{b}	16902 ± 1.25^{b}
5	9.8 ± 0.02^{b}	0.234 ± 0.001^{c}	84.3 ± 0.65^{c}	359 ± 1^{c}

Different lowercase letters between rows indicate significant differences between samples ($p < 0.05$). The data are presented as mean ± standard deviation.

3.1.2. Frequency Sweeps Test

In this test, the storage modulus (G') and loss modulus (G") were examined as functions of angular frequency (Figure 1). In all samples, both parameters, G' and G", showed a parallel relative increase with increasing frequency. The elastic modulus (G') was higher than the loss modulus (G") across all frequencies, indicating a predominantly elastic behavior in the samples.

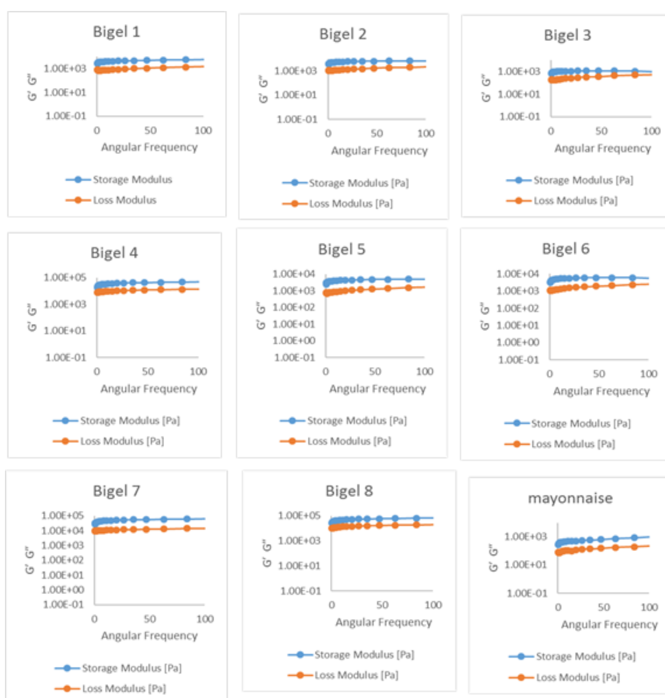

Figure 1. Frequency Sweep Test for all treatments

The frequency dependence of the elastic modulus was examined using the Bohlin model, with parameters shown in the Table 3. Among all samples, the highest value of A (3443.8 Pa·s·rad−1) was observed in treatment 8, which is a bigel based on 0.8% HPMC containing 6%beeswax and 2% saffron petals. The reported b values were all between zero and one, indicating pseudoplastic behavior of the material. The findings from examining changes in the loss modulus and storage modulus of different bigel treatments with increasing frequency are presented in the figure. The viscoelastic properties indicate that the storage modulus was higher than the loss modulus across all treatments and frequencies, suggesting that all produced bigels, regardless of formulation, exhibit viscoelastic solid characteristics. The storage and loss moduli of different bigel treatments were significantly influenced by the amount of hydrogel and oleogel. An increase in beeswax oleogel content significantly increased the storage modulus, indicating that increasing wax in the bigel formulation enhances semi-solid properties. Similar to these findings, Frenandes et al. (2023) reported that bigels exhibit semi-solid behavior regardless of the hydrogel-oleogel ratio, with more pronounced semi-solid properties at higher oleogel ratios (27). In another study, Zheng et al. (2020) reported that bigels derived from HPMC hydrogel exhibit semi-solid behavior, with samples having higher hydrogel ratios showing lower storage modulus among all bigel treatments (28). Patel et al. (2015) also reported that the storage modulus significantly increases with a higher oleogel share in bigel systems (29).

Table 3. Rheological parameters obtained from fitting the Bohlin model to frequency Sweeps test data

Sample	R^2	B	A
1	0.99	0.120± 0.01 [a]	3060.9± 0.85[a]
2	0.98	0.123± 0.005 [a]	422.31± 0.94[b]
3	0.97	0.086± 0.05 [b]	803.13± 0.32[c]
4	0.99	0.146± 0.03 [c d]	2349.5± 0.57[d]
5	0.99	0.113± 0.04 [a]	503.57± 0.15[e]
6	0.98	0.126± 0.02 [a c]	561.3± 0.18[e]
7	0.99	0.126± 0.01 [c a]	3227.7± 0.5[f]
8	0.98	0.163± 0.02 [d]	3443.8 ± 0.5[g]
9	0.99	0.146± 0.06 [c]	336.57± 0.44[h]

Different lowercase letters between rows indicate significant differences between samples ($p < 0.05$). The data are presented as mean ± standard deviation.

Based on the results, samples 5 and 6, which most closely resemble the rheological properties of mayonnaise, were selected as the best samples and were each compared separately with mayonnaise. After conducting the strain Sweeps test and determining the Linear Viscoelastic Region (LVR), a frequency Sweeps test was performed. The frequency Sweeps test examines the material's stability over time, both in the short and long term. In this test, the frequency is varied within the linear viscoelastic region, and the elastic and storage moduli are evaluated.

In this study, the frequency Sweeps test was conducted in the linear viscoelastic region at a constant shear strain of 0.05% to determine changes in the elastic modulus (G') and viscous modulus (G") over a frequency range of 1 to 100 radians per second at 25°C. The purpose of these tests is to understand the material's microstructure and its dependence on frequency changes. The resulting curves indicate the microstructure, including chain connectivity, network structure, chain length in polymeric materials, particle size, and molecular weight distribution.

Another important parameter obtained from this test is the material's behavior over time. Low frequencies indicate the long-term behavior of the bigel, while high frequencies reflect short-term behavior, which is crucial for food production, transportation, and storage. In all samples and frequency ranges, G' was greater than G", indicating a solid-like structure. The slope of G' changes was steeper than G", and G" remained relatively constant, showing elastic behavior dominance over viscous behavior. Similarly, Alkabaa et al. (2024) reported that in bigels composed of glycerol monostearate oleogels and chia seed gum, gelatin, and whey protein concentrate hydrogels, the storage modulus was greater than the loss modulus, indicating semi-solid behavior (30). Shakeel et al. (2021) found that increasing the oleogel portion increased both the storage and loss moduli, suggesting that higher oleogel concentration facilitates the compression of the dispersed phase, strengthening the gel's intrinsic structure (31, 32).

Han et al. (2024) examined the rheological properties of bigel systems based on mixed oleogelators (rice bran wax and glycerol monostearate) and sodium alginate hydrogels. For all bigel treatments, the storage modulus exceeded the loss modulus, indicating semi-solid behavior. The viscoelastic properties were influenced by the oleogel-to-hydrogel ratio, with increased oleogel improving both moduli(33). Other studies have shown that the viscoelastic properties of water-in-oil bigels are highly dependent on the oleogel portion (34, 35). Increasing the oleogel portion can lead to bigels with a stronger crystalline network structure, thus increasing the storage modulus. Additionally, interactions between the dispersed and continuous phases can affect the gel network's strength. As the frequency increased from 0.1 to 10 Hz, both moduli increased, indicating partial frequency dependence. The storage modulus is directly related to food stiffness, so increasing the oleogel ratio can enhance the mechanical strength of bigel samples.

In similarity to the results of this study, Jiang et al. (2024) reported in their frequency Sweeps test on whey protein and polysaccharide-based bigels that all samples exhibited a higher storage modulus than loss modulus across all frequencies, with minimal frequency dependence. This characteristic indicates a structured behavior and strong mechanical integrity of the system(36). Additionally, Zheng et al. (2020) and Rehman et al. (2014) reported that the storage modulus of bigels slightly increased with frequency, more pronounced in systems with higher hydrogel content. These observations suggest that bigels with higher hydrogel content tend to increase viscosity and decrease elasticity compared to those with lower hydrogel content (28, 37).

3.1.3. Steady state properties

The changes in apparent viscosity of different bigel treatments with increasing shear rate are presented in the Figure 2. As observed, the apparent viscosity of all treatments decreased with increasing shear rate, indicating pseudoplastic or shear-thinning behavior. Results showed that despite similar flow behavior across treatments, Treatment 1 (bigel based on 0.4% hydroxypropyl methylcellulose with 3% beeswax) had the lowest viscosity at all shear rates, while Treatment 8 (bigel based on 0.8% hydroxypropyl methylcellulose with 6% beeswax and 2% saffron petals) had the highest viscosity. Figure 2. illustrates the changes in apparent viscosity with increasing shear rate across various bigel and mayonnaise treatments.

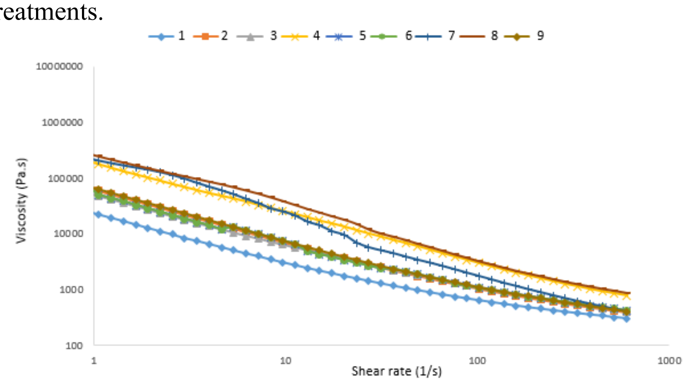

Figure 2. Shear rate- apparent viscosity plot of the bigels.

The apparent viscosity of mayonnaise also demonstrated non-Newtonian shear-thinning behavior with increasing shear rate. To further examine the effects of different wax and HPMC concentrations on rheological behavior, data from this test were fitted using the Herschel-Bulkley and Power Law models (Table 4). The R^2 values for the Herschel-Bulkley model were above 0.85 for all samples, indicating a better fit compared to the Power Law model. Consequently, the Herschel-Bulkley model was used to fit the viscosity-shear rate data, with the summary of fitted model parameters provided in the Table 4. Since the coefficient of determination for the Power Law model was lower than that for the Herschel-Bulkley model, indicating a poorer fit for describing the rheological behavior, data related to this model were not reported.

The Herschel-Bulkley model was utilized to determine the consistency index and flow behavior index of the bigels, with the results presented in the Table 5. According to the findings, the highest consistency coefficients were obtained for samples 7 and 8, with values of 86.56 and 92.16, respectively. The elevated consistency coefficient may be attributed to the increased percentages of wax and hydroxypropyl methylcellulose (HPMC), while the lowest value was associated with treatment 2. The results indicated that an increase in the wax content in the bigel formulation significantly enhanced the consistency coefficient. Conversely, an increase in the hydrogel content in the bigel formulation also led to an increase in the consistency coefficient. As shown in the table, the flow behavior index of all bigels was less than 1, indicating a shear-thinning or pseudoplastic behavior. The flow behavior index (n) reflects the flow behavior of emulsions, where $n=1$ corresponds to Newtonian fluids, $0<n<1$ indicates pseudoplastic fluids, and $n>1$ denotes dilatant fluids. In line with the results of this study, Singh et al. (2014) reported that the flow index of bigels based on guar gum and sesame oil was less than 1(38). McClements described the pseudoplastic behavior of emulsions, stating that at low shear rates, the emulsion droplets exhibit a three-dimensional and random arrangement, which becomes organized as the shear rate increases, leading to flow and layering. Consequently, the resistance to stress decreases, resulting in a reduction in the viscosity of the emulsion(39).

Table 4. The correlation coefficients obtained from fitting shear stress-shear rate data in the Herschel-Bulkley and Power law models

sample	Hershel-Bulky R^2	Power law R^2
1	0.998	0.87
2	0.99	0.8
3	0.988	0.84
4	0.95	0.92
5	0.999	0.89
6	0.999	0.89
7	0.85	0.71
8	0.87	0.74
9	0.998	0.91

The consistency coefficient is a measure of viscosity, and in comparison, to the power law model, the yield stress is also included. Understanding yield stress is crucial for the optimal design of food processing systems, especially during thermal processes. The highest yield stresses were observed in samples 7 and 8, with values of 238.74 and 189.72, respectively. A very high yield stress can hinder the flow of the sample, while a very low yield stress is also undesirable. The differences in yield stress among all samples were significant, with samples 5 and 6 exhibiting yield stress values closer to that of mayonnaise

In this study, the bigels exhibited shear-thinning behavior with increasing shear rates, confirming their pseudoplastic nature. This behavior can be explained by the deformation of flocs and the breakdown of the gel network in emulsions under shear, leading to a reduction in viscosity (40). At higher shear rates, larger droplets may break into smaller ones, which could account for the decrease in viscosity with increasing shear rate. Shear-thinning behavior is a desirable characteristic for semi-solid formulations, facilitating ease of application with maximum efficiency, thus requiring less material to achieve optimal results (38). The pseudoplastic behavior of various bigels has been reported by several researchers. In one study, Li et al. (2024) investigated the rheological properties of bigels based on walnut oil oleogel and chitosan hydrogel, reporting that in bigels formulated with different compositions, the apparent viscosity decreased with increasing shear rate, indicating the pseudoplastic behavior of the treatments(41). In another study, Jiang et al. (2024) also noted that whey protein and sodium alginate-based bigels exhibited shear-thinning behavior with increasing shear rates (37). Similarly, Ghiasi and Golmakani (2021) reported non-Newtonian and shear-thinning behavior in bigels based on ethyl cellulose oleogel and HPMC hydrogel at various oleogel ratios. A higher amount of hydroxypropyl methylcellulose hydrogel enhances hydrogen bonding in the bigel's polymer network, resulting in increased stiffness and viscosity(42). In line with these findings, Dhal et al. (2024) reported that increasing the hydrogel content in bigel formulations raised viscosity(43). Additionally, Andonova et al. (2017) found that increasing oleogel content in bigel formulations also increased viscosity (44).

In Liu Y et al.'s study, the viscosity of double emulsions increased with higher percentages of HPMC, which can be attributed to the presence of high molecular weight molecules in the flow path, increasing flow resistance. The alignment of large molecules does not occur easily, leading to increased viscosity (45). According to the results, the bigel based on 0.8% hydroxypropyl methylcellulose containing 6% beeswax and 2% saffron petals exhibited the highest viscosity. The higher amount of hydroxypropyl methylcellulose hydrogel strengthens hydrogen bonds in the bigel's polymer network, thereby increasing stiffness and viscosity. Consistent with this study, Dhal et al. (2024) reported that increasing hydrogel content in bigel formulations raised (43). Similarly, Andonova et al. (2017) reported that increasing oleogel content in bigel formulations also increased viscosity (44). The apparent viscosity of mayonnaise samples also indicated non-Newtonian shear-thinning behavior with increasing shear rates, aligning with findings by Shamsai et al. (2017), who reported that mayonnaise exhibited pseudoplastic non-Newtonian behavior with increasing shear rate (46). In this context, Mandala et al. (2004) and İbanoğlu (2002) reported similar results for white sauces (47, 48).

The Herschel-Bulkley model was identified as the most suitable model for describing flow behavior, with data fitting performed accordingly. One of the parameters obtained was the yield stress. Based on the results, the high yield stress of the samples indicates that a greater shear stress is required for flow. As the gel structure in mayonnaise becomes more robust, the yield stress increases. This property prevents the aggregation of oil droplets in the liquid phase, thereby inhibiting the mayonnaise from separating into two phases. Mayonnaise is classified as a non-Newtonian, pseudoplastic fluid with yield stress (49)

Table 5. Rheological parameters obtained from fitting the Herschel-Bulkley model to test data

sample	R^2	n	K ($Pa.s^n$)	τ0 (Pa)
1	0.998	0.53 ± 0.005 a	1.86 ± 0.22 a	41.93 ± 0.81a
2	0.99	0.72 ± 0.004 b	1.6 ± 0.34 b	59.61 ± 0.57b
3	0.988	0.7± 0.005 c	31.77±0.24b	20.97 ± 0.84c
4	0.95	0.34± 0.005 d	34.40 ± 0.34 c	160.25 ± 0.71d
5	0.999	0.32± 0.007 e	55.65 ± 0.3 d	55.54 ± 0.5e
6	0.999	0.35± 0.006d	59.02 ± 0.45 e	51.56 ± 0.51f
7	0.85	0.62± 0.005 f	86.56 ± 0.6 f	189.72 ± 0.28g
8	0.87	0.17± 0.006 g	92.16 ± 0.51 g	238.74 ± 0.67h
9	0.998	0.26± 0.005 h	68.13 ± 0.4 h	48.14 ± 0.44i

Different lowercase letters between rows indicate significant differences between samples ($p < 0.05$). The data are presented as mean ± standard deviation.

2.1. Investigation of the Antioxidant Effect of Saffron Petals on Bigels

During the extraction and processing of fats, as well as the storage of fat-containing foods, lipid oxidation may occur. This process leads to the formation of primary and secondary oxidation products (50). Lipid oxidation can lead to unpleasant flavors and odors, as well as changes in nutritional quality, color, aroma and texture, ultimately reducing food quality. Therefore, ensuring the oxidative stability of oils is a critical issue. In this study, peroxide, anisidine, and rancidity were measured to indicate the oxidation level.

2.1.1. Examination of Peroxide Index in Bigel Treatments During Storage

Peroxides are primary oxidation products in oils, and their presence indicates the onset of oxidation; thus, a lower peroxide index signifies better oil quality(51). Results from the examination of the peroxide index in bigel treatments over a 90-day storage period are presented in Table 6. The treatments included bigel based on 0.8% HPMC and 3% beeswax, and bigel containing 0.8% HPMC, 3% beeswax, and 2% saffron petal powder. Results indicated that as storage time increased, the peroxide index of the treatments also increased, with the final peroxide index for the bigel without petals and the bigel containing 2% saffron petal powder being 30.5 and 48.3 meq/kg , respectively. Statistical findings showed that the addition of saffron petal powder had no significant effect on the peroxide index of the bigel on day zero, while on days 45 and 90, the peroxide index in the treatment containing saffron petal powder was significantly lower ($p \leq 0.05$) than in the bigel without it. The lower peroxide index in the bigel with saffron petal powder is attributed to its phenolic compounds. Saffron petals are a rich source of polyphenols. Furthermore, a relationship was established between the antioxidant activity of plant sources and their phenolic compounds (20).

Table 6. Changes in the peroxide index of bigel treatments during the storage period

Storage time (day)			
90	45	0	day
235^{aA}/0±5.30	075^{bA}/0±3.57	005^{cA}/0±2.30	bigel
345^{aB}±٠/3.48	080^{bB}/0±2.89	005^{cA}/0±2.29	Bigel+2% saffron petal

Different lowercase letters in each row indicate a significant difference over the storage period ($p < 0.05$). Different uppercase letters in each column indicate a significant difference between samples ($p < 0.05$).

In line with the results of this study, Baltuonytė et al. (2022) reported that the addition of lingonberry pomace to bigel reduces the peroxide index over the storage period (48). The researchers attributed this decrease in the peroxide index to the presence of phenolic compounds in lingonberry pomace(52). Ansari et al. (2019) also reported that microemulsified green tea extract reduces the peroxide index of canola oil(23). Additionally, Mirahmadi et al. (2005) reported that the use of green tea leaf extract prevents the oxidation of sunflower oil (53).

3.2.2. Anisidine Value Analysis of Bigel Treatments During Storage

The peroxide value is an indicator of the presence of primary oxidation products and does not indicate the production of secondary oxidation products. Therefore, A test, such as the determination of anisidine value, which indicates the extent of oxidation and the production of secondary products from this reaction, is necessary. The Table 7, shows the changes in the anisidine value of bigel treatments, with increasing storage time. The results indicate that with the increase in storage time, the anisidine value increased, however, the increase in the sample with saffron petal powder was significantly lower than in the bigel sample without it. The results showed that on days 45 and 90 of storage, the anisidine value of the treatment containing saffron petal powder was significantly ($p \leq 0.05$) lower than that of the sample without saffron petal powder. The increase in the anisidine value over the storage period indicates the progression of the oxidation reaction and the increase in secondary products, resulting from the decomposition of hydroperoxides and carbonyl compounds over time. These results were consistent with the peroxide test results. The reduction in the anisidine value with the addition of saffron petal powder can be attributed to the presence of antioxidant compounds in saffron petals that have anti-oxidative effects.

Table 7. Changes in anisidine number of bigel treatments during the storage period

Storage period (day)			
90	45	0	day
01aA/0±4.8	01bA/0±3.53	0.001cA±3.01	bigel
01aB/0±4.25	01bB/0±3.06	0.001bA±3.00	Bigel+2% saffron petal

Different lowercase letters in each row indicate a significant difference over the storage period ($p < 0.05$). Different uppercase letters in each column indicate a significant difference between samples ($p < 0.05$).

In line with the results of this study, Gharekhani et al. (2009) and Okhli et al. (2020) stated that the addition of natural antioxidants to oil leads to a reduction in the anisidine value due to the reaction of these compounds with free radicals (54, 55). In this context, Ansari et al. (2019) reported that the addition of microemulsified green tea extract to canola oil reduces the anisidine index(23). In a study conducted by Jafarpoor et al. (2021), the anisidine index in creams increased over time; however, with the increase in the concentration of saffron petal extract, this value decreased. Specifically, until the fourteenth day of storage, the anisidine index remained low, but after the fourteenth day, the index in cream samples without saffron petal extract increased sharply, while this increase was moderate in samples treated with saffron petal extract(56). In the study by Azizkhani et al. (2006), the lowest anisidine value was reported in the margarine sample with the highest percentage of natural antioxidant mixtures, which aligns with the current study's findings(57).

3.2.3. Evaluation of Rancimat Index of Bigel Treatments During Storage

The stability of oil, as measured by the Rancimat method, indicates oxidative resistance changes in oils. Comparing the degree of degradation of different oils during heating can be achieved by measuring this parameter. The sensitivity of bigels to oxidation was measured using the Rancimat test. Figure 3 illustrates the findings from the evaluation of stability changes using the Rancimat method for bigel. At the beginning of the storage period, the addition of saffron petal powder had no significant effect on the stability of the bigel, while on days 45 and 90 of storage, the stability of the bigel treatments containing saffron petal powder was significantly ($p \leq 0.05$) higher than bigel treatments without saffron petal powder. According to another part of the statistical results, with the increase in storage time, the stability of the bigel treatments significantly decreased ($p \leq 0.05$). As mentioned, the increased stability of the bigel with the addition of saffron petal powder may be may result from the phenolic compounds it contains, which delay oxidation and consequently enhance the stability of the bigel.

Changes in stability using the Rancimat method for bigel treatments during the storage period. Different lowercase English letters indicate significant changes during the storage period at a significance level of $p < 0.05$. Different uppercase English letters indicate significant differences between different treatments at the same point in the storage period at a significance level of $p < 0.05$.

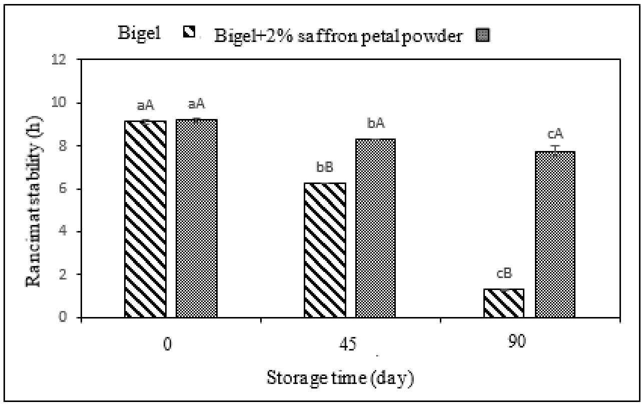

Figure 3. Comparison of Oxidative Stability index During Storage: Bigels with and without Saffron Petal Powder

Different lowercase letters indicate a significant difference over the storage period ($p < 0.05$). Different uppercase letters indicate a significant difference between two samples ($p < 0.05$).

In a study by Ein Afshar et al. (2023), the effect of ethanolic saffron petal extract with various chemical types on the oxidative stability of refined soybean oil was compared, showing that saffron petal extract acts as a natural antioxidant enhances oxidative stability of oils or related emulsions. The addition of ethanolic saffron petal extract to soybean oil and its emulsion can increase its oxidative stability and serve as a suitable alternative to chemical antioxidants with adverse effects. This study examined oxidative stability using the Rancimat method at a temperature of 120° C.The results indicated that ethanolic saffron petal extract at a concentration of 1000 ppm, without significant differences from BHT, had the highest induction period of 4 hours and was able to slow down the oxidation process similar to a synthetic antioxidant(58).

3.3. Sensory Evaluation

A 5-point hedonic scale was utilized for sensory evaluation (1 = very bad, 5 = very good). The bigel's appearance, taste and aroma were evaluated by 7 evaluators. The sensory characteristics assessment results (acceptability of aroma, color, and texture) of various bigel treatments are presented in Table 8. The investigations indicate that there was no significant difference in aroma acceptability among the different bigel treatments as perceived by the panelists. The findings revealed that the highest color acceptability scores for the bigels were associated with the treatments 5 (0.8% HPMC 3% wax) and 6 (0.8%, HPMC 3% wax and 2% saffron petals), which received significantly higher scores ($p \leq 0.05$) compared to other treatments. Conversely, the lowest color acceptability was noted for the bigel treatment 8 (based on 0.8% HPMC ,6% beeswax and 2% saffron petals). In the study by Ziaei Rizi et al. (2024), the bread samples containing saffron petal extract exhibited an unusual color (grayish-purple), which evaluators considered a negative factor compared to other samples, assigning them lower scores ($P \leq 0.05$) relative to other treatments, consistent with the results of the present study (59). The texture acceptability findings from the evaluators indicated that the highest texture acceptability was associated with the bigel treatment based on 0.8% HPMC and 3% beeswax, which showed a statistically significant difference ($p \leq 0.05$) compared to other treatments. Notably, among the treatments examined, the lowest texture acceptability scores were recorded for the bigel treatments 4 (based on 0.4% HPMC, 6% beeswax and 2% saffron petals) and bigel treatment 8(based on 0.8% HPMC, 6% beeswax and 2% saffron petals).

Table 8 .Comparison of sensory characteristics of different bigel treatments

sample	Texture (1-5)	Color (1-5)	Odor (1-5)
1	49^{ab}/0±42/4	0.45^{a}±71/4	45^{a}/0±71/3
2	35^{bc}/0±85/3	0.45^{b}±71/2	75^{a}/0±00/3
3	50^{ab}/0±28/4	0.49^{a} ±/42/4	45^{a}/0±71/3
4	75^{d}/0±00/3	0.49^{bc}±57/2	75^{a}/0±00/3
5	34^{a}/0±85/4	45^{a}/0±71/4	0.45^{a}±71/3
6	72^{ab}/0±28/4	69^{b}/0±71/2	0.75^{a}±00/3
7	49^{cd}/0±57/3	49^{a}/0±57/4	0.45^{a}±71/3
8	75^{d}/0±00/3	75^{c}/0±00/2	0.75^{a}±00/3

Different lowercase letters between rows indicate significant differences between samples ($p < 0.05$). The data are presented as mean ± standard deviation

4. Conclusion

this research successfully developed bigels using HPMC hydrogel and beeswax oleogel, demonstrating significant improvements in rheological properties and antioxidant activity. In rheological tests, all all bigel formulations demonstrated non-Newtonian pseudoplastic behavior. Notably, Samples 5 (bigel formulated with 0.8% HPMC and 3% beeswax) and 6 (bigel with 0.8% HPMC, 3% beeswax, and 2% saffron petal powder) were identified as the best treatments in comparison to mayonnaise. The addition of saffron petal powder improved the antioxidant properties, resulting in a significant decrease in peroxide and anisidine levels, furthermore, it enhanced oil stability, which makes these bigels suitable for extended shelf life. Sensory evaluations highlighted that the bigel with 0.8% HPMC and 3% beeswax received the highest ratings for texture and color, suggesting high consumer acceptability due to the preferred attributes identified. These findings highlight the versatility of bigels in the food industry, demonstrating their ability to provide functional benefits like improved texture and stability, as well as sensory enhancements such as flavor and aroma. So, Bigels are a promising solution for reducing the consumption of unhealthy fats, such as saturated and trans fats, while preserving desirable food characteristics.

Declaration of Competing Interest

The authors have no conflict of interest to declare.
Ethics approval and consent to participate

This research has approval ID, IR.IAU.SRB.REC.1402.096, from Research Ethics Committees of Islamic Azad University-Science and Research Branch.
The online link:
https://ethics.research.ac.ir/IR.IAU.SRB.REC.1402.096

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