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Research Article

Oleogel From Basil Seed Oil for Quercetin Delivery: Physicochemical, Rheological, Thermal, and In Vitro Digestion Properties

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This study is aimed at developing and characterizing functional oleogels (OGs) based on *Ocimum basilicum* L. seed oil (a rich source of α-linolenic acid) structured with beeswax (5%–20%) for the effective delivery of quercetin. The formulation's physicochemical properties, structural integrity, and release behavior under simulated gastrointestinal conditions were evaluated. Gel setting time was significantly ($p < 0.05$) reduced from 8.44 to 6.25 min by increasing the beeswax concentration from 5% to 20%, and oil binding capacity exceeded 99% in formulations containing ≥ 10% beeswax. As the wax concentration increased, the firmness of OGs increased significantly ($p < 0.05$) from 14.8 to 130 g, with the highest hardness observed at 20% beeswax. Rheological analysis confirmed gel-like viscoelastic behavior and formation of well-structured OGs using more than 5% oleogelator. FTIR spectra indicated physical interactions between quercetin and the wax–oil matrix. XRD analysis revealed β'-polymorphic crystals ($d = 4.18$ and 3.76 Å), desirable for food applications. DSC confirmed enhanced thermal stability and absence of quercetin crystallinity in the OG matrix. In vitro release studies showed that oil structuring resulted in lower release of loaded bioactive in harsh gastric conditions (36% and 22% for OGs containing 5% and 20% oleogelator, respectively), thus promoting intestinal delivery (up to 71%). The release data were best fitted with the Kopcha kinetic model ($R^2 \geq 0.96$). These results highlight the potential of basil seed oil–based OGs as sustainable carriers for bioactive delivery in food and nutraceutical applications.

Keywords: beeswax structuring agent; bioactive delivery; controlled release; functional oleogel; *Ocimum basilicum* L.; omega-3 enrichment

1. Introduction

Lipid-based bioactive delivery systems have garnered considerable interest in food science and nutraceutical industries due to their capability to encapsulate health-promoting compounds such as antioxidants, vitamins, and polyunsaturated fatty acids and enhance their bioavailability, stability, and controlled release in the gastrointestinal tract [1, 2]. However, oils rich in polyunsaturated fatty acids, particularly omega-3 and omega-6, despite having high nutritional value, are highly susceptible to oxidative degradation during processing and storage, which severely compromises their nutritional quality and functional efficacy. This oxidative vulnerability poses a significant

challenge in developing stable formulations that retain bioactivity over time.

Ocimum basilicum L. (basil) seed oil is distinguished by its remarkable content of α-linolenic acid (ALA, ω-3), which imparts substantial nutritional benefits, including anti-inflammatory and cardioprotective effects. The favorable n-3/n-6 ratio in basil seed oil and the presence of natural antioxidants contribute to its potential as a functional lipid in food-grade applications [3, 4]. Nonetheless, its high degree of unsaturation makes it susceptible to rapid oxidation, necessitating innovative stabilization strategies to preserve its quality during processing and storage.

Quercetin, a flavonoid widely recognized for its potent antioxidant, anti-inflammatory, and health-promoting properties, has attracted considerable attention for incorporation into food and pharmaceutical systems [5, 6]. By scavenging free radicals and inhibiting lipid peroxidation, quercetin plays a pivotal role in protecting unsaturated systems from oxidative deterioration. Yet, its practical application is hindered by poor aqueous solubility, low bioavailability, and instability under food processing conditions, necessitating an efficient delivery system to maximize its functional benefits [6].

Oleogels (OGs) are viscoelastic, self-supporting, thermoreversible, anhydrous liquid oil matrices structured by oleogelators (or organogelators), designed to immobilize liquid oils into semisolid networks [7, 8]. These systems have emerged as promising carriers for both oil stabilization and bioactive encapsulation, offering advantages such as enhanced oxidative stability, improved texture, and controlled release of active compounds. Among various oleogelators, including phospholipids, mono- and diglycerides, polysaccharides, and fatty alcohols, natural waxes have demonstrated superior gelation efficacy and applicability [9]. Beeswax, in particular, is a good structuring agent for edible OGs due to its high gel strength, biocompatibility, ability to form stable and reproducible networks, and low cost [10–12].

Several recent studies have explored the use of OGs to stabilize different edible oils (including omega-3 oils) using beeswax as oleogelator [13–15] and also to encapsulate and stabilize bioactive compounds such as curcumin, coenzyme Q10, and polyphenols [14, 16, 17]. The encapsulation of polyunsaturated oils in OGs has been shown to significantly reduce their oxidative degradation, preserving their nutritional benefits and extending their shelf life [14]. These studies highlighted the ability of OGs to provide a protective matrix that reduces the exposure of sensitive bioactives to destructive environmental factors such as light and oxygen, which are responsible for their rapid degradation [18].

Although the gelation behavior of beeswax with various vegetable oils has been extensively studied, reports focusing on basil seed oil–based OGs remain scarce. Moreover, despite numerous investigations into OGs as bioactive carriers, limited data exist on the delivery of quercetin within such systems, particularly with oils rich in ω-3 fatty acids.

Therefore, the present study is aimed at (1) developing a novel OG based on *O. basilicum* L. seed oil (rich in omega-3 fatty acids) and beeswax as a carrier for quercetin, (2) comprehensive evaluation of physicochemical properties of OGs and instrumental characterization (differential scanning calorimetry [DSC], Fourier transform infrared [FTIR], x-ray diffraction [XRD], and textural and rheological analysis) to clarify their potential to use in food formulations and product development, and (3) at investigating the in vitro release behavior and kinetics of quercetin incorporated into the OGs, confirming their suitability and feasibility as effective oral delivery system for food and pharmaceutical applications.

2. Materials and Methods

2.1. Materials. High purity quercetin (≥ 98%, HPLC grade) was obtained from Sigma-Aldrich (Germany). Food-grade yellow beeswax (melting point 61°C–65°C) was supplied by Espadana Mokamel Co. (Isfahan, Iran). Analytical grade solvents and reagents including n-hexane, ethanol, hydrochloric acid, sodium hydroxide, sodium bicarbonate, pepsin, pancreatin, and bile salts were purchased from Merck (Darmstadt, Germany). Basil seeds (*O. basilicum* L.) were purchased from a certified local herbal supplier in Isfahan, Iran, and stored in airtight containers at room temperature in the dark until further use.

2.2. Basil Seed Oil Extraction and Evaluation of Its Physicochemical Properties. Prior to extraction, basil seeds were cleaned manually to remove debris and then air-dried at 40°C for 6 h to standardize moisture content (~7%). No dehulling was required due to the fine pericarp. The oil was extracted via cold-pressing using a screw press machine (Machinery Equipment Co., Ltd., Zhejiang, China) under optimized conditions: pressure ~30 MPa and pressing temperature maintained below 45°C to prevent thermal degradation. The extracted oil was filtered to remove impurities and stored in dark glass bottles under a nitrogen atmosphere at −18°C to minimize oxidation.

The peroxide value (PV) was determined using a titration process with sodium thiosulfate (0.1 N) based on the AOAC method (965.33, 1990). The refractive index (RI) of the oil was determined using a prismatic benchtop refractometer (BPTR100, Iran) at 25°C according to the AOAC method (921.08, 1990) [19]. The oil's viscosity was measured using a Brookfield viscometer (DV-II, United States) at 20°C fitted with Spindle No. 3 at 100 rpm.

2.3. Preparation of Quercetin-Loaded OGs. Four formulations of OGs were prepared by dissolving a fixed amount of quercetin (0.1% *w*/*w*) directly into basil seed oil. The beeswax concentration, determined by scientific literature [10, 12], was as follows: 5%, 10%, 15%, and 20% *w*/*w*. The wax and oil were heated in a water bath at 80°C (slightly above the melting temperature of beeswax). The mixture was continuously stirred at 80°C for 10 min to ensure complete dissolution and uniform blending. The homogenized mixture was poured into cylindrical molds and allowed to cool down to room temperature (~25°C) for 1 h, followed by storage at 20°C for 24 h before further analysis [12].

2.4. Fatty Acid Analysis of Basil Seed Oil by Gas Chromatography. The methyl esters of fatty acids were prepared using the method proposed by Jafari et al. [20]. Briefly, 100 μL of sodium methoxide (0.5 M) as a catalyst was added to 50 μL of oil dissolved in 1 mL n-hexane. The mixture was shaken for 15 min at room temperature, and then, the hexane phase containing fatty acid methyl esters was removed. Fatty acid analysis was conducted using an Agilent 6890 N gas chromatograph equipped with an HP-88 column (100 m, 0.25 mm internal diameter, and 0.2 μm film thickness). In the following, 1 μL was injected with a 1:40 split ratio. The carrier gas was hydrogen, maintained at a constant 1 mL/min flow rate. The oven temperature program was set as follows: initial hold at 150°C for 1 min, followed by a ramp to 190°C at a rate of 5°C/min, then held at 190°C for 2 min, and finally raised to 240°C at 5°C/min and maintained for an extra 8 min. Throughout the process, the FID detector and injector were kept at 250°C and 150°C, respectively.

2.5. Gel Setting Time Measurement of OGs. About 5 g of each OG was transferred into the tubes and completely melted in a water bath set at 70°C, subsequently cooled down to ambient temperature (20°C) while recording the time. The gelation time was considered at the time at which no flow was observed when the tubes were inverted [12].

2.6. Oil Binding Capacity (OBC). The OBC of the OGs was determined according to the method described by Babu et al. [16], with slight modifications. Briefly, 1 mL of each melted OG formulation was poured into 1.5 mL Eppendorf tubes and allowed to solidify at 20°C for 24 h. After solidification, the tubes were centrifuged at 10,000 rpm for 15 min using a microcentrifuge (MC-24, Benchmark Scientific, United States). After centrifugation, the tubes were immediately inverted to drain any released oil. The OBC was estimated using Equation (1):

$$\text{OBC\%} = 1 - \left(\frac{m1 - m2}{m1 - m}\right) \times 100, \quad (1)$$

where m is the mass of the empty tube, $m1$ is the mass of the tube with OG before centrifugation, and $m2$ is the mass after centrifugation.

2.7. Color Evaluation. The color of OGs was measured using a HunterLab (Color Flex EZ colorimeter, HunterLab, United States). Color values were reported in terms of International Commission on Illumination (CIE) units: $L*$ (white or light/dark), $a*$ (red [+]/green [−]), and $b*$ (yellow [+]/blue [−]).

2.8. OG Firmness Evaluation. Firmness was measured using a texture measuring device (Stable Micro Systems, TaTx plus model, England) with a 20 kg load cell and a cylindrical probe of 10 mm diameter. The probe penetrated the cylindrical sample (18 mm high and 45 mm diameter) to a depth of 6 mm (1/3 of sample height) at a speed of 100 mm/min, and firmness was taken as the force (N) required to puncture the samples [21].

2.9. OG Rheological Measurements. The rheological characteristics of OGs were assessed utilizing a rotary rheometer equipped with a temperature control system (MCR301, Anton Paar, Austria). The distance between the plates was 1000 μm on a parallel plate with a 40 mm diameter. A strain sweep ranging from 0.01% to 100% at a frequency of 1.0 Hz was conducted to determine the linear viscoelastic region (LVR). Furthermore, a frequency sweep test ranging from 0.1 to 10 Hz was conducted at 25°C to ascertain the G' (storage modulus) and G'' (loss modulus). Shear rates ranging from 0.1 to 100 s^{-1} were employed to evaluate the flow properties of the OGs [22].

2.10. DSC Analysis. The thermal behavior of OGs, beeswax, basil seed oil, and quercetin was determined using a differential scanning calorimeter (SL 800 Irsa, China) calibrated with indium. About 20 mg of the samples was placed in an aluminum pan and tightly sealed. An empty pan was used as a reference. The samples were heated from room temperature to 160°C at 5°C/min and kept in isothermal condition. SPICO 1.0.0 software was used to plot and analyze the thermal data [12].

2.11. FTIR Spectroscopy. The FTIR spectrophotometer (Thermo Scientific, United States) was utilized to analyze the OG samples, pure quercetin, and basil seed oil. Each sample underwent 20 scans, with an average taken at a resolution of 0.5 cm^{-1}, within the wavenumber range of 4000–400 cm^{-1}. The spectrogram of the empty cell (air) served as the reference (background). The baseline of all spectrograms was corrected using the OriginPro 2023b software.

2.12. XRD. The sample's crystalline structure was assessed utilizing an x-ray diffractometer (D8 Phaser, Bruker AXS GmbH, Germany) equipped with a Cu-based X-ray radiation source. The OGs were placed flat in the groove of the test piece and analyzed at 25°C. The analysis was carried out at a scan speed of 0.03 s/step (with a step size of 0.3), using an emission and antireflection slit of 1.0 mm and a reception slit of 0.1 mm, covering a diffraction angle range from 5 to 60 (2θ) [22].

2.13. In Vitro Simulated Digestion for OGs. The in vitro release of quercetin from OGs was investigated under simulated gastrointestinal conditions, based on the standardized INFOGEST 2.0 protocol with minor modifications to better mimic physiological environments [23, 24]. Dialysis bags (MWCO 10–12 kDa, Sigma-Aldrich) were used for diffusion-based release of quercetin from OGs. Prior to use, the bags were presoaked in deionized water overnight at room temperature to remove residual preservatives.

The OG samples were loaded into the dialysis bags and immersed in 30 mL of simulated gastric fluid (SGF), consisting of 0.32% (w/v) pepsin (2000 U/mL) in 0.03 M NaCl, with pH adjusted to 1.2 using HCl. The digestion was conducted in a shaking incubator at 37°C and 100 rpm for 2 h. Samples were withdrawn at 30 min intervals, and the sampled volume was replaced with fresh buffer solution to maintain sink conditions.

Following gastric digestion, the pH of the medium was adjusted to 5.7 using 1 M $NaHCO_3$ and incubated for 20 min at 37°C. The pH was then further increased to 6.8 with 1 M NaOH. Simulated intestinal fluid (SIF), composed of 10 g/L pancreatin (100 U/mL) and 12 g/L bile salts in 0.1 M $NaHCO_3$, was added, and incubation continued for another 2 h under identical conditions. Samples were collected every 30 min, reaching a total digestion time of 240 min. Quercetin release was quantified by measuring absorbance at 370 nm using a UV-Vis spectrophotometer (DR6000, Hach, United States), and concentrations were calculated based on a calibration curve ($R^2 = 0.99$).

In addition, the release kinetic of quercetin was studied based on the Kopcha (Equation 2), zero-order (Equation 3), and Higuchi (Equation 4) models.

$$Mt = At^{0.5} + Bt, \tag{2}$$

$$M0 - Mt = k0 \times t, \tag{3}$$

$$Q = kHt^{0.5}, \tag{4}$$

where $M0$, Mt, t, and $k0$ are the initial concentrations of quercetin in OGs and the concentration of quercetin in OGs at time t, respectively. $k0$ is the zero-order release constant and where k_H is the Higuchi dissolution constant.

2.14. Statistical Analysis. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was conducted using SPSS Version 28.0.1 (IBM Corp., Armonk, New York, United States). One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test was applied to assess significant differences ($p < 0.05$) among means. Release kinetic models were compared using regression parameters and error values (RMSE).

3. Results and Discussion

3.1. Physicochemical Properties of Basil Seed Oil. The cold-pressed basil seed oil extracted for this study exhibited desirable physicochemical properties suitable for oleogelation and bioactive delivery systems (Table 1). The specific gravity of basil seed oil was 0.91, which is comparable to some common vegetable oils such as soybean oil (0.91), sesame oil (0.91), and olive oil (0.90) [25]. The RI was measured as 1.4856 at 25°C, consistent with high levels of unsaturation, and close to values reported for commercial oils like peanut oil (1.38) and canola oil (1.35) [26].

The PV of the freshly cold-pressed extracted oil was 2.1 meq O_2/kg, indicating low primary oxidation and acceptable oxidative stability for food applications. This value falls well within the acceptable range defined by the Codex Alimentarius Commission for crude oils (10 meq O_2/kg) and is comparable to other cold-pressed extracted oils used in OG systems, such as chia (2.5 meq O_2/kg) and linseed oils (2.3 meq O_2/kg) [13, 27] but lower than basil seed oil extracted by Soxhlet apparatus [4].

The viscosity calculated for basil seed oil was 40.44 cp. As reported in previous studies, the proportion of unsaturated fatty acids can affect the viscosity; therefore, regarding to the degree of unsaturation, basil seed oil viscosity is comparable to commercial oils such as flaxseed oil (53 cp) [28], sunflower oil (63 cp), and corn oil (60 cp) [12]. Viscosity is directly related to gel strength and texture, resistance to syneresis, mouthfeel, sensory attributes, and also gel setting time of OGs [1]. According to Calligaris et al. [28] study, the slowest gel formation was observed in the oil with higher viscosity (i.e., triolein; 48 and 4 h for 10% and 20% gelator, respectively). So, based on the present results, it is expected that valuable basil seed oil provides an acceptable gelation speed in the OG system.

TABLE 1: Physicochemical properties of basil seed oil.

Oil properties	Value
Specific gravity	0.91 ± 0.01
Refractive index	1.47 ± 0.02
Peroxide value (meq O_2/kg)	2.10 ± 0.01
Viscosity (cp)	40.44 ± 0.9

Taken together, these data confirm that basil seed oil possesses the physicochemical attributes necessary for stable OG formation and nutraceutical applications, especially when combined with wax-based structuring agents.

3.2. Fatty Acid Composition of Basil Seed Oil. The results of the GC analysis of basil seed oil are presented in Table 2. The amount of saturated fatty acids was low (approximately 13%) while the oil was rich in unsaturated ones (86.36%). The primary unsaturated fatty acids were linolenic acid (49.60%), followed by linoleic acid (22.25%) and oleic acid (14.51%(. Fatty acids are the main nutritional components in edible oilseeds, and growing evidence suggests that n-3 fatty acids (linolenic acid, ALA) and n-6 fatty acids (linoleic and arachidonic fatty acids) in the diet can provide human health benefits. In Table 2, basil seed oil is compared to some other important high linolenic oils. Linolenic acid in basil seed oil was close to flax and perilla oils but lower than *D. kotschyi* oil [29–31]. Although the ratio of n-3 to n-6 in basil seed oil is lower than the other three oils but considering the ratio of 2.23, it can be concluded that basil oil–based OGs may be a good candidate for commercial vegetable fat replacement along with enrichment of food products with ω-3 fatty acids such as spreads.

3.3. OBC and Gel Setting Time. The results for the OBC and gelling time of OG systems (OGs) are presented in Table 3. The stability of OGs is associated with the ability to bind oil, oxidative, and thermal stability [32]. Generally, considering that OGs contain a large amount of oil (on average, more than 90%), it is necessary to have a high OBC for their successful application as solid fats [33].

The present findings demonstrated the excellent capacity of the beeswax crystalline structure to effectively entrap basil seed oil, and the presence of long-chain triglycerides in the basil oil could be one of the influential factors in this regard [34]. It was established that a minimum beeswax

TABLE 2: Fatty acid composition of basil seed oil compared to high linolenic oils.

Fatty acid composition (%)	Basil seed oil	Flaxseed oil [29]	Perilla oil [30]	*Dracocephalum kotschyi* [31]
Palmitic acid (C16:0)	8.89 ± 0.25^{d}	6.1	5.21	4.75
Stearic acid (C18:0)	4.16 ± 0.3^{e}	4.5	2.30	2.31
Oleic acid (C18:1)	14.51 ± 0.63^{c}	20.3	14.70	18.11
Linoleic acid (C18:2)	22.25 ± 0.50^{b}	13.0	14.11	13.56
Linolenic acid (C18:3)	49.60 ± 0.45^{a}	54.9	51.52	61.23
SFA	13.05 ± 0.25	10.6	7.51	7.07
USFA	86.36 ± 0.46	78.2	80.33	92.91
n-3/n-6 ratio	2.23	4.2	3.65	4.5

Note: Values are presented as means ± standard deviation ($n = 3$). Data with different superscript letters within each column are significantly different ($p \leq 0.05$).

TABLE 3: Oil binding capacity and gel setting time of basil seed oil/beeswax OGs loaded with quercetin and high n-3 oils.

Oleogelator concentration	Oil binding capacity (%)	Gel setting time (min)
5%	96.49 ± 1.05^{b}	8.44 ± 0.35^{a}
10%	99.54 ± 0.30^{a}	7.14 ± 0.17^{b}
15%	99.97 ± 0.04^{a}	5.80 ± 0.37^{c}
20%	99.98 ± 0.02^{a}	6.25 ± 0.05^{c}
Linseed oil OGs	Less than 70% with 6% beeswax [13] 91.4% with 9.1% beeswax [15]	9.2 min with 5% beeswax [32]
Fish oil OGs	99.27% and 78.20% for 15% and 5% beeswax, respectively [33] 100% for 6% beeswax [13]	Incomplete setting within 1 h with 20% beeswax [33]

Note: Values are presented as means ± standard deviation ($n = 3$). Data with different superscript letters within each column are significantly different ($p \leq 0.05$).

concentration of 10% is required to form a stable network structure and maintain its structural integrity even after centrifugation. Given that the quercetin content was consistent across all OG samples, the observed differences among samples were mainly attributed to the amount of oleogelator used as already mentioned in previous studies [12].

When compared with previous studies on omega-3 oil-based OGs such as those formulated with linseed and fish oil (Table 3), it is evident that, at nearly the same beeswax concentration, the combination of basil seed oil and beeswax produced OGs with a stronger structure and reduced oil loss (approximately 3.5% for 5% beeswax) compared to linseed oil [13, 15] and fish oil–based OGs [35]. However, Frolova et al. [13] reported zero oil loss for fish oil–based OGs containing 6% beeswax, which may be due to a more compact crystal network and no impurities in oil. On the other hand, we found that the stability of basil seed oil OG was significantly better compared to other unsaturated vegetable oils such as hemp, sunflower, and olive oils at similar beeswax concentrations [36].

According to the data presented in Table 3, increasing the beeswax concentration up to 15% significantly decreased the gel formation time ($p < 0.05$), consistent with previous literature reporting that higher supersaturation in the system leads to shorter gelling times [37]. The presence of quercetin in OGs did not significantly affect the gel setting time (data not shown).

As previously noted, oil viscosity plays a crucial role in gel formation, and the low viscosity of basil seed oil positively influences this process. So, in all tested OGs, the gels set in less than 10 min. These findings confirm the capability of beeswax to gel basil seed oil effectively.

Despite the limited number of studies on the setting times of omega-3–rich oil-based OGs, Ramadhan et al. [35] observed the gradual setting of fish oil OGs after 1 h of gelation at room temperature (Table 3). Singh et al. [38] reported a gel setting time of 9.2 min at 5% beeswax concentration for flaxseed oil, which is longer than the setting time observed for basil seed oil (Table 3). Additionally, Calligaris et al. [28] reported a prolonged gelling time (exceeding 1 h) for highly viscous lipids such as triolein and castor oil when using β-sterol and sterol ester as structuring agents.

3.4. Color Measurement of OGs. The color characteristics of the OG samples are shown in Table 4. The lightness value ($L*$) of oil was higher than that of the OGs containing wax. Among the OGs, the samples containing 5% and 20% beeswax showed the highest and lowest brightness, respectively. The wax concentration significantly affected the lightness of OGs as the concentration of beeswax increased from 5% to 20%, and the $L*$ value decreased from 48.3 to 42.14, respectively. Yellowness ($b*$) of the OGs decreased significantly with the increasing proportion of wax ($p < 0.05$), while redness ($a*$) increased significantly ($p < 0.05$).

TABLE 4: Color measurement of basil seed oil/beeswax OGs.

Oleogel samples	Color indices		
	$L*$	$a*$	$b*$
OG 5%	48.34 ± 0.02^{a}	-6.77 ± 0.01^{a}	24.60 ± 0.03^{a}
OG 10%	47.24 ± 0.01^{b}	-3.97 ± 0.03^{c}	24.02 ± 0.01^{b}
OG 15%	46.12 ± 0.02^{c}	-3.10 ± 0.02^{d}	23.06 ± 0.01^{d}
OG 20%	42.14 ± 0.04^{e}	-2.71 ± 0.02^{e}	21.35 ± 0.02^{e}
Beeswax	23.42 ± 0.05^{f}	2.13 ± 0.03^{f}	9.87 ± 0.02^{f}

Note: Values are presented as means ± standard deviation ($n = 3$). Data with different superscript letters within each column are significantly different ($p \leq 0.05$).

The visual difference between OGs containing the lowest and highest amounts of wax can be seen in Figure 1. Yi et al. [39] reported that $L*$ and $a*$ values increased in grape seed oil OGs as the proportion of beeswax increased, whereas the $b*$ value did not change significantly ($p > 0.05$). By contrast, as reported by Yi et al. [39], yellowness ($b*$) of the OG samples with canola oil and carnauba wax significantly increased with the increasing proportion of wax ($p < 0.05$), whereas redness ($a*$) did not change significantly ($p > 0.05$). Therefore, color indices of OGs depend on both the type of constituent oil and oleogelator.

3.5. Texture Analysis. The hardness of OG is one of the most critical parameters for delivery application, as it influences the gel breakdown, which is highly related to the digestion and release of quercetin. Figure 2 shows the hardness of OGs, and as can be seen, all samples showed statistically significant differences in hardness ($p < 0.05$). Given that the same oil, basil seed oil, was used for all OGs, the differences in textural properties could be caused by the structuring agent concentration. So, the OGs tended to be firmer with increasing levels of beeswax. A literature review reveals that the hardness of basil seed oil OGs (according to present results) is lower than other n-3-based OGs such as linseed/flaxseed and fish oil OGs [13, 40].

In accordance with the present study, Pang et al. [40] showed that the firmness of OGs prepared with different vegetable oils increased with the beeswax in a concentration-dependent manner. However, using different oils made the hardness of OGs different, even with the same amount of oleogelator as follows: soybean oil > flaxseed oil > sunflower oil > -camellia oil. Calligaris et al. [28] reported that the combined effect of structural concentration and oil viscosity could affect the gel network formation, and the influence of oil type was increasingly noticeable with the reduction in oleogelator concentration. Based on present findings, the hardness of the OGs prepared from basil seed oil and beeswax was less than that of the OGs prepared from the mentioned oils. Many factors can directly or indirectly affect the textural properties of OGs such as fatty acid profile, storage temperature, cooling rate, and polar component present in the oil. Based on evidence, the presence of certain fatty acids or polar matters affects the crystal morphology, thereby changing the textural properties of OGs. On the other hand, it has been confirmed that the presence of polar compounds decreases critical gelation concentration and aids to network formation [41].

3.6. Rheological Properties. Viscoelastic properties of OGs are influenced by the degree of crystallization and crystal morphology, which represent their mechanical strength [42]. The elastic modulus (G') indicates the solid-like behavior of the system, while the viscous modulus (G'') reflects liquid-like behavior.

Frequency sweep tests (Figure 3a) were used to investigate the deformation behavior of basil seed oil OGs within the LVR. In all samples, G' values were consistently higher than G'' values across the studied frequency range (0.1–10 Hz), confirming the gel-like solid behavior of the OGs [42]. Slight frequency dependence was observed in most samples except for the OG containing 5% beeswax, where this dependence was more pronounced, especially at higher frequencies, indicating a weaker and less developed network structure in this formulation. This observation aligns with the lowest crystallization intensity seen in the XRD patterns for this sample [33].

An increase in OG strength was evident as beeswax concentration increased from 5% to 20%, with G' values rising significantly ($p < 0.05$) from approximately 1750 ± 45 to 8780 ± 122 Pa at 1 Hz, as shown in frequency sweep curves (Figure 3b) and Table 5. The LVR decreased with increasing beeswax content, suggesting that OGs with higher wax concentrations are more rigid and brittle than those with lower wax content. These results align with previous studies on rice bran oil–based OGs with rice bran wax [43] and pumpkin and sunflower oil–based OGs with beeswax and carnauba wax [44].

OGs containing more than 5% beeswax exhibited higher G' and G'' values, consistent with a more compact crystalline association and a stronger gel network capable of effectively entrapping basil seed oil. High G' modulus values are typically associated with smaller crystals that form a well-organized and uniform network with excellent oil binding properties [33]. The 5% beeswax OG showed the lowest G' and G'' values, indicating a less strong network structure that may not have been well developed despite gel formation.

From a network theory perspective, the viscoelastic behavior of OGs can be attributed to the spatial organization and extent of intercrystalline junction zones, which restrict molecular mobility and impart solid-like features [40]. The increase in G' values with wax content suggests a higher degree of network crosslinking, resulting in more effective oil phase entrapment and increased structural integrity.

Phase angle ($\tan\delta$) values ranged from 0.41 to 0.50 (Table 5), and according to viscoelastic theory, $\tan\delta$ values below 0.5 indicate true gel behavior, supporting the robustness of the internal network at room temperature [42, 45]. Taken together, the rheological findings establish that basil seed oil–based OGs exhibit strong, frequency-independent elastic properties, and their mechanical strength can be fine-tuned through beeswax concentration. These characteristics are essential for applications requiring mechanical stability, controlled release, and specific textural qualities, such as oral delivery systems or fat replacers in food matrices [22, 42, 44, 46].



Figure 1: Visual color of basil seed oil/beeswax OGs containing 5% and 20% oleogelator.

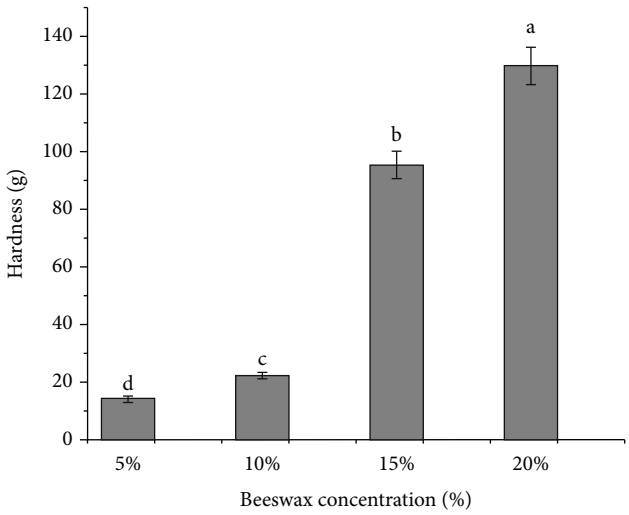


Figure 2: Firmness of quercetin-loaded OGs containing different amounts of beeswax. Values are presented as means ± standard deviation ($n = 3$). Data with different letters are significantly different ($p < 0.05$).

3.7. Differential Scanning Calorimetric of OGs. Table 6 presents the thermal behavior of beeswax and OG samples, while Figure 4 displays the melting profiles of OGs with varying wax concentrations. The thermal properties of the OGs were influenced by the concentration of wax, which has been observed in previous studies [40, 44, 47]. The melting onset and peak (T_p) temperatures slightly increased with increasing wax concentration. Moreover, the melting enthalpy (ΔH) demonstrated a proportional increase with wax concentration, ranging from 1.32 to 14.38 J/g for OGs containing 5% and 20% wax, respectively. This phenomenon can be attributed to forming a robust crystalline network and the thermal stability of the OGs at higher wax concentrations [48]. Generally, the lower melting enthalpy values indicate that less energy is required for thermal destruction of the crystalline network; however, the apparent heat of fusion of such mixtures of crystalline structures with liquids (oil in the OGs) is a combined effect of crystal melting (endothermic) and dissolution of solute constituents (exothermic) [10].

These findings were in agreement with previous works on oleogelation of vegetable oils with carnuba wax, beeswax, and monoglyceride [39, 49]. It is worth noting that the melting ΔH and T_p values of the OGs were generally lower than those of crude beeswax due to the colligative properties and solubility of the wax components in the basil seed oil. Previous literature has reported that the type of oil used can influence the thermal behavior of OGs, as a higher ratio of saturated to unsaturated fatty acids can enhance the structural integrity and reduce the solvency effect of liquid oils, resulting in the formation of a greater mass of crystalline wax. The DSC curve of quercetin showed two main endothermic peaks that have been reported previously by Borghetti and Galli [50]. The first peak at 118.4°C corresponds to the water loss and release of incorporated water in the crystal lattice. The second peak at 321.5°C is related to the melting point of quercetin. However, the DSC analysis of OGs did not exhibit the quercetin melting peaks, confirming the absence of the quercetin crystalline form and suggesting the protective effect of the OG matrix on quercetin.

3.8. FTIR Spectrum. The FTIR spectrum has the potential to offer insights into the existence of various functional groups, and it is beneficial for studying molecular interactions in the prepared OG formulations (Figure 5). In the OG spectrum, all typical basil seed oil and beeswax peaks were visible, confirming successful structuring by oleogelator. The OGs displayed peaks at 1000–1300 cm^{-1} (C–O stretching vibration of ester groups and CH2 bending vibration), 1735–1750 cm^{-1} (stretching vibration of carbonyl groups C=O of esters and free fatty acids), about 2920 and 2850 cm^{-1} (correspond to the asymmetric and symmetric CH_2 stretching of alkane groups of the basil seed oil and the beeswax hydrocarbons, respectively), and =C–H stretching vibrations in the range 3000–3100 cm^{-1}[51].

According to the literature, intramolecular hydrogen bonds appear in the 3540–3570 cm^{-1} wave number range and intermolecular hydrogen bonds in the 3450–3550 cm^{-1} wave number range [52]. Based on present findings, it can be inferred that networks formed by beeswax and its mixture in basil seed oil may be mainly attributed to van der Waals interactions. Similar findings were also reported on beeswax OGs containing hazelnut oil [52] and pomegranate seed oil [12]. The slight changes in the positions of the bands for asymmetric and symmetric CH_2 in OGs toward lower wavenumbers indicate a decrease in the vibration energy of these bonds and the formation of more ordered structures of the hydrocarbon chain. Figure 5 also presents the FTIR

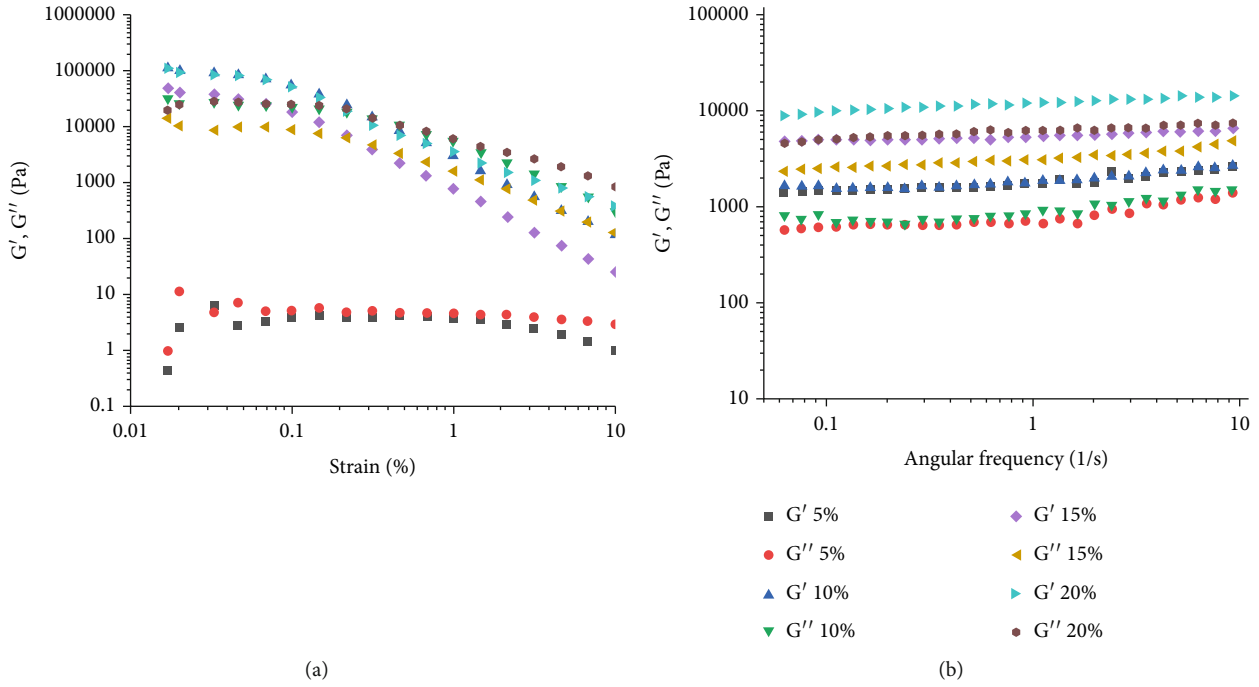


FIGURE 3: (a) Strain sweep and (b) frequency sweep curves of quercetin-loaded OGs containing 5%–20% beeswax.

TABLE 5: Storage modulus (G'), loss modulus (G''), tan δ, and complex viscosity ($\eta*$) of basil seed oil–based OGs containing 5%–20% oleogelator and 0.1% quercetin after 24 h.

Oleogel samples	G'	G''	Tanδ	$\eta*$ (Pa s)
OG 5%	1750 ± 45^{d}	669 ± 133^{d}	0.41 ± 0.01^{b}	1680 ± 236^{d}
OG 10%	1920 ± 115^{c}	934 ± 186^{c}	0.48 ± 0.02^{a}	1910 ± 282^{c}
OG 15%	8780 ± 122^{a}	4460 ± 250^{a}	0.50 ± 0.02^{a}	8810 ± 325^{a}
OG 20%	8160 ± 190^{b}	3980 ± 235^{b}	0.48 ± 0.01^{a}	8130 ± 370^{b}

Note: Values are presented as means ± standard deviation ($n = 3$). Data with different superscript letters within each column are significantly different ($p \leq 0.05$).

spectrum of pure quercetin, where its characteristic bands are detected, as reported by Catauro et al. [53]. The spectrum reveals the presence of C=C aromatic ring stretch bands at 1610, 1560, and 1510 cm^{-1}. Additionally, the absorption of the C=O aryl ketonic stretch is evident at 1666 cm^{-1}. The in-plane bending band of C–H in aromatic hydrocarbon is detected at 1317 cm^{-1}, while out-of-plane bending bands are observed at 933, 820, 679, and 600 cm^{-1}. Other notable bands include those at 1263, 1200, and 1165 cm^{-1}, corresponding to the C–O stretching in the aryl ether ring, the C–O stretching in phenol, and the C–CO–C stretching and bending in ketone, respectively. The stretching of free phenolic OH groups is detected at 3406 and 3283 cm^{-1}, resulting in a distinct and broad peak in this region. In the spectra of OGs, the broad O–H peak of quercetin at 3410 cm^{-1} became attenuated and slightly shifted, suggesting the presence of hydrogen bonding between hydroxyl groups in quercetin and polar sites within the wax network. FTIR evidence of hydrogen bonding interactions supports the barrier properties of the crystalline network in protecting quercetin from acidic gastric degradation. Moreover, the characteristic C=O stretching peak of quercetin at 1655 cm^{-1} was either masked or significantly diminished, which may imply the physical entrapment of quercetin molecules within the OG matrix rather than their complete molecular dissolution. The absence of any new covalent bond signals further supports that quercetin was incorporated through noncovalent interactions, primarily van der Waals forces and hydrogen bonding. Additionally, the lack of sharp crystalline peaks associated with quercetin indicates its amorphous or well-dispersed state in the wax–oil matrix, confirming a stable physical integration within the OG structure.

3.9. XRD Patterns. To obtain more information about the polymorphic nature of the crystals, the XRD patterns of the OGs were measured. XRD is usually used as an index of fat performance. Figure 6 illustrates the XRD patterns of basil seed oil–based OGs loaded with quercetin. The XRD patterns of the basil seed oil revealed a smooth, amorphous peak, suggesting the presence of liquid oil (data not shown). This finding aligns with previous studies by Monto et al. [49], which also observed similar smooth and broad peaks in the XRD patterns of liquid oils. All OG samples showed two clear peaks at $2\theta = 21^{\circ}$ and 24° with d spacing values 4.18 and 3.76 A°, respectively, indicating an orthorhombic prependicular subcell packing crystals. Our findings are in agreement with the observation from sunflower, flaxseed/linseed, perilla, rice bran, and even a mixture of sunflower and linseed oil/wax OGs [10, 54], which signifies the occurrence of the β' polymorphic form. As a result, all the OGs encompass crystal forms of the β' polymorphic structure.

TABLE 6: Thermal behavior (melting) of basil seed oil OGs, beeswax, and quercetin.

Samples	T_{on}(°C)	T_{off}(°C)	T_p(°C)	$\triangle Hm$(J/g)
OG 5%	48.50 ± 0.45^{f}	62.50 ± 0.13^{f}	56.07 ± 0.19^{g}	1.32 ± 0.35^{g}
OG 10%	51.25 ± 0.32^{d}	61.48 ± 0.37^{g}	57.04 ± 0.28^{f}	2.44 ± 0.37^{f}
OG 15%	51.72 ± 0.47^{d}	65.16 ± 0.42^{e}	59.71 ± 0.12^{e}	8.08 ± 0.87^{e}
OG 20%	50.74 ± 0.57^{e}	66.52 ± 0.28^{d}	60.59 ± 0.38^{d}	14.38 ± 0.43^{d}
Beeswax	52.89 ± 0.45^{c}	67.48 ± 0.71^{c}	64.32 ± 0.42^{c}	147.05 ± 2.35^{b}
Quercetin	93.48 ± 0.80^{b}	126.55 ± 0.50^{b}	118.34 ± 0.26^{b}	334.05 ± 3.40^{a}
	317.74 ± 1.50^{a}	324.02 ± 0.75^{a}	321.51 ± 0.54^{a}	123.63 ± 6.32^{c}

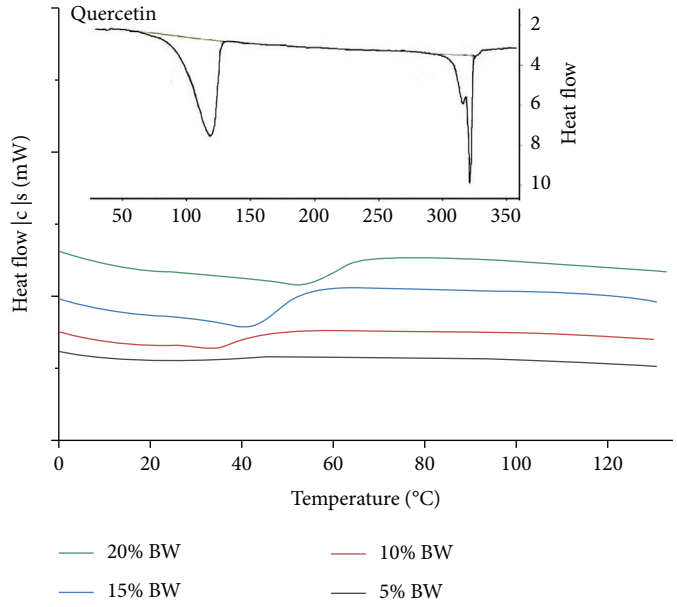


FIGURE 4: Thermograph of quercetin and OGs with beeswax contents of 5%–20%.

Among the three main types of polymorphs (α, β', and β), β' had a medium melting point, intermediate density, fine and creamy texture, strongest plasticity, best spreadability, and mouthfeel. It is worth mentioning that our results confirm the suitability of OG prepared from basil seed oil and beeswax (even loaded with bioactive compounds) to partially replace margarine and shortening. Our results are in agreement with previous studies on seed oil OGs loaded with bioactives such as α-tocopherol and lutein [27, 49].

Another noteworthy point as shown in Figure 6 is that the peak intensity increased as the wax concentration increased from 5% to 20%, which was also found elsewhere for other seed oils [16, 40]. This confirms the more intense effect of wax on polymorphism in beeswax-based OGs. In some cases, the type and concentration of selected gelator showed an important role in the gradual transformation of crystals to β-form over time which is not suitable for food application [45].

The gelation behavior of wax-based systems is profoundly influenced by parameters such as cooling rate and wax concentration. A rapid cooling regime typically results in the formation of smaller, less ordered crystals, which compromise the mechanical strength and integrity of the resulting gel network. In contrast, slower cooling promotes the development of larger, more ordered crystalline domains, thereby enhancing the structural robustness of the gel. Increasing the wax concentration accelerates nucleation onset and intensifies the nucleation rate, culminating in a denser and mechanically stiffer crystalline framework [10].

3.10. In Vitro Simulated Digestion. One of the main goals in preparing OGs is to protect the bioactive compounds from the harsh conditions of the stomach and transport them to the intestinal section at the absorption site. Therefore, the bioactive-loaded OGs were subjected to in vitro simulated digestion conditions using SGF and SIF to know the percentage release of quercetin at different time intervals, and the data is shown in Figure 7. The amount of quercetin released in SGF after 90 min was observed to be about 36%, 31%, 27%, and 22% for OGs containing 5%–20% beeswax, respectively, and no significant change was observed in the release of quercetin even after 120 min in SGF. According to Figure 7, the percentage of release decreased with the increase in the concentration of oleogelator (beeswax). Then, the OG with the lowest beeswax (5%) had the highest release

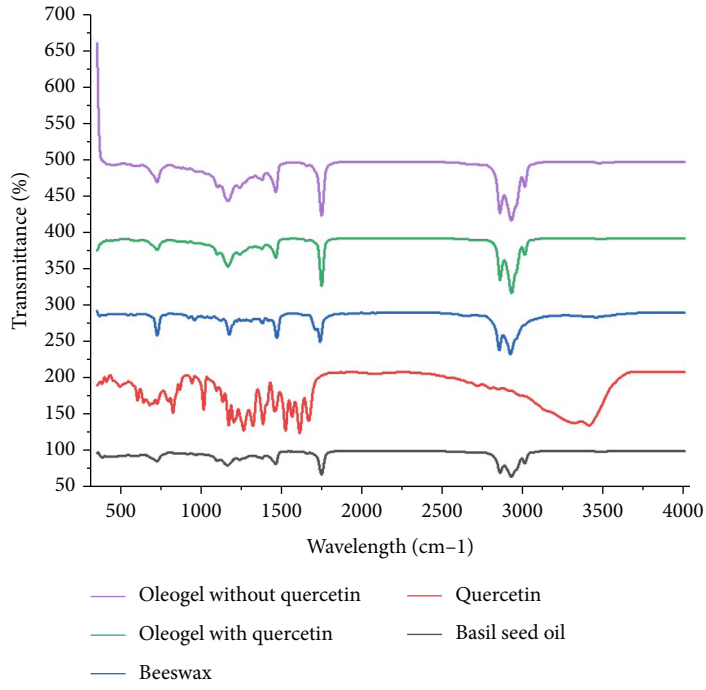


Figure 5: FTIR spectrum of basil seed oil, beeswax, quercetin, and OGs.

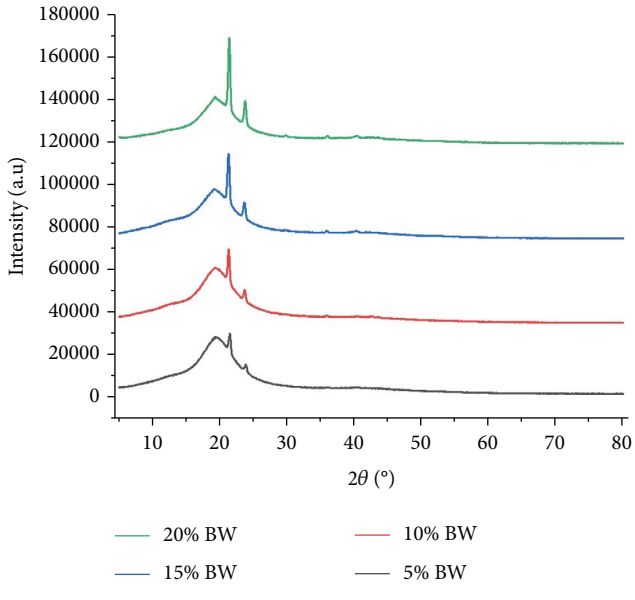


Figure 6: X-ray diffraction (XRD) patterns of quercetin-loaded OGs with different amounts of beeswax.

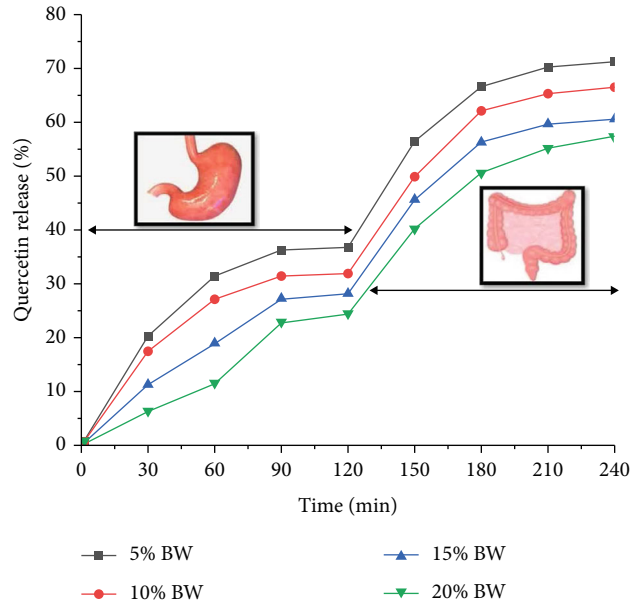


Figure 7: Release behavior of quercetin from OGs with beeswax contents of 5%–20%.

of quercetin, probably due to the weaker gel strength of the sample, as confirmed by the textural, rheological, and OBC tests. Generally, developed OGs protect the loaded quercetin from gastric conditions and promote the targeted release in the intestine.

The crystalline network, formed by the self-assembly of gelators like beeswax in OGs, creates a dense three-dimensional matrix that physically entraps and encapsulates quercetin molecules and acts as a physical barrier against the harsh environment of the stomach. Also, interaction between quercetin and components of the gel matrix (which was confirmed by FTIR) can stabilize quercetin conformation against acid-induced structural changes. The delay in releasing quercetin can be attributed to the denser crystalline structure of higher wax OGs, as confirmed by XRD, rheological (higher G' values), and textural analysis as well as limited permeability. Moreover, the lack of crystalline quercetin (as shown by DSC and FTIR) supports its entrapment in an amorphous or

TABLE 7: Different kinetic models fitted for quercetin release.

Kinetic models	Treatments	*R* -square	Adjusted *R* -square	RMSE
Kopcha model	OG 5%	0.96	0.96	4.68
	OG 10%	0.96	0.95	4.76
	OG 15%	0.97	0.96	4.08
	OG 20%	0.97	0.97	3.53
Zero-order model	OG 5%	0.90	0.90	7.39
	OG 10%	0.93	0.93	6.11
	OG 15%	0.96	0.96	4.16
	OG 20%	0.97	0.97	3.48
Higuchi model	OG 5%	0.93	0.93	6.07
	OG 10%	0.91	0.91	6.77
	OG 15%	0.88	0.88	7.67
	OG 20%	0.82	0.82	9.16

molecularly dispersed state, which is more responsive to matrix erosion rather than direct dissolution.

During the digestion in simulated intestinal condition, the release percentage of quercetin was significantly increased to 71% in OG 5%, 66% in OG 10%, 61% in OG 15%, and 57% in OG 20% after 240 min. The release percentages were decreased with the increase in beeswax concentration, and the OG with 20% oleogelator had the lowest release of quercetin. Considering the OG composition, a larger volume of oil in the OG structured by a low concentration of oleogelator facilitates lipid digestion.

During the initial 60 min of intestinal digestion, the quickest quercetin release was observed across all samples because most lipid digestion occurred during this period. A robust gel network appeared to impede the action of lipase and, consequently, the release of quercetin. These results indicate that intestinal digestion is the primary trigger for quercetin release, likely due to bile salt–mediated destabilization of the wax–oil network and partial enzymatic degradation by pancreatin.

It is worth noting that the bioaccessibility in OG systems can be affected by various factors, especially the type of oleogelator, as it has been reported that rice wax and monoglycerides reduce the bioavailability of cocuminoids to values of ~30%, but for beeswax OGs, it was ~60% [15, 28]. Therefore, investigating the effect of different oleogelators on the release of bioactive compounds and nutraceuticals from basil seed oil OGs will complement the present study.

The overall release pattern suggests a hybrid mechanism involving both diffusion and matrix erosion. In low-wax systems, release is dominated by diffusion, facilitated by a looser microstructure. In contrast, high-wax OGs resist both enzymatic penetration and fluid ingress, requiring gradual erosion of the matrix for release to proceed.

The release data were fitted with the Kopcha, zero-order, and Higuchi models, and the resulting parameters were summarized in Table 7. The release rate of quercetin in the intestine from all formulations follows the Kopcha kinetic model. The correlation coefficient of the Kopcha model for OGs containing 5%, 10%, 15%, and 20% beeswax was 0.96, 0.96, 0.97, and 0.97, respectively.

4. Conclusion

This comprehensive study highlights the promising potential of basil seed oil as a valuable source of omega-3 fatty acids (notably reflected by the 2.23 ratio of n-3 to n-6) for enriching OG formulations and serving as a sustainable replacement for solid fats in food products. The physicochemical characterization of basil seed oil confirmed its compatibility for OG preparation, exhibiting properties comparable to widely utilized oils. The incorporation of beeswax at varying concentrations significantly influenced the textural, rheological, thermal, and structural attributes of the OGs, with higher beeswax content correlating to increased hardness and a solid-like viscoelastic behavior. The prepared OGs showed superior OBC and reduced setting time compared to OGs formulated with other omega-3–rich oils reported in the literature, with these parameters improving progressively up to 10% wax concentration. Notably, the hardness of basil seed oil OGs was lower than those formulated with linseed/flaxseed, sunflower, and fish oils, suggesting favorable sensory and processing attributes. DSC analysis confirmed the effective encapsulation and protection of quercetin within the OG matrix, while FTIR spectroscopy suggested that the network formation between beeswax and basil seed oil predominantly involves van der Waals interactions. The β' polymorphic crystal form observed in all OGs is particularly suitable for applications in margarine or spread formulations; however, possible transformation in crystals over time requires careful consideration. Moreover, the OG matrix modulated the release profile of quercetin, reducing its release under gastric conditions and favoring targeted release in the intestinal environment. This controlled release behavior underscores the potential application of basil seed oil OGs in nutraceutical and functional food sectors. Additionally, further investigation into the bioaccessibility of various bioactives influenced by different oleogelators is recommended to enhance the efficacy of these functional delivery systems.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Soosan Karimi: methodology, investigation, funding acquisition, software, conceptualization, statistical analysis, data curation, and formal analysis. Maryam Jafari: writing—review and editing, supervision, conceptualization, visualization, and data curation. Mohammad Goli: review and

editing. Nafiseh Jahanbakhshian: review and editing, modeling, and data curation.

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