LWT - Food Science and Technology 204 (2024) 116369

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Fabrication of novel oleogel enriched with bioactive compounds and assessment of physical, chemical, and mechanical properties and storage stability

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ARTICLE INFO

Keywords:
β-carotene
β-sitosterol
Fish oil
Rice bran oil
Sesame oil

ABSTRACT

Amidst evolving dietary challenges, developing oleogels as functional foods enriched with bioactive compounds is gaining dramatic attention. The utilization of fish oil (a source of omega-3 fatty acids) in food is greatly restricted due to its susceptibility to oxidation and the study on the use of fish oil in oleogel is also scanty. β-Carotene and β-sitosterol are lipophilic compounds with several health benefits. Therefore, in this study, oleogels enriched with fish oil, β-carotene, and β-sitosterol were developed and examined for their properties. Oleogels were developed from a basic formula comprising a blend of rice bran oil and sesame oil (4:5, w/w) and a synergistic mixture of beeswax and stearic acid (3:1, w/w) as oleogelators. Fish oil and β-sitosterol were incorporated at 10% (w/w) and 5% (w/w), respectively of the total oil mixture, while β-carotene was included at 0.1, 0.25, 0.5, and 1 % (w/w). The oils and oleogels were evaluated for their properties and storage stability in terms of rheological stability, oxidative stability, and β-carotene content until 4 months of storage. The incorporation of fish oil did not cause any significant changes in the properties of the oleogels. The incorporation of β-carotene resulted in a more uniform microstructure and increased gel strength, whereas oil binding capacity and thermal and molecular properties of the oleogels were not influenced by β-carotene. Oleogels exhibited higher oxidative stability and less β-carotene loss during storage than the oils. The incorporation of β-carotene enhanced the oxidative stability of the oleogels. The findings of this study suggested that there is a potential for preparing oleogels incorporated with bioactive compounds with good stability.

1. Introduction

There has been an increased demand for functional foods in recent years driven by changing dietary habits due to several reasons such as changing lifestyles, increased health consciousness, an aging population, and a rise in chronic diseases (Vos et al., 2020). Functional foods are specifically formulated or modified to provide health benefits beyond basic nutrition because of bioactive compounds, such as nutrients, dietary fiber, phytochemicals, probiotics, or other substances (Temple, 2022). Regular consumption of functional foods can help promote overall health and well-being. Oleogelation has gained immense interest within the field of food science and the growing body of recent literature reports the potential of preparing functional oleogels (Martins et al., 2020; Perța-Crișan et al., 2023).

Fish oil is an excellent source of omega-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6), which are protective against cardiovascular diseases, memory impairment, and cognitive impairment (Temple, 2022). β-Carotene belonging to the class of carotenoids is a natural, orange-colored pigment and it is the precursor of vitamin A (provitamin A).

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https://doi.org/10.1016/j.lwt.2024.116369
Received 2 February 2024; Received in revised form 5 June 2024; Accepted 16 June 2024
Available online 18 June 2024
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β-Carotene is recognized for its ability to quench singlet oxygen and exhibit potent antioxidant properties (Black et al., 2020). The antioxidant activity could contribute to enhancing the oxidative stability of the oil and provide health benefits. β-Carotene plays a vital role in supporting vision health, strengthening the immune system, lowering the likelihood of cardiovascular issues, and protecting against cancer (Black et al., 2020). The human body cannot synthesize β-carotene, therefore humans depend on external sources as the sources of β-carotene (Anand et al., 2022). Some characteristics of β-carotene such as poor water-dispersibility, chemical stability, and bioavailability limit its incorporation into all kinds of functional foods (Chen et al., 2017). However, the lipophilic nature of this compound makes it a suitable candidate to be incorporated into lipid-based systems such as oleogels. β-Sitosterol is a phytosterol with a plethora of health benefits including anti-inflammatory, anticancer, hepatoprotective, antioxidant, cardioprotective, and antidiabetic effects (Khan et al., 2022). It possesses the capacity to lower low-density lipoprotein cholesterol (LDL-C), a significant factor in the development of atherosclerosis, by competing with cholesterol for absorption in the intestine (Fumeron et al., 2017). Besides providing health benefits, the incorporation of minor components such as β-carotene and β-sitosterol could enhance the microstructure and resulting properties of oleogels (Barragán-Martínez et al., 2022; Martins et al., 2017; Sivakanthan et al., 2024). Considering the above factors, this study aimed to develop oleogels enriched with these compounds and comprehensively analyze their properties including storage stability over a period of 4 months.

Preparation of β-carotene incorporated oleogel has been reported by Jeong et al. (2021), Barragán-Martínez et al. (2022), Martins et al. (2017), Li et al. (2021), and O'Sullivan et al. (2017) and fish oil oleogel has been reported by Zhang et al. (2021), Gómez-Estaca et al. (2019), and Liu et al. (2020). Since bioactive compounds such as β-carotene and fish oil are highly susceptible to degradation, it is crucial to examine the storage stability in terms of oxidative stability and physical stability. However, to the best of the authors' knowledge, there are no studies that reported the storage stability of fortified oleogels including physical (rheological) stability, oxidative stability, and β-carotene retention over a long period of storage. Li et al. (2021) studied the β-carotene content of β-carotene incorporated oleogels over 120 days of storage, however, the study did not focus on the influence of β-carotene content on the oxidative stability and shelf life of the oleogels.

Therefore, this study was designed to develop and characterize the properties and storage stability of functional oleogels enriched with omega-3 fatty acids, β-carotene, and β-sitosterol. For this purpose, oleogels incorporated with fish oil, β-carotene, and β-sitosterol were developed based on sesame oil and rice bran oil using beeswax and stearic acid as oleogelators. This study was carried out as a continuation of our previous study (Sivakanthan et al., 2023) which optimized the oil and oleogelator mixture to produce oleogel with properties similar to commercial margarines. The optimized oleogel mixture consisted of rice bran oil and sesame oil (4:5, w/w) and a synergistic mixture of beeswax and stearic acid (3:1, w/w) and this mixture was used as the basic formula in the present study. Sesame oil and rice bran oils were used due to their healthy fatty acid profile and bioactive properties (Devarajan et al., 2016). The oleogels were characterized for their rheological properties, thermal properties, molecular properties, microstructure, and storage stability up to 4 months of storage. The findings of this study can provide new knowledge on approaches for developing oleogels incorporated with bioactive compounds with good stability and shelf life.

2. Materials and methodology

2.1. Materials

Oil samples such as sesame oil (Changs, Thailand), rice bran oil (Alfa one, USA), and three different brands of commercial margarines were sourced locally in Brisbane, Australia. Fish oil was purchased from Melrose Laboratories Pty Ltd, Australia. Beeswax (refined), stearic acid, β-sitosterol ≥70%, β-carotene (synthetic, ≥93% (UV), powder) and β-carotene pharmaceutical secondary standard, standards for GC-MS (Supelco 37 component FAME mix, linoleic acid methyl ester mix, heneicosanoic acid, methyl nonadecanoate), and other chemicals, reagents were purchased from Sigma Aldrich, Australia.

2.2. Preparation of oleogel

Six oleogel formulations were prepared based on a basic formula developed in our earlier studies consisting of an oil blend of rice bran oil and sesame oil (4:5, w/w) and a synergistic mixture of beeswax and stearic acid (3:1, w/w) as oleogelators (Sivakanthan et al., 2023). Additionally, 5% of β-sitosterol was also added to the formula to enhance the bioactive properties and the rheological properties of the oleogels, aligning with the findings of our previous study (Sivakanthan et al., 2024). Fish oil was used at 10% (w/w) of the total oil mixture and β-carotene was used at 0.1, 0.25, 0.5, and 1 % (w/w) of the total oil mixture. The details of the oleogel formula are provided in Table 1. The oleogels were named COG (control 1 – oleogel made using rice bran oil and sesame oil blend without fish oil and β-carotene), BCCOG (control 2 - oleogel made using rice bran oil and sesame oil blend with fish oil and without β-carotene), and BCOG1, BCOG2, BCOG3, and BCOG4 (oleogels made using rice bran oil and sesame oil blend with fish oil and β-carotene at 0.1%, 0.25%, 0.5%, and 1%, respectively) and respective oils (the oils used to make the above-mentioned oleogels) were named as CO, BCCO, BCO1, BCO2, BCO3, and BCO4, respectively.

Enrichment of oil with β-carotene was performed as explained by Martins et al. (2017) with slight modifications. Firstly, the range of concentration of β-carotene to be added to the oleogels was determined from preliminary experiments. For this purpose, different concentrations of β-carotene such as 0.1%, 0.25%, 0.5%, 1%, 1.5%, and 2% (w/w) of the oil were added separately to rice bran oil-sesame oil blend and heated at 80 °C while stirring at 300 rpm until a clear solution is obtained. The oils added with 0.1% and 0.25% of β-carotene yielded clear solutions after heating for 20 min while the oil samples added with 0.5% and 1% yielded lear solutions after heating for 30 min. The oils added with 1.5% and 2% of β-carotene did not yield a clear solution even after heating for up to 40 min. Therefore, considering the negative effect of prolonged heating on the quality of the oil, concentrations ranging from 0.1 to 1% and a heating time of 20 min for the samples with 0.1% and 0.25% of β-carotene and 30 min for the samples added with 0.5% and 1% of β-carotene were selected for further experiments.

Table 1
Composition of oleogels.

Sample label	Oil blend[a] (g)	Fish oil (g)	Oleogelators[b] (g)	β-Sitosterol (g)	β-Carotene (mg)
COG (Control 1)	88.26	–	11.74	5	–
BCCOG (control 2)	79.43	8.83	11.74	5	–
BCOG1	79.43	8.83	11.74	5	88.26 (0.1% of oil)
BCOG2	79.43	8.83	11.74	5	220.65 (0.25% of oil)
BCOG3	79.43	8.83	11.74	5	441.30 (0.5% of oil)
BCOG4	79.43	8.83	11.74	5	882.60 (1% of oil)

The oils of oleogels COG (Control 1), BCCOG (control 2), BCOG2, BCOG3, and BCOG4 were named CO, BCCO, BCO1, BCO2, BCO3, and BCO4, respectively.
[a] Rice bran oil: sesame oil – 4:5, w/w.
[b] Beeswax: stearic acid 3:1, w/w.

The oil blend (rice bran oil: sesame oil) was preheated to 60 °C in a magnetic stirrer and an accurate amount of β-carotene was added and continued heating at 80 °C while stirring at 300 rpm. Then the oils were cooled and stored at 4 °C until used for preparing oleogel within two days. Oleogels were prepared by directly dispersing oleogelators in the oil. The oil, oleogelators, and β-sitosterol were weighed into clean Erlenmeyer flasks as shown in Table 1, and heated at 80 °C for 10 min in a magnetic stirrer. After heating, the mixture was allowed to cool at ambient conditions and stored at 20 °C for 48 h before analysis. All the procedures involving β-carotene were performed by covering it with an alminium foil to minimize exposure to the light.

2.3. Analysis of oils and oleogels

2.3.1. Polarity determination

The polarity of the oil samples was measured using an oil tester (Testo 270, Testo Inc., Germany). The samples were preheated, and the amount of total polar compounds was measured at 45 °C (±4 °C). The amount of polar compounds was reported in percentage.

2.3.2. Acid value determination

The acid value of the oils was determined according to AOCS Official Method Cd 3d-63 (AOCS, 2017). Briefly, about 5 g of the sample was accurately weighed into an Erlenmeyer flask and dissolved completely in 100 mL of isopropyl alcohol: toluene (1:1, v/v) and titrated against 0.01 g/L ethanolic KOH solution using phenolphthalein indicator. A blank titration was also performed. The acid value of the sample was calculated as mg KOH/g of oil.

2.3.3. Peroxide value determination

The peroxide value of the oils was assessed in accordance with AOCS Official Method Cd 8–53 (AOCS, 2003) with some modifications. Briefly, 5 ± 0.05 g of sample was weighed into a 250 mL Erlenmeyer flask. Subsequently, 30 mL of acetic acid: chloroform (3:2, v/v) was added and mixed well to dissolve the sample. Then, 0.5 mL of saturated potassium iodide was added, stoppered, and left undisturbed for 1 min in darkness with intermittent shaking. Following this, 30 mL of distilled water was added and titrated with 0.01 g/L sodium thiosulfate using the starch indicator. A blank determination also was conducted in parallel. The results were expressed as meq O_2/kg of oil or oleogel.

2.3.4. β-Carotene content analysis

β-Carotene content of oil/oleogel samples was evaluated as reported by Abad and Shahidi (2020) with some modifications. The oil/oleogel was dissolved in a solution of hexane and acetone (70:30, v/v) (1:1, w/v) by vortexing for 1 min. Then the mixture was filtered through a 0.22 μm PTFE syringe filter and 200 μL of the solution was transferred to a 96-well plate. The absorbance of the solution was read at 430 nm using a plate reader (Synergy HTX multi-mode reader). The content of β-carotene was quantified from a standard curve of β-carotene (β-carotene pharmaceutical secondary standard) prepared under the same condition.

2.3.5. Oxidation induction temperature analysis

The oxidation induction temperature of the samples was derived from the thermogravimetric analysis (TGA) curves obtained for a non-isothermal heating experiment (30–700 °C at a heating rate of 20 °C per min) using a Simultaneous Thermal Analyzer (Jupiter STA 449 F^3, Netzsch, Germany) as described by Sivakanthan et al. (2023).

2.3.6. Microscopic analysis

The oleogels were examined for their microstructure using polarized light microscopy (Nikon Eclipse LV100ND, Nikon Instruments Inc., USA) equipped with a Nikon DS-Fi2 digital camera. A droplet of the molten oleogel was placed on a preheated glass slide, covered with a cover slip, and allowed to set at 20 °C for 48 h before imaging. Bright-field images were captured at a magnification of 200 × at room temperature (20 °C). Images were processed using ImageJ software (ImageJ 1.53e; Java 1.8.0_172, National Institutes of Health, USA). The images were converted into 8-bit grayscale images to measure fractal dimension using the tool "fractal box-counting" after converting the images to 8-bit binary images.

2.3.7. Oil binding capacity determination

The determination of the oil binding capacity of the oleogels was conducted following the procedure outlined by Sivakanthan et al. (2023).

2.3.8. Rheological measurements

The rheological characteristics of the oleogels were assessed using amplitude sweep, frequency sweep, and thixotropy as explained by Sivakanthan et al. (2023) using Rheometer (Anton Paar MCR302 Rheometer, Austria). Sand-blasted parallel plate geometry (PP50–S) was used with a normal force of 0.1 N to ensure contact between the plate and the sample. All experiments were conducted at 20 °C and after 48 h of storage of the oleogel at 20 °C to ensure the complete formation of the gel structure. An amplitude sweep experiment was conducted at the strain values ranging from 0.01 to 100% and a constant frequency of 1 Hz. Consequently, frequency sweep experiment was performed at frequencies ranging from 0.01 to 100 Hz at a strain value within the Linear Viscoelastic Range (LVR) determined by amplitude sweep. Temperature ramp test was performed from 20 to 70 °C at a rate of 2 °C min^{-1} at the frequency of 1 Hz and a strain value within LVR to determine the gel point/crossover temperature ($G' = G''$). An initial fixed gap of 0.5 mm was set for all experiments. The thixotropic behavior (structural recovery ability) of the oleogels was determined by a 3-interval thixotropy (Rot-Rot-Rot) test as explained by Sivakanthan et al. (2023).

2.3.9. Thermal analysis

Thermal characteristics such as the onset of crystallization, onset of melting, peak melting, and peak crystallization temperatures of oleogels were analyzed according to Sivakanthan et al. (2023) using Differential Scanning Calorimetry (DSC 204 F1 Phoenix, Netzsch, Germany).

2.3.10. Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectra of the samples were obtained within the range of 4000 to 400 cm^{-1} employing a Fourier transform infrared spectrometer (Nicolet iS50 FT-IR, Thermo Scientific, USA) set at a resolution of 4 cm^{-1}. A total of 64 scans were collected at room temperature (20 °C) and the peaks were analyzed.

2.3.11. Fatty acid composition analysis

The fatty acid profile of oleogels samples was analyzed using gas chromatography-mass spectrometry (GC-MS). Fatty acid methyl esters (FAMEs) were prepared according to the method by WHO (WHO, 2020). Briefly, the sample (50 mg) and recovery standard (heneicosanoic acid) were taken in a Teflon-lined screw-capped glass test tube and dissolved in toluene (1 mL). Then 2 mL of BF_3 in methanol (7% v/v) was added. Tubes were heated at 95 °C for 45 min in a water bath. The tubes were removed from the water bath after 45 min and allowed to cool to room temperature. Then, distilled water (5 mL), hexane (1 mL), and sodium sulfate (1 g) were added and vortexed. FAMEs in the hexane layer were collected and filtered through a 0.22 m PTFE syringe filter. Internal standard (methyl nonadecanoate) was added to the FAMEs and 1 μL of FAMEs was used to inject into the GC-MS system (Shimadzu GCMS TQ-8040) equipped with a capillary column (Rtx-2330, 60 m × 0.25 mm, 0.20 μm) to identify and quantify the fatty acids. The reference standards such as Supelco 37 component FAME mix, and *cis* and *trans* linoleic acid methyl ester mix were used to identify and quantify the fatty acids using calibration curves. The carrier gas (helium) was set at the flow rate of 1 mL min^{-1}. The inlet temperature was set at 240 °C. The split ratio was 22:1. The initial column oven temperature was

maintained at 100 °C for 1 min, followed by 10 °C min^{-1} ramp to rise the temperature to 140 °C, 6 °C min^{-1} ramp to rise the temperature to 175 °C, 10 °C min^{-1} ramp to increase the temperature to 200 °C, and 5 °C min^{-1} ramp to increase the final temperature to 250 °C with a hold time of 4 min. The interface temperature was 260 °C and the ion-source temperature of GCMS-TQ was 230 °C. The mass spectrum was obtained in the range of 45–600 m/z. Identification and quantification of the detected components was carried out by GCMS Solution and LabSolutions Insight software.

2.3.12. Storage stability study

Storage stability of the oils and oleogels at 5 °C was evaluated in terms of rheological properties, β-carotene content, peroxide value, and oxidation induction temperature. For this purpose, the samples were stored at 5 °C for 4 months and analyzed. The same amount of samples were stored in identical clear containers.

2.3.13. Comparison of developed oleogel and commercial margarines

Based on the findings of the analysis of oleogel incorporated with different amounts of carotene, the best formula was selected and the properties such as oil binding capacity, fatty acid profile, rheological properties, and thermal properties of the selected oleogel were compared with those of three different brands of commercial margarines.

2.4. Statistical analysis

The results were statistically analyzed using Minitab 21.4.0.0. (Minitab, LLC, USA). Values were presented as mean ± standard deviation. Statistical significance was assessed through One-way ANOVA and Tukey's test at a 95% confidence level ($p < 0.05$). The correlation between various responses was examined using the pairwise Pearson correlation method with a 95% confidence interval.

3. Results and discussion

Both oils and oleogels were evaluated for their properties to understand the relationship between the physical and chemical characteristics of oil and the physical and chemical characteristics and storage stability of oleogel. As a photosensitive molecule, β-carotene degrades upon exposure to light (Limbo et al., 2007). Therefore, the exposure to light was minimized during all experiments. The acid value quantifies the amount of free fatty acids that are generated by hydrolysis and oxidation of triglycerides triggered by exposure to heat, light, and oxygen. The acid values of BCO3 and BCO4 (2.34 ± 0.34 and 3.30 ± 0.22 mg KOH/g of oil, respectively) were significantly higher than those of other oils (1.03 ± 0.02, 1.01 ± 0.12, 1.04 ± 0.12, and 1.10 ± 0.04, mg KOH/g of oil for CO, BCCO, BCO1, and BCO2, respectively) indicating a greater degree of degradation of triglycerides and oxidation. This is because of the prolonged heating time required for BCO3 and BCO4 to dissolve the β-carotene. The prolonged heating time leads to a higher degree of degradation of oil (Bhat et al., 2022). During the heating of oils, polar compounds such as mono and diacylglycerols, and free fatty acids are generated by the breakdown of nonpolar triacylglycerols. The level of total polar compounds is used as an indicator of the degradation of frying oils and the maximum permissible limit set by most countries ranges from 24% to 27% (Flores et al., 2021). Even though the amounts of total polar compounds were within this set value, the amount of polar compounds was significantly higher for BCO3 and BCO4 (9.45 ± 0.07% and 11.05 ± 0.07%, respectively), whereas BCO1 and BCO2 had similar values (8.10 ± 0.00% and 8.20 ± 0.00%, respectively) as those of both controls such as CO and BCCO (8.05 ± 0.07% and 8.05 ± 0.00%, respectively). Similar to the acid value, the higher values reported for BCO3 and BCO4 could be attributed to the prolonged heating time required to dissolve the β-carotene.

3.1. Oil binding capacity of oleogels

The oil binding capacity of oleogels indicates the connection between the strength of the oleogel and the capability of the oleogels to hold the oil in its structure when exposed to an external force (Martins et al., 2017). It is a critical parameter that influences the stability, functionality, and sensory attributes. All oleogels had an oil binding capacity of above 99% and there were no significant changes in the oil binding capacities during storage. This observation indicated that either incorporation of fish oil or β-carotene or storage did not influence the oil binding capacity of the oleogels. From these results, it could be interpreted that the oil binding capacity of the developed oleogels depends on the oleogelators. Because the concentration (11.74%) and proportion of oleogelator mixture (beeswax and stearic acid (3:1) used in this study was optimized in our previous study (Sivakanthan et al., 2023) and the optimized formula had a similar oil binding capacity as reported in the present study. Martins et al. (2017) found that the oil binding capacities of oleogels made with 2% and 4% (w/w) of beeswax and incorporated with β-carotene were significantly different whereas the oleogels made with 6% and 8% of beeswax and incorporated with β-carotene did not show any significant differences among them with values near to 99% as reported in our study.

3.2. Microstructure of oleogels

Fig. 1 illustrates the microstructure of oleogels observed under a polarized light microscope. The microstructure of the oleogels was investigated using the size, shape, and spatial arrangement (using fractal dimension). The fractal dimension is a mathematical parameter used in image analysis to quantify the microstructure (spatial arrangement). The shape of the crystals of all oleogels was inconsistent with our previous study for the beeswax and stearic acid oleogels (Sivakanthan et al., 2023). As can be seen in Fig. 1 as well as based on fractal dimensions, it can be understood that the incorporation of fish oil, β-sitosterol, and β-carotene did not have any influence on the crystal shape, however, they influenced the spatial distribution of the crystals.

Compared to both controls, all β-carotene incorporated oleogels exhibited a notable difference in the arrangement of the crystals. BCOG1 and BCOG2 exhibited consistent appearance and significantly higher fractal dimensions (2.093 ± 0.030 and 2.084 ± 0.055, respectively) than both controls such as COG and BCCOG (1.966 ± 0.005 and 1.970 ± 0.010, respectively), whereas BCOG3 and BCOG4 (1.825 ± 0.020 and 1.774 ± 0.022, respectively) had the lowest. The fractal dimension provides a clear idea of the spatial arrangement of crystals in the oleogels. A higher fractal dimension indicates well-organized crystals with less cavities (Trujillo-Ramírez et al., 2022). Therefore, significantly higher fractal dimensions in BCOG1 and BCOG2 than in both controls indicated that the incorporation of β-carotene positively influenced the crystal arrangements, which is in accordance with the results reported by Martins et al. (2017) and Ramezani et al. (2024) for the beeswax oleogels and glyceryl stearate-lecithin oleogel, respectively. β-Carotene molecules may interact with the oleogelator molecules (such as beeswax and stearic acid) through physical or chemical interactions. These interactions may promote the formation of a more interconnected gel network. β-Carotene may act as a nucleating agent, facilitating the formation of smaller and more uniform crystals within the gel matrix and reducing the spacing between crystal arrangements (Martins et al., 2017; Ramezani et al., 2024) resulting in a finer and more homogeneous microstructure and higher fractal dimension. Contrary to this observation of the positive influence of β-carotene on the microstructure of the oleogel, the lowest fractal dimensions reported for BCOG3 and BCOG4 could be due to the undissolved crystals of β-carotene (Fig. 1 (E and F)). The undissolved β-carotene crystals can disrupt the connectivity of the gel network by creating a void space and acting as physical barriers that hinder the formation of continuous pathways between gelator molecules. This may result in the irregular arrangement of the crystals and,

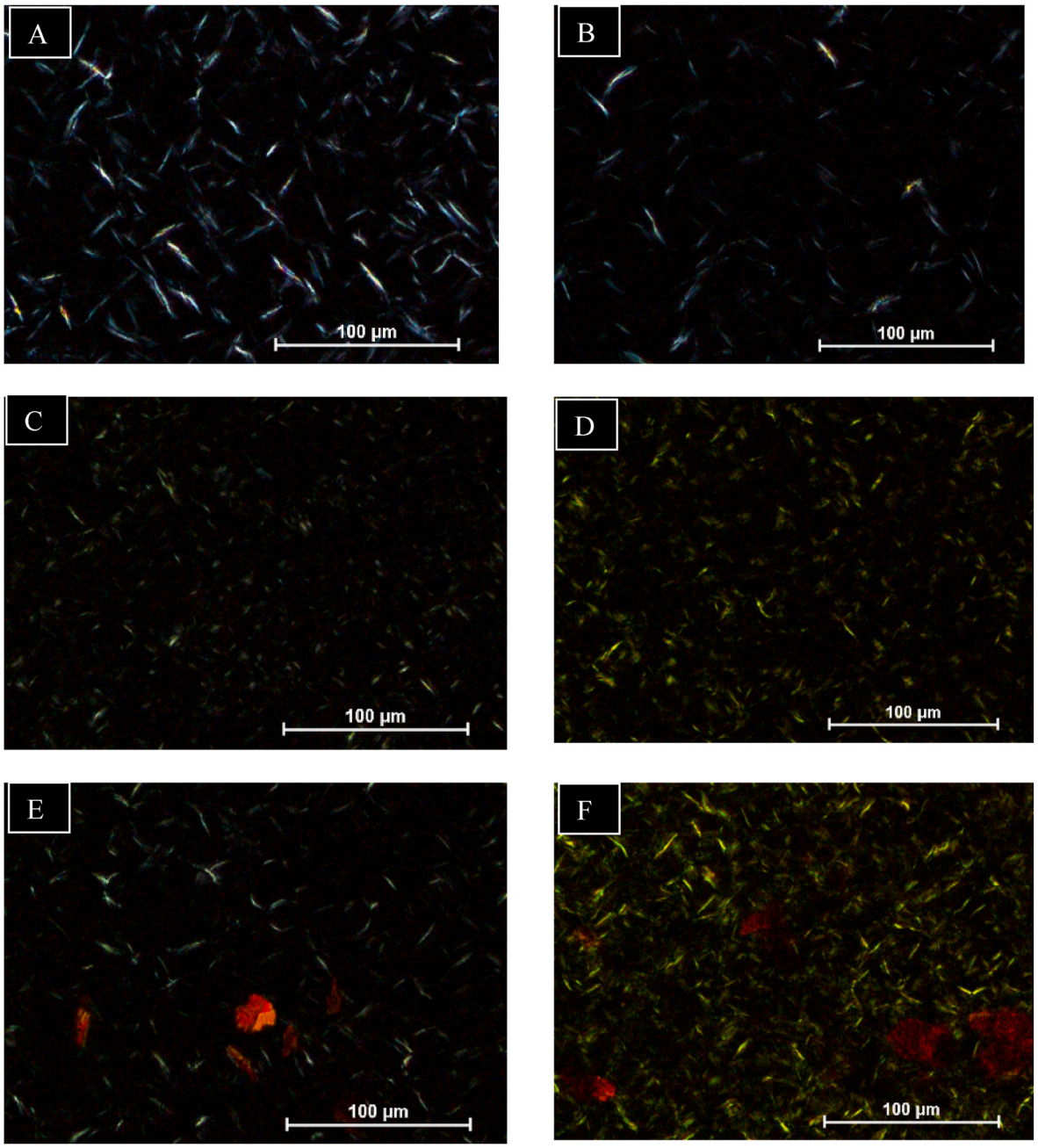


Fig. 1. Bright-field polarized microscopy images of oleogels (A: COG, B: BCCOG, C: BCOG1, D: BCOG2, E: BCOG3, and F: BCOG4) acquired at a magnification of 200 × at 20 °C. Scale bar: 100 μm.

thus, lower fractal dimension. These results were in line with the results reported in the literature for the β-carotene incorporated oleogels. Barragán-Martínez et al. (2022) also observed occasionally dispersed spots of undissolved β-carotene crystals in oleogel incorporated with 0.4% of β-carotene. Additionally, Fayaz et al. (2017) as well as Li et al. (2021) noted that the oil used did not affect the crystal shape. Li et al. (2021) further mentioned that the addition of β-carotene did not alter the crystal shape, whereas it changed the crystal arrangement in the candelilla wax oleogels. Lu et al. (2022) found that the addition of β-carotene facilitated the formation of much denser gel networks in zein-based emulsion gels. Barragán-Martínez et al. (2022) studied the effects of the incorporation of β-carotene (0.025%–0.4%) on the properties of canola oil/beeswax oleogel and their findings demonstrated that β-carotene addition negatively affected the structure and the oil binding capacity of the oleogels. However, our present study has reported a positive influence of β-carotene addition up to the concentration of 0.25% on beeswax-stearic acid-based oleogels produced from a blend of rice bran oil, sesame oil, and fish oil.

3.3. Rheological characteristics of the oleogels

The oleogels were characterized for their rheological properties using amplitude and frequency sweeps, temperature ramp, and thixotropy experiments. In the case of amplitude and frequency sweeps, and temperature ramp experiments, the storage modulus (G′) and loss modulus (G″) were measured as a function of shear strain, frequency, and temperature, respectively. The G′ represents the energy that remains stored in the system during oscillation and the G″ represents the viscous properties (Martins et al., 2017). The three-interval thixotropy test is used to study how the gel structure changes over time as it experiences

periods of deformation and rest. It measures the change in viscosity over time with controlled shear. Rheological analysis of oleogels is vital in order to get the desired texture, stability, and functionality for the intended food application as the food applications of oleogels involve various mechanical operations such as spreading, mixing, etc.

Fig. 2 (A) illustrates the amplitude sweep of oleogels. All samples showed similar G′ and G″ behavior with increasing shear strain. After the LVR, both G′ and G″ decreased with increasing strain values. Table 2 shows the parameters derived from amplitude sweep such as G′ at LVR, LVR, and loss factor. The G′ at LVR of all oleogels incorporated with β-carotene was significantly higher than both controls, while the amount of β-carotene did not show any significant impact on G′ at LVR. G′ at LVR indicated the strength of the gel structure (Scharfe et al., 2022). Therefore, it could be interpreted that β-carotene contributed to enhancing the strength of the gel structure potentially due to the physical interactions among the oleogelators and β-carotene. This was further supported by the higher fractal dimensions of the oleogels with β-carotene (BCOG1 and BCOG2) as detailed in the microstructure section above. Based on the FTIR analysis (explained below), there was no evidence for the formation of any new bonds among the molecules. Martins et al. (2017) similarly noted that the addition of β-carotene at a 0.01% level resulted in increased G′ at LVR in beeswax oleogels. A nonsignificant difference in the G′ at LVR of both control oleogels suggested that the incorporation of fish oil did not have any effect on the gel strength.

Even though the G′ at LVR of the oleogels differed significantly, LVR and loss factor did not show any significant differences among them. The LVR is the range of strain value within which the G′ remains constant (non-destructive deformation). The loss factor is a measure of energy dissipation during deformation. A high loss factor suggested that the gel structure is less stable, and the oleogel may experience flow or deformation more easily. The nonsignificant LVR and loss factor of the oleogels indicated that even though the strength of the oleogels is influenced by the addition of β-carotene, their behavior against the applied stress is similar. The behavior of G′ and G″ with shear strain was consistent with the behavior of complex viscosity which showed a shear thinning behavior (Fig. 2 (B)). Such behavior is a desirable property in spreadable products.

Table 2
Initial rheological parameters of the oleogels.

Sample	LVR (%)	G′ at LVR (Pa)	Loss factor	Structure recovery (%)	Flow point (°C)
COG (Control 1)	0.047 ± 0.003^{a}	107605 ± 1450^{c}	0.147 ± 0.008^{a}	34.43 ± 0.78^{a}	59.99 ± 0.76^{a}
BCCOG (control 2)	0.046 ± 0.003^{a}	108555 ± 106^{bc}	0.144 ± 0.002^{a}	33.32 ± 0.10^{a}	60.41 ± 0.06^{a}
BCOG1	0.046 ± 0.001^{a}	117915 ± 530^{ab}	0.141 ± 0.002^{a}	33.82 ± 0.25^{a}	60.03 ± 0.44^{a}
BCOG2	0.047 ± 0.003^{a}	126500 ± 707^{a}	0.150 ± 0.002^{a}	34.17 ± 0.60^{a}	60.04 ± 0.83^{a}
BCOG3	0.047 ± 0.001^{a}	121500 ± 4949^{a}	0.139 ± 0.001^{a}	29.29 ± 0.07^{b}	61.17 ± 1.66^{a}
BCOG4	0.047 ± 0.001^{a}	122000 ± 2828^{a}	0.149 ± 0.001^{a}	29.09 ± 0.57^{b}	59.79 ± 0.76^{a}

All results are presented as the mean ± standard deviation based on three replicates. Different superscript letters (a-c) in the same column show a significant difference ($p < 0.05$).

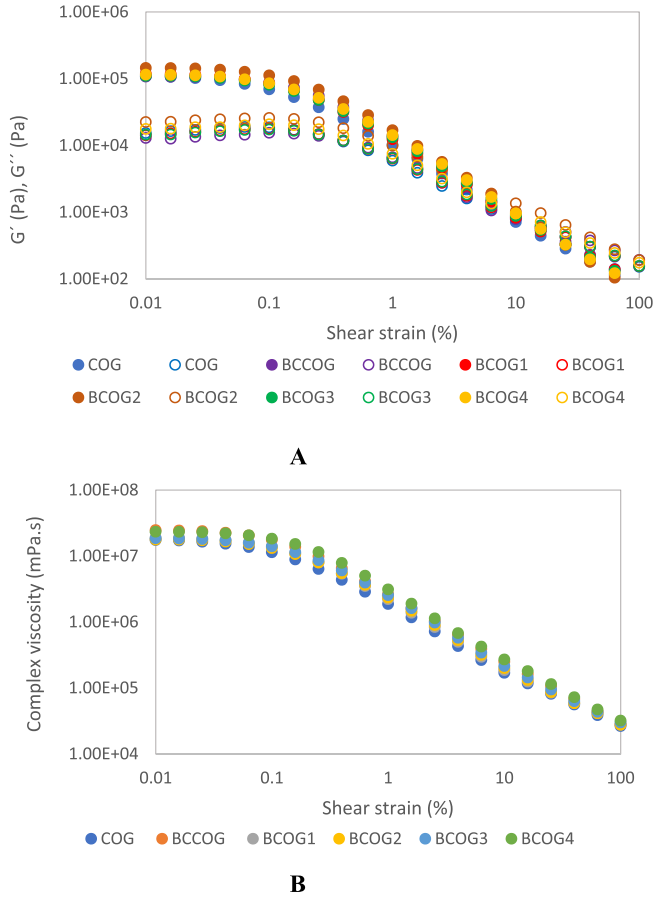


Fig. 2. Amplitude sweep (A) and complex viscosity (B) of oleogels. In the amplitude sweep, closed series markers refer to the G′ and open series markers refer to the G″.

Fig. 3 illustrates the frequency sweep of the oleogels. Throughout the experiments, all samples showed higher G′ than G″ indicating the solid-like behavior of the oleogels. However, as the frequency increased, both G′ and G′ exhibited a rise, indicating a frequency-dependent nature across all samples. Compared to the change in G′, both controls (COG and BCCOG) exhibited a greater increase (about 70–80% increase from the initial value) than the oleogels containing β-carotene (which exhibited a 55–60% increase from the initial value). These findings suggested that β-carotene incorporated oleogels had a more stable gel network demonstrating less susceptibility to frequency-induced changes than the control oleogels. This could be attributed to the role of

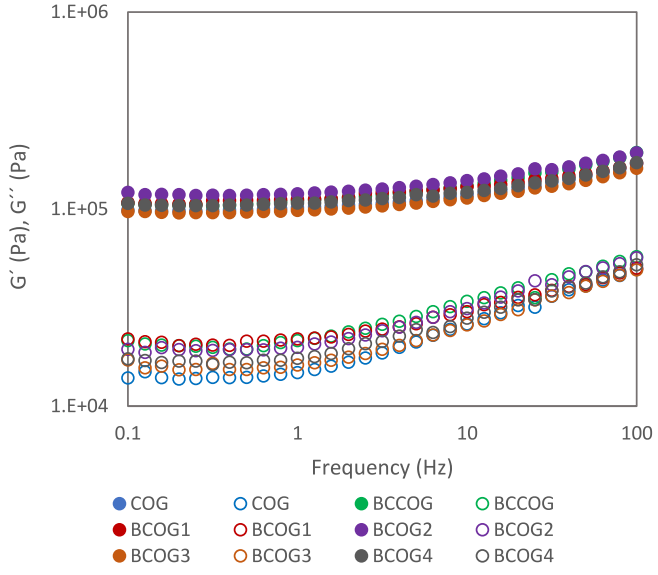


Fig. 3. Frequency sweep of oleogels. Closed series markers refer to the G′ and open series markers refer to the G″.

β-carotene in enhancing the connectivity within the gel network. Consequently, it suggested that the oleogels incorporated with β-carotene can withstand applied stresses without significant deformation or flow compared to the oleogels of the same formulation without added β-carotene. No significant differences were observed among the β-carotene incorporated oleogels in relation to frequency. In line with these observations, Cui et al. (2019) also reported the frequency-dependent behavior of monoglyceride oleogels incorporated with β-carotene.

The thixotropic behavior of oleogels is shown in Fig. 4 and the corresponding structure recovery percentages are shown in Table 2. Despite similar initial viscosities across all samples, BCOG3 and BCOG4 recorded significantly lower structure recovery abilities, unlike the remaining samples, which displayed similar structure recovery abilities. The lower structure recovery ability of BCOG3 and BCOG4 could be due to the presence of undissolved β-carotene crystals as depicted in the microscopy images (Fig. 1). These undissolved aggregates could have hindered the ability of the gel to recover.

Temperature ramp curves are shown in Fig. 5 and flow point temperatures acquired from the temperature ramp experiment are presented in Table 2. All oleogels showed similar behavior with increasing temperature and there were no significant differences in the flow points of the oleogels. These results demonstrated that the incorporation of fish oil and β-carotene did not exert any noticeable influence on the behavior of oleogels with the temperature.

3.4. Thermal properties of oleogels

No significant variations in the thermal characteristics were observed between the control oleogels and those incorporated with varying quantities of β-carotene. The onset and peak of melting ranged from 25.45 ± 1.77 to 26.35 ± 0.21 °C and 48.95 ± 0.21 to 49.40 ± 0.42 °C, respectively. These results indicated that the incorporation of fish oil and β-carotene at the concentrations used in this study did not have any significant influence on the melting and crystallization behavior of the oleogels. This observation aligned with the observation by Martins et al. (2017) who reported that the incorporation of β-carotene (0.01%) did not influence the thermal parameters for higher beeswax concentrations (6% and 8%). However, contrasting results were reported by Cui et al. (2019) indicating that the incorporation of β-carotene at a concentration of 10 g/100g significantly reduced the melting temperature of monoglyceride oleogels. The disparities between

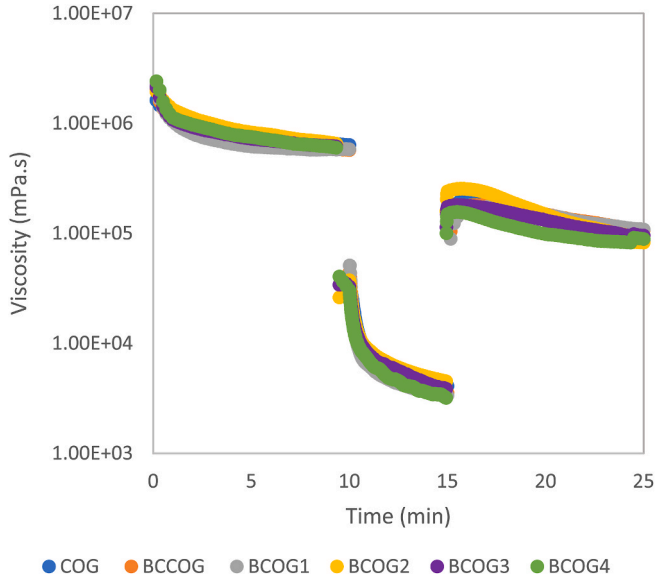


Fig. 4. Thixotropic behavior of oleogels.

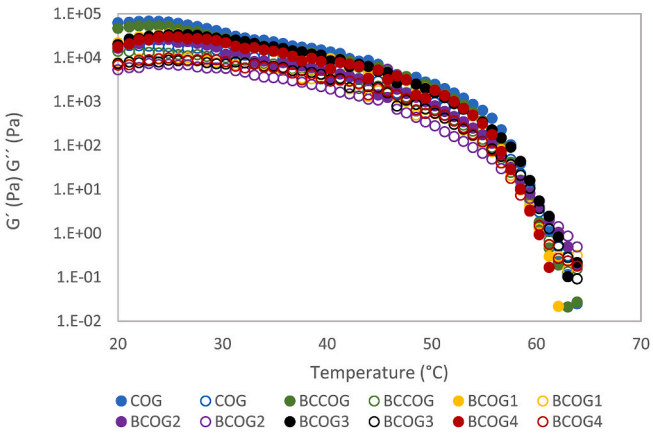


Fig. 5. Temperature ramps of oleogels.

the observations in the present study and the findings of Cui et al. (2019) could be due to the differences in the concentration of the β-carotene used in the respective studies.

3.5. FTIR analysis of oleogels

Examining the FTIR spectrum of the oleogel could provide valuable insights into the molecular interactions among its constituents. The FTIR spectra of the oleogels are shown in Fig. 6. In the functional group region of the spectra of the oleogels, prominent sharp peaks were observed at ~2922 cm^{-1} (symmetric CH_2 stretching), ~2852 cm^{-1} (asymmetric CH_2 stretching), and ~1744 cm^{-1} (ester carbonyl group), and a less prominent peak was observed at 3008 cm^{-1} (C–H stretching of the alkenyl group) (Li et al., 2022). The oleogels containing β-carotene displayed no peak shifts or new peaks, suggesting that β-carotene likely engaged with other oleogel components solely through physical interactions. This differed from the findings of Barragán-Martínez et al. (2022) who noted an impact on the C═O stretching band upon β-carotene addition. Notably, other researchers who investigated β-carotene's integration into oleogels did not provide FTIR analysis in their studies (Cui et al., 2019; Jeong et al., 2021; Martins et al., 2017).

3.6. Storage stability of the oleogels

The assessment of the oleogels' storage stability was conducted over a duration of 4 months at 5 °C, evaluating parameters such as oil binding capacity, rheological properties, β-carotene content, total phenolic content, as well as oxidative stability measured by peroxide value and oxidation induction time. Given that the typical storage for oleogels involves refrigeration at temperatures ranging from 2 to 5 °C, this study focused on assessing storage stability under the same conditions. Therefore, the samples were stored at 5 °C throughout the evaluation period. The oil binding capacity of the oleogels did not show any significant differences through the storage, whereas significant changes in the rheological properties were reported for all oleogels after 4 months of storage at 5 °C. As depicted in Fig. 7 (A and B), noticeable reductions were observed in both G′ at LVR and LVR limit in all oleogels after three months of storage. These changes indicated a microstructural change in the gel network during storage leading to the weakening of the gel structure.

To gain a comprehensive understanding of the oleogel, β-carotene content and the oxidative stability of the corresponding oils stored under the same conditions were also evaluated. Table 3 shows the β-carotene content, peroxide value, and oxidation induction temperature up to 4 months of storage. β-Carotene is highly sensitive to heat, light, and oxygen (Zhou et al., 2018). Therefore, studying the stability of β-carotene in the oleogel system is crucially important to develop oleogels for the

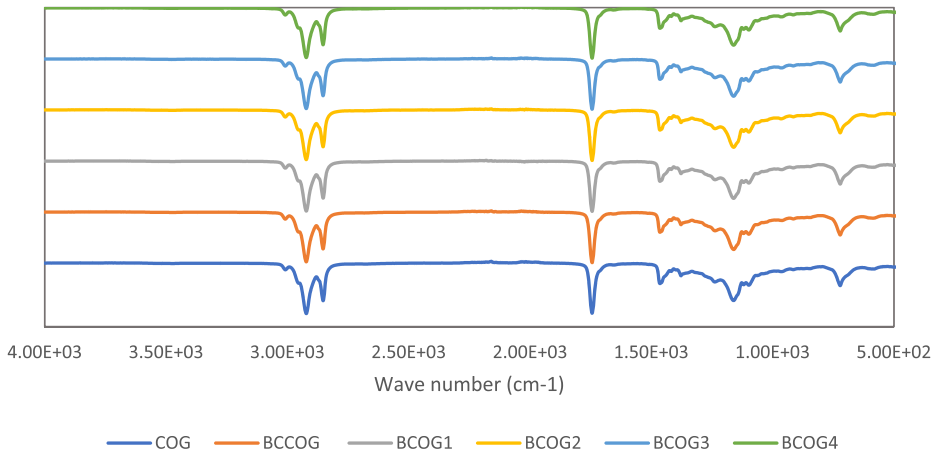
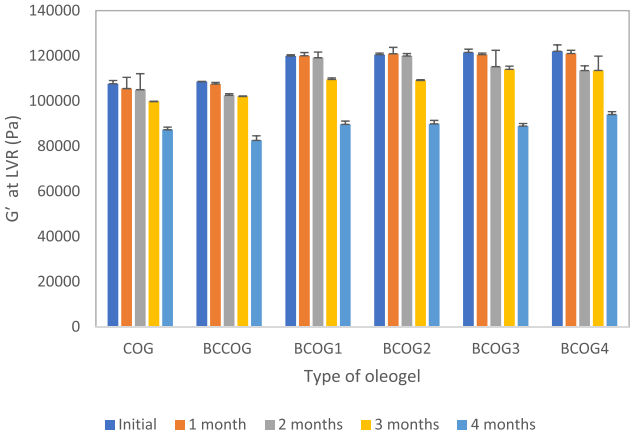
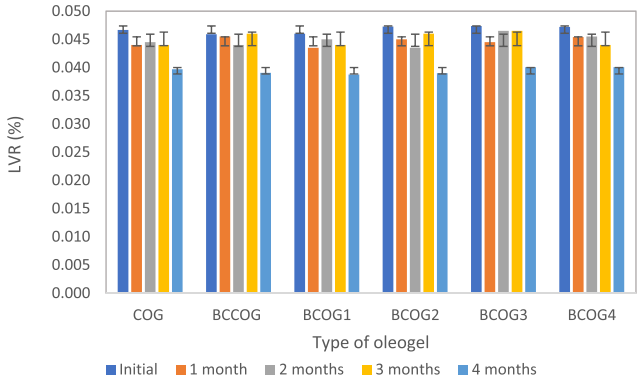


Fig. 6. FTIR spectra of the oleogels.



A



B

Fig. 7. G′ at LVR (A) and LVR (B) of the oleogels stored for up to 4 months at 5 °C.

effective delivery of β-carotene. This study evaluated the incorporation of β-carotene into the oleogel at four different levels (0.1, 0.25, 0.5, and 1 g/100g of oil). When comparing the initial β-carotene concentrations (Table 3) in the oils with those of added concentrations, the loss of β-carotene in BCO1 was negligible, whereas BCO2, BCO3, and BCO4 reported approximately 34%, 54%, and 60% loss. The length of time required to dissolve the β-carotene at the concentrations of 0.1% and 0.25% (BCO1 and BCO2, respectively) at 80 °C was about 20 min, whereas the time required to dissolve the β-carotene at 0.5% and 1% (BCO3 and BCO4, respectively) was about 30 min. Comparatively higher loss reported for the BCO3 and BCO4 could be attributed to the prolonged heating time. Further loss of β-carotene was observed in all oleogels except for both controls which could be due to the presence of only a minute quantity of β-carotene in both controls compared to the oleogels incorporated with β-carotene. However, throughout the storage, all oleogels added with β-carotene retained a significantly higher amount of β-carotene compared to the respective oils and at the end of 4 months of storage, the amount of β-carotene in the oleogels was higher than in the respective oils. This could be due to the protection of the β-carotene from external factors such as access to oxygen in the gel matrix of the oleogels. Despite a consistent decline in β-carotene content in all samples, the rate of reduction was more pronounced during the initial months of storage compared to the later stages. Li et al. (2021) similarly reported a sharp decrease in the β-carotene content of oleogels during the first 60 days followed by a slower degradation after storage for 60 days at 4 °C. The reason for this observation could be that the β-carotene in the outer network destroys first, leading to an initial rapid decline, while the interior β-carotene might remain protected within the gel network, leading to gradual degradation (Li et al., 2021). While some studies addressed β-carotene stability in oleogels (Li et al., 2021), to the best of the authors' knowledge, no studies focused specifically on the stability of β-carotene in the oils as control stored under the same condition as oleogels. Therefore, the outcomes of this study hold potential value in formulating oleogels incorporated with β-carotene with enhanced stability.

The peroxide value serves as a critical parameter in evaluating the quality and freshness of edible oils. It quantifies the hydroperoxides formed as the primary product during the oxidation of unsaturated fatty acids. The initial peroxide values, as well as those after one month of storage, displayed significantly higher levels in oils such as BCO3 and BCO4 and corresponding oleogels such as BCOG3 and BCOG4 than those of other oils and oleogels. This indicated a heightened degree of oxidation potentially associated with extended heating. Following two months of storage, the peroxide values of oils and oleogels either remained the same or exhibited a slight decrease. Peroxides are the primary oxidation products generated during the oxidation of lipids and these peroxides are unstable compounds and can decompose into various secondary oxidation products (aldehydes, ketones, alcohols, and hydrocarbons) (Grebenteuch et al., 2021). The reduction in peroxide value over time can be attributed to the decomposition of peroxides into these secondary oxidation products. Notably, there were no significant differences observed in peroxide values between the oils and their corresponding oleogels.

Oxidation induction temperature is a critical parameter in evaluating the oxidative stability of edible oils as it measures the resistance of oils to

Table 3
β-Carotene content, peroxide value, and oxidation induction temperature of oils and oleogels stored at 5 °C for up to 4 months.

Oil/ Oleogel	Initial	1 Month	2 Months	3 Months	4 Months
β-Carotene content (mg/kg)					
CO	7.03 ± 0.13^{hA}	6.91 ± 0.11^{hA}	5.50 ± 0.10^{gB}	5.00 ± 0.25^{gB}	5.06 ± 0.09^{hB}
COG	7.76 ± 0.35^{hA}	6.26 ± 0.13^{hB}	6.87 ± 0.07^{gB}	6.83 ± 0.16^{gB}	5.13 ± 0.06^{hC}
BCCO	6.56 ± 0.17h^{A}	5.99 ± 0.10^{hB}	5.85 ± 0.07^{gB}	5.92 ± 0.13^{gB}	5.07 ± 0.20^{hC}
BCCOG	9.76 ± 0.09^{hA}	6.35 ± 0.26^{hB}	6.28 ± 0.29^{gB}	6.48 ± 0.30^{gB}	5.59 ± 0.09^{hB}
BCO1	1009.83 ± 9.22^{gA}	936.73 ± 11.51^{gA}	732.44 ± 35.31^{fB}	659.48 ± 11.28^{fB}	552.45 ± 13.75^{gC}
BCOG1	940.71 ± 4.10^{gA}	917.45 ± 3.30^{gAB}	914.31 ± 4.31^{eB}	907.06 ± 7.76^{eB}	881.14 ± 8.82^{fC}
BCO2	1654.98 ± 25.13^{eA}	1276.94 ± 10.54^{fB}	1075.67 ± 11.17^{dC}	952.75 ± 5.08^{eD}	815.32 ± 13.44^{fE}
BCOG2	1499.56 ± 20.52^{fA}	1422.99 ± 10.52^{eA}	1319.06 ± 25.01^{cB}	1303.41 ± 5.13^{dB}	1286.07 ± 38.67^{dB}
BCO3	2320.85 ± 20.11^{cA}	1923.26 ± 9.67^{dB}	1401.67 ± 44.21^{cC}	1225.74 ± 8.88^{dD}	980.70 ± 36.78^{eE}
BCOG3	2209.50 ± 15.83^{dA}	2116.90 ± 21.48^{cAB}	2155.37 ± 18.21^{bAB}	2071.09 ± 47.87^{cB}	1870.92 ± 20.70^{cC}
BCO4	4068.48 ± 63.67^{aA}	3479.44 ± 49.10^{aB}	3077.76 ± 97.51^{aC}	2600.96 ± 82.41^{bD}	2128.97 ± 41.20^{bE}
BCOG4	3380.91 ± 17.84^{bA}	3217.25 ± 24.28^{bB}	3146.06 ± 23.27^{aB}	3034.62 ± 43.71^{aC}	2898.63 ± 18.62^{aD}
Peroxide value (meq O_2/kg)					
CO	5.81 ± 0.68^{cA}	5.33 ± 0.36^{cdA}	5.89 ± 0.30^{abA}	6.51 ± 0.70^{aA}	6.72 ± 1.01^{aA}
COG	6.58 ± 0.71^{bcAB}	4.88 ± 0.64^{dAB}	7.21 ± 0.62^{abA}	4.56 ± 0.44^{aB}	5.13 ± 0.63^{abcdAB}
BCCO	5.48 ± 0.54^{cA}	7.01 ± 0.02^{bcdA}	6.47 ± 0.02^{abA}	6.06 ± 0.48^{aA}	6.62 ± 0.57^{abA}
BCCOG	6.37 ± 0.32^{bcA}	5.01 ± 0.14^{dB}	7.47 ± 0.39^{aA}	4.59 ± 0.47^{aB}	5.03 ± 0.20^{abcdB}
BCO1	5.38 ± 0.68^{cA}	5.60 ± 0.69^{cdA}	5.74 ± 0.42^{abA}	5.33 ± 0.36^{aA}	3.90 ± 0.13^{dA}
BCOG1	6.42 ± 0.39^{bcA}	5.92 ± 0.63^{bcdA}	5.67 ± 0.01^{abA}	3.40 ± 0.37^{aB}	4.59 ± 0.73^{cdAB}
BCO2	5.78 ± 0.43^{cAB}	6.72 ± 0.15^{bcdA}	5.19 ± 0.46^{abAB}	4.84 ± 0.64^{aB}	4.71 ± 0.24^{bcdB}
BCOG2	6.94 ± 0.76^{bcA}	5.64 ± 0.11^{cdAB}	5.76 ± 1.04^{abAB}	3.38 ± 0.44^{aB}	3.75 ± 0.16^{dB}
BCO3	9.41 ± 0.81^{aA}	8.22 ± 0.29^{abcA}	6.04 ± 0.05^{abBC}	4.75 ± 0.03^{aC}	6.53 ± 0.35^{abcB}
BCOG3	8.78 ± 0.39^{abA}	7.14 ± 1.33^{bcdAB}	5.18 ± 1.00^{abB}	4.13 ± 0.29^{aB}	4.09 ± 0.29^{dB}
BCO4	9.67 ± 0.16^{aAB}	11.23 ± 1.53^{aA}	6.81 ± 1.09^{abBC}	4.67 ± 0.21^{aC}	5.32 ± 0.20^{abcdC}
BCOG4	10.55 ± 0.91^{aA}	8.81 ± 1.27^{abA}	5.00 ± 0.29^{bB}	4.87 ± 0.01^{aB}	3.63 ± 0.55^{dB}
Oxidation induction temperature (°C)					
CO	340.85 ± 0.78^{fgA}	340.00 ± 1.13^{fAB}	340.05 ± 1.63^{dAB}	336.65 ± 0.64^{fBC}	333.90 ± 0.42^{fC}
COG	341.60 ± 2.26^{efgA}	340.70 ± 0.99^{efA}	341.35 ± 1.06^{bcdA}	339.80 ± 0.42^{deA}	340.85 ± 0.92^{bcA}
BCCO	339.90 ± 0.57^{gA}	340.60 ± 1.56^{efA}	340.60 ± 1.56^{cdA}	337.15 ± 0.49^{efAB}	333.05 ± 0.78^{fB}
BCCOG	341.25 ± 0.35^{fgA}	340.65 ± 0.64^{efA}	340.00 ± 0.28^{dA}	338.50 ± 0.99^{defA}	339.35 ± 1.20^{cdA}
BCO1	346.65 ± 0.21^{bcdA}	346.80 ± 0.99^{abcA}	345.45 ± 1.34^{abA}	344.85 ± 0.49^{abAB}	339.20 ± 0.42^{cdB}
BCOG1	345.10 ± 1.27^{cdefA}	341.30 ± 0.28^{defB}	341.55 ± 1.34^{bcdAB}	340.70 ± 0.71^{cdB}	342.20 ± 0.42^{abB}
BCO2	349.50 ± 1.41^{bcA}	344.95 ± 0.49^{bcdB}	345.55 ± 0.92^{abB}	341.00 ± 0.28^{cdC}	337.90 ± 0.42^{deC}
BCOG2	358.00 ± 1.41^{aA}	348.25 ± 0.49^{abAB}	345.05 ± 0.64^{abcB}	343.80 ± 0.99^{abB}	343.35 ± 1.06^{abB}
BCO3	344.95 ± 0.92^{defA}	344.35 ± 1.20^{cdeA}	341.40 ± 1.56^{bcdAB}	338.85 ± 0.35^{defBC}	335.20 ± 0.57^{efC}
BCOG3	349.20 ± 1.41^{bcdA}	344.30 ± 1.56^{cdeB}	344.45 ± 1.20^{abcdB}	342.80 ± 0.99^{bcB}	342.95 ± 0.21^{abB}

Table 3 (*continued*)

Oil/ Oleogel	Initial	1 Month	2 Months	3 Months	4 Months
BCO4	345.80 ± 0.57^{bcdeA}	342.55 ± 0.35^{defB}	340.20 ± 0.85^{dC}	336.95 ± 0.21^{fD}	334.45 ± 0.35^{fE}
BCOG4	350.10 ± 0.85^{bA}	349.85 ± 0.49^{aAB}	347.35 ± 0.35^{aBC}	345.95 ± 0.78^{aCD}	343.65 ± 0.64^{aD}

All results are presented as the mean ± standard deviation based on three replicates. Different superscript simple letters (a-h) in the same column show a significant difference ($p < 0.05$). Different superscript capital letters (A-E) in the same row show a significant difference ($p < 0.05$).

oxidative degradation. It represents the temperature at which the oil begins to undergo significant oxidation when exposed to air or other oxidizing agents. Therefore, a higher oxidation induction temperature indicates that the oil is more resistant to oxidation. All oils incorporated with β-carotene showed significantly higher initial oxidation induction temperatures than both controls (CO and BCCO). It indicated that the incorporation of β-carotene increased the resistance of the oils against oxidation and the incorporation of fish oil did not cause any changes to the oxidation induction temperature.

Among the oils incorporated with β-carotene, BCO1, and BCO2 exhibited significantly higher oxidation induction temperatures compared to BCO3 and BCO4 despite the higher concentration of β-carotene BCO3 and BCO4. This could be due to the prolonged heating of BCO3 and BCO4 during solubilization of β-carotene. Since these two oils reported higher polar compounds, these products could have acted as prooxidants (Choi et al., 2018) to decrease the oxidation induction temperature of these two oils. Surprisingly, all oleogels incorporated with β-carotene except for BCOG1 exhibited significantly higher oxidation induction temperatures than corresponding oils. A similar observation was reported throughout the storage. Moreover, during the course of storage, all oleogels containing added β-carotene exhibited significantly higher oxidation induction temperatures than both controls. The role of β-carotene in improving the oxidative stability of oils has been reported in the literature (Fakourelis et al., 1987; Shadyro et al., 2020). β-Carotene exhibits a dual nature, acting both as an antioxidant and a prooxidant, with its effect being dependent upon the concentration of β-carotene. Shadyro et al. (2020) reported that β-carotene at concentrations above 10 mg/100 g induced oxidation in flaxseed oil. In our study, the maximum concentration of β-carotene used was 1 g/100g and all concentrations studied showed an antioxidant effect. Both control oleogels (COG and BCCOG) displayed notably higher oxidation induction temperatures compared to their respective control oils (CO and BCCO) following a 4 months storage period. This observation suggested that the gel network offered prolonged protection to the oil against oxidation. Several other studies also concluded that oleogelation can improve oxidative stability compared to respective liquid oil (Jeong et al., 2021; Li et al., 2019; Pan et al., 2021). This enhancement could be attributed to the entrapment of liquid oil within the 3D structure formed by the oleogelator, which potentially serves as a protective barrier, limiting the exposure of oil to oxygen. Similarly, Pandolsook and Kupongsak (2019) also demonstrated that rice bran wax oleogels exhibited increased oxidative stability when stored at temperatures of 4 °C and 30 °C, compared to their respective control (rice bran oil) stored at the same temperatures.

3.7. Comparison of bioactive enriched oleogel with commercial margarine

Based on the findings of rheological properties, microstructure, β-carotene content, peroxide value, and oxidation induction temperature, the oleogel incorporated with β-carotene at the concentration of 0.25% was chosen as the best oleogel formula. As already explained, the incorporation of β-carotene resulted in a stronger gel despite the amount of β-carotene added and a more uniform crystal arrangement. Even though the oleogels incorporated with β-carotene did not show

significant differences in other rheological properties such as LVR, loss factor, and flow point, the structure recovery of BCOG3 and BCOG4 was significantly less than other oleogels. Moreover, even though BCOG3 and BCOG4 exhibited similar oxidation induction temperatures at the end of 4 months of storage as BCOG2 and BCOG2, the loss of β-carotene was comparatively higher in BCOG3 and BCOG4 compared to the initial amount of β-carotene. Based on these observations, the formula of BCOG2 was selected as the best among all formulas examined in this study. Therefore, the fatty acid profile, oil binding capacity, rheological properties, and thermal properties of BCOG2 and three different commercial brands of margarines were compared (Table 4).

BCOG2 had significantly less saturated fatty acid content and higher polyunsaturated fatty acid content compared to commercial margarines. Further, the developed oleogel had zero *trans* fatty acids and omega-3 fatty acids (2.5%) whereas commercial margarines contained *trans* fatty acids ranging from 0.5%–6.4%. According to the recommendation by the WHO, the consumption of saturated fatty acids should be limited to <10% of total energy intake, and *trans* fatty acids should be limited to <1% of total energy intake. It has been reported that replacing saturated fatty acids with polyunsaturated fatty acids decreases Low-Density Lipoprotein (LDL) cholesterol concentration and the total/High-Density Lipoprotein (HDL) cholesterol ratio (FAO, 2010). Therefore, by comparing the fatty acid profile of oleogel and commercial margarines, it can be concluded that the oleogel developed from this study had a healthy fatty acid profile.

The oil binding capacity, G′ at LVR, and LVR of the oleogel were significantly higher than the commercial margarines. These results indicated that the oleogel had a stronger gel structure compared to the commercial margarine. However, the oleogel had a poor structural recovery ability compared to commercial margarine. Therefore, further modifications in the gel structure to improve the structure recovery ability should be considered. The oleogel exhibited a similar flow point (gel point) and peak melting point as the commercial margarine. Overall, it can be concluded that the developed oleogel exhibited similar mechanical properties as the commercial margarines. Considering the functional properties of the oleogel and commercial margarine, the oleogel formula is superior to commercial margarine because the developed oleogel formula contains β-sitosterol, β-carotene, and omega-3 fatty acids (EPA and DHA).

4. Conclusions

The study assessed the impact of integrating fish oil and β-carotene into oleogels based on beeswax and stearic acid over a 4-month storage period, evaluating physical, microstructural, rheological, molecular, thermal, and oxidation properties. The findings demonstrated that the incorporation of 10% fish oil did not have any significant influence on the physical and mechanical properties and oxidative stability of the oleogel. However, β-carotene notably influenced the strength of the gel network without altering thermal or molecular aspects positively impacting crystal arrangement. The incorporation of β-carotene enhanced the oxidative stability of the oleogels, displaying superior stability and β-carotene retention compared to liquid oils during storage. Considering all properties of the oleogels, the oleogel formula that contained β-carotene at the concentration of 0.25% (BCOG2) can be selected as the best. When comparing the properties of the oleogel developed from this study (BCOG2) with those of commercial margarines, the oleogel had significantly less saturated fatty acid content and higher polyunsaturated fatty acid content. In addition to the healthy fatty acid composition, the developed oleogel can be considered superior as it is enriched with omega-3 fatty acids, β-carotene, and β-sitosterol. Overall, BCOG2 possesses superior nutritional and functional properties and similar mechanical properties to commercial margarines. From this study, it can be concluded that oleogels with enhanced functional properties and shelf life could be produced by incorporating fish oil, β-carotene, and β-sitosterol.

Table 4
Comparison of properties of BCOG2 and commercial margarines.

Parameter	BCOG2	Commercial margarines[a]
Saturated fatty acids (%)	18.86 ± 0.62	50.01–52.07
Monounsaturated fatty acids (%)	34.41 ± 1.62	32.63–45.45
Polyunsaturated fatty acids (%)	47.04 ± 1.43	6.63–11.25
EPA (%)	1.40 ± 0.42	ND
DHA (%)	1.12 ± 0.06	ND
Trans fats (%)	ND	0.53–6.41
Oil binding capacity (%)	99.91 ± 0.06	70.13–79.65
G′ at LVR (Pa)	1.26E+05	7.86E+04–1.01E+05
LVR (%)	0.047 ± 0.003	0.022–0.034
Structure recovery (%)	34.17 ± 0.60	44.15–52.43
Flow point (°C)	60.04 ± 0.83	57.10–61.05
Peak crystallization temperature (°C)	45.85 ± 0.64	15.40–16.20
Peak melting temperature (°C)	49.10 ± 0.00	51.9–61.8

All results are presented as the mean ± standard deviation (SD) based on three replicates.
ND – not detected.
[a] The values are a range of values of three commercial samples.

CRediT authorship contribution statement

Subajiny Sivakanthan: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Sabrina Fawzia:** Writing – review & editing, Supervision, Resources, Conceptualization. **Sagadevan Mundree:** Writing – review & editing, Supervision, Conceptualization. **Terrence Madhujith:** Writing – review & editing, Supervision, Conceptualization. **Azharul Karim:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

We like to declare that the authors have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

This work was financially supported by Queensland University of Technology, Brisbane Australia and Accelerating Higher Education Expansion and Development (AHEAD): a World Bank-funded Sri Lankan government operation. The authors would like to acknowledge the laboratory facilities from the Central Analytical Research Facility (CARF) and the Centre for Agriculture and the Bioeconomy (CAB) of the Queensland University of Technology, Australia.

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