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Development of non-animal chicken fat using faba bean protein-based emulsion gels

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ABSTRACT

Increasing awareness of the risks associated with overconsumption of saturated fats has focused attention on healthier fat replacers that can be incorporated into alternative protein foods as non-animal fat. A key technological challenge is in developing non-animal fats that are: (1) relatively high in melting point, (2) low in saturated fat, and (3) able to combine with proteins in a food matrix. In our study, protein-based emulsion gels (PEGs) consisting of faba bean protein concentrate, olive oil and water at different protein (7–10% w/w) and oil (40–60% w/w) concentrations, were created using high-pressure processing (HPP) technology. The PEGs were characterized and compared to chicken fatty deposits based on their appearance, color, oil-holding capacities, freeze-thaw stability, and rheological properties. We found that PEGs with lower protein concentrations, particularly 7% protein, 40% oil (7%P,40%O) exhibited the closest rheological properties and gel strength G' (4465.07 ± 76.12 Pa) to chicken fatty deposits (2933.60 ± 763.21 Pa). PEGs with 10% protein had better long-term oil-holding capacities after the second freeze-thaw (for 10%P,60%O: 42.062 ± 0.530%), in comparison to chicken fat (45.362 ± 5.928%). Freeze-thaw treatments disrupted the stability of the PEG, signifying the importance of temperature control during gel storage and transportation. These results provide valuable insight into the effect of protein and oil concentrations on measured attributes in the PEGs. This will enable us to utilize PEGs to create and mimic animal fats such as chicken fat.

1. Introduction

Fats are major macronutrients in foods that are a key source of energy, but also impart taste, aroma, texture, and juiciness to foods. However, diets rich in saturated fats have been associated with elevated risk of cardiovascular diseases and increased health complications (Siri-Tarino, Sun, Hu, & Krauss, 2010). Increasing awareness of risks associated with overconsumption of fats (particularly saturated fats) has focused attention on healthier fat replacers that have traditionally been used in foods with high fat content, such as baked food products or confectionery (Colla, Costanzo, & Gamlath, 2018). The increasing usage of alternative protein foods has also created the opportunity for fat replacers to be incorporated as non-animal fat. Using suitable non-animal fat replacers allows the desirable physicochemical and sensory properties of animal fats to be mimicked while lowering the saturated fat content in foods. This can be highly useful in the plant-based food market, whose growth is expected to reach $160 billion by 2030 (Mintel, 2023).

Existing plant-based meats are mostly made with comminuted/ground meat analogs that contain a limited range of vegetable fat options such as coconut and palm fat. These fats have relatively low melting points, causing lipids to leak out of the food matrix during processing/cooking (Cho, Bae, Lee, & Choi, 2023; López-Pedrouso, Lorenzo, Gullón, Campagnol, & Franco, 2021). Considerable technological challenges exist in developing plant-based non-animal fats that are: (1) relatively high in melting point, (2) low in saturated fat, and (3) able to combine well with proteins in a food matrix (Gumus-Bonacina, McClements, & Decker, 2024).

Developing commercial fat substitutes to reduce saturated fat intake began with the launches of fat-based replacers such as Olestra (Olean®), Caprenin (Caprenin®) and Salatrim (Benefat®), that are based on mixtures of fatty acids or structured/modified lipids (Lin & Appleby, 2012;

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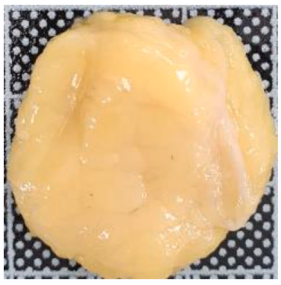
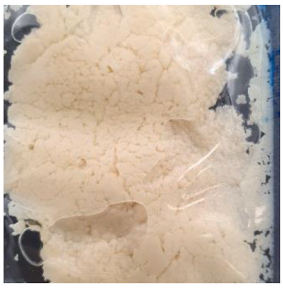
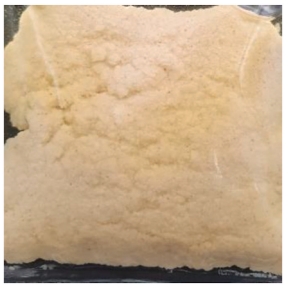
Table 1
Visual appearance, of (a) chicken fat, (b) 7% protein, 40% oil (7%P,40%O), (c) 10% protein, 40% oil (10%P,40%O), at FTC0 and FTC2. FTC refers to the number of freeze-thaw cycles.

Sample	FTC 0	FTC 2
(a) Chicken fat		
(b) 7%P,40%O		
(c) 10%P,40%O		

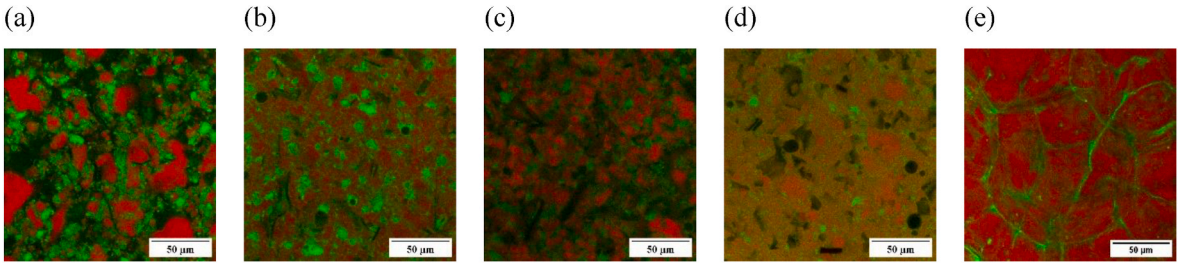


Fig. 1. Confocal laser scanning micrographs of samples at FTC0 from left to right (a) 7% protein, 40% oil (7%P,40%O); (b) 7% protein, 60% oil (7%P,60%O); (c) 10% protein, 40% oil (10%P,40%O); (d) 10% protein, 60% oil (10%P,60%O); (e) Chicken fat. Oil was stained with Nile Red and appeared as red components, while protein was stained with FITC and appeared as green components. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

O'Sullivan, 2016). Some of these substitutes come with drawbacks such as potential detrimental gastrointestinal effects and potential inhibition of fat-soluble vitamins and mineral absorption (Jones & Jonnalagadda, 2006; Kew, Holmes, Stieger, & Sarkar, 2020). As a result, newer fat substitutes seek to retain the natural lipids and rely on oil structuring (Abdullah, Liu, Javed, & Xiao, 2022). These oil-structuring techniques include the formation of oleogels (Flöter, Wettlaufer, Conty, & Scharfe, 2021; Manzoor, Masoodi, Naqash, & Rashid, 2022), emulsion gels (emulgels) (Silva, Barrera-Arellano, & Ribeiro, 2021), high-internal phase emulsions (Y. Zhang, Lu, Zhang, Gao, & Mao, 2021), lipid interesterification, organogels and biphasic systems (Jimenez-Colmenero et al., 2015; Patel & Dewettinck, 2016). These systems exhibit solid-like properties, and are formed by a combination of dispersed phase (consisting of dispersed or dissolved hydrocolloids such as proteins and polysaccharides) and a continuous phase (Abdullah et al., 2022; Devi, Buckow, Hemar, & Kasapis, 2013). The continuous phase in an oleogel is an edible oil. In emulgels, the phases are primarily water and oil, where either the dispersed or continuous phase forms a 3-dimensional network structure (Abdullah et al., 2022). Variations of emulgels can exhibit different physicochemical and sensory properties, depending on the nature of the dispersed and continuous phase.

Emulgels show great potential as fat replacers as they can easily be formed with natural food-grade biopolymers, such as proteins and polysaccharides (Abdullah et al., 2022; Silva et al., 2021). Varying oil

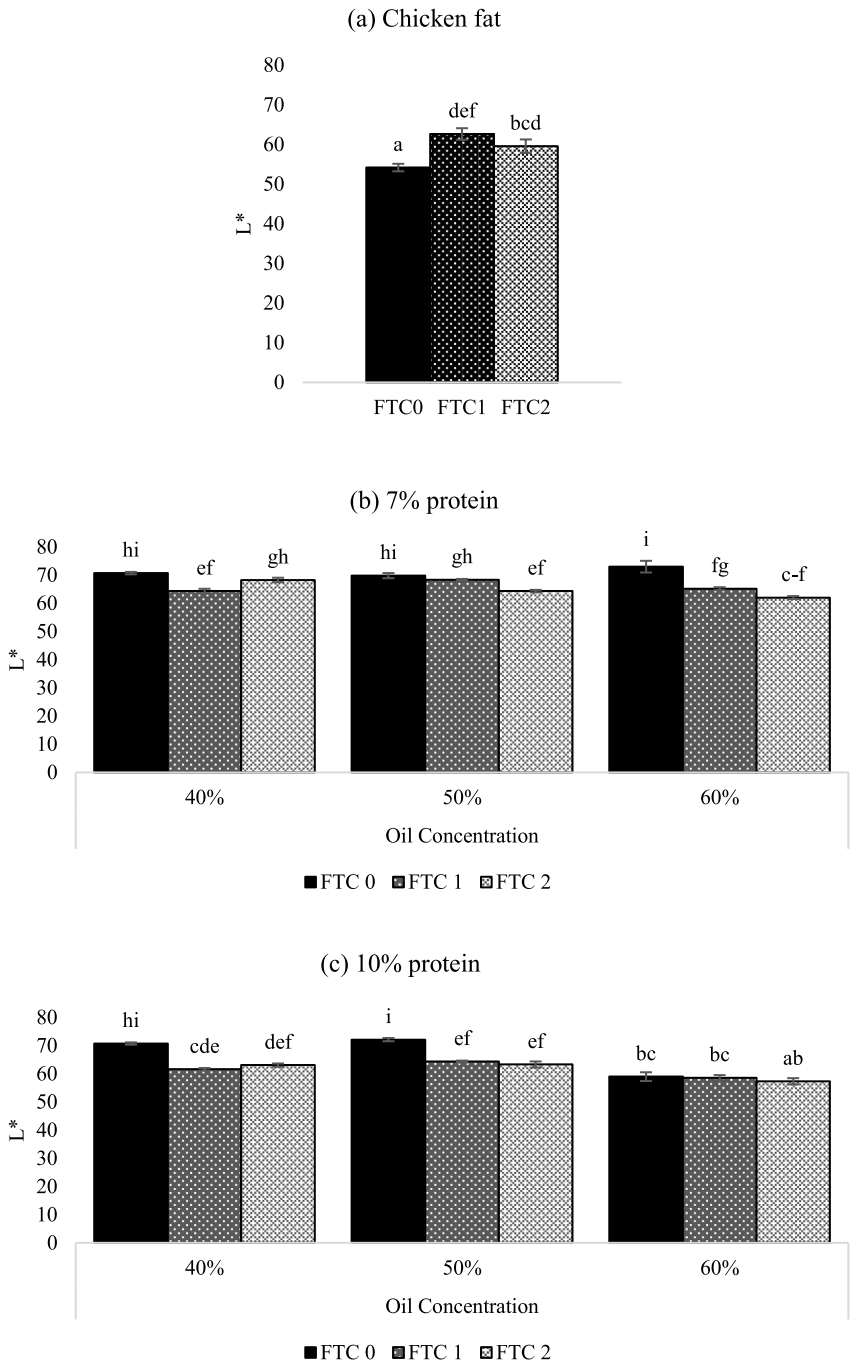


Fig. 2. Lightness (L*) readings of emulsion gel with (a) chicken fat; (b) 7% protein and (c) 10% protein across FTCs in comparison to chicken fat. FTC refers to the number of freeze-thaw cycles. Different lowercase letters indicate significant differences across the three graphs ($p < 0.01$).

and water fractions, as well as changing the biopolymers used can create desirable rheological characteristics depending on the intended use of the fat replacers. The use of proteins as structuring agents poses an added advantage over other surfactants such as gums and waxes, in terms of their ability to be functionalized for various applications and their nutritional profile that better aligns with animal proteins. Many studies have been conducted on foods formed from emulgels with animal-based proteins, such as cheese (casein-based), sausages, and meats (Abdullah et al., 2022; Nacak, Öztürk-Kerimoğlu, Yıldız, Çağındı, & Serdaroğlu, 2021; C. Paglarini et al., 2020; Xi, Liu, McClements, & Zou, 2019), with more recent ones looking into using plant-based emulgels as animal fat replacers (X. Hu & McClements, 2022; Ren et al., 2022).

Until recently, most plant-based proteins studied for such use have been soy and wheat proteins (Funami, Nakano, Maeda, Yamasaki, & Nakauma, 2023; Liu et al., 2018; Xi et al., 2019). Due to concerns about soy and wheat being major food allergens (Komei, 2011), newer alternatives such as lentils and pulses have been explored (Teng & Campanella, 2023). Among pulses, faba beans (*Vicia faba* L.), are being recognized as a good protein source for plant-based gel systems, with them having a high protein content of up to 32.4%, comparable to soy (40%) and higher than other sources such as chickpeas (19.53%) and lentils (22.15%) (Y. Hu, Cheng, Lee, & Yang, 2023; Martineau-Côté, Achouri, Karboune, & L'Hocine, 2022). Faba beans are cultivated extensively worldwide, making them a familiar food ingredient for most (Çalışkantürk Karataş, Günay, & Sayar, 2017; Dangi et al., 2022). Faba

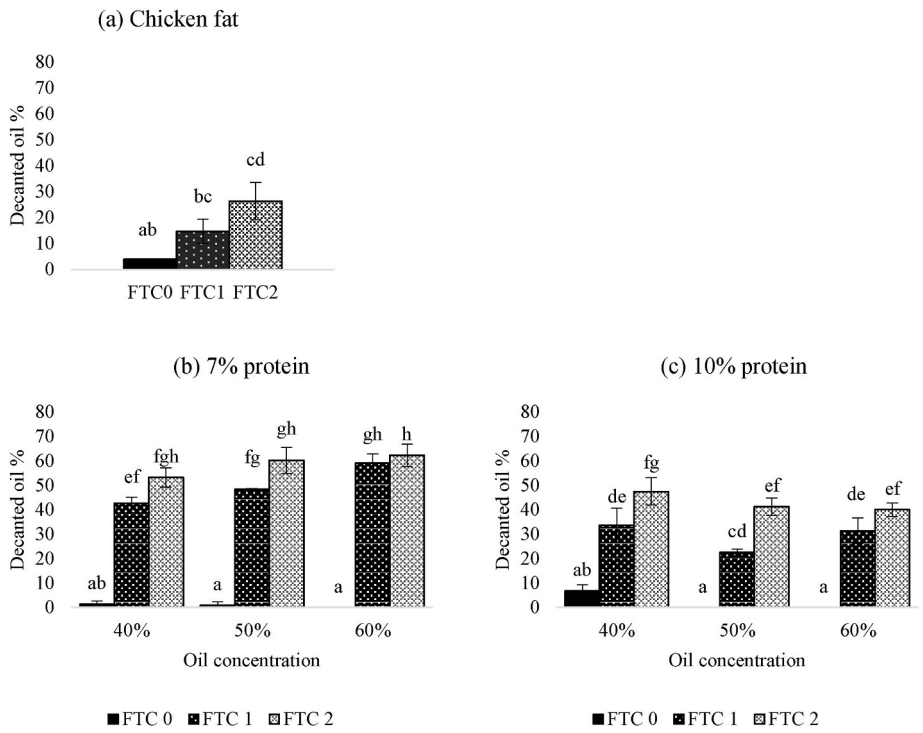


Fig. 3. Decanted oil amount (%) after each FTC for (a) chicken fat; (b) emulsion gels with 7% protein; and (c) emulsion gels with 10% protein. FTC refers to the number of freeze-thaw cycles.

beans are also sustainably grown, non-allergenic, high in protein, and have a well-balanced amino acid profile, making them a good candidate for developing plant-based gel systems (Martineau-Côté et al., 2022). There are currently only a handful of studies looking into the formation of faba bean gel and faba bean emulsion gels (Y. Hu et al., 2023; Jiang, Wang, Stoddard, Salovaara, & Sontag-Strohm, 2020; Langton et al., 2020). The limited studies, combined with the nutritional profile of faba beans, highlight their potential as a unique alternative legume-based protein source, well suited to meet the rising demands of the growing plant-based market (Martineau-Côté et al., 2022).

Protein-based emulsion gels (PEG) have often been produced through methods involving heating, addition of salts or pH adjustments, which denature proteins and allow conformational changes that lead to gelation (Abdullah et al., 2022; Xi et al., 2019). While these methods have been effective in achieving gelation, they limit the incorporation of temperature- and pH-sensitive flavor and bioactive molecules (Dangi et al., 2022; Devi et al., 2013; Mozhaev, Heremans, Frank, Masson, & Balny, 1996). Non-thermal methods, such as High-Pressure Processing (HPP), offer opportunities to overcome these challenges. HPP involves the quasi-instantaneous treatment of high pressures on the food, typically ranging from 200 to 600 MPa (Queirós, Saraiva, & da Silva, 2018; S. Y. J. Sim, Srv, Chiang, & Henry, 2021). Plant-based HPP-induced gels have been studied as mimics of existing food products: such as pea protein gels to mimic the texture of jellies and tofu (S. Zhang, Han, & Chen, 2023), and plant-based protein gels for mimicking yoghurt (Sim et al., 2020). The effectiveness of HPP at temperatures below 40 °C makes it a more desirable method for retaining the nutrient and sensory properties of foods (Devi et al., 2013). Despite its advantages, using HPP to develop plant protein-based emulsion gels and plant-based foods, specifically from faba bean protein, remains poorly studied.

Although beef fat has been used as an animal fat reference for the development of non-animal fat (X. Hu, Zhou, & McClements, 2022), we use chicken fatty deposits (or chicken fat) as a reference for our PEGs studied due to its widespread availability and its reputation for being less healthy (Buege et al., 1998). Our study aims to develop a PEG that simulates chicken fatty deposits, using faba bean protein concentrate, olive oil and water via HPP processing method. We also aim to characterize the visual, thermal, and rheological behaviors, as well as the freeze-thaw stability of the gels developed at different oil (40%, 50%, 60%) and protein concentrations (7%, 8%, 9%, 10%). The oil and protein concentration in the PEGs studied were determined through preliminary studies (refer to supplementary information, Appendix A, Tables A1 and A2). A 60% oil concentration was chosen as the upper limit of oil due to protein agglomeration at higher oil concentrations. As we are developing emulsion gels to mimic chicken fat, which typically contains high lipid profiles, we have decided not to investigate oil concentrations below 40%. 7% protein was selected as the lower limit of protein concentration, due to signs of phase separation after HPP for protein concentrations below 7%. The protein concentrations were selected to be below and above the critical gelling concentration of faba bean protein. Our study on using emulgel as an animal fat substitute can help develop healthier foods with a lower fat content (Abdullah et al., 2022).

2. Methods and materials

2.1. Materials

In our study, the PEGs consist of three main components – water, olive oil and faba bean protein concentrate as the protein source. Compared to the protein isolate, faba bean protein concentrate was obtained through dry fractionation, a more sustainable processing method that better retains the protein's structural and functional properties (Sharan et al., 2021; Vogelsang-O'Dwyer et al., 2020). Additionally, faba bean concentrate has been shown as a promising plant-based emulsifier (Gumus, Decker, & McClements, 2017). Plant-based oils were chosen as they are generally considered healthier due to their lower saturated fat and trans-fat content (Abdullah et al., 2022). In particular, olive oil contains higher proportions of antioxidants and monounsaturated fatty acid (MUFA). These antioxidants reduce the susceptibility of oil to oxidation. The primary MUFA in olive oil is oleic acid, an omega-9 fatty acid which has the benefits of

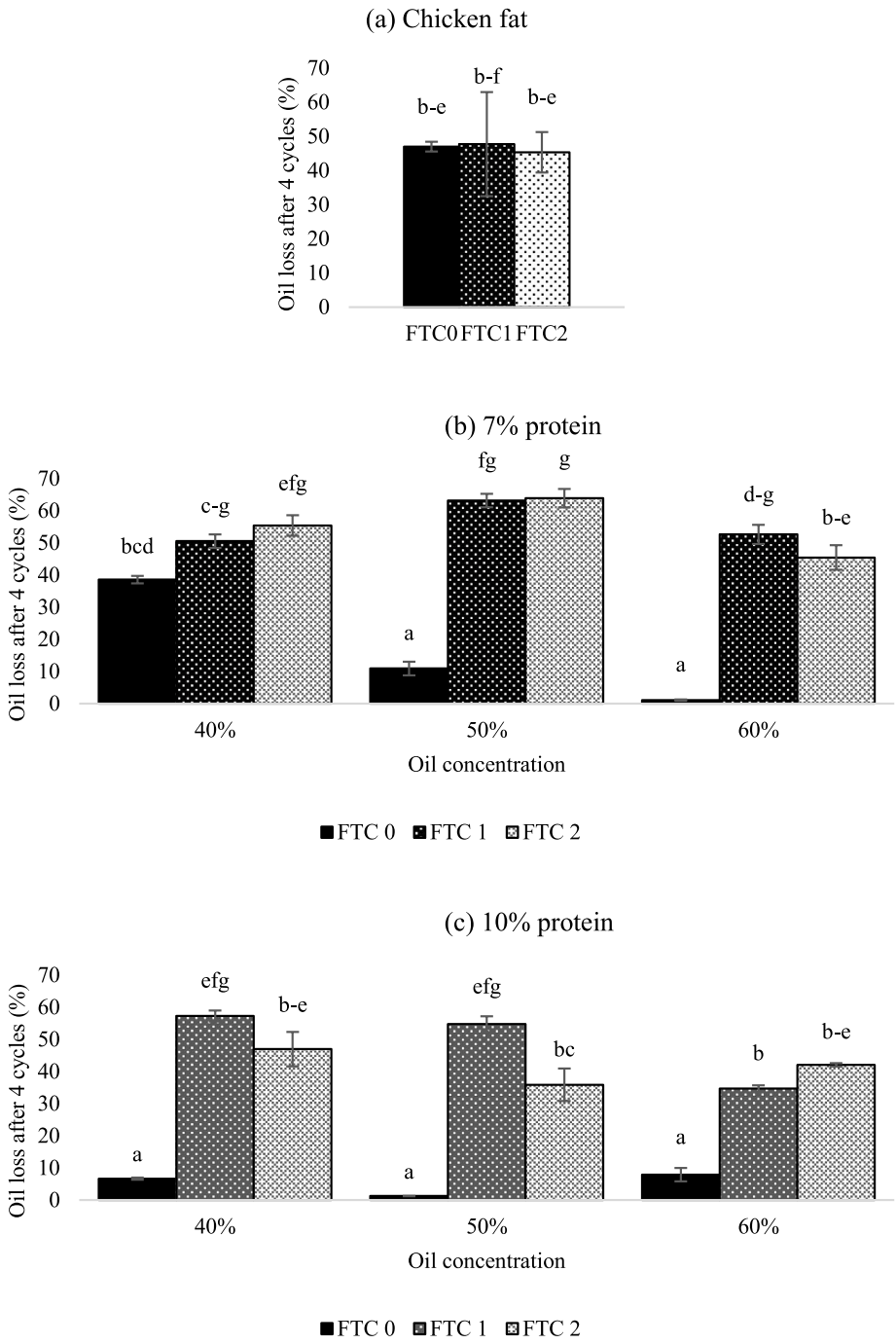


Fig. 4. Oil loss (%) after 4 rounds of centrifugation at 14 000 rpm, 30 min, for each FTC (a) chicken fat; (b) emulsion gels with 7% protein; (c) emulsion gels with 10% protein. FTC refers to the number of freeze-thaw cycles. Different lowercase letters indicate significant differences across the three graphs ($p < 0.01$).

anti-inflammatory and cholesterol-lowering properties (Revelou et al., 2021).

Faba bean protein concentrate (VITESSENCE® Pulse 3600) was obtained from Ingredion Inc. (Westchester Illinois, USA). Olive oil (*Naturel*) was purchased from a local supermarket. Chicken fatty deposits (chicken fat) were collected from a local market and used as a reference. Distilled water was used to prepare all aqueous phases in this experiment. Nile Red (cat. No. 72485) and fluorescein isothiocyanate (FITC) (cat. No. 46950) were both purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2. Crude fat analysis of chicken fat

Triplicates of chicken fat deposits were sent to Eurofins Food Testing Singapore Pte Ltd to analyze the crude fat amount with hydrolysis. The average crude fat by hydrolysis was reported as 88.13 g (±0.95 g) per 100 g fat deposit and was used to calculate the oil-holding capacity of chicken fat.

2.3. Preparation of protein emulsion gel

2.3.1. Preparation of aqueous phase

Before aqueous phase preparation, the total protein in the faba bean concentrate was determined using the protein analyzer (C. Gerhardt

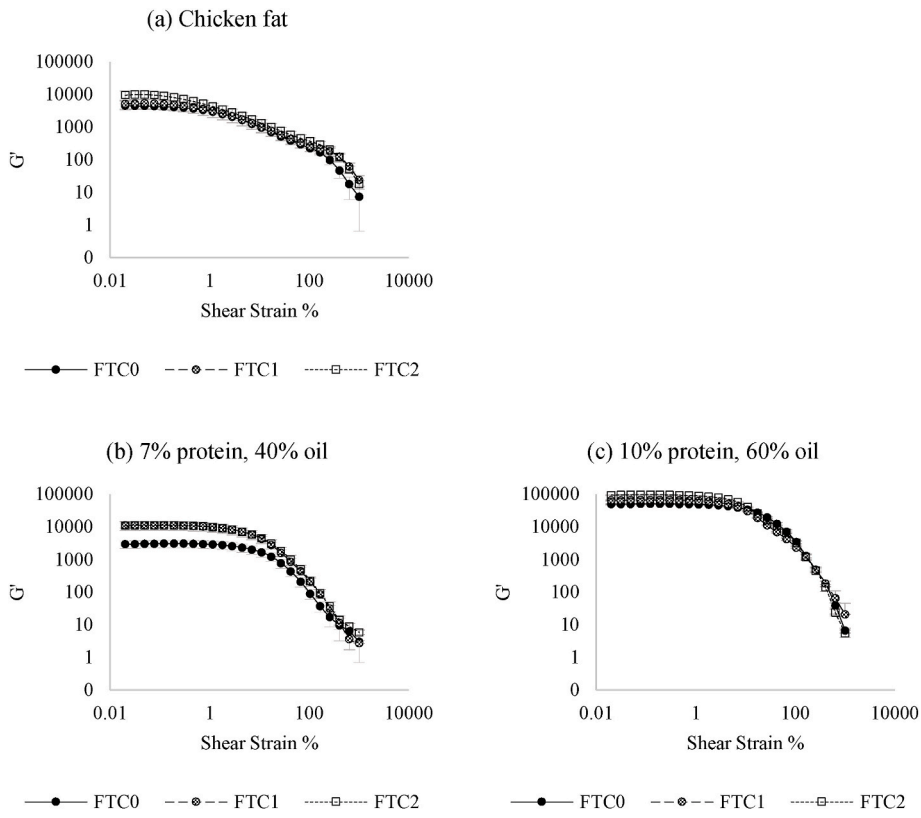


Fig. 5. Storage modulus (G′) for (a) chicken fat; (b) emulsion gel with 7%P,40%O (7% protein, 40% oil); (c) emulsion gel with 10%P,60%O (10% protein, 60% oil). FTC refers to the number of freeze-thaw cycles.

GmbH & Co. KG, Germany) with the DUMAS method and was found to be 54.48%.

Preliminary trials were carried out to design a suitable range of protein and oil concentrations for the experiment. Oil concentrations of 40%, 60% and 80% were initially tested. As PEGs with 80% oil caused protein agglomeration, 60% was chosen as the upper concentration limit of oil in the PEG. PEGs formulated with protein concentrations lower than 7% (w/w) at all oil concentrations tested (40–80% oil) showed signs of phase separation immediately after HPP. Studies have identified the critical gelling concentration of faba protein gel formation to be 15–15.5% in the aqueous phase (Dangi et al., 2022), which translates to ~6% (w/w) protein concentration at 60% oil, and ~9% (w/w) protein concentration at 40% oil in our PEG system. The solubility limit of protein in PEG was found to be around 10.5% (w/w) at the upper limit of oil concentration, as identified in our preliminary studies (Appendix A, Tables A1 and A2). With the limits identified above, our study investigated PEGs with a protein range of 7–10% (w/w) and an oil range of 40–60%.

We labeled the composition of PEGs studied as %P and %O, with %P indicating overall protein concentration and %O indicating overall oil concentration. Faba bean protein concentrate was sieved through 250-μm mesh before being dispersed in water with a stirrer (IKA Eurostar 20 digital, IKA Works Inc, Staufen, Germany). The aqueous phase was left overnight for complete hydration at 4 °C.

2.3.2. Preparation of gel through HPP

HPP technology provides an advantage of being a non-thermal method for protein gel formation. (Balasubramaniam, Martínez-Monteagudo, & Gupta, 2015; Shaun Y.J. Sim & Moraru, 2020). PEGs were prepared by blending different weights of oil with their corresponding aqueous phases using a high-shear homogenizer (Ultra-Turrax® T25 digital homogenizer, IKA Works Inc, Staufen, Germany) at 10 000 rpm. For every 10 g of emulsion prepared, 1 min was assigned to mixing, as modified from Hu et al. (X. Hu et al., 2022). The emulsions were packed in vacuum bags and molded to 0.3–0.8 cm in height. They were high-pressure processed once using a 300 L HPP unit (Hiperbaric 300, Hiperbaric, Burgos, Spain) at 600 MPa at 5 °C for 5 min to form PEGs. This treatment parameter is commonly used for commercial microbial inactivation in foods such as dairy products (Devi et al., 2013), and has been used in HPP treatments for plant-based meat to improve their physiochemical properties (Janardhanan, Huerta-Leidenz, Ibañez, & Beriain, 2023).

2.3.3. Freeze-thaw cycling

The gels were subjected to two rounds of freeze-thawing to mimic harsh storage temperature fluctuations. Samples were kept for at least 22 h at −20 °C. They were then thawed in a 37 °C thermostat water bath for 2 h (Yu, Wang, Li, & Wang, 2022). Measurements of different parameters were taken fresh, after the first and second rounds of freeze-thaw treatment (FTC0, FTC1, FTC2).

2.4. Visual appearance and microstructure

Samples with height between 0.3 and 0.5 cm were cut using a 3 cm × 3 cm metal ring mold and photographed in a lightbox.

2.4.1. Confocal laser scanning microscopy (CLSM)

Nile Red was used to stain the oil portions, while FITC was used to stain the protein portions of the gel. PEG samples at FTC0 were cut into thin slices and loaded onto a microscope slide. Nile Red and FITC dissolved in ethanol (0.5 and 1 mg/ml respectively) were applied to the sample on the slide. The sample was covered with a coverslip (22 × 22 mm, thickness No. 1.5H) and sealed on the sides to prevent moisture loss.

The microstructures of the PEGs were imaged with a confocal laser scanning microscope (Leica Stellaris 8 with White Light Laser) with 63x

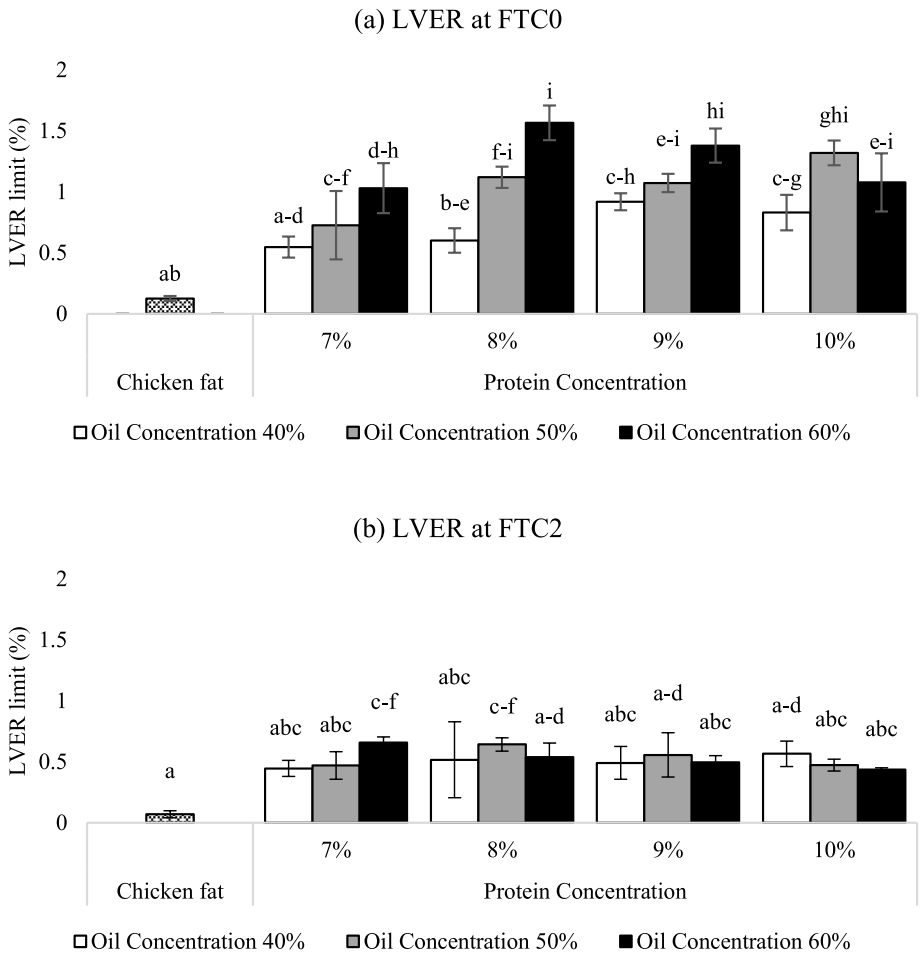


Fig. 6. Linear Viscoelastic Region (LVER (%)) of samples at (a) FTC0; (b) FTC2. FTC refers to the number of freeze-thaw cycles. Different lowercase letters indicate significant differences across the FTCs ($p < 0.01$).

water objective (63×/1.20 motCORR Plan APO). Excitation and emission wavelengths of 555 nm and 575–750 nm were used for Nile Red, while wavelengths of 495 nm and 505–545 nm were correspondingly used for FITC. Images from two channels were acquired sequentially. Laser power was adjusted based on the signal levels to ensure no saturation, and the brightest signal was at around 80% of the dynamic range for both channels. The microstructures of freeze-thawed samples were not imaged as their structures were destroyed after freeze-thaw treatments.

2.5. Color

The color of the samples was measured using a benchtop spectrophotometer (CM-5 Spectrophotometer, Konica Minolta, Tokyo, Japan). The lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) of the samples were measured. Three sample points were measured per sample, with triplicates taken at each point.

2.6. Oil holding capacity

The oil holding capacity (OHC) of the PEG was determined through two steps: decanting and centrifugation. The oil present in emulsion gel packets was first decanted and its weight was recorded. This represents the short-term stability of the gel. We used a centrifugation procedure for long-term gel stability testing. The remaining samples were divided into three 2 mL centrifuge tubes and were centrifuged based on procedures modified from Blake and Marangoni (Blake & Marangoni, 2015), at a rotary speed of 14 000 rpm for 30 min per cycle (Eppendorf 5424R, Eppendorf AG, Hamburg, Germany). The topmost oil layer was removed, and oil loss (OL) was calculated by the difference in weight of the tubes before and after oil removal. A total of four centrifuge cycles were carried out. OL after each centrifugation cycle was calculated based on the formula of Blake and Marangoni (Blake & Marangoni, 2015):

$$\textit{Oil loss after each cycle } (OL_i) = \frac{\textit{Sum of oil loss after i cycle}}{\textit{Theoretical amount of oil in sample after decanting}} \times 100\%$$

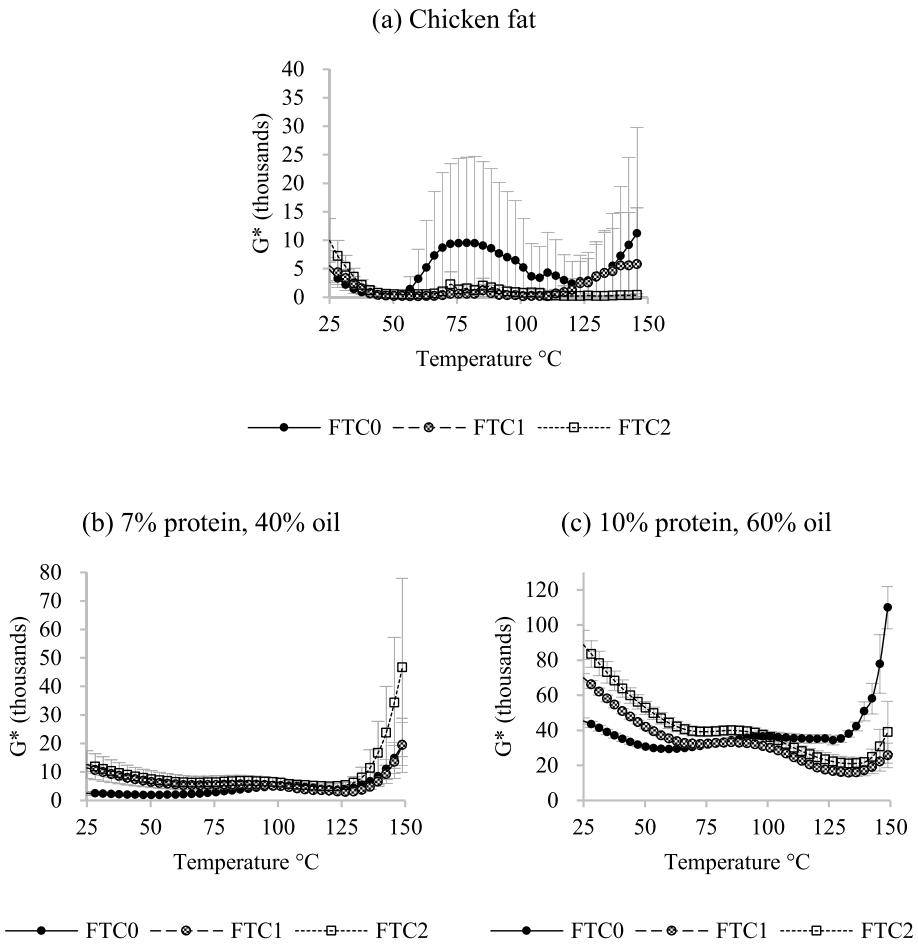


Fig. 7. Complex modulus (G*) for (a) chicken fat; (b) emulsion gel with 7%P,40%O (7% protein, 40% oil); (c) emulsion gel with 10%P,60%O (10% protein, 60% oil). FTC refers to the number of freeze-thaw cycles.

2.7. Oscillatory Rheology

The rheological properties of the PEGs were measured using a MCR302 rheometer. The rheometer was controlled by RheoCompass Software (Anton Paar GmbH, Graz, Germany). A 20 mm profiled parallel plate upper geometry was used to reduce sample slippage. Samples were loaded on a standard Peltier plate with a running gap at 1000 μm and a trim gap at 1050 μm. The solvent trap was placed on the Peltier plate for all runs to minimize water loss. Strain sweeps were conducted from 0.02% to 1000%, at a frequency of 1 Hz and a temperature of 25 °C. Temperature sweeps were conducted within the viscoelastic range of 0.1% strain, at a frequency of 1 Hz. The heating rate was 5 °C/min, from 25 °C to 150 °C. The heating curves were plotted for analysis.

2.8. Statistical analysis

Triplicate readings were taken for each sample, and results were reported as mean ± standard deviation. Statistical analyses were conducted in SPSS (Version 29, IBM SPSS Statistics Inc., Chicago, IL, USA) and Microsoft Excel (Version 2211, Microsoft Corporation, Redmond, WA). One-way analysis of variance (ANOVA) was used to determine the presence of significant differences between the PEG variations. The significance was established with the Tukey HSD post-hoc test. Regression analysis was conducted to further analyze the presence of significant effects from the variables. A p-value of 0.01 was used to determine significant difference.

3. Results and discussion

3.1. Appearance and microstructure

The appearance and microstructure of the different gels were compared visually and with CLSM respectively. Images for the different gel variations can be found in Appendix B, Tables B1 and B2.

3.1.1. Appearance

In general, 7% protein gels were brittle and easily broken compared to 10% protein gels. As samples with 7%P, 40%O were too brittle to shape in the mold described in Section 2.4 (Table 1), they were kept in a transparent bag. Samples with higher oil and protein concentrations could be molded and retained their circular shapes. This is a result of more extensive and stable networks forming in gels with higher protein concentrations. Furthermore, the denser packing of oil droplets in gels with higher oil concentrations improves the gel's ability to retain its shape (Xi et al., 2019). Sample shrinkage was more obvious in gels that underwent freeze-thaw cycling. This is due to the destruction of the protein network with each freeze-thaw cycle, which increased the brittleness, moisture, and oil loss.

Chicken fat was more malleable and flexible compared to all PEGs before and after freeze-thaw treatments. There were white fibrous-like layers surrounding portions of the fatty deposits, which gave the fatty deposits their malleable nature.

3.1.2. Microstructure through CLSM

In the micrographs, oil portions were stained red and protein

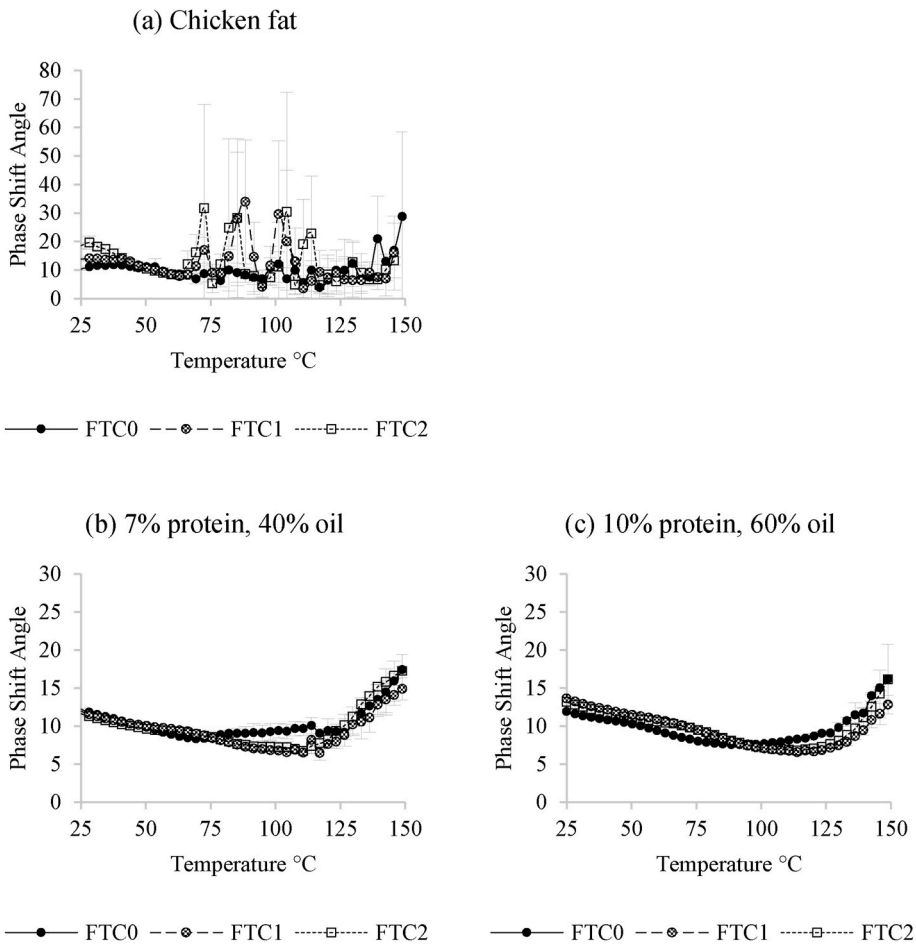


Fig. 8. Phase shift angle for (a) chicken fat; (b) emulsion gel with 7%P,40%O (7% protein, 40% oil); (c) emulsion gel with 10%P,60%O (10% protein, 60% oil). FTC refers to the number of freeze-thaw cycles.

portions stained green. PEG micrographs at lower protein concentration showed smaller and closer packing of their oil droplets with increasing oil concentration, as seen in PEGs with 60% oil compared to those with 40% oil (X. Hu et al., 2022; Lu, Mao, Cui, Yuan, & Gao, 2019). There were also more gaps (unstained portions) present in the micrographs of PEGs with lower protein concentrations than in higher protein concentrations, which indicated a less dense protein network (Xi et al., 2019). These gaps in the protein network allow small oil droplets to move and coalesce to form larger droplets (Fig. 1) (X. Hu & McClements, 2022). Overall, the denser packing of oil droplets at high protein and oil concentrations improved the ability of PEGs to retain their shape and correlated well with the visual appearances of the samples (Table 1) (Xi et al., 2019).

Most of the PEGs had oil droplets of varying sizes entrapped in a protein network. The proteins in the PEGs appeared more particulate in structure and more scattered, indicative of a particulate gel network (Fig. 1). At high oil and protein concentrations (i.e. 10%P,60%O), oil seemed to be incorporated into the protein structure, producing a uniform orange-brown component through the overlay channel on the confocal microscope. This is similar to the situation seen in micrographs of whey protein emulsion gels, which explains the incorporation and binding of oil droplets to the protein network (Cheng et al., 2019).

The chicken fat microstructure exhibits similarities with PEG microstructures in terms of the entrapment of lipid droplets in the protein network. Green outlines might indicate the protein-rich lamellar layers or fibril-like connective tissues, which encapsulate the red triacylglycerol-rich lipid droplets, an appearance similar to that reported for beef adipose tissue (X. Hu et al., 2022). There were also fewer unstained portions in chicken fat compared to PEGs, with unstained regions in the latter indicating either the absence of both protein and oil or air pockets.

3.2. Color

The average readings of three sample points per PEG sample and chicken fat were recorded, with triplicate readings taken at each sample point. The PEGs with 7%P,40%O and 10%P,60%O decrease in lightness when compared with each other. There were no significant differences in L* readings for samples with FTC0, with the exception of that for the sample with 10%P,60%O. We speculate that this may be due to the variation of two factors affecting color across the samples, making the trend less easily observable. The PEG with 10%P,60%O was the dullest among all PEGs at FTC0 in terms of both color readings at 58.98 ± 1.56 (Fig. 2) and visual appearance (Table 1). This is possibly due to this formulation having the highest protein and oil concentration, in which oil is incorporated into the protein structure (Fig. 1). This may have lowered the light scattering ability, resulting in a dull appearance (Xi et al., 2019). Similarly, the PEG with 10%P,60%O displayed a stronger yellowish tinge (b* reading of 20.77 ± 0.34 at FTC0; Appendix C; Table C1), likely also due to its highest protein and oil concentration. In general, the color of PEGs became duller (lightness L* decreased) with FTC. This may be due to larger oil droplets developing at later FTCs, which affected the scattering of light and in turn the lightness of the gel (X. Hu et al., 2022). There were no other significant differences in a* and b* values, and they can be found in supplementary information, Appendix C, Table C1.

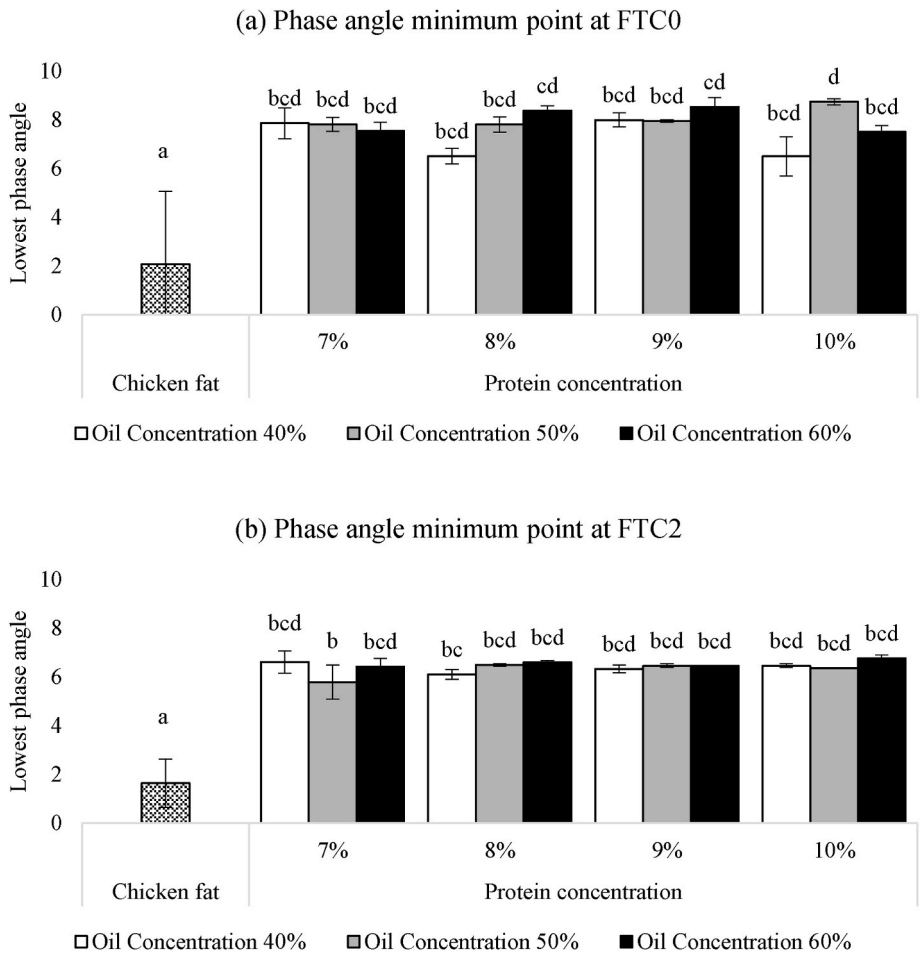


Fig. 9. Phase angle minimum point of samples at (a) FTC0; (b) FTC2. FTC refers to the number of freeze-thaw cycles. Different lowercase letters indicate significant differences across the FTCs ($p < 0.01$).

The natural color of chicken fat is dependent on multiple factors, such as the nutritional content and source of diet of the chicken, their physiological characteristics (gender, breed) and living environment (Palmer, 1915; Peña-Saldarriaga, Fernández-López, & Pérez-Alvarez, 2020; Pikul, Leszczynski, & Kummerow, 1985; Wang, Kim, Cline, & Gilbert, 2017). Most have a yellowish hue due to the consumption of cereals high in carotenes and xanthophylls, such as maize (Peña-Saldarriaga et al., 2020). In this study, the lightness of chicken fat increased after freeze-thaw treatments. This may be explained by the formation of small fat crystals in the fat cells during freezing, and their subsequent melting and exudation from the cells to increase the chicken fat's light scattering (X. Hu & McClements, 2022). The formulated PEGs resulted in a similar L* when compared to chicken fat, ranging from +54 to +63. L* with the overall visual appearance of the chicken fat in terms of color resembling that of PEG with 10%P,60%O the most.

3.3. Oil-holding capacity

3.3.1. Short-term stability

In this study, the short-term stability of a PEG is characterized as the amount of free oil easily separated through freeze-thaw cycling. This free oil after freeze-thaw treatment can be determined through the amount of oil decanted after each cycle (Fig. 3). Increasing oil content led to more oil being decanted, which agrees with our expectations. However, PEGs with 10% protein did not follow this trend, with 6.80 ± 2.51% oil decanted at FTC0 for 10%P,40%O, compared to no free oil decanted at FTC0 for 10%P,50%O and 10%P,60%O. This is likely due to their increased gel network strength with high effective protein concentration in the aqueous phase. The oil and protein components obtained from micrographs (Fig. 1) also explain this observation. PEGs at FTC0 were largely stable with no free oil removed, unlike PEGs which underwent freeze-thaw treatment. This can be explained by the change in the microstructure of PEGs during the freezing and thawing process. The forming and melting of ice crystals may have damaged the particulate gel network structure, resulting in notable oil loss through decanting for FTC1 and FTC2 (Freschi, Doran, Malumba, & Blecker, 2014).

Chicken fat showed similar trends for oil loss, with increasing oil decanted from more FTCs (3.95 ± 0.21%, 14.70 ± 4.67%, 26.34 ± 7.22% for FTC0, FTC1 and FTC2 respectively). Compared to the situation for the PEGs, chicken fat lost less oil through decanting after freeze-thaw treatment, possibly due to the presence of strong oil binders and proteins, such as fatty acid binding proteins (Shi, Wang, Zhang, Leng, & Li, 2010). Another possibility is the lower water content present in chicken fat, which lowers destruction in microstructure from ice crystal formation and melting during the freeze-thaw cycles (X. Hu & McClements, 2022). In this study, the oil loss values of chicken fat were calculated based on a total fat percentage of 88.13%. Similar to the situation for color, the lipid content in chicken fat varies as a result of many factors, such as breed, diet, and rendering conditions. (Farmani, Roshani, & Ghaboos, 2016; Marx et al., 2016; Peña-Saldarriaga et al., 2020; Pikul et al., 1985).

3.3.2. Long-term stability

We used centrifugation as a method for accelerated shelf-life testing, with the oil loss values obtained used to indicate the long-term shelf

stability of the gels. This method of testing oil loss is simple and effective, and has been done in existing literature (Blake & Marangoni, 2015). Oil loss values were calculated based on the theoretical amount of oil left after the previous decanting step. A large increase in oil loss was observed for all PEGs after a cycle of freeze-thaw treatment (Fig. 4). For instance, oil loss for 10%P,60%O PEG increased from 7.90 ± 2.09% to 34.67 ± 1.02% with a single freeze-thaw cycle. Besides the destruction of the particulate gel network from the freeze-thaw treatment, another possible explanation for this observation may be due to gaps left in the protein network after water loss from thawing. These gaps can compromise the oil-holding strength of the protein network in PEGs, resulting in greater oil loss after freeze-thaw treatment (Zhou et al., 2019). Apart from the three PEG formulations (7%P,40%O; 7%P,50%O and 10%P,60%O), oil loss from all FTC1 samples were higher than all FTC2 samples. This is due to more free oil being removed during the decanting step for FTC2 samples, which in turn had a lower proportion of oil removal from centrifugation.

The oil loss for chicken fat at FTC0 was notably higher than those for all PEGs at FTC0, at 47.02 ± 1.43%. This may be due to a smaller surface area for proteins on the fat cell membranes of chicken fat for interaction with lipid droplets, retaining the latter in the fat cells. In contrast, the smaller-sized oil droplets and the particulate protein gel network in PEGs provide a higher protein surface area to oil ratio for more protein-lipid interactions, holding the oil droplets in the PEGs at FTC0. Chicken fat showed a non-significant difference in oil loss (between 45 and 48%) across freeze-thaw treatments. This may be due to minimal changes in the fat cellular structures after freeze-thaw treatments, with strong protein-protein interactions within the fat cell membranes (X. Hu & McClements, 2022).

3.4. Rheological measurements by oscillatory rheology

3.4.1. Strain sweep

The G′ at low strain values indicate sample strength. We found G′ to increase as oil and protein concentrations increased (Fig. 5). This corroborated the results of previous studies that indicate a firmer gel forming from the denser packing of oil droplets and protein aggregates (Fig. 1) (X. Hu et al., 2022; Lu et al., 2019; Mleko, 1999; Teng & Campanella, 2023; Xi et al., 2019; Zetzl, Marangoni, & Barbut, 2012). Chicken fat had low gel strength in comparison to most PEG formulations, with its G′ at FTC0 (4465.07 ± 76.12 Pa) closest to that of the PEG formulation with 7%P,40%O (2933.60 ± 763.21 Pa). We postulate this to be partly due to the difference in microstructure between chicken fat and PEGs (Fig. 1). Since the fat content in chicken fat is 88.13%, the total solid content in it is lower than that of the lowest PEG sample at 7% protein. This may result in the lower G' and gel strength of the chicken fat.

G' increased after freeze-thaw treatments for all PEG samples regardless of protein and oil concentrations. This corroborates the results of similar gel stability studies and can be explained by syneresis and oil loss that packs the protein aggregates closer to increase the density of gels (Shiroodi, Rasco, & Lo, 2015; Yu et al., 2022). Similarly, G" values (not shown) increased with freeze-thaw treatments and did not exceed G' within the linear viscoelastic range (LVER) for all PEG samples, highlighting their predominantly elastic nature.

Although G' at lower strain increased with each FTC, the LVER limit strain percentage decreased (Fig. 6). This suggests that the gel became stronger but more brittle with each FTC. The decrease in the LVER limit after freeze-thaw treatments may be due to the formation and thawing of ice crystals which weakens the gel microstructure (X. Hu & McClements, 2022). This is coupled with the small oil droplets coalescing with larger droplets which further disrupts the microstructure stability, in turn leading to the decrease in LVER. Regression analysis show increasing oil and protein concentration to increase the strain limit, which agrees with trends observed in previous studies (Dickinson, 2012). The freeze-thaw treatment significantly affected strain limit. Interestingly at 10% protein, an increase in oil content from 50% to 60% led to a decrease in LVER from 1.32 ± 0.10% to 1.08 ± 0.24% at FTC0. Xi et al. suggested that this might be due to a change in gel behavior to be more like a particle gel (Xi et al., 2019). Chicken fat exhibited lower LVER than those for the PEG samples, suggesting that it can be more easily deformed.

3.4.2. Temperature sweep

All G′ values were stable and consistently higher than G" (Appendix E, Figure E3), indicating the PEGs were more elastic than liquid-like across the temperature sweep. G′ values were relatively stable throughout a temperature range of 25 °C to about 100 °C, highlighting the heat stability of the PEGs formed. Freeze-thawed PEGs generally had higher G′ than their counterparts at FTC0, and most samples showed a decrease in G' as temperature increased. This may be explained by the softening of PEGs and weakening of local interactions under heat (Lu et al., 2019).

Temperature sweep simulates the cooking process of the chicken fat and PEGs, with the rheological changes of the samples measured between 25 °C and 150 °C. The results for the temperature sweep were also represented with G* and phase angle, following conventions used in previous studies (X. Hu et al., 2022). Our observations on the effect of oil content agreed with the results of previous studies on emulsions, in which G* increased with oil concentration due to the denser packing of oil droplets (X. Hu et al., 2022; Lu et al., 2019). At lower temperatures between 25 °C and 70 °C, G* also increased as protein concentration increased (Fig. 7), due to the denser protein network and aggregates formed (X. Hu et al., 2022; Lu et al., 2019; Mleko, 1999; Teng & Campanella, 2023; Xi et al., 2019; Zetzl et al., 2012). Some freeze-thawed gels displayed a decrease in G* before a steep increase, such as 7%P, 40%O. This may be due to the initial softening of samples with heat before they hardened at higher temperatures. Higher G* at lower temperatures for freeze-thawed PEGs can be explained by syneresis and oil loss, leading to stiffer gels (Shiroodi et al., 2015; Yu et al., 2022). The G* for chicken fat was low across all temperatures, being most similar that of the PEG formulation with 7%P and 40%O.

The phase angle of the PEGs studied decreased before increasing. This initial decrease in phase angle, which represented more solid-like behavior, may be due to further denaturation of faba bean protein and strengthening of the gel network (C. d. S. Paglarini, Martini, & Pollonio, 2019), since the denaturation point of this protein has been reported to be between 75 and 95 °C (Kimura et al., 2008; Nilsson et al., 2023). Regardless, the phase angles of the PEGs were largely stable at a temperature range of 25 °C–125 °C compared to chicken fat.

The phase angle minimums of the PEGs at FTC0 are slightly higher than those of their freeze-thawed counterparts, indicating that the PEGs were more solid-like at higher temperatures after FTCs. For instance, the minimum phase angle reached for 7%P,40%O at FTC0 was 7.86 ± 0.64° while it was 6.62 ± 0.46° at FTC2 (Fig. 9). This is due to the gel structure of PEGs at FTC0 being maintained during the temperature sweep. The destruction of gel network and moisture loss through freeze-thaw treatments may have caused the phase angle minimums to be reached at a higher temperature. Regression analysis showed that oil concentration and the presence of freeze-thaw treatment affect the phase angle minimum. Interestingly, protein concentration did not have a major effect on the phase angle minimum reached, unlike the correlation established between protein content and the elasticity of the gel (Mleko, 1999).

The G* and phase angles of chicken fat fluctuated greatly across temperature sweep, unlike those for the PEG samples. This could be due to the wide melting-point range of chicken fat from 22 °C to 40 °C (Bartov, Lipstein, & Bornstein, 1974; Hrdinka, Zollitsch, Knaus, & Lettner, 1996; Mohammadnezhad & Farmani, 2022), with the melting of fat solids possibly leading to G* and phase-angle fluctuations across increasing temperatures. Nonetheless, the phase angles of chicken fat across the temperature sweep remained predominantly below 45°,

highlighting its elastic nature. This may be due to the presence of the protein-rich laminar layers or cell membranes (Fig. 1), which confer solid-like properties and mechanical rigidity to the chicken fat (X. Hu & McClements, 2022). PEGs did not have oil crystals in the gel structure before the samples were loaded on the Peltier plate, resulting in the absence of large fluctuations in G* and phase angles as temperature increased to 150 °C (Fig. 8) (X. Hu et al., 2022).

4. Conclusion

This study showed the potential for PEG to mimic chicken fatty deposits, as well as the use of HPP to develop non-animal fats. Both protein and oil proportions affected the visual appearances, rheological behavior, and stabilities of the PEGs. Gels with lower protein concentrations (such as gels with 7% protein and 40% oil content) exhibited gel strengths most similar to those of chicken fatty deposits. However, gels with higher effective protein concentrations (such as those with 10%P and 60%O) had better oil-holding capacity and were closer in color to chicken fat. Freeze-thaw treatments disrupted the structural stability of the PEG, manifested by changes in appearance, increases in gel strength and brittleness, and decreases in oil-holding capacities. This highlights the importance of temperature control during gel storage and transportation. Modifications in PEG formulations or protein microstructures are needed to improve the freeze stability of the PEG and its resemblance to chicken fat. One promising way is to incorporate suitable stabilizers and hydrocolloids, such as starches and sodium alginate into the PEGs, before HPP treatment. Future work will also involve the sensory evaluation of PEGs in plant-based meat applications, in terms of flavor and texture.

Although HPP reduces processing time and is a non-thermal method compared to conventional thermal processes of forming gels, large-scale implementation of this processing method is still costly and difficult to fully implement for commercialization (Nabi et al., 2021). Future cost optimization from areas such as packaging and maintenance is required to improve the commercial feasibility of PEG production using HPP (Cacace, Bottani, Rizzi, & Vignali, 2020). When successfully formulated and upscaled, PEG can be used as a healthier animal fat replacement that can withstand high-temperature treatments.

CRediT authorship contribution statement

Yan Kang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Shu Min Ng:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation. **Umamaheshweri Aruchunan:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Xiaoxiao Ma:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **Shaun Yong Jie Sim:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

All data used are reported in supplementary materials.

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