Ultrasound-assisted gelation of β-carotene enriched oleogels based on candelilla wax-nut oils: Physical properties and in-vitro digestion analysis

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\textbf{A B S T R A C T}

This study investigated the effects of high-intensity ultrasound (HIU, 95 W, 10 s) on the physical properties, stability and in vitro digestion of β-carotene enriched oleogels. Candelilla wax (3 wt%) and nut oils (peanut, pine nut and walnut oil) without or without β-carotene were used to form oleogels. HIU improved the storage modules (G') of peanut, pine nut and walnut oleogels without β-carotene from 11048.43 ± 728.85 Pa, 38111.67 ± 11663.98 Pa and 21921.13 ± 1011.55 Pa to 13502.40 ± 646.54 Pa, 75322.47 ± 9715.25 Pa and 48480.97 ± 4109.64 Pa, respectively. Moreover, HIU reduced oil loss of peanut, pine nut and walnut oleogels without β-carotene from 23.98 ± 2.58%, 17.14 ± 0.69% and 24.66 ± 1.57% to 17.60 ± 1.10%, 13.84 ± 0.74% and 18.72 ± 3.47%, respectively. X-ray diffraction patterns showed that HIU did not change the form of the crystal (β-polymorphic and β'-polymorphic) but increased the crystal intensity. Polarized light microscope images indicated that all oleogels showed more visible crystals after HIU. After 120 d of storage, HIU decreased the degradation of β-carotene for peanut oil and walnut oil samples (the contents of β-carotene in peanut and walnut oleogels without HIU after 120 d of storage were 897 ± 2 μg/g and 780 ± 1 μg/g, respectively, and those of sonicated samples were 1070 ± 4 μg/g and 932 ± 1 μg/g, respectively). Furthermore, HIU reduced the release of β-carotene in intestinal digestion. In conclusion, HIU could improve the functional properties of wax-nut oils oleogels and their β-carotene enriched oleogels.

\section{1. Introduction}

Oils and fats promote the mouthfeel and texture of many food products. Saturated fat and trans fats can provide desirable properties (hardness and elasticity) for certain foods, such as shortenings, butter and other processed foods [1]. However, widespread consumption of hydrogenated trans fats can cause several adverse consequences to human health, such as obesity, cardiovascular diseases, endothelial dysfunction, oxidative stress, and inflammation [2]. With the increase of consumers’ awareness, hydrogenated trans fats have become less appreciated by consumers. Therefore, healthier fat-rich products with more unsaturated fatty acids and desirable textural properties need to be introduced [3,4]. Oleogels are semi-solid systems prepared by liquid oil oleogelation using oleogelators [5]. Since the oleogelation does not change the structure of unsaturated fatty acid in the oil, it is considered as an important process in the oil industry [1,4,6]. Currently, oleogels are widely applied in various applications such as food products (cookies, creams, and trans-fat replacers), encapsulation and control release of bioactive components [7–10]. Moreover, nut oils are known for their high content of unsaturated fatty acids, so there is a potential to build nut oils-based oleogels to expand their scope of application [11]. Different oleogelators, including saturated glycerides [12,13], sodium steaoryl lactate [14], cellulose [15–17] and sucrose esters [18], have been used recently to improve the stability and physical properties of oleogels. Among all the oleogelators, wax has attracted much attention due to its low cost, wide availability, high gelation efficiency, and acceptable sensorial properties [13,19–22]. Wax is a natural mixture comprising hydrocarbon, free fatty acid, fatty alcohol, wax ester, ketone and sterol. Moreover, wax is considered as a promising alternative to saturated fat, providing some desirable properties in some foods such as...
cookies and cakes [23,24]. Candellila wax (CLX) is a wax extracted from the Candellila shrub leaves and approved by the US Food and Drug Administration (FDA) as a food additive [25]. CLX has a high melting point (>60 °C) and is used as an oleogelator to replace hydrogenated fats and shortening in baked foods [24,26–28]. The crystallization process and the success of oleogels applications rely on the ability of the oleogelator to form a strong network to hold the liquid oil. The processing conditions during the crystallization (cooling rate, stirring and shearing) and the material conditions (type and quantity of solute and solvent) both affect the crystallization process [13,19]. Thus, controlling the lipid crystallization to achieve desirable properties is crucial. In the food industry, improving the stability of oleogels to prevent oil migration is a potential aspect to broaden the application of oleogels [29]. Therefore, highly effective green technologies are needed to control lipid crystallization to produce stable oleogels.

Ultrasound refers to an acoustic wave beyond the human hearing threshold level (>20 kHz) [30]. As a green and energy-efficient technology, studies on the application of ultrasound in various fields are increasing rapidly. High-intensity low-frequency ultrasound (HIU, 16–100 kHz) technology has been widely used for many applications, including extraction, emulsification, microbial inactivation, modifying protein structures and drying [31–40]. The major effect of ultrasound arises from acoustic cavitation, the formation and collapse of air bubbles in a liquid [41]. The formation of microscopic crystals (nucleation) and the growth of these crystals are the main steps of the crystallization process [42]. The process of applying ultrasound during the crystallization process is called sonocrystallization [43]. The mechanism of ultrasound effects on the crystallization process is still not well established. However, it is thought that HIU could increase both nucleation and crystal growth rates by generating nucleation sites. This could be due to that the collapse of cavitation bubbles may act as nuclei for crystal growth. Moreover, HIU could increase the number of nucleation sites by disrupting the crystals in the medium [44]. The effects of HIU on the crystallization process and properties of wax-based oleogels are still not clear, and further investigations are needed. Recently, HIU showed a potential to increase the oil binding capacity of oleogels [45,46]. Sharif et al. [45] studied the effects of HIU on the properties of propolis wax/olive oil oleogel (organogel). They found that HIU facilitated the formation of small crystals, enhancing the gel strength and oil binding capacity of the gel system. Jana and Martini [47] concluded that HIU can delay the oil migration in wax/oil systems. Giacomozzi et al. [48] noticed that HIU encouraged the packing of monoglycerides crystals during the growth of crystals in a more organized structure. However, studies investigating the effects of HIU on the crystallization process and properties of CLX/different nut oils oleogel systems are scarce. Furthermore, to the best of our knowledge, few works used CLX/nut oils oleogels to encapsulate β-carotene under HIU conditions. Moreover, the stability and digestibility of β-carotene enriched CLX/different nut oils oleogel induced by HIU are not clear. It could be hypothesized that different oils could produce different crystal morphology during the crystallization process. This can affect the functional properties of wax-based oleogels (or β-carotene enriched wax-based oleogel) induced by HIU.

Therefore, this study aimed to access the effects of different types of nut oils on the physical properties of HIU-induced CLX oleogels. The physical properties of different oleogels, including morphology, mechanical properties and stability, were characterized. Moreover, oleogels were used to encapsulate β-carotene and the stability as well as in vitro digestion of β-carotene enriched oleogels were investigated. The results obtained from this study could help better understand the effects of HIU and different nut oils on the properties of oleogels systems. This could increase the utilization of oleogel systems and ultrasound technology, as an eco-friendly technology, in the food and pharmaceutical industries.

2. Materials and methods

2.1. Materials

Oleogels were prepared by mixing 3 wt% CLX with 3 kinds of nut oils, respectively (24.25 g oil + 0.75 g CLX, 25 g in total). The mixtures were heated at 80 °C for 20 min under continuous stirring to ensure the complete melting of mixtures [45]. The homogenous liquid oil mixture was cooled at room temperature for 5 min and stored at 4 °C for 24 h before characterization. For sonicated samples, oleogels were taken out from 80 °C and kept at room temperature for 5 min, then a 20 kHz ultrasound processor (JY92-IIDN; Ningbo Xinzhi Biotech Co., Ningbo, China) with a 6.36 mm probe was used to treat oleogels at 95 W (10% amplitude) for 20 s (2 s on, 2 s off, the actual applied sonication time was 10 s). The ultrasound instrument’s power drawn (measured by a power meter; Shenzhen Northmeter Co., Ltd., Shenzhen, China) was around 55 W. The ultrasound intensity was measured calorimetrically, and its value was 36.92 ± 2.30 W cm⁻² (0.47 ± 0.03 W/mL). The sonicated samples were transferred to different containers and stored at 4 °C for 24 h. Oleogel containing 3 wt% CLX with peanut oil, pine nut oil and walnut oil were named as P, PN and W, respectively. P, PN and W after HIU treatment were named as HP, HPN and HW, respectively.

2.2. Preparation of oleogel

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2.3. Polarized light microscopy

The crystal morphology was analyzed by a light microscope in polarized mode (M43; Mshot, China) with a digital camera (MS60; Mshot, China). A small amount of gel was placed onto a glass slide and then covered with a glass sliding [23]. The gel was flattened as much as possible. The crystalline microstructure was observed and photographed using the Mshot Main software for Windows.

2.4. X-ray diffraction

X-ray diffraction (XRD) patterns were acquired by using an X-ray diffractometer (D8 ADVANCE; Bruker AXS GmbH). The angular scanning region was from 0° to 50° (2θ), and the sweep rate was 4°/min according to a previous study with some modifications [7].

2.5. Rheological measurement

The storage modulus (G’) and loss modulus (G’’) were measured by a DHR2 Rheometer (Waters, US) with air as the purge gas at 30 psi. A 40 mm standard steel with a gap of 1000 μm was used, and the strain (%) varied from 1.0 × 10⁻³ to 100% at 1 Hz. The linear viscoelasticity region was chosen to represent G’ and G’’ [26].

2.6. Firmness

The firmness of oleogels was measured by a Texture Analyzer (TA-XT2; Stable Microsystems, UK) at room temperature according to our previous studies with some modifications [49]. A P/0.25 S probe was used to compress the oleogels (before and after HIU treatment) in cylinder containers at a depth of 10 mm and a speed of 1 mm/s under 5 g.
compression. Firmness was determined by analyzing the force-distance curves from the penetration test. The firmness of the oleogels was defined as the maximum force (N) required to penetrate the oleogels.

2.7. Oil loss

Oil loss was analyzed to determine the oil binding capacity of oleogels [26]. The oleogel was formed in a cylindrical container and cut into small regular columns. Then the columns were transferred to filter papers (Whatman #1, 125-mm diameter) supported by a Petri plate. The oleogels were left in the filter paper for 24 h at room temperature. The oil loss was determined by using Eq. (1):

\[
\text{Oil loss} \left(\%\right) = \frac{w_{tf}(g) - w_{t0}(g)}{w_{sam}(g)} \times 100
\]

where \(w_{t0}\) is the weight (g) of paper and Petri dish without oleogel before the storage experiment, \(w_{tf}\) is the weight (g) of the paper and Petri dish after the storage time (1 and 7 days), and \(w_{sam}\) (g) is the weight of the oleogel.

2.8. \(\beta\)-carotene encapsulation

\(\beta\)-carotene was added to three kinds of nut oils (peanut, pine nut and walnut oils) at 1 mg/g (for in vitro digestion analysis) and 2 mg/g (for storage evaluation). \(\beta\)-carotene was dissolved in nut oils according to a previous study [50]. The \(\beta\)-carotene enriched oleogels were prepared as mentioned in section 2.2. P, PN and W containing \(\beta\)-carotene were named as CP, CPN and CW, respectively. HIU treated CP, CPN and CW were named as HCP, HCPN and HCW, respectively.

2.9. Characterization of \(\beta\)-carotene enriched oleogels

Microstructure, X-ray diffraction patterns, rheological properties, firmness and chromaticity of \(\beta\)-carotene enriched oleogels were characterized according to the methods introduced above (in sections 2.3, 2.4, 2.5, and 2.6).

Stability of \(\beta\)-carotene enriched oleogels

2.10. Oil loss

The oil loss was examined based on the method in section 2.9.

2.10.1. Storage ability

Oleogels containing \(\beta\)-carotene were stored in a fridge at 4 °C for 60 and 120 days. The content of \(\beta\)-carotene in oleogels was measured to examine the stability of \(\beta\)-carotene in oleogels.

2.10.2. In vitro digestion

The digestion solutions were prepared according to Minekus’s digestion model [51]. The extraction of \(\beta\)-carotene was referred to a previous study with some modifications [52]. Gastric and intestinal digestion phases were performed independently according to a previous study [53]. The digested substrate was separated every half hour during the gastric digestion stage and the intestinal digestion phase. Then, a mixture of hexane and ethanol (hexane: ethanol; 3:2) was added to the separated substrate, shaken vigorously for 1 min, left for 5 min to separate. Then, the supernatant (containing \(\beta\)-carotene) was taken and the absorbance value was measured at 450 nm using a UV spectrophotometer (Nanodrop 2000C; Thermo Fisher Scientific) to evaluate the destruction and release of \(\beta\)-carotene.

2.11. Statistical analysis

The experiments were carried out in triplicates. The significance of the results was analyzed by SPSS software (version 24), and graphics were drawn by Origin 9.0. Significant differences were determined with
3. Results and discussion

3.1. Physicochemical properties of oleogels

3.1.1. Visual observation

Fig. 1a and b show the visual observation of oleogels before (liquid mixture) and after (semi-solid) crystallization. Before gelation, all the samples were transparent liquid, while the oleogels became turbid and semi-solid after gelation. Fig. 1c and d represented the macrographs of the oleogel inversion. All the oleogels did not flow, indicating gels were formed [26]. Toro-Vazquez et al. [54] also found that 3% (w/w) of CLX was efficient in forming stable oleogels. According to the visual observation, the colors of oleogel systems were different. As the concentration of CLX was the same, the color of oleogel was mainly attributed to the initial color of the oil. There was a little difference between the oleogels induced with (Fig. 1d) and without (Fig. 1c) HIU treatment.

3.1.2. X-ray diffraction and morphology of oleogels

The polymorphism of oleogels’ crystals was provided by X-ray diffraction (XRD) patterns. The d-spacing of the crystal was calculated based on 2θ. Fig. 2a and b displayed the XRD patterns of CLX-based oleogels prepared with peanut, pine nut and walnut oils with and without HIU treatment, respectively. In our study, all the oleogels formed by different oils with and without HIU showed three major peaks around 3.8, 4.2 and 4.6 Å with varying intensity as the type of oil and the HIU treatment changed. Wide-angle region peaks at around 3.8 and 4.2 Å indicated the presence of β’-form crystals, and the peaks around 4.6 Å
meant the presence of β-form crystals [23,55]. The presence of both β’-polymorphic form and β-polymorphic indicated that the oil type and HIU treatment could not change the form of crystals. Fayaz et al. [56] used 4 kinds of oils (canola, sesame, sunflower and flaxseed oils) and propolis wax to form oleogels and found that oil type did not change the forms of crystals. They also found that HIU treatment did not influence the crystal forms of olive oil oleogels formed with propolis wax [45].

Before HIU application, the oleogels formed with pine nut oil and walnut oil showed higher intensities than the peanut oil sample. According to our previous research (Table S 1) [57], the concentrations of C 18:0 in all the nut oils were low (< 4%), but the amount of C 16:0 in peanut oil was higher than pine nut and walnut oils. Previous studies showed that the crystalline network of oleogels is mainly formed by the interaction of long-chain fatty acids with n-alkanes [26]. Moreover, the gel formation needed a large amount of high melting fats [58]. The size and morphology of the crystals in high melting point fats played a decisive role in gel formation, producing larger crystals [59]. This could be the reason for the low intensity of peanut oil oleogel, which had the lowest content of 18-carbon fatty acids (C18). After HIU application, the crystal intensities of all 3 kinds of oleogels (peanut, pine nut and walnut oleogels) increased. As HIU treatment was proved to have the potential to create more sites for nucleation [46,47,60], it might also facilitate crystal arrangement and crystal interactions which may result in the increase of crystal intensity of oleogels after HIU application.

The crystal morphology of the oleogels is shown in Fig. 3. The images of polarized light microscope (PLM) showed that the crystals were in the shape of microplatelets, which was consistent with previous studies [26,61]. From Fig. 3, it is observed that oil type did not influence the shape of crystallization of oleogels. Fayaz et al. [56] reached similar conclusions in their study on canola/sesame/sunflower/flaxseed oil-propolis wax oleogels. It was found that peanut and walnut oleogels showed fewer crystals than pine nut oleogel (Fig. 3). All types of oils used in this study consisted mainly of C16:0 and C18:0 (Table S 1). Peanut oil had higher C 16:0 than pine nut oil which meant that its 18-carbon fatty acid content was lower than that of pine nut oil (18-carbon fatty acids dominated the crystallization process). This might explain the lower quantity of crystals showed in peanut oleogel compared to pine nut oleogel. The C 16:0 and C 18:0 contents of walnut and pine nut oil were similar, but C 18:1 of walnut oil was lower than pine nut oil. This means that walnut oil had fewer high melting long-chain fatty acid, which may account for the lower number of visible crystals in walnut oleogel under PLM.

Compared to before HIU treatment, PLM images clearly demonstrated that all the oleogels presented more crystals after sonication (Fig. 3). This change could be attributed to the acoustic cavitation effects of sonication. The bubbles created by ultrasound collapsed and formed cavities that could generate more sites for nucleation. Thus, these crystallization sites influenced the formation and distribution of crystals [45–47,60,62–64]. Due to increasing the crystallization sites, more crystals’ clusters were formed gradually as crystals grew and thus could be seen under the PLM.

### 3.1.3. Rheology and texture analysis of oleogels

Rheological and texture data were used to represent the mechanical strength of the oleogels. It was found that all the elastic modules (G’) were higher than viscous modulus (G’’), indicating the solid behavior of waxy oleogels (Fig. 4a) [26,65]. Before HIU application, pine nut and walnut oleogel showed higher G’ and G’’ values than peanut oleogel (Fig. 4a and b), which was consistent with the trend in the crystal strength (Fig. 2a). Peanut oleogel had the lowest G’ and G’’, and it also
had the lowest crystallization intensity (Fig. 2a). According to previous studies, the strength of oleogels was connected to the interactions between fatty acids and n-alkanes. High melting point fatty acids (long-chain saturated fatty acids and long-chain monounsaturated fatty acids) could form stronger gel networks than fatty acids with low melting points (medium-chain fatty acids and long-chain polyunsaturated fatty acids) [26,58]. All the nut oils in this study were mainly composed of fatty acids with 18 carbons, and most of them were unsaturated fatty acids (C 18:1 and C 18:2) (Table S1). Peanut oil had a high content of C 16:0 and low content 18-carbon fatty acids, this could be the reason for the low G’ value of the peanut oleogels. For pine nut oil and walnut oil, as the concentrations of C 16:0 (5% and 6.7%, respectively) and C 18:0 (2.4% and 2.6%, respectively) were similar, C 18:1 might dominate the crystallization process. The higher C 18:1 in pine nut oil (27.08%) compared to walnut oil (15.33%) may result in higher G’ and G” of pine nut oleogel than those of walnut oleogel.

The G’ and G” values of all the oleogels increased significantly after HIU treatment. To be noted, the G’ of HIU-treated pine nut and walnut oleogels increased from 38111.67 ± 11663.98 Pa, 21921.13 ± 1011.55 to 75322.47 ± 9715.25 Pa, 48480.97 ± 4109.64 Pa, respectively. The G’ and G” values of peanut oleogels increased from 11048.43 ± 728.85 Pa and 1548.52 ± 84.33 Pa to 13502.40 ± 646.54 Pa and 1892.05 ± 195.84 Pa, respectively. The C 18:0 content in all three oils were similar while the concentrations of C 18:1 and C 18:2 were different between pine nut oil and walnut oil. To be noted, pine nut and walnut oils contained high levels of polyunsaturated fatty acids (C 18:2) than peanut oil (Table S1). Since saturated fats could influence the physical strength of the crystallization network, unsaturated fatty acids might play an auxiliary role in the process of network construction. As peanut oil had the lowest content of C 18:2 (33.74%) among all the three oils, the G’ value of peanut oleogel increased by 22.21 % after HIU treatment. The C 18:2 content of PN and W was 45.96 and 61.65%, respectively. The G’ values of PN and W gels after HIU treatment increased by 97.64 and 121.16%, respectively, compared to PN and W without HIU. Previous studies reported that the weaker gel network would attach to the stronger gel network to reinforce the whole network [20]. Based on this, it was hypothesized that the high-melting fatty acids might form stronger networks that can affect the physical structure of oleogels. Moreover, the weaker network, which was formed mainly with low-melting fatty acids, could act as an additional skeleton attached to the stronger network. The effect of sonication may be more pronounced with weaker networks.

The firmness of oleogels is presented in Fig. 4d. Similar to rheological results, the firmness of oleogels formed with peanut, pine nut and walnut oils increased significantly after HIU application. The upward trend showed in hardness was consistent with that of rheological data, confirming the possibility of using HIU to improve the gel strength. Also, the sonication effects on the oleogel firmness were influenced by the types of oils.

3.1.4. Oil binding capacity of oleogels

The oleogels were cut into small columns and placed on filter papers for 24 h at 25 °C [26]. As shown in Fig. 5a, all samples could be cut into regular columns without collapsing, proving that the oleogels had a strong network with a stable texture. The oil loss of oleogels was calculated according to formula (1) to evaluate their ability to bind oils, and the results are shown in Fig. 5b. The peanut and walnut oleogels without HIU presented the highest oil loss among all the oleogels. The oil loss of oleogels formed with pine nut oil was much lower compared to that of peanut and walnut oleogels, indicating that pine nut oleogel had good oil binding capacity. It was found that oil loss was highly correlated with the G’ values and thus, the strong networks with high elasticity improved oil binding capacity [26]. In our study, the G’ value of pine nut oleogel was the highest, and therefore it had the lowest oil loss. The oleogels formed by peanut and walnut oils had lower G’ values and higher oil loss than pine nut oleogel. After HIU treatment, all the oleogels showed significant decreases in the oil loss (Fig. 5b), which meant that HIU application could enhance the crystalline networks of oleogels. The trend of oil diffusion diameter on the filter paper was consistent with oil loss (Fig. 5c). According to previous speculations [26], the
enhancement of oil-binding property was correlated with the increase of G’ value. In this study, the G’ values of all the oleogels increased significantly after HIU application (Fig. 4b) which could explain the decreasing oil loss of oleogels after HIU application (Fig. 5b). And we further analyzed this phenomenon in terms of crystallization behavior. The images in Fig. 3 showed the appearance of more crystals after HIU application. Several researchers proved that increasing the number of crystals in oleogels could result in tighter crystalline networks that hold more liquid oil [45,46].

3.2. Physicochemical properties of β-carotene enriched oleogels

3.2.1. Mechanical properties

Gel systems were proved to have potential application in the formation of bioactive substance delivery systems [66], so the overall performance of the β-carotene enriched oleogel system was evaluated comprehensively in this study. Peanut, pine nut and walnut oil were used to form this system based on the results of previous experiments (section 3.1).

Fig. 6 shows the images of inverted oleogels containing β-carotene enriched oils (peanut, pine nut and walnut oils) with (Fig. 6a) and without HIU treatment (Fig. 6b). The oleogels were inverted and did not flow, proving that all samples formed semi-solid gels. All oleogels were red in color after the addition of β-carotene and little difference was found under direct observation.

X-ray diffraction (XRD) patterns of oleogels containing β-carotene are shown in Fig. 7. There were wide-angle region peaks at around 3.8, 4.2 and 4.6 Å, indicating the presence of both β-form and β’-form.
crystals, consistent with the results shown in Fig. 2. Moreover, the appearance of these three major peaks (d = 3.8 Å, d = 4.2 Å and d = 4.6 Å) indicated that the addition of β-carotene did not influence the polymorphism of oleogels. Previous studies showed that the addition of β-carotene could induce a stronger laminar crystal network [67]. This special network might contribute to the higher crystallization intensity showed in oleogels with β-carotene (Fig. 2) than that in oleogels without β-carotene. This could explain that oleogels formed with peanut and pine nut oils showed little difference before and after HIU application as β-carotene plays a dominant role in crystallization. However, the intensity of the oleogel formed with walnut oil increased significantly after sonication. Previous studies showed that the microscopic network of rice bran wax/sunflower wax – berry wax – rice bran oil was composed of weak network (formed with low-melting-point substance) and strong network (formed with high-melting-point substance) [20]. They noticed that the weak network could attach to the strong network to facilitate the gel formation [20]. As walnut oil had the highest concentration of C 18:2 which may contribute to a large weak network. This weak network could attach to the main frame formed with β-carotene and C 18:1. Furthermore, HIU treatment might affect the weak network and increase in crystal intensity.

The polarized light microscope (PLM) images are shown in Fig. 8. The shape of crystals in all oleogels did not have significant differences after adding β-carotene, indicating that although β-carotene could change the arrangement of crystals, it could not change the shape and form of crystals (Fig. 7 and Fig. 8). All oleogels exhibited an increase in the number of crystals after HIU treatment. This was consistent with the

![Fig. 8: Polarized light microscopy images of crystals (100×) obtained for β-carotene enriched oleogel systems at 25 °C without (left column) and with (right column) HIU.](image)

![Fig. 9: Rheological and texture data of β-carotene enriched oleogel systems prepared using peanut, pine nut and walnut oils at 25°C. (a) Strain scan curves; (b) G’ values of different oleogels; (c) G” values of different oleogels; (d) Firmness of different oleogels. The different lowercase letters indicate that values are significantly different (α = 0.05) between gels formed by the same oil without and with HIU; the different capital letters indicate that values are significantly different (α = 0.05) among all the gels.](image)
PLM images of oleogels without β-carotene (Fig. 3).

The G’, G” and firmness values are indicators of the mechanical strength of oleogels. Consistent with the rheological properties of oleogels without β-carotene (Fig. 4a), all the G’ values of β-carotene oleogels were higher than G” values (Fig. 9a), indicating that all β-carotene oleogels had good tolerance to deformation [23,26]. β-carotene enriched pine nut oleogel (CPN) showed the highest G’ value before HIU application. β-carotene-enriched oleogels made of peanut and walnut oils exhibited lower G’ values, which was consistent with the findings of β-carotene free oleogels (Fig. 4b). After HIU treatment, the G’ and G” of all the oleogels increased significantly. The G’ values of β-carotene enriched peanut oleogel (CP), CPN and β-carotene enriched walnut oleogel (CW) increased from 9730.22 ± 1501.45 Pa, 53718.10 ± 8086.62 Pa and 27583.73 ± 4093.77 Pa to 14192.14 ± 2094.48 Pa, 61922.57 ± 9842.91 Pa and 32990.07 ± 5032.14 Pa, respectively. All the oleogels showed more visible crystals after HIU application (Fig. 8). More visible crystals indicated stronger aggregation and interactions between crystals [45,68,69], which would have an impact on the structure of the oleogels and resulted in the improvements in rheological properties. The firmness of oleogels was presented in Fig. 9d. HIU treatment increased the firmness of all β-carotene oleogels. These three oleogels showed a considerable increase in physical strength after ultrasound treatment, proving that ultrasound also could enhance the strength of the embedding system.

3.2.2. Storage and in vitro digestion

As shown in Fig. 10a, it could be seen that CPN without HIU treatment had the weakest oil-binding capacity. Before HIU treatment, CW showed the highest stability among all β-carotene enriched oleogels. After HIU treatment, the oil binding capacity of all oleogels improved significantly. This might be due to the crystal aggregation induced by HIU (Fig. 8). da Silva et al. [64] pointed out that oil migrated from the oleogel system could be divided into 3 parts: (1) the amount of oil moved out of the gel network due to the effect of gravity; (2) the amount of oil drawn out by different kinds of materials such as filter papers; (3) the oil moving between multi-layer gel networks. Gravity and other external forces (material adsorption, etc.) may cause oil to seep out of the gel network. The process of gel formation was that the gel agents formed a three-dimensional network of multiple layers in the mixture and bound the oil in the network [1,4,6,70]. Therefore, the oil seepage would be inhibited by this network structure. Parts (1) and (2) were produced through part (3). Ultrasound may help to form more dense networks and improve the ability of gels to trap liquid oil through multi-layered action.

The β-carotene content over time is shown in Fig. 10b. Oleogels with and without HIU treatment were stored in the fridge (4 °C) for 60 and 120 d away from light. This was because β-carotene could be easily destroyed due to the exposure to high temperatures, light, and oxygen [71]. This experiment was performed to evaluate the oxygen-inhibition ability of β-carotene enriched oleogels with and without HIU treatment. As shown in Fig. 10b, the concentrations of β-carotene of all the oleogels showed a sharp decrease in the first 60 d of storage, especially the β-carotene content in CW without HIU. During the next 60 d, most oleogels showed a slower trend of degradation. As the interactions...
between solvent and solute constructed the crystalline network, \( \beta \)-carotene as a substance dissolved in the oil should also be involved in forming this network [9]. Therefore, the \( \beta \)-carotene in the outer network could be destroyed firstly, leading to an initial rapid degradation (0–60 d), while the \( \beta \)-carotene in the inner network could be protected and resulted in a slower degradation after the first 60 d (60–120 d). Inter- estingly, it was observed that HIU inhibited the degradation of \( \beta \)-caro- tene in CP and CW during 120 d of storage. Moreover, HIU treatment of CPN showed a trend of slower degradation of \( \beta \)-carotene during the last 60 d. These protective phenomena might be because HIU promoted crystal aggregation, which might cause denser networks and could improve the binding of \( \beta \)-carotene within the oleogel network.

The concentrations of \( \beta \)-carotene in oleogels during the gastric phase are shown in Fig. 10c. Results showed that the low-pH-environment (pH = 2.5) could break the \( \beta \)-carotene in oleogels. The final content of \( \beta \)-carotene in oleogels prepared with and without HIU application had no significant differences after 2 h. The concentration of \( \beta \)-carotene transferred to the intestinal micelle layer over time is shown in Fig. 10d. The \( \beta \)-carotene content of the micelle layer gradually increased over time. Two hours after the intestinal digestion phase, the amount of \( \beta \)-carotene transferred to the micelle layer was significantly higher in non-sonicated oleogels compared with HIU-induced oleogels. Compared with non-sonicated samples, sonicated samples also showed a tendency to inhibit the release of \( \beta \)-carotene. This demonstrated that the wax-oil-based network with HIU could effectively reduce the release of \( \beta \)-caro- tene in the intestinal digestion phase. Calligaris et al. [9] found that crystalline networks had the ability to inhibit the release of curcuminoi- d in oleogels. They hypothesized that the presence of crystals might inhibit the binding of enzyme to the active site. In our study, oleogels showed more visible crystals under polarized light microscope after HIU treatment. According to previous studies [45,46,60], more crystals may indicate denser networks and thus higher oil binding capacity of oleo- gels. Based on this, it was assumed that this denser network could not only inhibit the oil release in network but also inhibit the enzyme binding to the oil in network.

4. Conclusion

High-intensity ultrasound (HIU) showed a great potential to improve the functional properties of waxy/nut oils gels. The effect of HIU modification on the gel crystallization of candelilla wax-based oil depended on the type of oil. HIU significantly improved the crystal strength and physical properties of peanut, pine nut and walnut oleo- gels. All HIU-treated oleogels showed more crystals under polarized light microscopy. HIU improved G’ values but reduced oil loss of peanut, pine nut and walnut oleogels. After 120 d of storage, HIU decreased the degradation of \( \beta \)-carotene for peanut oil and walnut oil samples. More- over, HIU reduced the release of \( \beta \)-carotene in intestinal digestion.

CRediT authorship contribution statement

Letian Li: Methodology, Investigation, Writing - original draft, Writing - review & editing. Ahmed Taha: Writing - review & editing. Mengjie Geng: Methodology, Writing - review & editing. Zhongli Zhang: Methodology. Hongchen Su: Methodology. Xiaoyun Xu: Methodology. Siyi Pan: Resources. Hao Hu: Conceptualization, Resources, Writing - review & editing. Funding acquisition.

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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