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Oil structuring using whey protein-based cryogel particles: Effect of gelation pH and feasibility as an ingredient in low-saturated fat cocoa spreads

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ABSTRACT

Cryogel particles were obtained by freeze-drying and grinding hydrogel monoliths made from 20 % (w/w) whey protein isolate (WP) suspensions prepared at different pH (pH 4.8, 5.7, and 7.0). The microstructure, porosity, and density of the cryogels were strongly affected by the starting pH of the suspension. At pH 4.8, corresponding to the isoelectric point, proteins assumed a globular form leading to a cryogel with the highest porosity and lowest density compared to those formed at higher pH values (5.7 and 7.0). Such morphological differences accounted for different oil structuring capabilities. When mixed with oil, the cryogel particles formed at the pI were capable of entrapping larger quantities of oil (~63 % w/w) than those obtained distant from the pI (~47 %, w/w), forming a spreadable material. In this system, as confirmed by confocal microscopy, WP particles were evenly distributed in oil forming a network connected by capillary bridges and surface hydrophilic interactions. Thus, the mixture of sunflower oil with cryogel particles formed at the pI allowed to obtain an oleogel, exploitable for fat replacement, as confirmed by the preparation of a cocoa spread prototype. Results highlighted the critical impact of protein hydrogel structure in determining the ability of the cryogel particles thereof to entrap oil and tune the oleogel characteristics. The potentialities of this innovative material as ingredient of low saturated fat food products were also demonstrated.

1. Introduction

In response to the nutritional and environmental issues emerging on fats, there has been an exponential growth of research in the field of oleogelation in the last decade. Oleogels are defined as semi-solid materials entrapping large quantities of oil thanks to the presence of a supramolecular network of selected molecules (Marangoni and Garti, 2018). These innovative materials are explored mainly as promising solutions to reduce trans and saturated fatty acids in foods such as spreadable creams, bakery products, and meat preparations (Co and Marangoni, 2018; Martins et al., 2018). The majority of these food products are staple foods, whose consumption is associated with an increase in the risk for the onset of many non-communicable diseases (*e.g.*, cardiovascular diseases, type II diabetes, obesity, stroke, metabolic syndrome, and other cholesterol-associated health issues) (EFSA, 2010). Thus, the improvement of the lipid profile of fat-rich foods could favor the transition to more "sustainable-healthy diets", i.e., dietary patterns that promote all dimensions of individuals' health and well-being, as clearly stated in strategic documents of many global entities (European Commission, 2020; FAO, 2012).

Up to now, several approaches have been proposed in the literature to obtain oleogels. Upon direct dispersion of lipophilic molecules into oil, under defined physicochemical conditions, three-dimensional networks able to entrap oil can be formed, leading to gel-like materials with macroscopic properties associable with those of plastic fats. This approach, known as direct oleogelation, involves simple unit operations, such as heating and cooling, to generate an oleogel. Examples of oleogelators applicable in this context are fatty acids, monoglycerides, fatty alcohols, waxes, ethylcellulose, and phytosterols (Patel and Dewettinck, 2016; Zhao et al., 2022). However, these molecules have many compulsory restrictions being listed among food additives.

To avoid the use of food additives moving toward clean labels,

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indirect oleogelation strategies have been proposed. In this case, hydrophilic biopolymers (*i.e.*, carbohydrates and proteins conventionally used in food formulations as ingredients) are structured in supramolecular networks entrapping oil (Patel, 2018). Among indirect oleogelation strategies, the dried-template approach is one of the most promising. It involves the conversion of the selected biopolymer into porous particles able to retain large quantities of oil (De Vries et al., 2017a); Plazzotta et al., 2020). To this aim, the biopolymer is firstly solubilized in water, properly treated to induce its structuration in water (e.g., pH shift, thermal treatment), and then water is removed. The resulting particles showed remarkable oil-structuring ability (Ciuffarin et al., 2023; De Berardinis et al., 2024; Manzocco et al., 2022; Wang et al., 2024). In this context, despite the drying template approach requires high energy inputs and is time-consuming (Sabet et al., 2023), the use of freeze-drying to remove water appears particularly suitable for a scaling-up, being a well-known process associated with a reduced collapse of the material structure, largely used by the food industry to produce manifold dried food products (Ratti, 2013). In particular, water removal from hydrated biopolymeric structures by freeze-drying leads to the so-called cryogels characterized by a high porosity and a low density (Li et al., 2023; Wei et al., 2022); Ciuffarin et al., 2024).

Cryogels made of proteins have already been studied for oleogelation, demonstrating good capability to entrap oil thanks to its absorption and adsorption into particles as well as physical entrapment of oil within a hydrophilic particle–particle network (Plazzotta et al., 2020; Plazzotta et al., 2021; Selmer et al., 2019). The possibility to obtain protein-based oleogels with tunable properties and clean-label appears of particular interest not only in the attempt to substitute fats with feasible alternatives having better lipid profile, but also to improve their health functionalities. Protein-based oleogels have actually been demonstrated to modulate both protein and lipid digestibility, while increasing the bioavailability of lipophilic bioactive molecules (Ciuffarin et al., 2023). Among proteins, whey proteins (WP) are of particular interest being already widely used as an ingredient in many food formulations given their nutritional value, clean taste, and versatile functionalities (*e.g.*, solubility, viscosity, gelation, aroma retention capacity, emulsifying, and foaming capacity) (Baldissera et al., 2011). Moreover, whey proteins derive from milk whey, which is a common residue of the cheese-making process, requiring novel strategies for up-cycling to increase sustainability in a circular-economy perspective. Our research group previously demonstrated the feasibility of using WP cryogel particles formed at pH 5.7 to entrap oil forming a weak oleogel containing up to 70 % oil (Plazzotta et al., 2020). Considering that the hydrogelation conditions could greatly affect the microstructure of the WP particles and thus their oil structuring ability, in this study we move forward to understand the effect of pH during WP hydrogelation on their ability to entrap oil when converted into cryogels. As well reported in the literature, pH is a critical factor that controls the net protein charge and the overall structuring during gelation (Betz et al., 2012; Clark et al., 2001; Errington and Foegeding, 1998; Sarkar et al., 2017). To this regard, WP dissolved at pH 7 are unfolded in fibrillar form due to the presence of ionized carboxylic groups (*i.e.*, from COOH to COO–) which favor repulsions among protein chains. These characteristics lead to the formation of a stranded network that appears transparent due to the low dimensions of fibrillar chains (Fan et al., 2019; Zhu et al., 2022). By progressively reducing the pH towards the isoelectric pH (pI), the charges distributed on the protein quaternary structure are increasingly neutralized and, under thermal gelation, proteins form globular aggregated structures, also called microgels. The latter exhibit dimensions larger than that of natural light wavelength, favoring light scattering and resulting in opaque hydrogel (Betz et al., 2012; Foegeding et al., 1995). The importance of protein gelation pH was recently demonstrated by Wang et al. (Wang et al., 2024) with reference to oleogels obtained by capillary suspension technique, *i.e.*, by adding small amounts of water in the system. Results support the need for a better understanding of the role of pH on the oil structuring behavior of freeze-dried WP particles.

The present study aimed to elucidate the role of the pH of the starting three-dimensional hydrogel in determining the oleogelation performance of WP cryogels by considering the dried-template approach. To this aim, WP hydrogels were obtained by thermal treatment of a WP suspension at 20% (w/w), adjusted at pH 4.8, 5.7, and 7.0 and characterized for appearance, firmness and, colour. Furthermore, cryogel particles were obtained by freeze-drying and grinding. Particle characterization included porosity, bulk density, microstructure by SEM, and oil structuring ability. The latter was studied by progressively adding oil to the cryogel particles, using native unstructured WP as control. The best-performing oil-cryogel mixtures were studied for rheological properties, spreadability, microstructure via confocal microscopy, and oil binding capacity. Finally, a proof-of-concept by producing cocoa spreads was performed. This product was chosen as an example of a food product in which fats are crucial ingredients for determining the quality providing spreadability and palatability. Results demonstrated the critical impact of the structure, as determined by the pH, of the starting hydrogel in determining the oil structuring capacity of WP-cryogel particles.

2. Materials and methods

2.1. Materials

In the study whey protein isolate (WP, 94.7 % protein content; 74.6 % β-lactoglobulin, 23.8 % α-lactalbumin, 1.6 % bovine serum albumin, Davisco Food International Inc., Le Sueur, MN, USA), Nile Red and Fast Green dyes, and HCl purchased from Sigma Aldrich (Milan, Italy) were used.

2.2. Samples preparation

2.2.1. Hydrogels

Whey protein hydrogels were prepared according to the method of Betz et al. (Betz et al., 2012). In particular, 20 % (w/w) of WP was dissolved in double-distilled water, under continuous stirring at room temperature for 24 h. The pH of the WP suspension was then adjusted to 4.8, 5.7, and 7.0 (HI5221, HANNA Instruments, Padua, Italy) using a 6 M HCl solution. Aliquots of 40 mL of WP suspensions were then introduced in 50 mL capacity and 2.5 cm in diameter plastic tubes and heated at 90 °C for 20 min in a temperature-controlled water bath to induce protein denaturation. Samples were finally cooled in ice water for 15 min, stored overnight at 4 °C, and cut into cylinders with a 1.5 cm height and 2.5 cm diameter.

2.2.2. Cryogel particles

Hydrogel cylinders were manually broken and added in a ratio of 2:1 v/v to water and homogenized by using a high-speed homogenizer (DI 25 Basic, IKA Werke, Staufen im Breisgau, Germany) at 14,000 rpm for 3 min. The obtained samples were placed in aluminum containers, frozen at −40 °C for 45 min in a blast chiller (FAB25 Electrolux, Italy), and freeze-dried (EPSILON 2–4 LSCplus, Del Tek, Naples, Italy), similarly to the method described by Ciuffarin et al. (2024). The freeze-dryer was set at 0.2 mBar at different time/temperature settings: 20 min at −30 °C, 24 h at −20 °C, 24 h at −10 °C, 8 h at 0 °C, 16 h at 10 °C, and finally at 20 °C for 8 h. The resulting cryogel particles were sieved by using a vibrating sieve (Digital Electromagnetic Sieve Shaker, Filtra Virbacion, Barcelona, Spain), and the < 100 μm was collected. Particles were stored in a desiccator at room temperature until use.

2.2.3. Mixtures of oil and cryogel particles

An amount of 1.0 g of WP cryogel particles or native WP was weighed, and sunflower oil (SO) was gradually added, under manual mixing, according to the method used by Jung et al. (2023). In particular, the oil was added droplet-by-droplet and after each droplet

addition, thoroughly manually mixed until no free oil was visible. In this way, mixtures containing WP particles increasing oil amounts from 30 to 70% (w/w) were obtained.

2.2.4. Cocoa spreads

Cocoa spreads were prepared by mixing WP cryogel particles (7%, w/w), cocoa powder (10%, w/w), icing sugar (53%, w/w), and sunflower oil (30%, w/w). Firstly, the oil and the cryogel particles were manually mixed, as previously reported in 2.2.3, and then the other powders were added and manually mixed with a spatula for 1 min until a homogeneous sample was obtained. Control samples were produced by using native WP instead of WP cryogel particles.

2.3. Analytical determinations

2.3.1. Image acquisition

Images were acquired by using an image acquisition cabinet (Immagini and Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon, Ota City, Tokyo, Japan) and 60 mm lens with 2.8 focal aperture (Canon, Ota City, Tokyo, Japan). The digital camera was placed on an adjustable stand positioned 40 cm in front of a black cardboard base where the sample was placed. The light was provided by four 23 W frosted photographic floodlights, in a position allowing minimum shadow and glare. Other camera settings were: shutter time 1/25 s, f/10, and ISO 100. Images were saved in jpg format.

2.3.2. Bulk density

The WP cryogel particles were carefully filled into a graded cylinder and weighed. The bulk density was then calculated from the particle mass (m) and the sample bulk volume (V_b) (Eq. (1):

$$\rho_b = \frac{m}{V_b} \quad (1)$$

2.3.3. Particle size distribution

An amount of 10 g of cryogel particles was sieved with a set of sieves with mesh sizes of 25 and 50 μm (FTS-0200, Filtra Vibracion, Barcelona, Spain). The amount of powder remaining in each sieve was weighed and expressed as a percentage of the initial powder weight.

2.3.4. Porosity

Cryogel powder porosity (%) was estimated based on the following equation (Eq. (2)(Druel et al., 2018):

$$Porosity(\%) = \left(1 - \frac{\rho_b}{\rho_t}\right) \bullet 100 \quad (2)$$

where ρ_b (g cm^{-3}) is the bulk density of the powder and ρ_t (g cm^{-3}) is its true density (i.e., skeletal density). In particular, ρ_t was considered as WP true density ($\rho_t = 1.35$ g cm^{-3}) (Fischer et al., 2009).

2.3.5. Scanning electron microscopy

The WP cryogel particles were placed on double-side conductive adhesive tape and gold-sprayed using an ion sputter Coater (108 Auto, Cressington, UK), and observed using a FEI Quanta 250 SEM at 1.0 kV voltage with a magnification of × 30,000.

2.3.6. Confocal microscopy

A 0.2% aqueous solution of Fast Green and Nile Red (Sigma Aldrich, Milan, Italy) was used to stain the proteins and the oil, respectively, by gently hand-mixing the oil/cryogel mixtures, according to the method of Jung et al. (2023). The stained samples were then placed on the microscope slide, covered with a cover slide, and observed using a confocal laser scanning microscope at 100 times magnification (Leica TCS SP8 X confocal system, Leica Microsystems, Wetzlar, Germany). Images were imported in jpeg format using the software LasX 3.5.5 (Leica Microsystems, Wetzlar, Germany).

2.3.7. Oil holding capacity

The oil holding capacity (OHC) of mixtures of oil/WP cryogel particles was determined by weighing about 1 g of sample into a microcentrifuge tube. Samples were centrifuged at 10,000 rpm for 15 min at 20 °C (Mikro 120, Hettich Zentrifugen, Andreas Hettich GmbH and Co, Tuttlingen, Germany) and the excess oil was decanted. The samples were weighed and OHC was expressed as the percentage of oil retained by the sample.

2.3.8. Rheological properties

The viscoelastic properties (moduli G′ and G″) of mixtures of oil/WP cryogel particles were tested using an MCR Anton Paar 302 (Anton Paar, Graz, Austria) with a Peltier system for temperature control. Measures were performed using a 25 mm sandblasted parallel plate geometry (PP25S) at 20 °C with a gap of 2.0 mm. Oscillatory sweep tests to identify the linear viscoelastic region (LVR) were performed increasing stress from 1.0 to 1.0×10^4 Pa at 1 Hz frequency. Critical stress (Pa) was identified as the stress value corresponding to a 10 % drop in G′ value. Frequency sweep tests were then performed increasing frequency from 0.1 to 10 Hz at stress values selected in the LVR.

2.3.9. Firmness

The firmness of the hydrogels was measured by a uniaxial compression test using an Instron 4301 (Instron Corporation, Norwood, Massachusetts, US). To this aim, 2 cm-height and 2.5 cm- diameter hydrogels were cut and analyzed using a 6.2 mm in diameter cylindrical probe mounted on a 1000 N compression head at a 25 mm min^{-1} crosshead speed.

The firmness of oil/WP cryogel particles was measured by a uniaxial compression test using a 5942 Instron TA 500 Texture Analyzer. In this case, a volume of 2 mL was transferred in a 2 mm-diameter cylindrical sample container and compressed for 30 s with a 10 g-cylinder. The mixtures were then analyzed using an 8.1 mm-diameter cylindrical probe mounted on a 500 N compression head at a 25 mm min^{-1} crosshead speed.

Force-distance curves were obtained from the compression tests and firmness was taken as the maximum force (N) required to penetrate the sample for 2 mm.

2.3.10. Spreadability

The spreadability of mixtures of oil/WP cryogel particles and cocoa creams was assessed using a 34TM-5 Instron machine equipped with a back extrusion food cell (S5405A, Instron), consisting of a moving head and a cup. The latter was filled with 25 g of sample, which was then compressed for 5 mm by the head at 25 mm/s, upon an auto-detected force of 1 N.

2.4. Data analysis

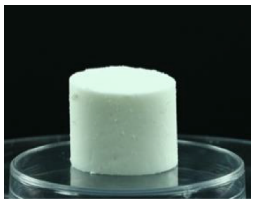
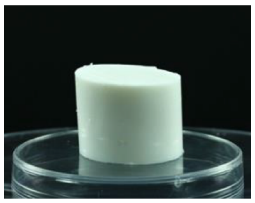
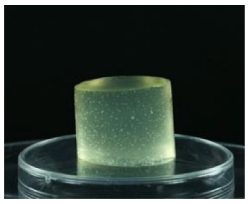
Data were expressed as the mean ± standard error of at least two measurements from two experimental replicates ($n \geq 4$). Statistical analysis was performed using R v. 4.0.3 (The R Foundation for Statistical Computing). ANOVA test was used to determine statistically significant differences between means ($p < 0.05$). Bartlett's test was used to check the homogeneity of variance ($p \geq 0.05$) and the Tukey test was used as a posthoc test ($p < 0.05$).

3. Results and discussion

3.1. Hydrogel and cryogel characterization

The appearance, color parameters, and firmness values of WP hydrogels resulting from the gelling of WP suspensions at pH 4.8 (corresponding to the isoelectric point, pI), 5.7, and 7.0 are reported in Table 1. As previously demonstrated in the literature, WP hydrogelation at different pH modifies proteins structuring by controlling their

Table 1
Appearance, firmness and color parameters of whey protein hydrogels at pH 4.8, 5.7, and 7.0.

pH	Appearance	Firmness (N)	Color		
			L*	a*	b*
4.8 (pI)		3.41 ± 0.12[b]	92.2 ± 0.0 [a]	−1.07 ± 0.05[c]	5.99 ± 0.03[b]
5.7		5.69 ± 0.14 [a]	92.1 ± 0.2 [a]	−1.36 ± 0.05[b]	3.56 ± 0.02[c]
7.0		2.43 ± 0.06[c]	38.9 ± 1.1[b]	1.51 ± 0.10 [a]	8.54 ± 0.21 [a]

[a-c] means indicated by different letters are significantly different *($p < 0.05$)*.

hydrophobic/hydrophilic landscape and the overall net charge (Betz et al., 2012; Clark et al., 2001; Errington and Foegeding, 1998; Sarkar et al., 2017). By gelling WP suspensions at pH 7.0, a transparent light-yellow hydrogel was obtained, while the hydrogels derived from the WP suspensions at pI (*i.e.*, 4.8) and pH 5.7 appeared white and opaque. These observations were confirmed by color parameters (Table 1). The hydrogels demonstrated also different firmness: the highest firmness was recorded for the sample gelled at pH 5.7, followed by samples obtained at pH 4.8 and finally, that obtained at pH 7.0.

According to the literature, pH 7.0 favors the unfolding of proteins that assume a fibrillar form mainly stabilized by covalent disulfide bridges, leading to the formation of a fine-stranded slightly yellow gel network (Fan et al., 2019). The reduction of the pH in the dispersion to 5.7 allowed the formation of non-covalent interactions in addition to disulfide bonds, leading to the formation of large aggregates, which resulted in a white gel with high firmness. At the pI, protein interactions result in a particulate gel, due to the null net charge on the protein surface (Sarkar et al., 2017).

These differences accounted for cryogel particles with different physical properties. In this regard, Table 2 shows the density and porosity of the particles. All the samples demonstrated lower values of density and higher porosity than the native WP (0.39 ± 0.03 g cm^{-3}; 71.1 ± 0.3 %, respectively), confirming the ability of hydrogelation followed by freeze-drying to generate porous materials. Depending on the hydrogel pH particle, density and porosity can be modulated. Specifically, the cryogel obtained from WP gelled at pH 7.0 displayed the highest bulk density and the lowest porosity, followed by the one gelled at pH 5.7, and finally 4.8. The pH of the initial suspensions also affected the particle size distribution. In particular, by changing the pH from 7.0 to the pI, a progressively higher fraction of small-dimension particles was obtained. These considerations were well confirmed by the observation of the cryogel particle microstructure (Table 2). The sample obtained at pH 7.0 displayed particles ranging from several tenths of micrometers to 100 μm, characterized by irregular shapes with flat surfaces and sharp edges. A similar particle dimension was imaged for the sample obtained at pH 5.7, but in this case, a pronounced surface roughness was noted. Conversely, at the pI smaller cryogel particles were produced, not exceeding 20–30 μm, characterized by spheroidal particles organized into aggregates.

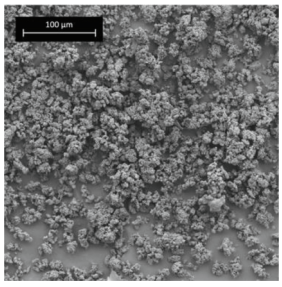
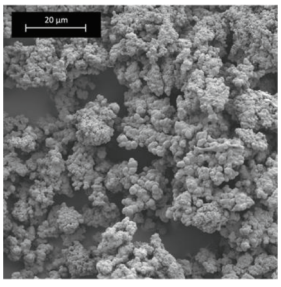
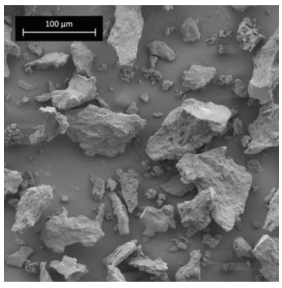
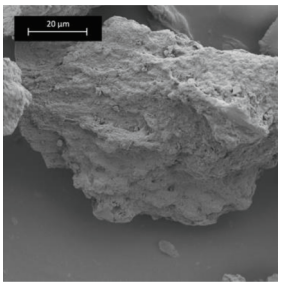
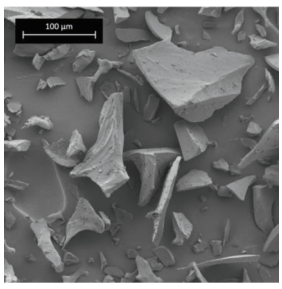
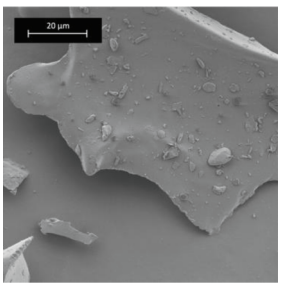
3.2. Oil structuring ability

The ability of the differently structured WP cryogel particles to form viscoelastic oleogels was assessed by adding an increasing amount of oil to the powders. Fig. 1 shows the visual appearance and firmness of the cryogel-oil mixtures having increasing oil content.

It should be noted that native WP showed no oil structuring capability. Independently on the oil content of the mixture, a particle suspension in oil was obtained with no oil retention capacity (data not shown). By contrast, all the cryogel samples displayed an oil structuring capability exhibiting a bell-shaped firmness curve (Fig. 1). At low-oil-content, grainy mixtures were obtained, since the oil was probably mainly absorbed into the particle pores, resulting in unconnected particulate systems with low firmness (Fig. 1). As the oil content increased, a homogeneous and self-standing material was obtained in correspondence with the firmness peak. In this situation, part of the oil occupied the spaces among the particles forming capillary oil bridges connecting the particles (Selmer et al., 2019). The latter was also demonstrated to interact *via* hydrogen bonding among the hydrophilic surface sites (De Vries et al., 2017b; Plazzotta et al., 2020; Jung et al., 2023). As a result, a network efficaciously embedding the oil was formed. As the oil content further increased, the particles were progressively detached upon dilution in oil, leading to the formation of a suspension of particles in the oil. In this condition, interactions among particles were eventually lost, resulting in firmness reduction, associated with a liquid-like behavior of the oil-cryogel mixtures (Fig. 1).

Despite the similar firmness bell-shaped behavior, the pH of the initial hydrogel strongly affected the range of oil content in which the peak firmness was obtained. Notably, for cryogel particles formed at pH 5.7 and 7.0, the firmness peak was detected at 47 % (w/w) oil content. In

Table 2
Bulk density, porosity, particle size distribution, and SEM microscopic images at different magnifications of whey protein cryogel particles obtained from hydrogels at pH 4.8, 5.7, and 7.0.

pH	Density ($g\ cm^{-3}$)	Porosity (%)	Particle size (%)		SEM microstructure	
			25–50 μm	50–100 μm		
4.8 (pI)	0.19 ± 0.01^{c}	85.3 ± 0.6^{a}	57.0 ± 0.4^{a}	43.0 ± 0.5^{c}		
5.7	0.22 ± 0.00^{b}	82.8 ± 0.6^{b}	50.7 ± 1.4^{b}	49.3 ± 0.8^{b}		
7.0	0.29 ± 0.02^{a}	78.6 ± 0.5^{c}	38.4 ± 0.2^{c}	61.3 ± 0.4^{a}		

[a-d] means indicates significant differences among samples ($p < 0.05$).

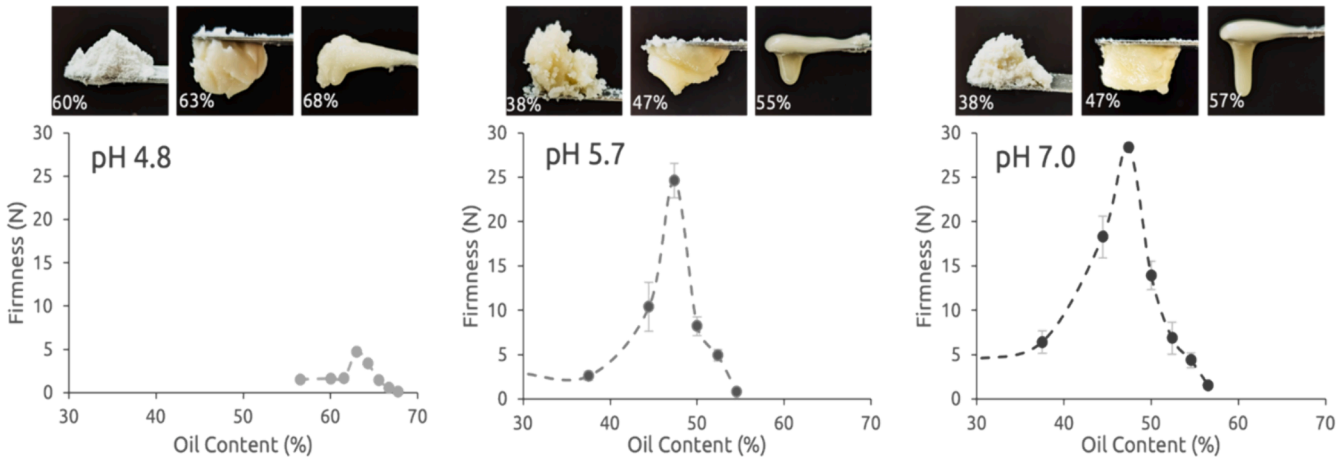


Fig. 1. Appearance and firmness of cryo-oleogels based on whey protein cryogel particles obtained from hydrogels at pH 4.8, 5.7, and 7.0. Percentages in the images refer to the oil content (%, w/w).

comparison, particles formed at the pI presented the maximum firmness at a remarkably higher oil content (63 %, w/w) (Fig. 1). This difference can be explained by observing the particle microstructural features (Table 2): being the particles obtained at pI smaller and more porous than those obtained at higher pH, they probably offered higher internal porosity for oil absorption and also a higher number of surface sites available for oil adsorption, particle hydrophilic networking, and oil bridging. Moreover, the higher oil-structuring capability of cryogel particles obtained at the pI can be attributed to their higher surface affinity with the oil as compared to that of particles obtained far from this

pH value. It is indeed well-known that thermal denaturation of WP suspensions at the pI results in the exposure of hydrophobic protein residues, highly increasing the surface hydrophobicity (Lam and Nickerson, 2015).

To better explore the effect of pH on the oil structuring capability of WP cryogel particles, the samples having the highest firmness, and thus corresponding to the peaks of the bell-shaped firmness curve were further characterized. Samples showed no oil separation during storage at ambient temperature for up to 5 months. This high physical stability was also confirmed by OHC values, which were always equal to 100 %. Table 3 reports the results of small-oscillation amplitude sweep tests of the three samples (Fig. 1).

The obtained rheological results allow the oil-cryogel mixtures to be classified as gels since the samples were characterized by an elastic modulus (G') higher than the viscous one (G''). As expected, the higher oil content structured by the cryogel particles obtained at the pI accounted for G' values lower than those detected for the mixture of oil with particles deriving from hydrogels formed at higher pH, confirming firmness values (Fig. 1). A similar trend was also observed for the critical stress, which is the stress value delimiting the linear viscoelastic region (LVR) where the material response is linear and reversible.

Since small-amplitude rheological parameters alone are insufficient for evaluating the suitability of a material as a fat replacer, the behavior of the oil-cryogel mixtures upon large deformation was assessed. The latter could mimic the deformations suffered by the material during its use as a food ingredient. For this reason, the oil-cryogel mixtures were subjected to spreadability analysis, which reflects the work required to spread a material under a given compressive deformation (Fig. 2). The typical profile of a plastic spreadable fat shows an initial increase of force with compressive displacement, followed by a progressive reduction of force dependence on the displacement, which indicates that a plastic, irreversible deformation is occurring (Guichard et al., 2018).

Both mixtures of oil with cryogels obtained at pH 5.7 and 7.0 did not show such behavior, evidencing instead a progressive force increase with the displacement. This indicates that these samples were not deformable but tended to compact under the spreading tool, accounting for a progressively increasing force opposing compression. By contrast, the sample containing cryogel particles formed at the pI showed the typical force–displacement profile associated with spreadable food fats (Fig. 2). This interesting behavior could be explained by considering the microstructure of the oil-cryogel mixtures, as evidenced by confocal microscopy (Fig. 3).

The images show the presence of large particle agglomerates for samples containing cryogels formed at a pH higher than pI. By contrast, the spheroidal cryogel particles formed at the pI appeared uniformly distributed in oil and interacting at the surface, supporting the hypothesis that a deformable particle–particle network embedding oil was formed. It can be hypothesized that particles formed at pI value were able to efficaciously interact on the surface, leading to the formation of an articulated three-dimensional network. The latter would be able to structure a large amount of oil while providing spreadability (Fig. 2). Thus, based on these results, only the sample obtained by gelling WP at pH 4.8 can be regarded as an oleogel intended for fat substitution.

Table 3
Rheological parameters (critical stress, G', and G'') of mixtures of sunflower oil and whey protein cryogel particles obtained from hydrogels at pH 4.8, 5.7, and 7.0., containing 63, 47, and 47% (w/w) oil content, respectively.

pH	Oil Content(%, w/w)	Critical Stress (Pa)	G' × 10^5 (Pa)	G'' × 10^5 (Pa)
7.0	47	355.5 ± 61.9 [a]	21.6 ± 3.2 [a]	0.9 ± 0.1 [a]
5.7	47	263.7 ± 23.8[b]	12.2 ± 1.3[b]	0.5 ± 0.1[b]
4.8	63	50.7 ± 13.6[c]	4.0 ± 0.8[c]	0.3 ± 0.0[c]

[ac] in the same column, different letters indicate significant differences ($p < 0.05$).

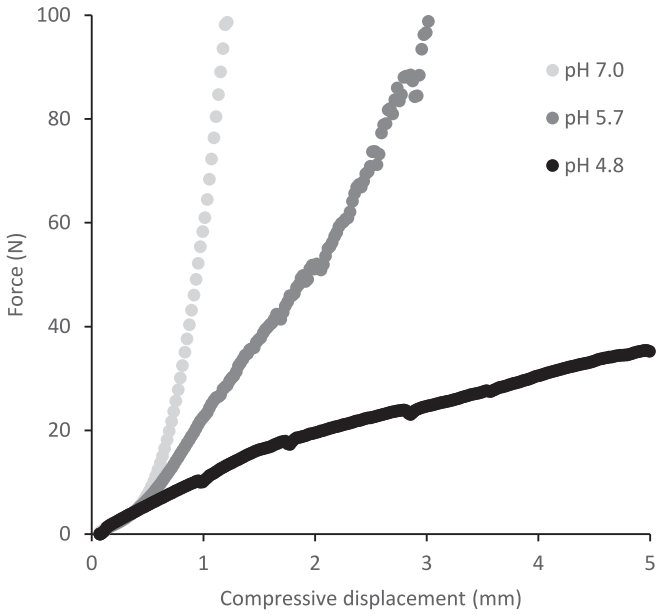


Fig. 2. Force-displacement curves of mixtures of sunflower oil and whey protein cryogel particles obtained from hydrogels at pH 4.8, 5.7, and 7.0, and containing 63, 47, and 47% (w/w) oil content, respectively.

3.3. *Proof of concept: Cocoa spreads*

As a demonstration of its potential, the practicality of employing a whey protein cryogel particle-based oleogel as a substitute for fat in a cocoa spread formulation was explored. It is noteworthy that, the typical spreadable structure of this product is provided by a high amount of fat rich in saturated fatty acids, such as palm and cocoa oils (Marra et al., 2023). Table 4 reports the physical properties of the cocoa spread prepared by using the oleogel structured by WP cryogel at pI and sunflower oil. As a control, cocoa spread prepared by using native WP instead of those structured in cryogel was also considered.

While the spread prepared with native WP (control) well-retained oil (OHC), it appeared as a fluid system. By contrast, when native WP were substituted with the porous cryogel particles prepared at the pI, a visibly more structured spread was obtained (Table 4). This can be inferred by the ability of the cryogel particles to interact with each other *via* capillary oil bridges, thus connecting the particles and resulting in a self-standing material. The rheological analysis confirmed these observations, with the cryogel-containing spread showing a significantly higher critical stress and G' value (Table 4). Spreads were also assessed for spreadability, as shown in Fig. 4.

As expected, the sample containing unstructured WP opposed minimum resistance to spreadability head movement, resulting in low force values typical of fluid samples. By contrast, a displacement-sensitive force curve characterized by higher values was obtained from the cryogel-containing spread, showing the typical profile of spreadable matrices.

4. Conclusions

This study highlighted the oil structuring potential of whey proteins in the form of cryogel particles, opening interesting opportunities in the development of novel ingredients from food residues such as those deriving from cheese-making. The gelling pH played a critical role in modifying the microstructure of the starting hydrogel and, finally, the oil structuring ability of the resulting cryogel particles. At pH near the pI, highly porous small globular cryogel particles were obtained, which showed the ability to retain large quantities of oil resulting in spreadable oleogels. Conversely, cryogel particles prepared from hydrogels

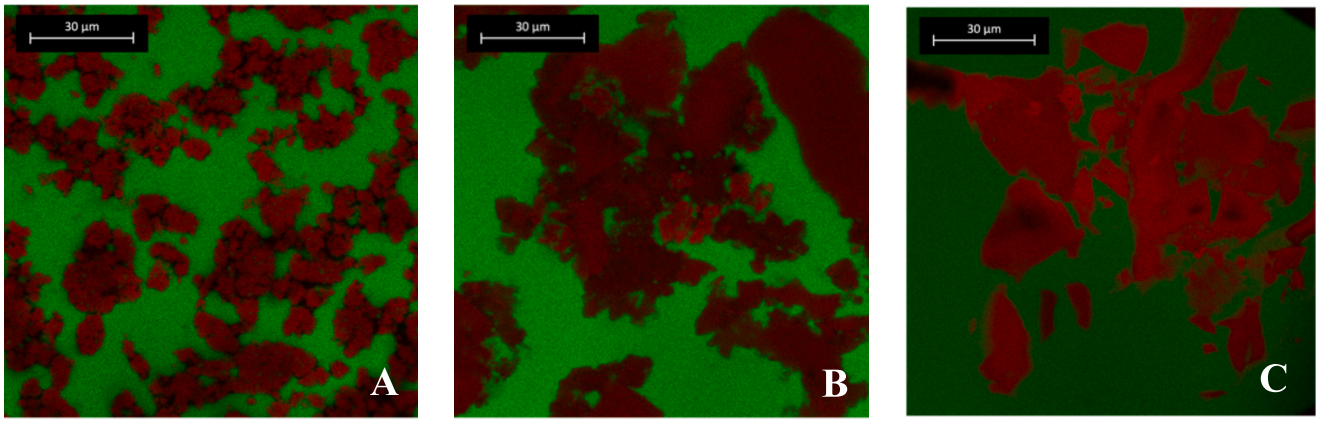


Fig. 3. Confocal microscopy of mixtures of sunflower oil and whey protein cryogel particles obtained from hydrogels at pH 4.8 (A), 5.7 (B), and 7.0 (C), containing 63%, 47%, and 47% (w/w) oil content, respectively.

Table 4
Appearance, oil holding capacity (OHC), and rheological parameters (critical stress and G' module) of cocoa spreads prepared using sunflower oil and native whey protein (control), or cryogel particles obtained from hydrogel at pH 4.8.

Sample	Visual Aspect	OHC (%)	Viscoelastic Properties	
			Critical Stress (Pa)	G' × 10^5 (Pa)
Control		96.3 ± 0.5[b]	0.31 ± 0.05[b]	0.078 ± 0.006[b]
Cryogel		98.9 ± 0.2 [a]	918.8 ± 114.7 [a]	41.1 ± 7.7 [a]

[a-b] in the same column, means indicated by different letters are significantly different ($p < 0.05$).

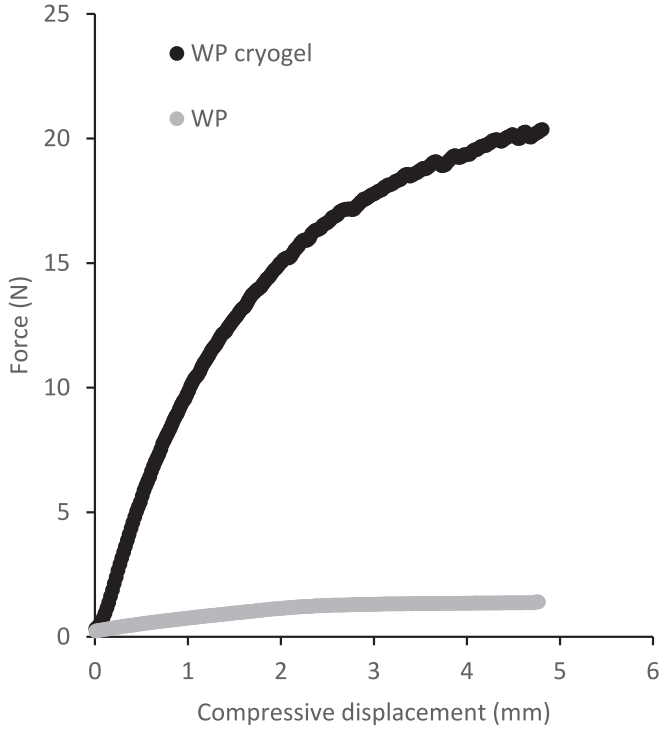


Fig. 4. Force-displacement curves of cocoa spreads containing sunflower oil and native whey protein (WP), or WP cryogel particles obtained from hydrogel at pH 4.8.

obtained at pH values far from the pI, demonstrated a lower oil structuring ability due to the higher particle size and reduced porosity. These results provided the demonstration of the high potentialities of the cryogel-template approach in obtaining protein-based oleogels upon a proper design of the hydrogel structure. Although the scaling up of the proposed oleogelation process would need a careful resetting of operational parameters and a detailed cost analysis, it is entirely based on technological steps that are well accepted by consumers and widely applied by the food industry.

Finally, in this study it was also proven the pivotal importance of particle structure in delivering functionalities in foods. The behaviour of WP cryogel particles in low-saturated fat food products was completely different from that of native proteins, enlarging the possibility to include structured proteins into complex food formulations. The use of protein-based oleogels could also address further nutritional challenges, including the delivery of both unsaturated fatty acids and proteins into foods. Interesting at this point would be the study of the chemical stability and thus the shelf life of reformulated foods since fat substitution with structured liquid oil might result in foods more prone to lipid oxidation, thus possibly impairing product sensory properties during food storage.

CRediT authorship contribution statement

Ciuffarin Francesco: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Plazzotta Stella:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Rondou Kato:** Writing – review & editing, Methodology, Investigation. **Van Bockstaele Filip:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Dewettinck Koen:** Writing – review & editing, Supervision, Conceptualization. **Manzocco Lara:** Writing – review & editing, Conceptualization. **Calligaris Sonia:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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References

Baldissera, A. C., Betta, F. Della, Penna, A. L. B., & De Dea Lindner, J. (2011). Alimentos funcionais: uma nova fronteira para o desenvolvimento de bebidas protéicas a base de soro de leite. *Semina:Ciencias Agrarias*, *32*(4), 1497–1512. 10.5433/1679-0359.2011v32n4p1497.

Betz, M., García-González, C. A., Subrahmanyam, R. P., Smirnova, I., & Kulozik, U. (2012). Preparation of novel whey protein-based aerogels as drug carriers for life science applications. *Journal of Supercritical Fluids, 72*, 111–119. https://doi.org/10.1016/j.supflu.2012.08.019

Ciuffarin, F., Alongi, M., Plazzotta, S., Lucci, P., Schena, P., Manzocco, L., & Calligaris, S. (2023). Oleogelation of extra virgin olive oil by different gelators affects lipid digestion and polyphenol bioaccessibility. *Food Research International, 173*, Article 113239. https://doi.org/10.1016/j.foodres.2023.113239

Ciuffarin, F., Plazzotta, S., Gelas, L., Calligaris, S., Budtova, T., & Manzocco, L. (2024). Cellulose cryogel particles for oil structuring: Mixture properties and digestibility. *Food Hydrocolloids, 110470*. https://doi.org/10.1016/j.foodhyd.2024.110470

Ciuffarin, F., Négrier, M., Plazzotta, S., Libralato, M., Calligaris, S., Budtova, T., & Manzocco, L. (2023). Interactions of cellulose cryogels and aerogels with water and oil: Structure-function relationships. *Food Hydrocolloids, 140*, Article 108631. https://doi.org/10.1016/j.foodhyd.2023.108631

Clark, A. H., Kavanagh, G. M., & Ross-Murphy, S. B. (2001). Globular protein gelation-Theory and experiment. *Food Hydrocolloids, 15*(4–6), 383–400. https://doi.org/10.1016/S0268-005X(01)00042-X

De Berardinis, L., Plazzotta, S., Magnan, M., & Manzocco, L. (2024). Hydrophilic or hydrophobic coating of whey protein aerogels obtained by supercritical-CO2-drying: Effect on physical properties, moisture adsorption and interaction with water and oil in food systems. *Innovative Food Science and Emerging Technologies, 91*, Article 103530. https://doi.org/10.1016/j.ifset.2023.103530

De Vries, A., Gomez, Y. L., Van der Linden, E., & Scholten, E. (2017a). The effect of oil type on network formation by protein aggregates into oleogels. *RSC Advances, 7*(19), 11803–11812. https://doi.org/10.1039/c7ra00396j

De Vries, A., Lopez Gomez, Y., Jansen, B., Van der Linden, E., & Scholten, E. (2017b). Controlling Agglomeration of Protein Aggregates for Structure Formation in Liquid Oil: A Sticky Business. *ACS Applied Materials and Interfaces, 9*(11), 10136–10147. https://doi.org/10.1021/acsami.7b00443

Druel, L., Niemeyer, P., Milow, B., & Budtova, T. (2018). Rheology of cellulose-[DBNH][CO2Et] solutions and shaping into aerogel beads. *Green Chemistry, 20*(17), 3993–4002. https://doi.org/10.1039/c8gc01189c

EFSA. (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal, 8*(3). 10.2903/j.efsa.2010.1461.

Errington, A. D., & Foegeding, E. A. (1998). Factors Determining Fracture Stress and Strain of Fine-Stranded Whey Protein Gels. *Journal of Agricultural and Food Chemistry, 46*(8), 2963–2967. https://doi.org/10.1021/jf980112y

European Commission. (2020). Farm to Fork Strategy. In *DG SANTE/Unit 'Food information and composition, food waste'''* (Issue DG SANTE/Unit 'Food information and composition, food waste'). https://ec.europa.eu/food/sites/food/files/safety/docs/f2f_action-plan_2020_strategy-info_en.pdf.

Fan, L., Ge, A., Chen, X. D., & Mercadé-Prieto, R. (2019). The role of non-covalent interactions in the alkaline dissolution of heat-set whey protein hydrogels made at gelation pH 2–11. *Food Hydrocolloids, 89*, 100–110. https://doi.org/10.1016/j.foodhyd.2018.10.035

FAO. (2012). Sustainable Diets and Biodiversity: Directions and Solutions for Policy, Research and Action (Food and Agriculture Organization and Bioversity International, Rome). *Biodiversity and Sustainable Diets United Against Hunger*, 309. http://www.fao.org/3/a-i3004e.pdf%0Ahttp://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/522860/%0Ahttp://www.fao.org/3/i3004e/i3004e.pdf.

Fischer, H., Polikarpov, I., & Craievich, A. F. (2009). Average protein density is a molecular-weight-dependent function. *Protein Science, 13*(10), 2825–2828. https://doi.org/10.1110/ps.04688204

Foegeding, E. A., Bowland, E. L., & Hardin, C. C. (1995). Factors that determine the fracture properties and microstructure of globular protein gels. *Topics in Catalysis, 9*(4), 237–249. https://doi.org/10.1016/S0268-005X(09)80254-3

Guichard, E., Galindo-Cuspinera, V., & Feron, G. (2018). Physiological mechanisms explaining human differences in fat perception and liking in food spreads-a review. *Trends in Food Science & Technology, 74*, 46–55. https://doi.org/10.1016/j.tifs.2018.01.010

Jung, I., Schroeter, B., Plazzotta, S., De Berardinis, L., Smirnova, I., Gurikov, P., & Manzocco, L. (2023). Oleogels from mesoporous whey and potato protein based aerogel microparticles: Influence of microstructural properties on oleogelation ability. *Food Hydrocolloids, 142*, Article 108758. https://doi.org/10.1016/j.foodhyd.2023.108758

Lam, R. S., & Nickerson, M. T. (2015). The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. *LWT-Food Science and Technology, 60*(1), 427–434.

Li, J., Zhao, S., Zhu, Q., & Zhang, H. (2023). Characterization of chitosan-gelatin cryogel templates developed by chemical crosslinking and oxidation resistance of camellia oil cryogel-templated oleogels. *Carbohydrate Polymers, 315*. https://doi.org/10.1016/J.CARBPOL.2023.120971

Manzocco, L., Plazzotta, S., Powell, J., de Vries, A., Rousseau, D., & Calligaris, S. (2022). Structural characterization and sorption capability of whey protein aerogels obtained by freeze-drying or supercritical drying. *Food Hydrocolloids, 122*. https://doi.org/10.1016/j.foodhyd.2021.107117

Marangoni, A. G., & Garti, N. (2018). Edible Oleogels. In *Edible Oleogels*. Elsevier. 10.1016/c2017-0-00541-4.

Marra, F., Lavorgna, A., Incarnato, L., Malvano, F., & Albanese, D. (2023). Optimization of Hazelnut Spread Based on Total or Partial Substitution of Palm Oil. *Foods, 12*(16), 3122. https://doi.org/10.3390/foods12163122

Martins, A. J., Vicente, A. A., Cunha, R. L., & Cerqueira, M. A. (2018). Edible oleogels: An opportunity for fat replacement in foods. In *Food and Function* (Vol. 9, Issue 2, pp. 758–773). 10.1039/c7fo01641g.

Patel, A. R. (2018). Structuring Edible Oils with Hydrocolloids: Where Do we Stand? In *Food Biophysics* (Vol. 13, Issue 2, pp. 113–115). Springer New York LLC. 10.1007/s11483-018-9527-6.

Patel, A. R., & Dewettinck, K. (2016). Edible oil structuring: An overview and recent updates. *Food and Function, 7*(1), 20–29. https://doi.org/10.1039/c5fo01006c

Plazzotta, S., Calligaris, S., & Manzocco, L. (2020). Structural characterization of oleogels from whey protein aerogel particles. *Food Research International, 132*, Article 109099. https://doi.org/10.1016/j.foodres.2020.109099

Plazzotta, S., Jung, I., Schroeter, B., Subrahmanyam, R. P., Smirnova, I., Calligaris, S., Gurikov, P., & Manzocco, L. (2021). Conversion of whey protein aerogel particles into oleogels: Effect of oil type on structural features. *Polymers, 13*(23). 10.3390/polym13234063.

Ratti, C. (2013). Freeze drying for food powder production. In *Handbook of Food Powders: Processes and Properties* (pp. 57–84). Elsevier.

Sabet, B. S., Pinto, T. C., Kirjoranta, S. J., Garcia, A. K., & Valoppi, F. (2023). Clustering of oleogel production methods reveals pitfalls and advantages for sustainable, upscalable, and oxidative stable oleogels. *Journal of Food Engineering, 357*, Article 111659. https://doi.org/10.1016/j.jfoodeng.2023.111659

Sarkar, A., Kanti, F., Gulotta, A., Murray, B. S., & Zhang, S. (2017). Aqueous lubrication, structure and rheological properties of whey protein microgel particles. *Langmuir, 33*(51), 14699–14708.

Selmer, I., Karnetzke, J., Kleemann, C., Lehtonen, M., Mikkonen, K. S., Kulozik, U., & Smirnova, I. (2019). Encapsulation of fish oil in protein aerogel micro-particles. *Journal of Food Engineering, 260*, 1–11. https://doi.org/10.1016/j.jfoodeng.2019.04.016

Wang, G. S., Chen, H. Y., Zhang, L. Y., Guo, J., Wan, Z. L., & Yang, X. Q. (2024). Facile formation of capillary whey protein oleogels with tunable mechanical and aesthetic properties and their applications as margarine alternatives. *Food Hydrocolloids, 154*, Article 110073. https://doi.org/10.1016/j.foodhyd.2024.110073

Wei, F., Lu, M., Li, J., Xiao, J., Rogers, M. A., Cao, Y., & Lan, Y. (2022). Construction of foam-templated oleogels based on rice bran protein. *Food Hydrocolloids, 124*. https://doi.org/10.1016/j.foodhyd.2021.107245

Zhao, W., Wei, Z., & Xue, C. (2022). Recent advances on food-grade oleogels: Fabrication, application and research trends. *Critical Reviews in Food Science and Nutrition, 62*(27), 7659–7676. https://doi.org/10.1080/10408398.2021.1922354

Zhu, P., Huang, W., & Chen, L. (2022). Develop and characterize thermally reversible transparent gels from pea protein isolate and study the gel formation mechanisms. *Food Hydrocolloids, 125*, Article 107373. https://doi.org/10.1016/j.foodhyd.2021.107373