Powder Technology 469 (2026) 121826

Contents lists available at ScienceDirect

Powder Technology

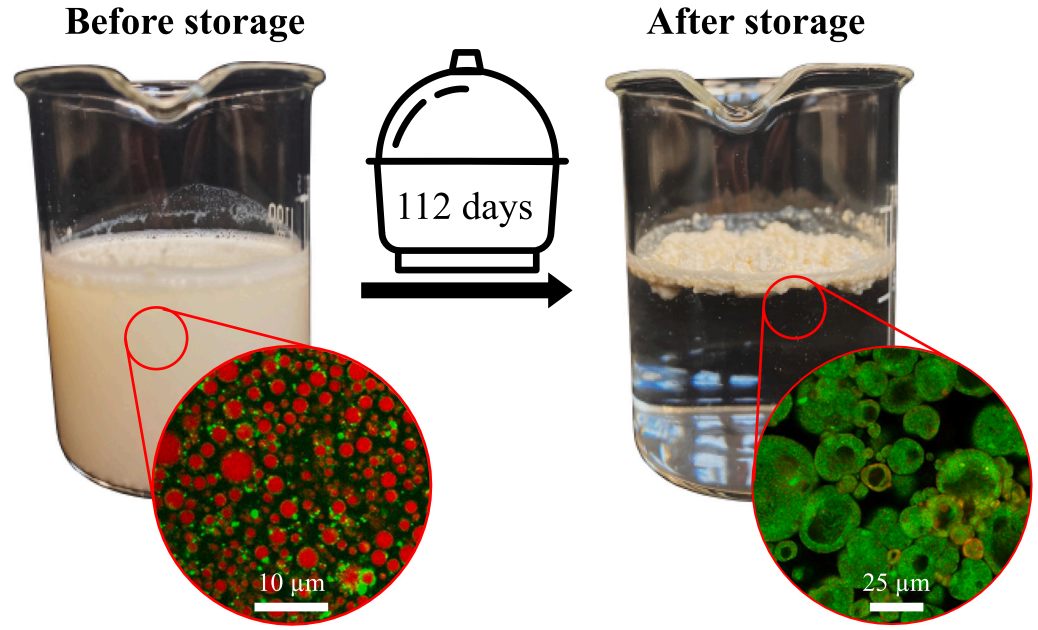
journal homepage: www.journals.elsevier.com/powder-technology

Storage-induced solubility loss in plant-based emulsion powders

T. Kurtz [a],*, K. Haas [b], C. Schmitt [b], C. Morgenegg [b], V. Meunier [b], S. Heinrich [a]

[a] *Institute of Solids Process Engineering and Particle Technology, Hamburg University of Technology, Denickestraße 15, Hamburg, 21073, Germany*
[b] *Nestlé Research, Nestlé Institute of Food Sciences, Route du Jorat 57, Lausanne 26, CH-1000, Switzerland*

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Storage induced insolubility in soy- and pea stabilized emulsion powders.
- Protein denaturation during storage lead to the formation of an insoluble network.
- Polymerization of free lipids lead to increase in hydrophobicity.

ARTICLE INFO

Keywords:
Plant protein
Spray drying
Solubility

ABSTRACT

Developing plant-based milk powder alternatives with similar nutritional profiles and functionalities to dairy powders poses significant challenges, particularly related to their rehydration and dispersion properties. In this first-of-its-kind study, factors affecting the solubility of plant-based emulsion powders during storage were investigated. To examine the impact of material properties and processing, model emulsions with varying

---

* Corresponding author.
E-mail address: teresa.kurtz@tuhh.de (T. Kurtz).

https://doi.org/10.1016/j.powtec.2025.121826
Received 5 May 2025; Received in revised form 30 September 2025; Accepted 23 October 2025

Emulsion

protein sources (pea, soy), protein content (2.4%, 20%), lipid type (sunflower, MCT), and processing conditions were spray-dried and stored under controlled conditions for 16 weeks. For the first time, storage-induced insolubility was shown to occur in plant-based emulsion powders, resulting in final powder insolubility from 7 to 51%, depending on the formulation. Storage further decreased protein solubility and free fat extractability in most samples. Protein content and the presence of lipids were found to be the most relevant factors influencing plant powder solubility during storage, while processing conditions and protein source were determined not to have a relevant impact. It is suggested that changes in protein conformation and enhanced protein–protein interactions due to oxidation and glycation contribute to the formation of an insoluble protein micro-gel network during storage, dramatically decreasing solubility. In addition, oxidative polymerization of unsaturated lipids like sunflower oil increased powder hydrophobicity, inhibiting wetting properties and the extractability of free fat. The current findings offer valuable insights into the structural changes occurring in plant-based emulsion powders during storage, highlighting the intricacies of protein–protein and protein–lipid interactions.

1. Introduction

With an annual production volume of more than 4 million tonnes, milk powder represents an important ingredient in a wide range of food products [1]. Its widespread use can be attributed to the excellent emulsifying properties of dairy proteins and the high degree of saturation of lipids, which contribute to a powder with high shelf stability that can be distributed worldwide. In recent years, the food industry has developed a growing interest in replacing dairy-based systems with plant-based alternatives to align with evolving consumer preferences, such as the demand for sustainable and animal-free products, while also reducing the environmental footprint of food manufacturing. However, plant and dairy proteins have notable differences in their composition and functionality [2]. Dairy proteins are mainly caseins and whey proteins, which are relatively small molecules (14–59 kDa), giving them the ability to be readily available at the emulsion interface and form small and highly stable emulsion droplets [3,4]. Plant proteins, such as pea and soy, on the other hand, are generally characterized by their higher molecular weight (150–440 kDa) and a rigid, globular structure and reduced solubility, which slows interfacial adsorption and reduces emulsion stability [5]. Therefore, transitioning to fully plant-based ingredients with a similar nutritional profile can introduce significant challenges, particularly with respect to processability, shelf-life, reconstitution properties, and overall functionality of the resulting powders.

An important functionality of milk powder to consider during shelf-life is reconstitution or dissolution properties that are closely related to the particle solubility of the powder [6–8]. Extensive research has been done on the changes in the solubility of dairy milk and protein powders during storage, including the effects of environmental conditions (e.g., temperature, humidity) and intrinsic powder characteristics [9–12]. Several mechanisms responsible for decreasing solubility during shelf-life of spray-dried emulsions are known and discussed in scientific literature on dairy powders, including the Maillard reaction [13–15], lactose crystallization that modifies protein structures [14,16], lipid and protein oxidation [17,18], and protein aggregation [19]. However, few studies have investigated changes in the solubility of plant protein powders during storage [20,21], and to date, no research has focused on the changes in solubility of plant-based emulsion powders, a category that is increasingly relevant for food applications. While existing knowledge from dairy systems provides valuable insights and it seems likely that similar mechanisms are affection plant-based powder solubility, the significant differences between dairy and plant proteins, such as protein composition, structural properties, and interactions, make it necessary to investigate these new systems independently. Furthermore, differences in oxidation stability of used lipids could impact lipid-protein interactions and co-oxidation phenomena.

Therefore, the present study aimed to understand what factors impact the formation of insoluble particles in plant-based emulsion powders over the course of storage. Model emulsions based on pea protein isolate, soy protein isolate, and milk protein concentrate were formulated to target specific research questions, namely the impact of protein source and content, protein–lipid interactions, and the influence of processing conditions, specifically pH and pre-homogenization of the protein slurry. Maltodextrin was used as a carrier material due to its well-known properties, good availability and tunable properties in terms of taste and viscosity. Emulsions were spray-dried and stored for 16 weeks under controlled conditions. To monitor changes, powders were regularly analyzed for their morphological properties and wetting behavior, and these findings were correlated with changes in overall solubility and protein solubility.

The findings of this study have significant implications for the design and optimization of plant-based emulsion powders, with potential applications in enhancing their shelf-life, functionality, and consumer acceptance. This research also aims to provide insights that bridge the gap between current knowledge of dairy and plant-based systems, ultimately supporting the development of high-quality, sustainable food products.

2. Materials and methods

2.1. Materials

In this study, a model system composed of protein, maltodextrin DE 21, and oil was used. Two plant-derived proteins (pea protein isolate, soy protein isolate) and one animal-based protein (milk protein concentrate) were compared. Pea protein isolate was used as reference material and investigated in two different concentrations (20%, 2.4%) while all other recipes contained 20% protein in total solids (TS). Additional emulsions containing pea protein were produced, containing either no oil (w/o oil) or MCT oil (stable oil) as a non-oxidizable oil. Pea protein isolate (PPI) (Pisane C9) was obtained from Cosucra (Warcoing, Belgium), Soy protein isolate (SPI) (Profam 974) from ADM (Chicago, IL, USA) and milk protein concentrate (MPC) from Fonterra (Auckland, New Zealand). Sunflower oil (SFO) was bought from Florin (Muttenz, Switzerland) and served as lipid phase. To investigate the impact of a non-oxidizable oil, medium chain triglyceride (MCT) oil from Oleon (Oelegem, Belgium) was used. The hydrophilic carbohydrate phase was composed of maltodextrin (MD) with a dextrose equivalent (DE) of 21 (Gluxidex 21) and obtained from Roquette (Lestrem, France). Analytical grad n-heptane was used for free fat extraction and purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). The composition of all recipes tested in this study can be found in Table 1.

2.2. Emulsion preparation

For the production of emulsions, for each formulation, protein was mixed with demineralized water, stirred for 30 min at 10 000 rpm using a L5M-A laboratory mixer (Silverson, Chesham, United Kingdom) and hydrated overnight at 7 °C. After hydration, maltodextrin powder was added and stirred for 5 min until total dissolution, before adding the lipid phase and shearing for 10 min at 10 000 rpm. Following the first emulsification, the pH of the formulations was adjusted to 7.5 using a 1 molar sodium hydroxide solution to guarantee sufficient distance to the isoelectric point of the proteins. The pH of one sample (high pH) was further increased to 8.5 to improve protein solubility and to investigate

Table 1
Composition based on % of total solids of different formulation produced by emulsification and spray-drying.

	MD	PPI	SPI	MPC	SFO	MCT oil
	(%)	(%)	(%)	(%)	(%)	(%)
Pea	52	20	–	–	28	–
Soy	52	–	20	–	28	–
Dairy	52	–	–	20	28	–
without (w/o) oil	80	20	–	–	–	–
Low protein	69.6	2.4	–	–	28	–
stable oil	52	20	–	–	–	28
high pH[a]	52	20	–	–	28	–
Pea prehomogenized[b]	52	20	–	–	28	–

[a] increase of emulsion pH from 7.5 to 8.5.

[b] use of homogenized protein slurry for emulsification.

weather concentration effects during spray drying could lead a rapid drop in pH and induce protein denaturation. Samples were further homogenized in a high pressure homogenizer (HPH) (Panda Plus, Gea, Düsseldorf, Germany) in two passages at 350/75 bar.

A sample with a prehomogenized pea protein dispersion was produced to test the impact of protein aggregate disruption prior to emulsification on long-therm stability. For this sample, the hydrated protein slurry was passed through the HPH at 350/75 in two passages before mixing and emulsification with the remaining ingredients. All formulations had a total solids (TS) content of 35% including lipid phase.

2.3. Drying

5 kg of emulsion for each formulation were spray-dried in a pilot-scale spray dryer (Niro minor, Søborg, Denmark) equipped with a two-fluid nozzle operated at 1 bar atomizing pressure. Inlet temperature was set to 190 °C and the flow rate was adjusted to maintain an outlet temperature of 70 °C. Powder was collected after cyclone separation. Powders were sealed in aluminum bags flashed with Argon and stored at −60 °C until further analysis.

2.4. Storage study

Powders were stored in a climatic chamber ICH260eco (Memmert, Schwabach, Germany) for a total of 16 weeks (112 days) in open plastic containers (9 × 9 × 13 cm) under controlled conditions of 35 °C and 20% rel. humidity and light exclusion. The temperature was selected to simulate accelerated shelf-life conditions while relative humidity was kept low to maintain powders in the glassy state and avoid collapse. Samples were taken and analyzed every 4 weeks.

2.5. Emulsion and powder characterization

Particle size distribution (PSD) of fresh and reconstituted emulsions was determined using laser diffraction (Mastersizer3000, Malvern Panalytical, Malvern, United Kingdom). Emulsions were added to a dispersion unit filled with demineralized water to an obscuration of around 5% at constant agitation speed and measured 5 times consecutively. PSD was calculated based on the Mie theory using a refractive index of sunflower oil of 1.6 and 1.33 for water. The distribution was described using surface mean ($d_{3,2}$) and volume mean diameter ($d_{4,3}$).

Powder particle size was determined by dynamic image analysis (Camsizer XT, Microtrac Retsch GmbH, Haan, Germany). Powder samples were mixed with 0.5% of silicon dioxide as flowing agent and dispersed with pressurized air and analyzed by two high speed cameras capturing large and small particles. The diameter of a circle with an identical area (x_{area}) was used for evaluation and median diameter ($d_{50,3}$) as well as surface mean diameter ($d_{3,2}$).

Residual moisture content (x_{res}) was measured in triplicates by weighing 2 g of powder into aluminum dishes and drying for 24 h at 103 °C. After cooling samples in a desiccator filled with silica gel, moisture content was calculated based on weight difference before and after drying.

Apparent density (ρ_a) and material density ($\rho_{material}$) were determined with a helium pycnometer (AccuPyc 1330, Micromeritics, Norcross, GA, USA) equipped with a 3.5 cm^3 sample cup at 1.38 bar and calculated using Boyle–Mariotte law. For ρ_a powder was measured as is, while the true material density was measured for powder that was previously ground in a mortal to destroy all internal cavities. The volume of closed pores (closed porosity) without connection to the particle surface, was calculated according to Eq. (1):

$$Closed\ porosity\,(\%) = \left(1 - \frac{\rho_a}{\rho_{material}}\right) \cdot 100\% \tag{1}$$

2.6. Free fat content

Free fat is an important parameter for powder quality, since it can largely impact powder flowability and storage stability. Following a protocol by Sarkar et al. [22] with some modifications, free fat was determined gravimetrically using heptane extraction. Approximately 2 g of powder (m_1) were weighed into screw cap glass bottles and mixed with 40 mL of heptane for 10 min using a lab shaker KL-2 (Edmund Bühler, Germany) to ensure good exposure of powder particles to solvent. The solvent was further decanted into 50 mL centrifuge tubes and centrifuged for 5 min at 2000 rpm. The clear supernatant was passed through a 45 nm syringe filter to remove all remaining particles. 25 mL of this solution were evaporated in weighed aluminum dishes (m_0) at 110 °C and further dried in a laboratory oven at 100 °C for 30 min to ensure total solvent removal. Samples were cooled in a silica desiccator for a minimum of 1 h and weighed (m_2). Free fat content was calculated based on a total lipid content (F) of 28% (Eq. (2)). Each sample was measured in triplicates.

$$Free\ fat\,(\%) = \frac{(m_2 - m_0) \cdot 40 \cdot 100}{m_1 \cdot 25} \cdot \frac{100}{F} \tag{2}$$

2.7. Insolubility index

The loss of solubility or insolubility of rehydrated powders was determined as “insolubility index”, based on the IDF standard 129A (1988) with some modifications: 2.5 g of each powder were weighed in a beaker, followed by addition of 42.5 mL of demineralized water at 20 °C. To ensure complete dissolution of the powder and obtain a uniformly homogenized system, the mixture was mixed using a rotor-stator disperser (ULTRA-TURRAX T18, IKA, Staufen, Germany) at 10,000 rpm for 3 min at room temperature. Subsequently, 15 mL of the mixture were transferred into pre-weighed 15 mL centrifuge tubes. The mixture was then centrifuged at 1000 g for 15 min at 20 °C. The supernatant was carefully removed from the centrifuge tube and the remaining pellet was dried at 100 °C for 48 h to ensure the complete removal of water from the sediment. Insolubility was calculated based on total dry matter in the reconstituted emulsion.

2.8. Protein solubility

In addition to total insolubility, protein solubility was measured to specifically determine which components are driving powder solubility. Protein solubility was determined using the Dumas method, using a Dumatherm® (Gerhardt GmbH&Co. KG, Königswinter, Germany). The method is based on the combustion of the sample in an oxygen-rich environment, the consecutive reduction of nitrogen oxides to elemental nitrogen in a copper coil, and the measurement of the nitrogen content by a thermal conductivity detector [23].

Powders were reconstituted for 1 h in demineralized water at a protein content of 2% (w/w). An aliquot of this solution was measured directly, while the remaining solution was centrifuged at 5000 g for 10 min at room temperature in a MiniSpin centrifuge (Eppendorf AG, Hamburg, Germany). After centrifugation, the supernatant as well as the creamed lipid phase were decanted and recombined using a Vortex mixer, and the protein content of one aliquot of this mixture measured. Protein solubility was calculated by the quotient of total and soluble protein.

2.9. Contact angle

The contact angle of the powders, which is an important indicator of powder wetting properties [24] was measured before and after 16 weeks of storage. In order to ensure an even surface and constant roughness throughout all samples, 4.5 g of powder were pressed into tablets using a PWV 300 laboratory press (Paul-Otto Weber GmbH, Remshalden, Germany) at a pressure of 200 MPa. Contact angle was measured in triplicate by depositing a 10 μL drop of water on the tablet surface and recording a video with a high speed camera at a maximum frame rate of 25 frames per second. The dynamic contact angle was determined using DSA4 software (Krüss, Hamburg, Germany), using the Laplace–Young model. The last 100 points of each measurement were taken and averaged for data display.

2.10. Changes in protein chemistry and aggregation state

2.10.1. Free amino groups determination by OPA method

To detect any potential chemical modification of the pea proteins upon storage of the powder, the amount of available amino groups present on the protein was determined using a modification of the o-phthaldialdehyde (OPA) method described earlier by Church et al. [25]. The OPA determination was carried out by mixing 200 μL of the rehydrated powder dispersion (at a protein concentration of 1.5 g/L in 50 mmol/L sodium phosphate buffer, pH 7.8), 2 mL of a reducing solution (480 mg of N-acetyl-L-cystein dispersed in 200 mL of 0.1 mol L-1 sodium borate buffer, pH 9.3), and 50 μL of a 20% (w/w) SDS solution. After mixing and incubation during 10 min at 50 °C, 50 μL of the OPA reagent (prepared by dispersing 170 mg of OPA in 5 mL of methanol) was added. The mixture was incubated for 30 min at 50 °C and then cooled to room temperature within 30 min before reading the absorbance at 340 nm using a Thermo Nicolet Evolution 100 spectrophotometer (ThermoFisher Scientific, Steinhausen, Switzerland). The calibration curve was obtained from an L-leucine standard ranging from 0.125 to 2.5 mmol/L. All results are mean values resulting from duplicate measurements. Results are expressed as a percentage of the initial available amino groups in the sample. The following products and reagents were used: boric acid, N-acetyl-L-cystein, OPA, from Fluka (Chemie GmbH, Buchs, Switzerland); sodium dihydrogen phosphate monohydrate, methanol, and sodium hydroxide from Merck (Darmstadt, Germany); and L-leucine from Sigma (St. Louis, MO).

2.10.2. Protein molecular weight distribution determined by gel electrophoresis

The distribution of the molecular weights of the pea protein fractions upon storage of the powder was investigated by gel electrophoresis using colloidal blue staining. Samples were analyzed using NuPAGE Novex 12% Bis-Tris-acetate gels (ThermoFisher) having a molecular mass resolution comprised of 15 and 260 kDa in MOPS buffer. Sample protein concentration was 0.3 mg/mL in a Tris-SDS-urea dissociating buffer with or without reduction treatment. The later was achieved by adding 50 μL of sample buffer (LDS) and 10 μL of Nu-PAGE sample reducing agent (NP0004) to 50 μL of protein solution followed by incubation for 10 min at 70 °C. After incubation, the samples were cooled to 25 °C, and 10 μL of the solutions was loaded on the gel as well as a molecular standard (5 μL) of a reduced unstained electrophoresis calibration kit (Mark 12, ThermoFisher). The running buffer for migration, NuPage MOPS SDS Running Buffer (20×) (NP0001), was diluted 20 times with MilliQ water, and migration was performed at a constant voltage of 200 V, for 45–50 min. Samples were deposited at a protein content of 15 g and staining was achieved with a colloidal blue staining solution. Gels were digitized using an Epson scanner at a resolution of 400 dpi.

2.11. Imaging

2.11.1. Confocal laser scanning microscopy (CLSM)

The spacial distribution of proteins and lipids in the emulsions and reconstituted powders was investigated with fluorescence microscopy. Proteins and lipids were stained with Fast Green (FG) and Nile Red (NR) respectively (Sigma–Aldrich, Zwijndrecht, The Netherlands). First, 10 μL of FG solution (1 mg/mL water) and then 10 μL of NR solution (0.25 mg/mL ethanol) were added to 1 mL of emulsion or rehydrated powder sample. One drop of sample was observed on a glass slide with a LSM710 upright confocal microscope equipped with an Airyscan detector (Zeiss, Oberkochen, Germany) using Plan-APOCHROMAT objectives (10×/0.45, 20×/0.8, 63×/1.4) and 488 nm and 633 nm excitation laser lines for NR and FG, respectively. Images were analyzed with the ZEN imaging software (Zeiss, Oberkochen, Germany).

2.11.2. Scanning electron microscopy (SEM)

Surface lipids before and after storage were visualized by staining powders with osmium tetroxide (OsO_4), which binds to double bonds of unsaturated fatty acids and thus increases the density between the lipid and non-lipid areas. This leads to increased contrast and lets lipid-rich areas appear brighter on SEM micrographs. Powder was attached to an aluminum stud with a conductive carbon sticker. To expose the inner structure, samples were lightly tapped with a razor blade and then placed in a dessicator and exposed to OsO_4 vapor over night. Scanning Electron Microscopy (SEM) was conducted with a Quattro S microscope (Thermo Fisher, Waltham, MA, United states) at an accelerating voltage of 10 kV using a back-scattered electron detector. Charging artifacts were reduced by employing low-vacuum mode.

To gain insights into the structure of rehydrated particles after storage, samples were prepared following a protocol by Mimouni et al. [26], with some modifications: Powder samples were placed in filter paper sachets and reconstituted in deionized water (3× for 1 h each) to remove soluble components. Samples were fixed in 4% glutaraldehyde in phosphate-buffered saline (PBS) solution at 4 °C overnight, rinsed in water (3×), and dehydrated in graded ethanol solutions (30%, 50%, 70%, 90%, and 100% (3×)). Samples were dried using a CO_2 critical point drier (CPD 300, Thermo Fisher Scientific). Dried powders were mounted on aluminum stubs with conductive carbon stickers, sputter-coated with 6 nm of gold to reduce charging artifacts, and imaged using a Quattro S scanning electron microscope (Thermo Fisher Scientific) at an accelerating voltage of 5 kV under high vacuum.

2.12. Statistical evaluation

Results are reported as mean ± standard deviation unless stated otherwise. Statistical analysis of selected data sets was carried out using one-way Analysis of Variance (ANOVA) with $\alpha = 0.05$.

3. Results

3.1. Plant-based powder insolubility

Powder insolubility gradually increased for all investigated samples over the course of storage (Fig. 1). The initial insolubility of powders with the three different proteins increased from dairy<soy<pea in the range of 1.2 ± 0.1 to $2.4 \pm 0.1\%$. For all three different protein sources (pea, soy, dairy), a similar increase in insolubility occurred over the course of storage, with a minimum 20-fold increase after 16 weeks. The insolubility of pea-based powders prepared with and without an additional homogenization step was similar, whereas the pea-based powder prepared at pH 8.5 exhibited slightly higher insolubility. Interestingly, the insolubility of the stable oil sample was highest at the beginning of storage, but lower than the pea reference after 16 weeks. The lowest insolubility was recorded for samples with low protein and w/o oil.

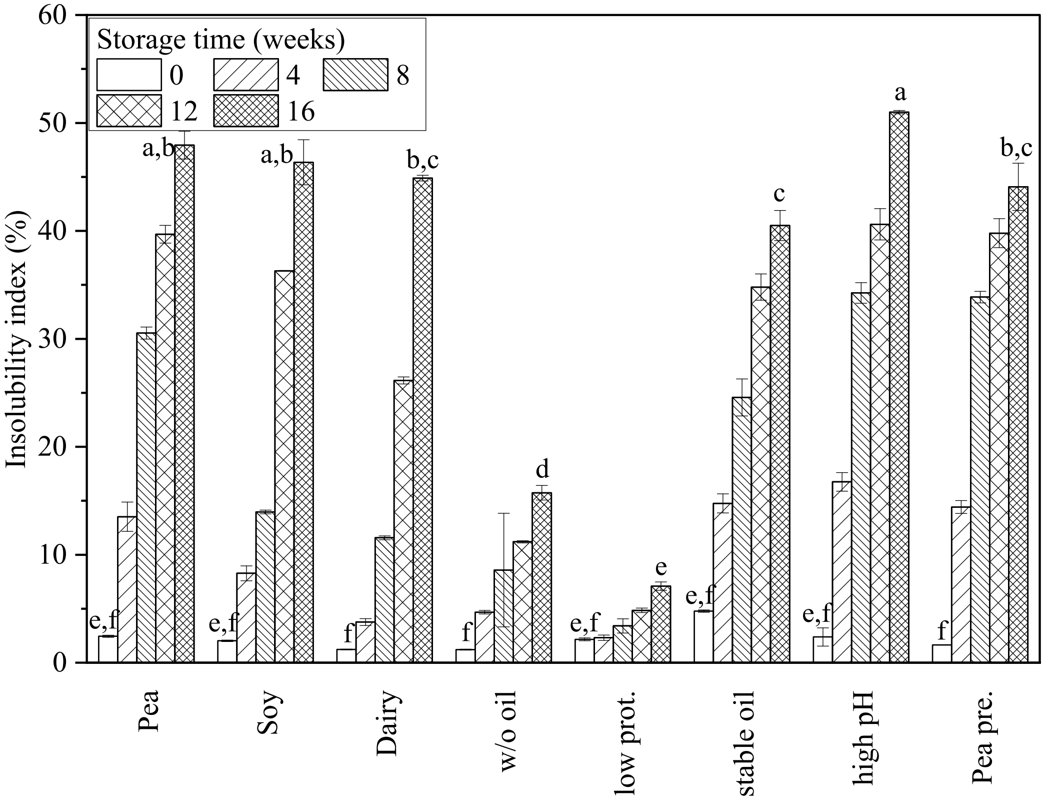


Fig. 1. Powder insolubility of different formulations before (week 0) and after 4, 6, 12 and 16 weeks of storage. Different letters indicate significant differences at 0.05 level.

Except for the low protein sample, percentage of insoluble mass was roughly equivalent to the quantity of non-maltodextrin components in the powder (protein and oil). However, for the low protein sample, the quantity of non-maltodextrin components was 30.4%, while the total insolubility at the end of storage was around 7%, notably lower than in powders with higher protein content.

3.2. Particle size analysis

$D_{3,2}$ values of the starting emulsions before spray-drying varied from 0.23 to 11.9 μm (Table 2), with samples containing MCT oil, high pH, and pre-homogenized protein exhibiting the largest values. However, since plant protein emulsions have a strong tendency to flocculate and form droplet clusters, interpreting the data remains challenging, as it cannot be guaranteed that only primary emulsion droplets were measured [27]. This issue becomes evident when comparing the particle sizes of rehydrated spray-dried powders at the beginning of storage (week 0), where $d_{3,2}$ and $d_{4,3}$ values were lower for said formulations compared to the starting emulsion, indicating that aggregated droplet clusters were broken up by the spray dryer nozzle during powder production [28,29]. Considering overall lowest measured $d_{3,2}$ values for all formulations, it can therefore be assumed that the primary droplet size was below 1 μm, with a greater tendency for aggregation in stable oil, high pH, and prehomogenized pea samples.

During storage, measured particle sizes of rehydrated powders increased across all samples. After 8 weeks of storage, only the dairy sample exhibited a $d_{3,2}$ below 5 μm, while after 16 weeks of storage, particle size further increased and even exceeded the size of primary particles after spray drying ($d_{3,2}$ = 18.3 ± 1.8 μm). These large particles may have resulted from either uneven dispersion in water prior to measurement or the formation of larger agglomerates. The lowest particle sizes at the end of storage were recorded for low protein and w/o oil samples, which only increased to 25.1 and 22.8 μm, respectively.

The gradual shift in the size distribution towards larger particles sizes can also be seen in the plotted volume distribution of the pea sample (Fig. 2). The size distribution of the starting emulsion was mostly monomodal, with a peak around 0.3 μm, likely representing the size of the primary oil droplets formed during emulsification. The extended tail of the distribution, which reached up to 12 μm, likely represents flocculated droplets and non-broken aggregates [30]. At the beginning of storage (week 0), this distribution becomes bimodal, with one peak remaining at 0.3 μm and a second one around 9 μm, possibly corresponding to large volume aggregates and droplet clusters. As storage progressed, the distribution further shifted towards larger particle sizes, culminating in a peak around 110 μm, with a broad span from 2–150 μm after 16 weeks.

Findings from particle size analysis are further supported by CLSM micrographs of rehydrated powders (Fig. 3). At the beginning of storage, all oil-containing samples successfully reconstituted back into an emulsion, with distinct oil droplets visible in the aqueous phase and no or few protein aggregates present. In contrast, the oil-free sample exhibited small dispersed protein aggregates, along with a few tiny oil droplets that likely remained in the protein isolate after extraction [31]. After 8 weeks of storage, low protein, dairy and soy powders showed strong aggregation in the hydrated state, and pea particles appeared to remain entirely intact, which is in accordance with results obtained from particle size analysis. After the 16 weeks storage period, no visual dissolution of primary spray-dried particles was observed across all protein sources (pea, soy, dairy). On the contrary, the particles remained distinguishable, with structural features like vacuoles visible. Some particles also appeared to be in an agglomerated state, which might explain the large particle sizes measured, which exceeded primary particle size. The same trends were observed in samples with stable oil, high pH, and pre-homogenized protein (Appendix A). The rehydrated state of these particles did not change over time, with no visual differences occurring after days or even weeks of dispersion in water. For low protein powders, however, no intact particles formed at

Table 2

$D_{3,2}$ and $d_{4,3}$ values of fresh emulsions of different formulations before spray-drying, and rehydrated powders at beginning (week 0), middle (week 8) and end (week 16) of storage.

Sample	$d_{3,2}$				$d_{4,3}$			
	Emulsion	Week 0	Week 8	Week 16	Emulsion	Week 0	Week 8	Week 16
Pea	0.23 ± 0	5.36 ± 3.5	22.9 ± 0.29	64.1 ± 1.27	0.66 ± 0.02	9.1 ± 2.65	41.7 ± 0.57	158 ± 3.71
Soy	0.24 ± 0	0.37 ± 0	10.2 ± 0.26	109 ± 2.08	2.22 ± 0.67	2.46 ± 0.1	18.2 ± 0.6	279 ± 7.16
Dairy	0.27 ± 0	0.21 ± 0.02	4.75 ± 0.13	126 ± 4.16	0.44 ± 0	0.98 ± 0.01	15.2 ± 0.25	340 ± 15.6
w/o oil	0.39 ± 0.02	0.15 ± 0.01	13.3 ± 0.84	22.8 ± 1.46	5.38 ± 0.8	5.41 ± 0.75	22.7 ± 1.71	133 ± 30.1
Low protein	0.74 ± 0.12	3.89 ± 0.09	15.7 ± 0.23	25.1 ± 0.07	3.05 ± 1.27	8.84 ± 0.59	26.3 ± 1.21	43.8 ± 1.11
Stable oil	10.1 ± 2.43	5.64 ± 8.71	14.1 ± 0.08	58.6 ± 9.72	12.5 ± 2.63	243 ± 24.3	31 ± 1.13	329 ± 11.9
High pH	8.54 ± 1.97	0.21 ± 0.02	15.8 ± 0.73	67.7 ± 6.41	10.7 ± 2.32	2.82 ± 0.28	33.1 ± 2.45	275 ± 23.1
Pea pre	11.9 ± 0.67	0.29 ± 0.09	18 ± 0.15	35.3 ± 0.67	14.4 ± 0.62	2.21 ± 0.64	39.1 ± 0.92	95.9 ± 5.12
Soy pre	0.28 ± 0	0.3 ± 0	11 ± 0.31	153 ± 5.79	0.67 ± 0.02	1.43 ± 0.1	23.3 ± 1.31	350 ± 9.38

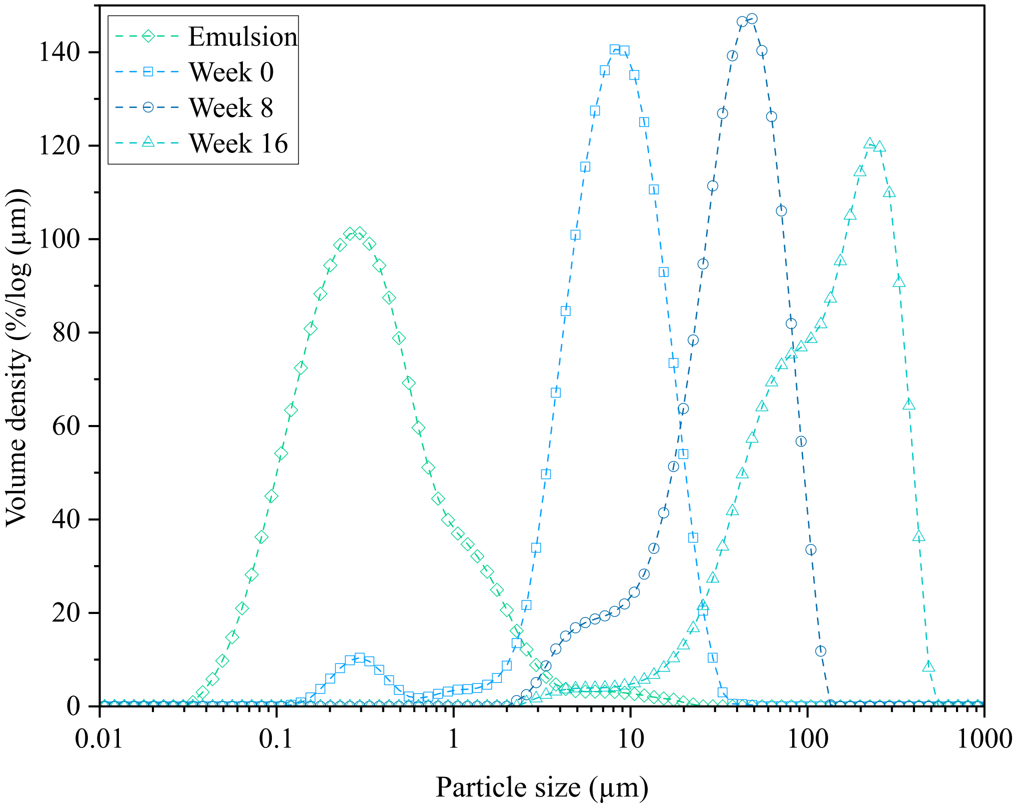


Fig. 2. Particle size distribution of emulsion before spray-drying and of reconstituted emulsion at beginning (week 0), middle (week 8) and end (week 16) of storage of pea sample.

the end of storage, but rather large protein aggregates with coalesced droplets, while for sample w/o oil, no visual changes from start to end of storage occurred, despite an increase in insolubility, suggesting a certain degree of aggregation.

3.3. Protein solubility

In contrast to total insolubility, protein solubility specifically examines changes in the solubility of nitrogen components. Protein solubility of samples over the course of storage is displayed in Fig. 4. Initial solubility at the beginning of storage (week 0) varied between 76.8 and 92.2%, where solubility for different protein sources increasing from pea to dairy to soy. Pre-homogenization slightly increased initial solubility of pea samples from 82.9 to 88.2%, which is in accordance with insolubility data (3.1). Except for low protein sample, protein solubility decreased during storage. For low protein samples, error bars are very large due to the low concentration of protein in the sample, which was close to the detection limit. After the low protein sample, the highest solubility after 16 weeks of storage was found in the sample w/o oil (44.7%), while all remaining samples were below 30%.

3.4. Free fat

The free fat content of samples at the beginning (week 0) and after 4, 8, 12 and 16 weeks of storage is summarized in Fig. 5. Since no fat was added to the samples w/o oil, they were excluded from this analysis. Initial free fat content varied from 2.6 to 13.5% of the total fat. For all samples containing sunflower oil, the measured free fat content dropped drastically after 16 weeks of storage to values as low as 0.7 to 3.1%, while for stable oil, on the other hand, an increase from 6.0 to 8.9% was measured.

Fig. 6 shows OsO_4 stained samples of pea powder before and after 16 weeks of storage. Before storage, large patches of free fat were visible, accumulating on the particle surface as well in fine wrinkles. Encapsulated lipid droplets are likewise visible in the inside of open particles. After storage however (Fig. 6 b)), particle surfaces appear dark with only very few light spots, while encapsulated lipid droplets remain easily recognizable. OsO_4 binds to the double bounds of unsaturated fatty acids. The almost complete disappearance of OsO4 binding

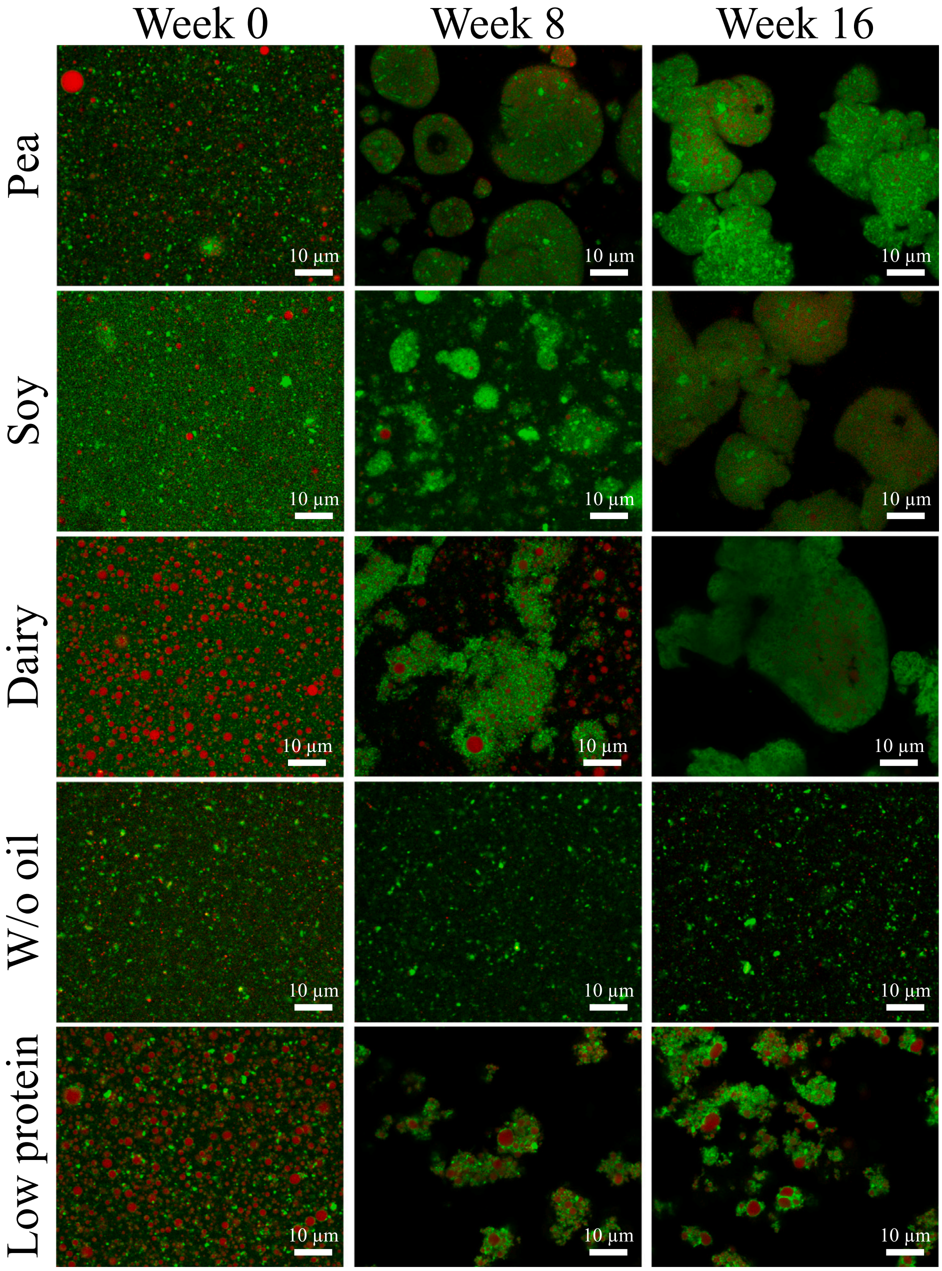


Fig. 3. CLSM images of reconstituted pea, soy, dairy, w/o oil and low protein emulsions at the beginning (week 0), middle (week 8) and end (week 16) of storage. Proteins are visualized in green, lipids are represented in red.

sites from the particle surface indicates a loss of exposed double bonds in this area.

3.5. Powder contact angle

Contact angle measurements of powders before and after storage are presented in Fig. 7. Lowest initial contact angle (25°) was observed for powder with low protein, while all other samples were in the range of 40 ± 3°, confirming that protein content is an important driver of powder hydrophobicity. Contact angle increased over storage, independent of sample formulation. For all samples containing sunflower oil, contact angle increased for at least 75% during storage, whereas the sample with MCT oil showed a lower increase of 56%. Lowest increase in contact angle was recorded for powder w/o oil. An increase of powder hydrophobicity is associated with slower water penetration into the bulk and particle pores, ultimately hindering powder rehydration [32]. The drastic changes in powder hydrophobicity can also be seen by a simple wetting experiment, in which powder is deposited on a water surface and monitored over time (Fig. 7 a and b).

3.6. Changes in protein chemistry and aggregation state

To investigate changes in the particle structure of insoluble particles, pea powder was re-dried after reconstitution according to the procedure described in Section 2.11. Fresh powder was imaged without a prior dissolution step since it would have disintegrated entirely during the reconstitution step. Images of powder before storage and after storage and reconstitution are displayed in Fig. 8. Unwashed powder before storage exhibited a smooth surface with wrinkles, whereas the inside of the particles was composed of a dense matrix in which oil droplets as well as small air enclosures were embedded. Washed powder after storage is still appearing as individual particles that

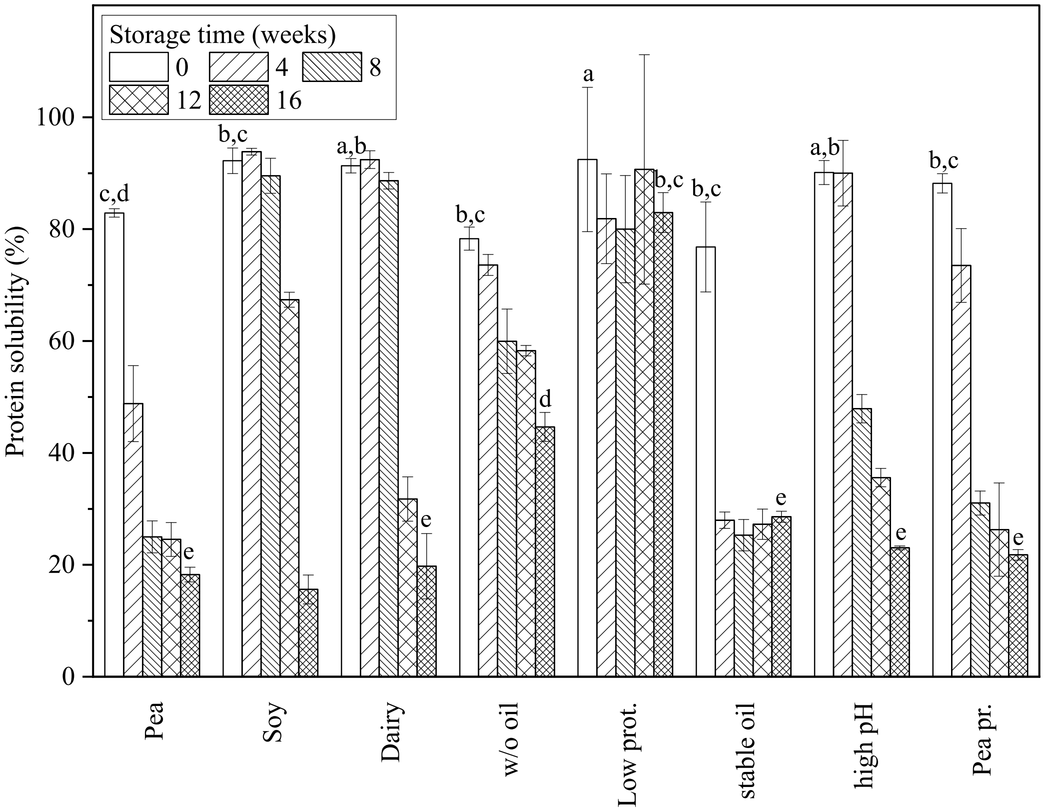


Fig. 4. Protein solubility of different formulations before (week 0) and after 4, 8, 12, and 16 weeks of storage. Different letters indicate significant differences at 0.05 level.

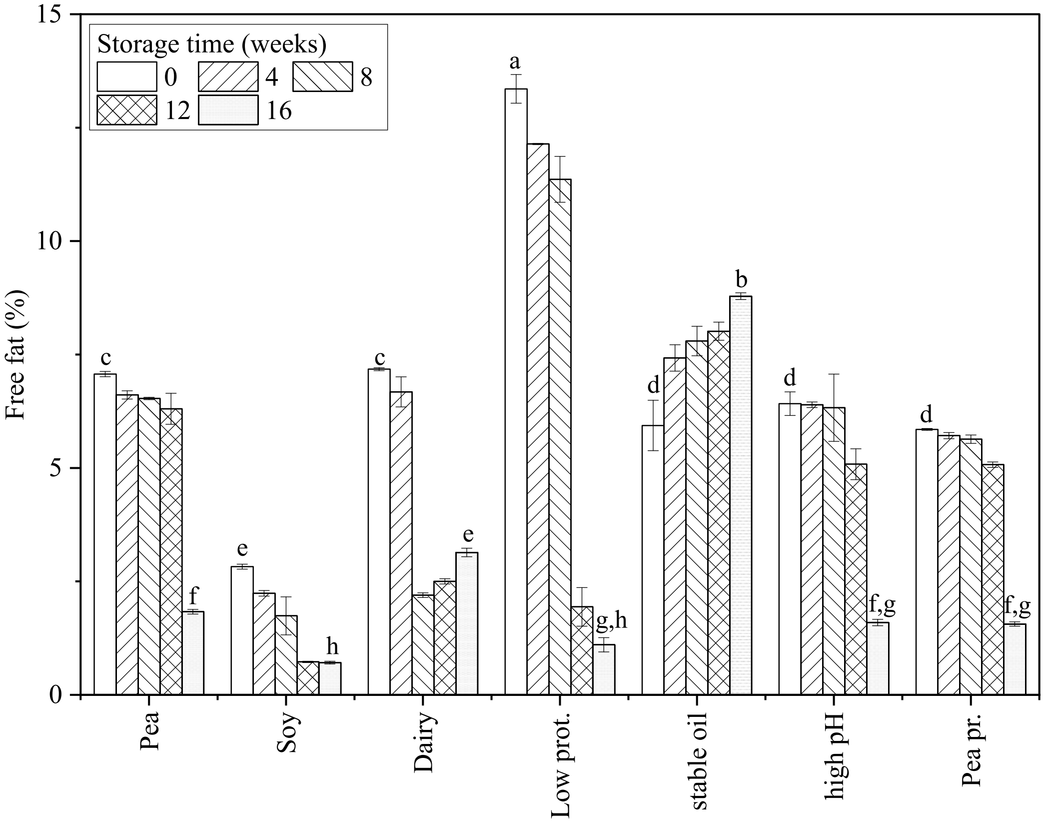


Fig. 5. Free fat content of different formulations before (week 0) and after 4, 8, 12, and 16 weeks of storage. Different letters indicate significant differences at 0.05 level.

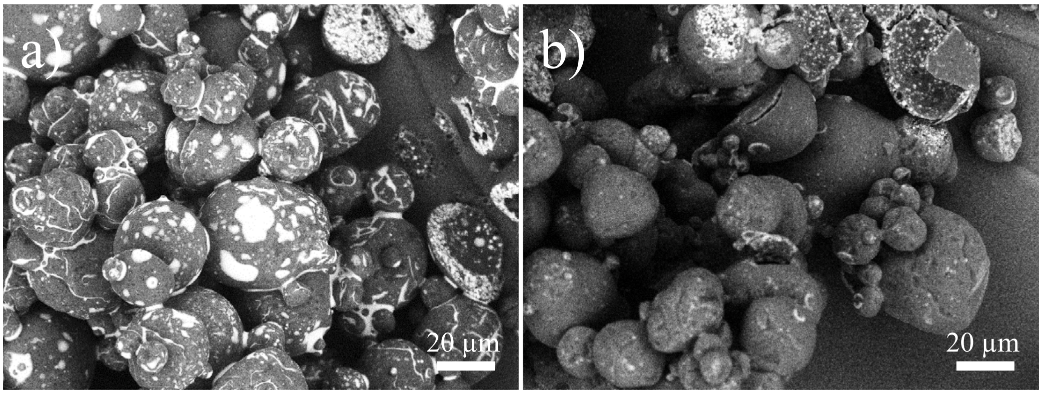


Fig. 6. SEM images of OsO_4 stained pea powder, before (a)) and after 16 weeks (b)) storage. Lighter areas mark an enrichment of unsaturated lipids.

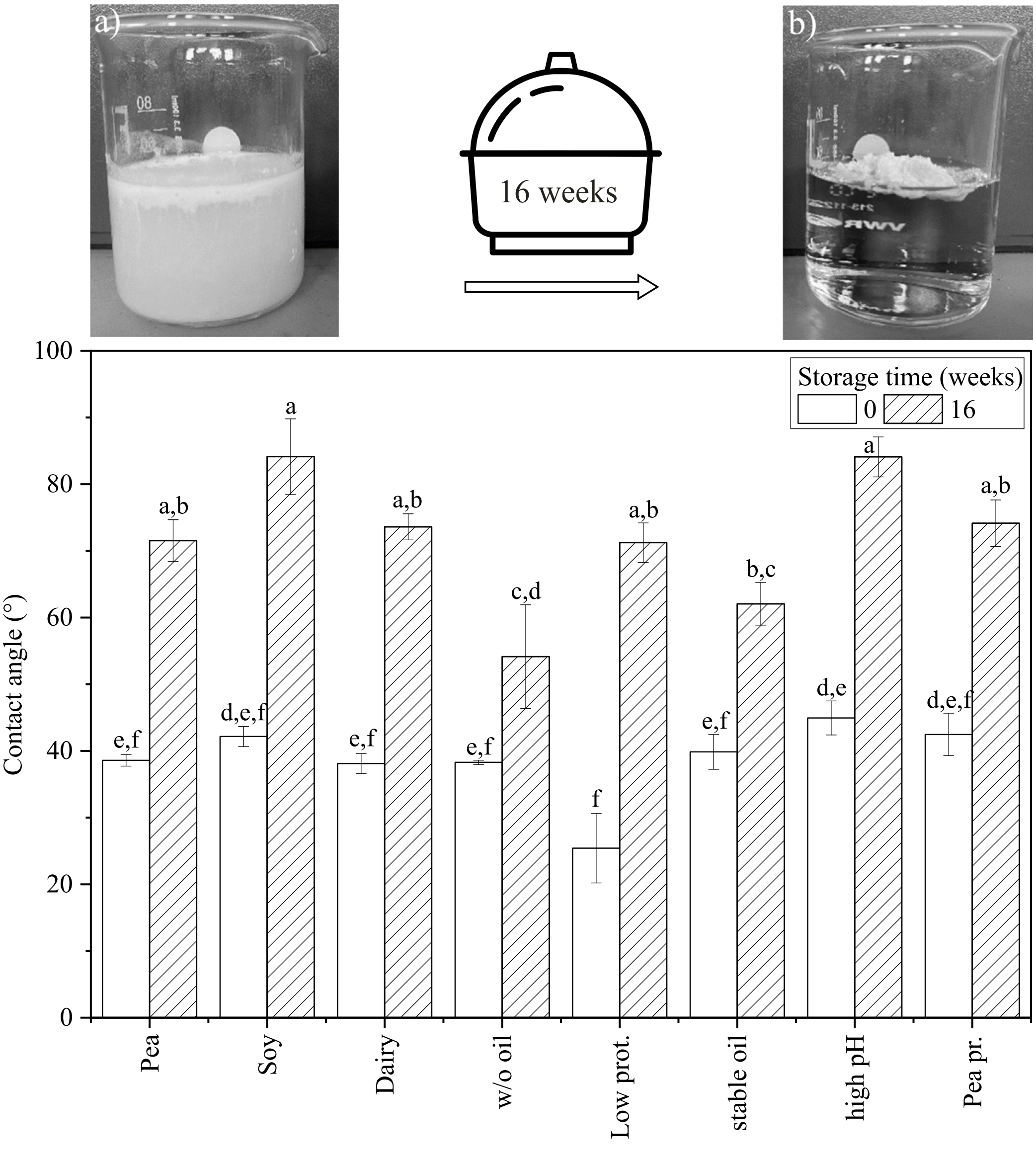


Fig. 7. Contact angle of water on powder tablets of different formulations before (week 0) and after (week 16) storage and powder wetting of pea sample before (a) and after (b) storage. Depicted contact angle values are averages of the last 100 data points of the measurement. Different letters indicate significant differences at 0.05 level.

maintained their integrity throughout the rehydration process as also seen in CLSM images. The particle surface as well as the particle inside were dominated by open pores, forming a highly porous, sponge-like structure. Measurement of the Brix value of the supernatant of

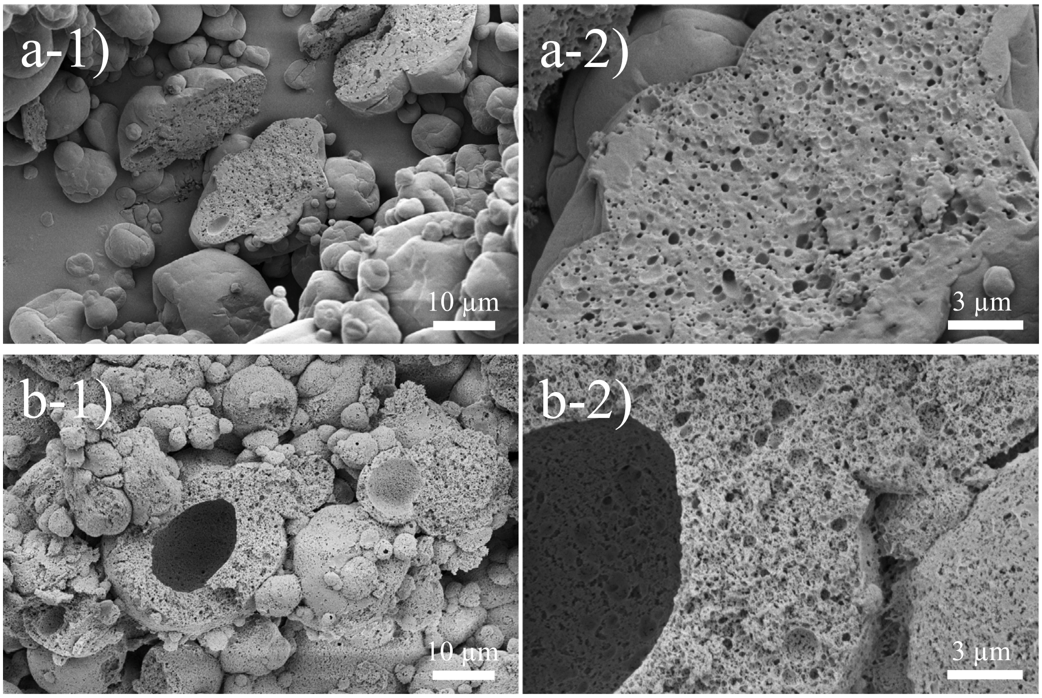


Fig. 8. A-1 - a-2: SEM images of the microstructure of initial powder (pea) before storage at different magnifications. B-1 - b-2: SEM images of the microstructure of rehydrated critical point-dried powder (pea) after 16 weeks of storage at different magnifications.

reconstituted and centrifuged pea sample after 16 weeks of storage gave a similar value compared to a solution of pure maltodextrin at same concentration as in the powder (5.71 and 5.17, respectively). This suggests that the insoluble matter was primarily composed of proteins with entrapped lipid droplets, while maltodextrin remained extractable, leading to an insoluble skeletal structure.

Free amino groups of pea reference powder decreased from 4.74 ± 0.27% at the beginning of storage (week 0) to 4.57 ± 0.68% (week 8) and further to 3.29 ± 0.44% after 16 weeks of storage, which signifies a reduction of 30% during 16 weeks of storage. Decrease in free amino groups is typically associated with either protein polymerization or the glycation of proteins with reducing sugars, as can be the case with some fractions present in the maltodextrin used in this study. From SDS-PAGE analysis (Fig. 9), an increase in accumulated material at the top of the well was observed over the course of storage in unreduced state, alongside fading bands in the lower region, confirming that some of the protein fractions initially present in the isolate increased in molecular weight and became insoluble [33]. In the reduced state, no strong differences between samples were observed, indicating partial polymerization of the protein via covalent bonds sensitive to chemical reduction, likely disulfide bonds.

4. Discussion

In the following, the impact of proteins, lipids, and processing conditions on the evolution of insolubility is discussed, based on the obtained results of the different sample formulations.

4.1. Impact of protein

4.1.1. Protein source

In the frame of this study, the impact of three different protein sources on the formation of insoluble particles was investigated. According to the presented data, no significant differences were detected between formulations with different protein sources when they were combined with the same oil and produced using the same homogenization protocol. Although the formation of insoluble particles seemed slightly slower for soy and dairy powders according to CLSM images, there were no strong differences in solubility and appearance of insoluble particles in reconstituted samples at the end of storage. Pea and soy protein are both mainly globular legume proteins with similar structural features, therefore, no major deviations between samples were expected. The high state of denaturation of plant proteins through their extraction process in addition to their high hydrophobicity makes legume proteins prone to aggregation and destabilization [2,34]. As this strong processing impact is characteristic of protein isolates, the behavior of less refined ingredients, such as protein concentrates or whole flours, in such systems might deviate. Guo et al. [20] investigated the storage stability of soy protein isolate powders and observed a decrease in protein solubility, as well as increased protein oxidation over the course of storage. They further observed that soluble aggregates present in fresh powder could be further transformed into insoluble aggregates during storage and thus reduce the powder quality. It is, however, surprising that changes in the dairy powder did not differ significantly from their plant-based counterparts. Despite the fact that insolubility in dairy milk powders has been intensively studied in recent decades, solubility losses were mostly described as large aggregates and other artifacts, such as white spots. Particles seemingly unaffected by the rehydration process, as in the present study, have only been seen in pure protein powders such as micellar casein powders [19,26]. Even though the composition of macronutrients in the formulations in this study is based on milk powder composition, different lipids and carbohydrates were used. In particular, sunflower oil is much more unstable and susceptible to oxidation than milk fat. Rapid oxidation of sunflower oil may increase co-oxidation with proteins and induce conformational changes in the protein structure, leading to the formation of an insoluble network. Unsaturated lipids may also promote protein–protein interactions and thus aggregation in both plant and dairy proteins [35].

4.1.2. Protein content

The superior solubility of low protein powder throughout storage in comparison to the other powders, makes it evident that changes in the protein structure must be highly relevant for the formation of insoluble

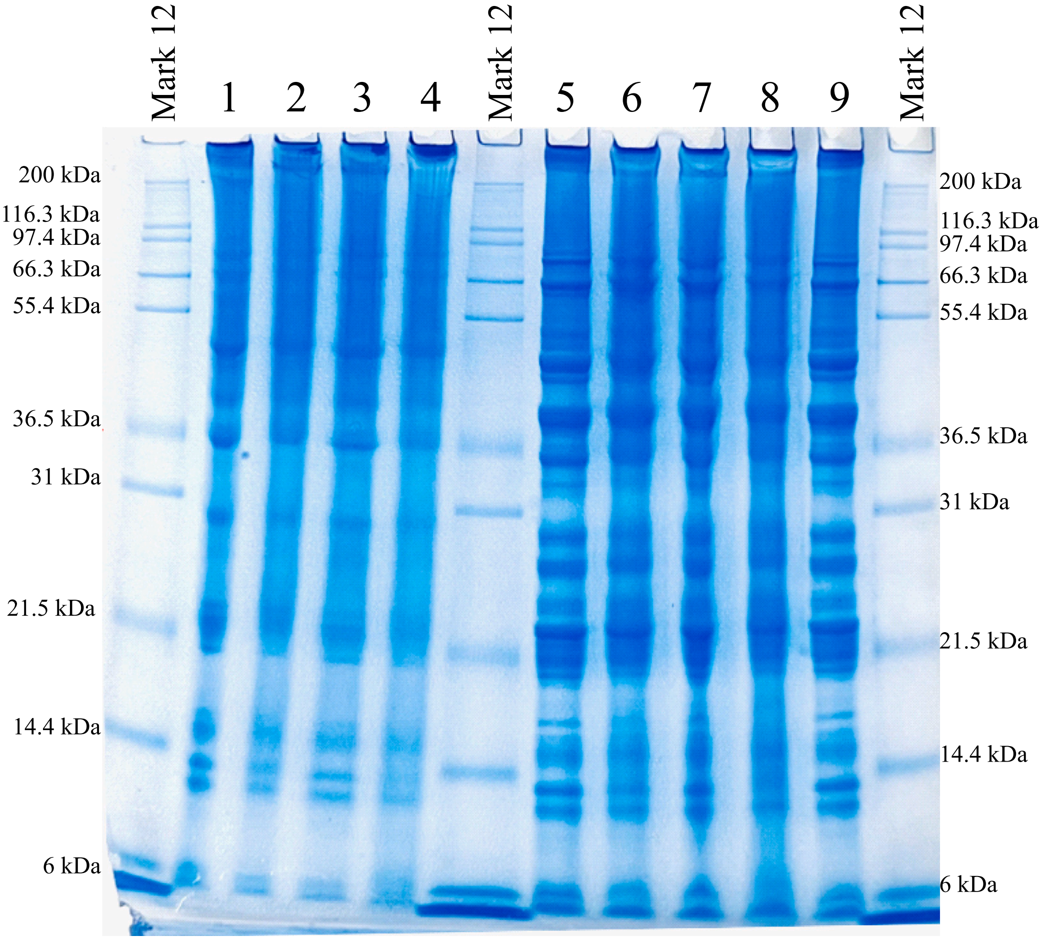


Fig. 9. SDS-PAGE of pea sample in non-reduced (1–4) and reduced state (5–9), after 0 (2,6), 8 (3,7) and 16 (4,8) weeks of storage compared to pure pea protein isolate (1,5,9).

particles and that a sufficient amount of protein is needed to build a continuous structure. The foam-like structure formed by insoluble samples with 20% protein at the end of storage (Fig. 8) strongly resembles a microgel structure, that is, a stable polymer network, cross-linked by covalent bonds or other strong intramolecular forces [36]. To form this stable network, a critical concentration of protein is needed in order to facilitate interactions between individual protein aggregates. A concentration of 2.4% of protein appears to be insufficient in this case, leading to single aggregates that fail to form a continuous network within the time frame of this study (Fig. 3). Changes in protein structure and protein–protein interactions during processing can be induced by several reactions, including heat, pH, and ionic strength. During storage at low water activity, changes appear much slower and are typically induced by chemical reactions such as oxidation and Maillard reaction [37].

From the reduction of free amino groups in the pea sample, as well as from SDS-PAGE, it can be assumed that increased glycation likewise induced changes in the protein structure of plant-based systems, possibly leading to the formation of disulfide bonds [38]. A lower amount of protein therefore reduces this effect and thus preserves initial powder properties. In addition to the Maillard reaction, protein oxidation may be one of the main mechanisms triggering protein–protein interactions and cross-linking during storage. Like lipid oxidation, protein oxidation is induced by a radical reaction, which makes it susceptible to co-oxidation with lipids and potentially accelerates due to rapid lipid oxidation [39,40]. However, samples with non-oxidizable MCT oil have undergone the same level of insolubility as samples with sunflower oil. This might be related to residual lipids remaining in pea protein isolate after purification, which can reach levels as high as 9% according to supplier information and is visible on CLSM images (Fig. 3). The oxidation of these residual lipids might suffice to induce oxidation and conformational changes in the protein structure [41].

According to [42], a high initial degree of denatured protein results in stronger exposure of sulfhydryl groups, which induces aggregate formation. Since plant proteins such as pea or soy protein undergo strong denaturation during their extraction process, this might also explain why insolubility is strongly pronounced in plant-based samples. From Fig. 8 it can be seen that a strong protein network is formed, from which water-soluble carbohydrates can still be extracted by water.

4.2. *Impact of lipids*

4.2.1. *Lipid type*

Generally, the reduction of free fat during storage is uncommon, as most studies report an increase in free fat due to the rearrangement of internal pores and the displacement of liquid lipids towards the particle surface [9,43–45]. This observation was likewise made in powders containing MCT oil, where free fat increased within a range similar to that observed in other studies [43]. The restructuring of the porous network should, however, not differ from powders containing sunflower oil, yet completely opposite trends have been observed. One potential explanation for the reduction of free fat during storage is the formation of an insoluble lipid-protein complex through mechanisms such as hydrophobic interactions, which could render lipids unextractable by solvents like heptane [46,47]. This mechanism may depend on lipid polarity, thus accounting for the differences between sunflower oil and

MCT oil samples. More plausibly, however, the reduction in free fat could result from polymerization of sunflower oil due to extensive oxidation on the particle surface. Storage of pure sunflower oil for one year has led to pronounced textural changes, resulting in a honey-like viscosity which may be linked to polymerization as a result of lipid oxidation (Appendix B) [48]. Even though the present powders were only stored for four months, the increased surface area of free fat can accelerate oxidation and polymerization compared to bulk oil [22]. The phenomenon of free radical polymerization is already well known and used specifically in other applications such as oil paint [49], but has yet to receive much attention in the food industry. Furthermore, it was found that this polymerized oil exhibited limited solubility in heptane when processed with the same protocol used for free fat (Appendix B). These conformational changes of polymerized lipids can further explain why free fat is no longer visible in osmium-stained images (Fig. 6). The polymerized oil will consequently be tightly bound to the particle surface, forming a viscous film, making it highly hydrophobic and preventing powder wetting as well as hindering the penetration of water into the particle pores. This is reflected in contact angle data, where a higher contact angle was recorded after storage for sunflower oil samples than for non-oxidizable MCT oil samples, from which lipids remained extractable.

Even though water penetration into the particle was likely slowed down by polymerized fat on the surface, insolubility also increased in powder with MCT oil, albeit with slightly lower values, which is likely explained by the maintained dispersibility of non-polymerized free fat after storage. This means that additional mechanisms other than free fat polymerization must be driving powder insolubility, such as changes related to protein structure. From contact angle data (Fig. 7), it becomes clear that powder hydrophobicity increased irrespective of oil type or even presence of oil, although to a lesser extent.

4.2.2. Presence of lipids

From CLSM images (Fig. 3) it can be observed that the sample w/o oil underwent the least visual changes from beginning to end of storage, except for the low protein sample, even though both insolubility and protein solubility markedly changed over the course of storage (Fig. 1, Fig. 4). This indicates that protein–lipid interactions must still be relevant for the formation of an insoluble network. When proteins adsorb to the oil-water interface during emulsification, they are assumed to change their orientation due to the more hydrophobic environment, which exposes hydrophobic regions and facilitates protein–protein interactions as well as the formation of an interfacial protein film [35,50]. The magnitude of these interactions depends on various factors, including processing conditions, the type of lipid, and the type of protein [51]. Using unsaturated lipids like sunflower oil can enhance both protein–lipid and protein–protein interactions and lead to the exposure of more hydrophobic groups compared to using milk fat [52,53]. Chen et al. [54] reported that an exposure of hydrophobic groups and enhanced aggregation of pea proteins occurred in the presence of fatty acids during high-moisture extrusion. The absence of lipids would therefore reduce protein–protein interactions or, rather, foster the formation of smaller individual aggregates instead of a continuous network as seen in Fig. 8.

4.3. Impact of processing conditions (pH, HPH)

Neither an increase in pH nor the addition of an additional homogenization step applied to the protein fraction led to any improvement in powder solubility throughout storage. On the contrary, while protein solubility slightly increased in the high pH sample, overall insolubility and contact angle were likewise increased at the end of storage. Increasing pH from 7 to 9 can generally increase the solubility of spray-dried pea protein, while surface hydrophobicity simultaneously increases [55]. However, the exposure of hydrophobic regions can, in turn, reinforce hydrophobic interactions and the formation of insoluble aggregates [34,56]. Guo et al. [20] further postulated that a higher pH led to increased protein aggregation and solubility loss in soy protein powders over the course of storage. The authors attributed these changes to the formation of disulfide bonds and hydrophobic aggregation. Additionally, increased pH can decrease the number of protonated amino groups, making them more reactive toward glycation [57].

Even though protein solubility of the pre-homogenized sample was slightly higher than that of the reference pea sample, solubility decreased at a similar rate and particles likewise became visually insoluble at the end of storage. It is likely that homogenization of the entire emulsion in two passages was already sufficient to break up aggregates, so structural differences were only minor when protein and water were additionally homogenized before emulsification. Furthermore, despite the improvement in functionality through HPH, proteins are not converted back to their native state, and their functionality remains impaired [58]. It may be more useful to compare the current results with a protein that was undergone less intense processing steps, such as a protein concentrate or flour.

5. Conclusion

This study highlights the critical role of proteins and lipids in the formation of insoluble aggregates of plant-based emulsion powders during storage. Despite variations in protein source and processing conditions, all formulations exhibited notable loss of solubility, leading to an insoluble fraction of 7 to 51% after 16 weeks of storage. The findings suggest that protein structure and interfacial film formation play a vital role in governing solubility throughout storage. A high protein content in the formulations led to the formation of a robust microgel network during storage that resisted the rehydration process, whereas a low protein content in powders led to the formation of large aggregates and coalesced oil droplets. While the introduction of non-oxidizable MCT oil mitigated some adverse effects, oxidative degradation of surface lipids further decreased the wetting behavior of sunflower oil-based formulations, likely through lipid polymerization. Surprisingly, no significant differences were found between plant-based and dairy systems, underscoring the importance of understanding protein denaturation and aggregation phenomena during storage. The insights gained lay the groundwork for future innovations in creating plant-based powders with enhanced solubility, offering potential pathways to develop high-quality, sustainable food systems aligned with evolving consumer preferences.

CRediT authorship contribution statement

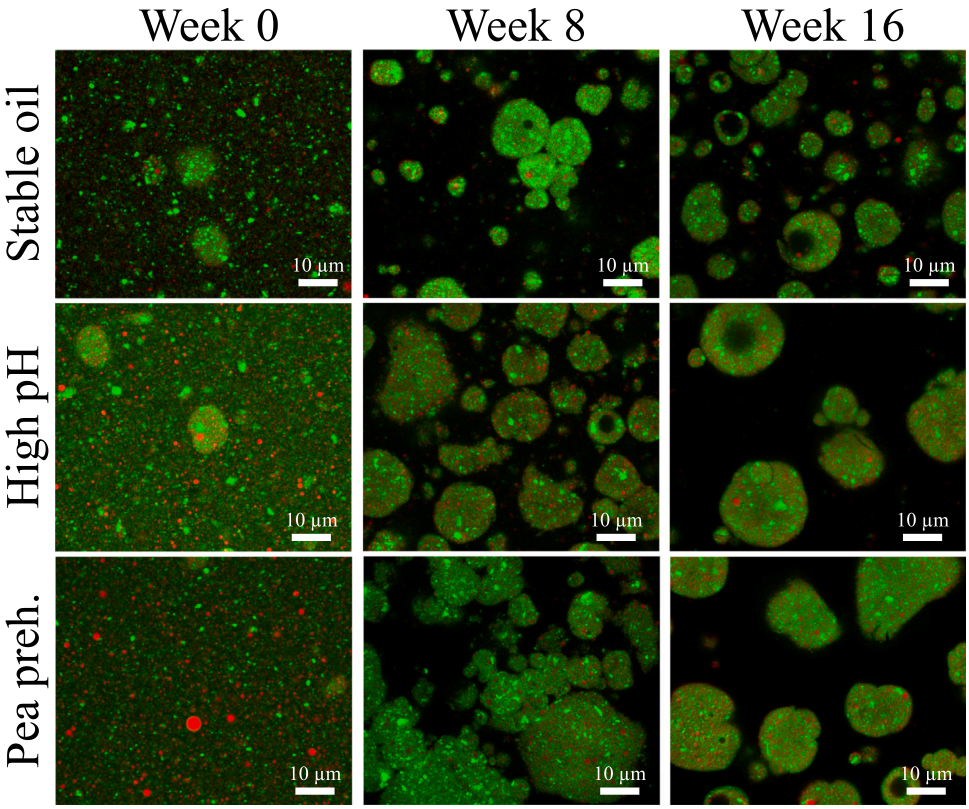
T. Kurtz: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **K. Haas:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **C. Schmitt:** Writing – review & editing, Methodology, Investigation. **C. Morgenegg:** Methodology, Investigation. **V. Meunier:** Writing – review & editing, Supervision, Project administration. **S. Heinrich:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

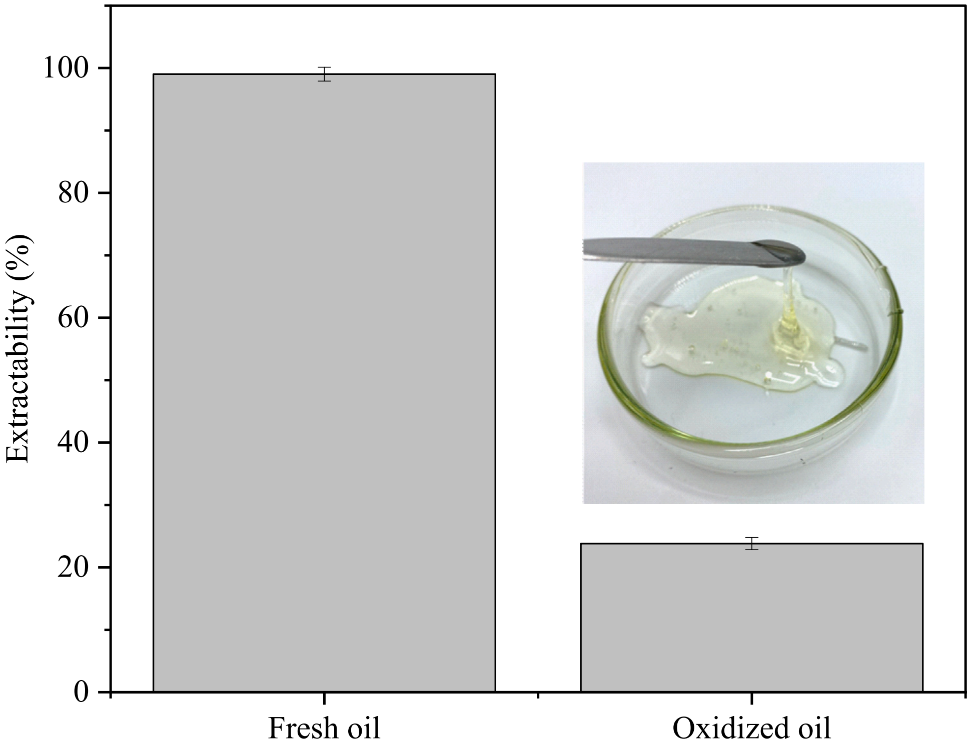
Declaration of competing interest

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.

Acknowledgments

The authors would like to thank Josep Busom Descarrega, Fikrey Andetsion, Claudine Bovay from Nestlé Research Lausanne for SEM imaging, SDS-PAGE and OPA measurements and Sweta Shamria for her support in sample analysis. The authors also thank Prof. Stefan Drusch and Marina Eichhorn from the Department of Food Technology and Food Material Science at Technische Universität Berlin for their support with protein solubility analysis.





Appendix A. CLSM images of powders with stable oil, high pH, and prehomogenized pea protein after 0, 8 and 16 weeks of storage.

See the above image.

Appendix B. Heptane solubility of fresh and oxidized sunflower oil

See the above image.

Data availability

Data will be made available on request.

References

[1] Statista, Dairy products: worldwide production by category 2023 | Statista, 2024, https://www.statista.com/statistics/869510/global-dairy-production-by-category/.

[2] C. Schmitt, L. Bovetto, J. Buczkowski, G. de Oliveira Reis, P. Pibarot, L. Amagliani, J. Dombrowski, Plant proteins and their colloidal state, Curr. Opin. Colloid & Interface Sci. 56 (2021) 101510, http://dx.doi.org/10.1016/j.cocis.2021.101510.

[3] R.S.H. Lam, M.T. Nickerson, Food proteins: a review on their emulsifying properties using a structure-function approach, Food Chem. 141 (2) (2013) 975–984, http://dx.doi.org/10.1016/j.foodchem.2013.04.038.

[4] W. Kim, Y. Wang, C. Selomulya, Dairy and plant proteins as natural food emulsifiers, Trends Food Sci. Technol. 105 (2020) 261–272, http://dx.doi.org/10.1016/j.tifs.2020.09.012.

[5] L. Day, J.A. Cakebread, S.M. Loveday, Food proteins from animals and plants: Differences in the nutritional and functional properties, Trends Food Sci. Technol. 119 (2022) 428–442, http://dx.doi.org/10.1016/j.tifs.2021.12.020.

[6] L. Forny, A. Marabi, S. Palzer, Wetting, disintegration and dissolution of agglomerated water soluble powders, Powder Technol. 206 (1–2) (2011) 72–78, http://dx.doi.org/10.1016/j.powtec.2010.07.022.

[7] Y. Fang, C. Selomulya, S. Ainsworth, M. Palmer, X.D. Chen, On quantifying the dissolution behaviour of milk protein concentrate, Food Hydrocolloids 25 (3) (2011) 503–510, http://dx.doi.org/10.1016/j.foodhyd.2010.07.030.

[8] V.L. Cenini, L. Gallagher, G. McKerr, N.A. McCarthy, D.J. McSweeney, M. Auty, B. O'Hagan, A novel approach for dynamic in-situ surface characterisation of milk protein concentrate hydration and reconstitution using an environmental scanning electron microscope, Food Hydrocolloids 108 (2020) 105881, http://dx.doi.org/10.1016/j.foodhyd.2020.105881.

[9] E.H.-J. Kim, X.D. Chen, D. Pearce, Surface composition of industrial spray-dried milk powders. 3. Changes in the surface composition during long-term storage, J. Food Eng. 94 (2) (2009) 182–191, http://dx.doi.org/10.1016/j.jfoodeng.2008.12.001.

[10] K.N. Fyfe, O. Kravchuk, T. Le, H.C. Deeth, A.V. Nguyen, B. Bhandari, Storage induced changes to high protein powders: influence on surface properties and solubility, J. Sci. Food Agric. 91 (14) (2011) 2566–2575, http://dx.doi.org/10.1002/jsfa.4461.

[11] E. Haque, B.R. Bhandari, M.J. Gidley, H.C. Deeth, A.K. Whittaker, Ageing-induced solubility loss in milk protein concentrate powder: effect of protein conformational modifications and interactions with water, J. Sci. Food Agric. 91 (14) (2011) 2576–2581, http://dx.doi.org/10.1002/jsfa.4478.

[12] S. Nasser, A. Moreau, R. Jeantet, A. Hédoux, G. Delaplace, Influence of Storage Conditions on the Functional Properties of Micellar Casein Powder, vol. 106, 2017, http://dx.doi.org/10.1016/j.fbp.2017.09.004.

[13] J. Burgain, R. El Zein, J. Scher, J. Petit, E.-A. Norwood, G. Francius, C. Gaiani, Local modifications of whey protein isolate powder surface during high temperature storage, vol. 178, 2016, http://dx.doi.org/10.1016/j.jfoodeng.2016.01.005.

[14] T.T. Le, J.W. Holland, B. Bhandari, P.F. Alewood, H.C. Deeth, Direct Evidence for the Role of Maillard Reaction Products in Protein Cross-Linking in Milk Powder During Storage, vol. 31, 2013, http://dx.doi.org/10.1016/j.idairyj.2013.02.013.

[15] E.-A. Norwood, S. Pezennec, J. Burgain, V. Briard-Bion, P. Schuck, T. Croguennec, R. Jeantet, C. Le Floch-Fouéré, Crucial role of remaining lactose in whey protein isolate powders during storage, J. Food Eng. 195 (2017) 206–216, http://dx.doi.org/10.1016/j.jfoodeng.2016.10.010.

[16] N. Yazdanpanah, T.A. Langrish, Comparative study of deteriorative changes in the ageing of milk powder, J. Food Eng. 114 (1) (2013) 14–21, http://dx.doi.org/10.1016/j.jfoodeng.2012.07.026.

[17] T. Cucu, B. Devreese, F. Mestdagh, B. Kerkaert, B. de Meulenaer, Protein–lipid interactions during the incubation of whey proteins with autoxidizing lipids, Int. Dairy J. 21 (6) (2011) 427–433, http://dx.doi.org/10.1016/j.idairyj.2011.01.003.

[18] F. Mestdagh, B. Kerkaert, T. Cucu, B. de Meulenaer, Interaction between whey proteins and lipids during light-induced oxidation, Food Chem. 126 (3) (2011) 1190–1197, http://dx.doi.org/10.1016/j.foodchem.2010.11.170.

[19] J. Burgain, J. Scher, J. Petit, G. Francius, C. Gaiani, Links between particle surface hardening and rehydration impairment during micellar casein powder storage, Food Hydrocolloids 61 (2016) 277–285, http://dx.doi.org/10.1016/j.foodhyd.2016.05.021.

[20] F. Guo, L. Lin, Z. He, Z.-P. Zheng, Storage stability of soy protein isolate powders containing soluble protein aggregates formed at varying pH, Food Sci. Nutr. 8 (10) (2020) 5275–5283, http://dx.doi.org/10.1002/fsn3.1759.

[21] Q. Zhao, J. Lin, C. Wang, L. Yousaf, Y. Xue, Q. Shen, Protein structural properties and proteomic analysis of rice during storage at different temperatures, Food Chem. 361 (2021) 130028, http://dx.doi.org/10.1016/j.foodchem.2021.130028.

[22] A. Sarkar, J. Arfsten, P.-A. Golay, S. Acquistapace, E. Heinrich, Microstructure and long-term stability of spray dried emulsions with ultra-high oil content, Food Hydrocolloids 52 (2016) 857–867, http://dx.doi.org/10.1016/j.foodhyd.2015.09.003.

[23] S. Jung, D.A. Rickert, N.A. Deak, E.D. Aldin, J. Recknor, L.A. Johnson, P.A. Murphy, Comparison of kjeldahl and dumas methods for determining protein contents of soybean products, J. Am. Oil Chemists' Soc. 80 (12) (2003) 1169, http://dx.doi.org/10.1007/s11746-003-0837-3, arXiv:https://aocs.onlinelibrary.wiley.com/doi/pdf/10.1007/s11746-003-0837-3.

[24] M. Lazghab, K. Saleh, I. Pezron, P. Guigon, L. Komunjer, Wettability assessment of finely divided solids, Powder Technol. 157 (1–3) (2005) 79–91, http://dx.doi.org/10.1016/j.powtec.2005.05.014.

[25] F.C. Church, H.E. Swaisgood, D.H. Porter, G.L. Catignani, Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins, J. Dairy Sci. 66 (6) (1983) 1219–1227, http://dx.doi.org/10.3168/jds.S0022-0302(83)81926-2.

[26] A. Mimouni, H.C. Deeth, A.K. Whittaker, M.J. Gidley, B.R. Bhandari, Investigation of the microstructure of milk protein concentrate powders during rehydration: alterations during storage, J. Dairy Sci. 93 (2) (2010) 463–472, http://dx.doi.org/10.3168/jds.2009-2369.

[27] M. Bernaschina, M.E. Leser, H.J. Limbach, P. Fischer, A. Roucher, Lentil protein stabilized emulsion - Impact of lecithin addition on emulsions properties, Food Hydrocolloids 147 (2024) 109337, http://dx.doi.org/10.1016/j.foodhyd.2023.109337.

[28] M.L. Taboada, T. Heiden-Hecht, M. Brückner-Gühmann, H.P. Karbstein, S. Drusch, V. Gaukel, Spray drying of emulsions: Influence of the emulsifier system on changes in oil droplet size during the drying step, J. Food Process. Preserv. 45 (9) (2021) e15753, http://dx.doi.org/10.1111/jfpp.15753.

[29] M.L. Taboada, D. Chutani, H.P. Karbstein, V. Gaukel, Breakup and coalescence of oil droplets in protein-stabilized emulsions during the atomization and the drying step of a spray drying process, Food Bioprocess Technol. 14 (5) (2021) 854–865, http://dx.doi.org/10.1007/s11947-021-02606-1.

[30] T. Kurtz, K. Haas, J. Busom Descarrega, V. Meunier, O. Schafer, S. Heinrich, The role of pea protein content and carbohydrate molecular weight in the structure and stability of spray-dried emulsions, Food Hydrocolloids 166 (2025) 111320, http://dx.doi.org/10.1016/j.foodhyd.2025.111320.

[31] T. Schumacher, T. Steinmacher, E. Köster, A. Wagemans, J. Weiss, M. Gibis, Physico-chemical characterization of ten commercial pea protein isolates, Food Hydrocolloids 162 (2025) 110996, http://dx.doi.org/10.1016/j.foodhyd.2024.110996.

[32] A. Bozon, L. Fries, J. Kammerhofer, L. Forny, G. Niederreiter, S. Palzer, A.D. Salman, Effect of heterogeneous hydrophobic coating on floating of insoluble particles, Powder Technol. 395 (2022) 592–603, http://dx.doi.org/10.1016/j.powtec.2021.10.015.

[33] P. Moll, H. Salminen, O. Seitz, C. Schmitt, J. Weiss, Characterization of soluble and insoluble fractions obtained from a commercial pea protein isolate, J. Dispers. Sci. Technol. 44 (13) (2023) 2417–2428, http://dx.doi.org/10.1080/01932691.2022.2093214.

[34] J.G. Fernandes, R.C. Rodrigues, L. Pereira, P.C. Stringheta, P.H. Campelo, E. Martins, Encapsulation of hydrophobic active ingredients in plant proteins: modulation of interfacial properties and encapsulation efficiency, Curr. Opin. Food Sci. 57 (2024) 101170, http://dx.doi.org/10.1016/j.cofs.2024.101170.

[35] Q. Wang, M.-h. Pan, Y.-s. Chiou, Z. Li, S. Wei, X. Yin, B. Ding, Insights from alpha-Lactoalbumin and beta-Lactoglobulin into mechanisms of nanoliposome-whey protein interactions, Food Hydrocolloids 125 (2022) 107436, http://dx.doi.org/10.1016/j.foodhyd.2021.107436.

[36] L. Amagliani, C. Schmitt, Globular plant protein aggregates for stabilization of food foams and emulsions, Trends Food Sci. Technol. 67 (2017) 248–259, http://dx.doi.org/10.1016/j.tifs.2017.07.013.

[37] M.E.C. Thomas, J. Scher, S. Desobry-Banon, S. Desobry, Milk powders ageing: effect on physical and functional properties, Crit. Rev. Food Sci. Nutr. 44 (5) (2004) 297–322, http://dx.doi.org/10.1080/10408690490464041.

[38] I. Kutzli, J. Weiss, M. Gibis, Glycation of plant proteins via maillard reaction: Reaction chemistry, technofunctional properties, and potential food application, Foods (Basel, Switzerland) 10 (2) (2021) http://dx.doi.org/10.3390/foods10020376.

[39] M. Hellwig, The chemistry of protein oxidation in food, Angew. Chem. (International Ed. English) 58 (47) (2019) 16742–16763, http://dx.doi.org/10.1002/anie.201814144.

[40] P. Duque-Estrada, K. Kyriakopoulou, W. de Groot, A.J. van der Goot, C.C. Berton-Carabin, Oxidative stability of soy proteins: From ground soybeans to structured products, Food Chem. 318 (2020) 126499, http://dx.doi.org/10.1016/j.foodchem.2020.126499.

[41] L.L. Chang, M.J. Pikal, Mechanisms of protein stabilization in the solid state, J. Pharm. Sci. 98 (9) (2009) 2886–2908, http://dx.doi.org/10.1002/jps.21825.

[42] A.J. Baldwin, J.D. Ackland, Effect of preheat treatment and storage on the properties of whole milk powder. Changes in physical and chemical properties, Neth. Milk Dairy J. (Netherlands) 45 (3) (1991).

[43] C. Jacquot, J. Petit, F. Michaux, E. Chávez Montes, J. Dupas, V. Girard, A. Gianfrancesco, J. Scher, C. Gaiani, Cocoa powder surface composition during aging: A focus on fat, Powder Technol. 292 (2016) 195–202, http://dx.doi.org/10.1016/j.powtec.2016.01.032.

[44] A. Masum, J. Chandrapala, T. Huppertz, B. Adhikari, B. Zisu, Influence of drying temperatures and storage parameters on the physicochemical properties of spray-dried infant milk formula powders, Int. Dairy J. 105 (2020) 104696, http://dx.doi.org/10.1016/j.idairyj.2020.104696.

[45] L. Dewulf, M.K. Hausmann, A. Bozon, G. Niederreiter, S. Palzer, A.D. Salman, Particle-based food systems subject to lipid migration – A review of measurement, modelling, and mitigation approaches, Powder Technol. 445 (2024) 120097, http://dx.doi.org/10.1016/j.powtec.2024.120097.

[46] I.I. Litman, U.S. Ashworth, Insoluble scum-like materials on reconstituted whole milk powders, J. Dairy Sci. 40 (4) (1957) 403–409, http://dx.doi.org/10.3168/jds.S0022-0302(57)94494-6.

[47] T. Heiden-Hecht, M.L. Taboada, M. Brückner-Gühmann, H.P. Karbstein, V. Gaukel, S. Drusch, Towards an Improved Understanding of Spray-Dried Emulsions: Impact of the Emulsifying Constituent Combination on Characteristics and Storage Stability, vol. 121, 2021, http://dx.doi.org/10.1016/j.idairyj.2021.105134.

[48] M.B. Kahsay, O.J. Nydal, Degradation of viscosity of vegetable oils employed for thermal energy storage due to oxidation in ambient temperature, J. Energy Storage 73 (2023) 108816, http://dx.doi.org/10.1016/j.est.2023.108816.

[49] G. DePolo, P. Iedema, K. Shull, J. Hermans, Comprehensive characterization of drying oil oxidation and polymerization using time-resolved infrared spectroscopy, Macromolecules 57 (17) (2024) 8263–8276, http://dx.doi.org/10.1021/acs.macromol.4c01164.

[50] J.-H. Shao, Y.-M. Deng, G.-H. Zhou, X.-L. Xu, D.-Y. Liu, A Raman spectroscopic study of meat protein/lipid interactions at protein/oil or protein/fat interfaces, Int. J. Food Sci. Technol. 50 (4) (2015) 982–989, http://dx.doi.org/10.1111/ijfs.12695.

[51] U. Aruchunan, C.J. Henry, S.Y.J. Sim, Role of protein-lipid interactions for food and food-based applications, Food Hydrocolloids 160 (2025) 110715, http://dx.doi.org/10.1016/j.foodhyd.2024.110715.

[52] Y. Gao, J. Zheng, S. Liu, L. Shi, J.-H. Shao, Effects of lipids on the properties of emulsified interfacial film of myofibrillar protein by Raman spectroscopy, Int. J. Food Sci. Technol. 57 (3) (2022) 1517–1526, http://dx.doi.org/10.1111/ijfs.15501.

[53] K. Li, L.-M. Wang, B.-B. Cui, B. Chen, D.-B. Zhao, Y.-H. Bai, Effect of vegetable oils on the thermal gel properties of PSE-like chicken breast meat protein isolate-based emulsion gels, Food Chem. 447 (2024) 138904, http://dx.doi.org/10.1016/j.foodchem.2024.138904.

[54] Q. Chen, J. Zhang, Y. Zhang, H. Liu, T. Li, Q. Wang, D.L. Kaplan, Microscopic insight into the interactions between pea protein and fatty acids during high-moisture extrusion processing, Food Chem. 404 (Pt A) (2023) 134176, http://dx.doi.org/10.1016/j.foodchem.2022.134176.

[55] T.G. Burger, I. Singh, C. Mayfield, J.L. Baumert, Y. Zhang, The impact of spray drying conditions on the physicochemical and emulsification properties of pea protein isolate, LWT 153 (2022) 112495, http://dx.doi.org/10.1016/j.lwt.2021.112495.

[56] B. Muhoza, Y. Harimana, E. Kayitesi, A. Uriho, Q. Liu, Insight into the effect of extraction and spray drying conditions on the nutritional and techno-functional properties of legume protein powder: A review, Food Bioprocess Technol. (2024) http://dx.doi.org/10.1007/s11947-024-03541-7.

[57] S.I. Martins, W.M. Jongen, M.A. van Boekel, A review of maillard reaction in food and implications to kinetic modelling, Trends Food Sci. Technol. 11 (9–10) (2000) 364–373, http://dx.doi.org/10.1016/S0924-2244(01)00022-X.

[58] J. Yang, R. Kornet, E. Ntone, M.G. Meijers, I.A. van den Hoek, L.M. Sagis, P. Venema, M.B. Meinders, C.C. Berton-Carabin, C.V. Nikiforidis, E.B. Hinderink, Plant protein aggregates induced by extraction and fractionation processes: Impact on techno-functional properties, Food Hydrocolloids 155 (2024) 110223, http://dx.doi.org/10.1016/j.foodhyd.2024.110223.