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Combining plant and dairy proteins to enhance the oxidative stability of microencapsulated lipids in spray-dried powders

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ABSTRACT

This study investigates the oxidative stability of spray-dried emulsion powders using single plant (pea or soy), dairy (milk), or hybrid (plant-dairy) protein systems for encapsulation. Emulsions were produced and spray-dried and powders stored for 91 days under controlled conditions. Lipid oxidation was monitored through peroxide value, free radical concentration (Miniature Electron Spin Resonance Spectroscopy), and GC–MS analysis. Hybrid protein systems showed superior protection of encapsulated lipids, with 15-times lower accumulation of hydroperoxides and 2-3 times lower free radical concentrations compared to single-protein formulations. Furthermore, the formation of total volatiles of hybrid samples plateaued after 28 days, while it continuously increased for all single protein formulations, demonstrating that also secondary lipid oxidation was reduced in hybrid samples. The findings suggest that the combination of plant and dairy proteins synergistically improved the oxidative stability of encapsulated lipids, laying a promising groundwork for the development of clean-label, shelf-stable food powers.

1. Introduction

Lipid oxidation is a primary concern in the food industry, leading to the degradation of nutritional value, the formation of off-flavors, and a reduction in overall product quality and shelf life. While some compounds from lipid oxidation are part of the desired aroma profile in freshly prepared products such as cooked meat or fried products, their accumulation over shelf-life usually leads to a loss of quality and consumer rejection (Dunkel et al., 2014; Guth & Grosch, 1990). Microencapsulation via spray drying is a widely employed technique to protect lipids from oxidation by encapsulating them within a protective matrix, typically composed of carbohydrates, proteins, or a combination thereof. Proteins stand out as encapsulation materials due to their inherent amphiphilic characteristics, which give rise to exceptional emulsifying capabilities, facilitating the formation of stable emulsions with lipids prior to spray drying (Gharsallaoui et al., 2007). Dairy proteins, such as whey protein isolate and sodium caseinate, have long been used for microencapsulation due to their excellent film-forming and emulsifying properties (Díaz-Montes, 2023).

More recently, plant proteins, derived from sources such as soy or pea, have gained interest as alternatives to dairy proteins due to their increasing availability and sustainability (Akbarbaglu et al., 2021). However, these alternative proteins often present limitations in their functional properties such as solubility, emulsification, and gelling capacity, shortcomings that can reduce their effectiveness for encapsulation and limit the oxidative protection of encapsulated lipids (Kurtz et al., 2025).

Hybrid protein systems that combine plant and dairy proteins have recently been identified as a promising strategy to overcome these challenges and develop products with improved sensory quality compared to pure plant-based systems while reducing the environmental impact of animal-derived ingredients. Recent research suggests that the combination of proteins of different origins can lead to synergistic effects, improving their physicochemical properties and functionality in food applications (Alves & Tavares, 2019; Hinderink et al., 2021; Ho et al., 2018). However, the potential for such combinations to enhance oxidative stability, particularly in lipid-rich spray-dried powders, remains underexplored.

This study therefore aimed to investigate the effects of blending plant and dairy proteins on the oxidative stability of encapsulated lipids in spray-dried powders. Although primary oxidation products such as peroxides and free radicals are highly relevant markers for lipid oxidation, their instability, as well as their taste and odor neutrality make them less suitable to characterize advanced lipid oxidation and changes in sensory profile. Consequently, secondary volatile compounds are mainly responsible for the deterioration of lipid-rich foods over shelf

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life. The volatile carbonyls formed usually have low odor thresholds and can induce fatty, rancid, fishy, green, or fruity odors depending on the fatty acid profile and the corresponding breakdown products (Grosch, 1982). In particular, the amount of mono and polyunsaturated fatty acids present in a product determines its stability and the formation of off-notes. In addition, the off-flavor formation in plant proteins such as pea and soy is mainly associated with lipid oxidation that leads to non-volatile bitter molecules, as well as volatile aroma compounds (Utz et al., 2022; Gläser et al., 2020). As in dairy alternatives or hybrid products, a more oxidation-sensitive vegetal oil is usually added to replace milk fat; understanding the chemical and physical ingredient interactions that drive lipid oxidation over shelf-life becomes even more relevant.

Combining chemical interactions with physical properties such as encapsulation efficiency, morphology, and oxidative degradation under controlled storage conditions, this work provides insight into protein-protein interactions and their implications for improving the functionality of lipid delivery systems. Understanding how plant and dairy proteins interact within a microcapsule matrix and how these interactions influence lipid oxidation could open new avenues for the development of clean-label, stable and environmentally friendly food powders.

2. Materials and methods

2.1. Materials

Pea protein isolate (PPI) (Pisane C9, 84% protein in dry matter) was sourced from Cosucra (Warcoing, Belgium), soy protein isolate (SPI) (Profam 974, 94% protein in dry matter) from ADM (Chicago, IL, USA), and milk protein concentrate (MPC) (MPC4861, 86% protein in dry matter) from Fonterra (Auckland, New Zealand). Sunflower oil (Peroxide value = 0.1 meqO_2/kg), obtained from Florin (Muttenz, Switzerland), was used as lipid phase. The hydrophilic carbohydrate phase consisted of maltodextrin (Glucidex 21) with a dextrose equivalent (DE) of 21, a well studied and characterized material that is commonly used for microencapsulation and spray drying. Maltodextrin was obtained from Roquette (Lestrem, France). N-heptane (≥ 99%) was used for free fat extraction and purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Chloroform and acetone for determination of the peroxide value were obtained from Merck (Darmstadt, Germany).

2.2. Powder production

In the present study, a model recipe made from maltodextrin DE 21 (52%), sunflower oil (28%) and protein (20%) as percentage of total solids (TS) was used. Three formulations with single protein sources (pea, soy, dairy) and two formulations containing a mixture of equal parts of pea and dairy (1:1), as well as soy and dairy protein, were produced.

Emulsification and spray-drying was carried out according to the protocol of Kurtz et al. (2026). For emulsion production, protein (350 g) was mixed with demineralized water (3250 g) using a L5M-A laboratory mixer (Silverson, Chesham, United Kingdom) for 30 min at 10,000 rpm. The dispersions were further hydrated overnight at 7 °C. After hydration, maltodextrin (910 g) was added and mixed until dissolution. Sunflower oil (490 g) was added and emulsified at 10,000 rpm for 10 min and the pH was adjusted to 7.5 using 1 M sodium hydroxide solution. The coarse emulsions were further processed in two passages in a high pressure homogenizer at 350/75 bar, maintaining the pH of 7.5. The total batch size of each emulsion was 5 kg and all emulsions had a total solid content (TS) of 35%.

Emulsions were spray-dried in a pilot-scale spray dryer (Niro minor, Søborg, Denmark) at an inlet temperature of 190 °C. The feed emulsion was atomized through a bi-fluid nozzle at an operating pressure of 1 bar and feed rate adjusted to maintain an outlet temperature of 70 °C. The dried powder was separated from the air stream and collected through the cyclone.

2.3. Storage test

Powders of the same batch were stored in duplicate for a total of 91 days in open plastic containers (9 × 9 × 13 cm) in a climate chamber ICH260eco (Memmert, Schwabach, Germany) under light exclusion. Storage conditions were controlled at 35 °C and 20% rel. humidity to maintain the powders in their glassy state while simulating accelerated shelf-life conditions. Samples were taken after 0, 28, 56 and 91 days and frozen at -60 °C in argon-flushed aluminum bags until further analysis. Additionally, some powder was filled into hermetically closed vials, stored under the same conditions, and analyzed after 91 days of storage. Each storage sample was analyzed twice per sampling point.

2.4. Emulsion and powder characterization

The produced emulsions were characterized by particle sizing and reconstituted powders by particle sizing and light microcopy. Particle sizes were expressed as surface mean ($d_{3,2}$) and volume mean ($d_{4,3}$) diameter, while ($d_{3,2;rec}$) and ($d_{4,3;rec}$) characterized the reconstituted samples. Particle size of liquid samples was measured in triplicate using laser diffraction (Mastersizer 3000, Malvern Panalytical, Malvern, United Kingdom). Dry powders were analyzed using dynamic image analysis (Camsizer XT, Microtrac Retsch GmbH, Haan, Germany) with a sample size > 1 million particles and expressed as mean diameter ($d_{50,3}$).

The residual moisture content (x_{res}) was determined gravimetrically in triplicate by drying 2 g of powder at 102 °C for 24 h. Free fat content of powders was determined in triplicate by dispersing 2 g of powder in 40 mL of heptane, separating the solvent from the particles and evaporating the solvent at 110 °C. For powder density and porosity, one sample was measured six times consecutively using a helium pycnometer (AccuPyc 1330, Micromeritics, Norcross, GA, USA) and the density calculated using the Boyle-Mariotte law.

Micrographs of spray-dried powders were taken by scanning electron microscopy (SEM) with a Quattro S microscope (Thermo Fisher, Waltham, MA, United states) at an accelerating voltage of 10 kV using a back-scattered electron detector and low-vacuum mode. To visualize non-encapsulated lipids, powders were exposed to (OsO_4) vapor prior to SEM analysis, which increased contrast between lipid-rich and lipid-poor areas.

A detailed description of emulsion and powder analytical methods has been provided in a previous study (Kurtz et al., 2025).

2.5. Peroxide value

Peroxide value was determined photometrically by determining the iron (III) complex. Briefly, approximately 0.5 g of powder were weighed in 50 mL centrifuge tubes and dispersed in 2 mL of distilled water. 10 mL of a mixture of chloroform and methanol in the ratio of 7:3 (v:v) were added, shaken for 10 s to extract the lipids, and centrifuged at 4000 g for 10 min (Multifuge X3R, ThermoFischer, Waltham, MA, USA) to accelerate phase separation. The chloroform-lipid phase was transferred into pre-weighed glass vials, dried under nitrogen flow, and the weight of the extracted lipids determined. The lipid phase was re-dispersed and diluted in chloroform-methanol mixture to stay within the calibration curve. 50 µL of iron (II) chloride solution were added, incubated for 5 min, and 200 µL of the solution were measured at 500 nm in a 96 wells glass plate using a Multiskan Go spectrophotometer (Thermo Scientific, Waltham, Wa, USA). Afterward, 50 µL of ammonium thiocyanate solution were added to the well, incubated for further 5 min, and iron (III) complex measured at 500 nm. The peroxide value was calculated using a calibration curve.

2.6. Free radicals

The presence of free radicals in the powder samples was analyzed using an X-band (9.815 GHz) miniature electron spin resonance spectrometer (microESR; Bruker, Billerica, MA, USA). Approximately 50 mg of powder was transferred to a quartz ESR tube and gently tapped to ensure uniform sample distribution and minimize air pockets. The loaded ESR tube was then sealed to prevent moisture uptake and external contamination. MicroESR spectra were recorded at room temperature (25 °C) using an internal calibration curve using the following parameters: modulation amplitude, 4.5 G; microwave power, 10 mW; sweep with, 70 G; conversion time, 5 ms. The obtained spectra were processed using Bruker Xenon ESR software, and the peak intensity was used as a relative measure of the radical concentration.

2.7. Volatile analysis

To monitor secondary oxidation products, a range of 16 volatiles known to derive from the breakdown of oleic, linoleic, and linolenic acid, respectively, have been monitored by GC/MS comprising linear aldehydes, monounsaturated aldehydes, polyunsaturated aldehydes, and unsaturated ketones (Belitz et al., 2005).

For the GC analysis 1 g (+/-50 mg) of each powder was reconstituted in a 50 ml falcon tube with addition of 19 g of water (50 °C) containing 16 mg/L of internal standard dimethoxytoluene (Sigmal-adrich). The solution was vortexed for 1 min and then 1 mL was transferred to GC vials for analysis. Vials were maintained at 10 °C in the cold rack of the autosampler prior to the following SPME headspace sampling process. After equilibration (30 °C, 10 min), the aroma compounds were extracted from the headspace for 10 min at 30 °C under agitation (350 rpm) using a Polydimethylsiloxane/Divinylbenzene fiber (PDMS/DVB; 1 cm; film thickness 65 µm; Supelco, Buchs, Switzerland). The extracted compounds were thermally desorbed for 5 min in a split/splitless injector maintained at 240 °C and operated in splitless mode. Chromatographic separation and peak detection was performed on a GC–MS 7890/5975C instrument from Agilent technologies. The GC was equipped with a capillary column DB-WAX (30 m, ID 0.25 mm, 0.25 µm film thickness). The oven temperature was held at 35 °C for 5 min, raised to 240 °C at 4 °C/min and then held for 10 min. Helium was the carrier gas and ran at a constant flow rate of 1.0 ml/min. MS acquisitions were achieved in EI ionization mode at 240 °C and at 70 eV from m/z 29 to 300 amu. Metalign software was used for data preprocessing of GC/MS raw data for baseline correction, peak-picking, and peak alignment to obtain a data matrix aligned with RT and m/z. Identification was achieved by comparing MS spectra and RI index with an external database (NIST/EPA/NIH Mass Spectral Library, version 2.0, Faircom Corporation, USA). All samples were randomized and analyzed in duplicates. Data were corrected for with the internal standard in case of response shift. Results of individual compounds were checked for linear response and expressed as peak areas.

2.8. Statistical analysis

Results were reported in mean ± standard deviation unless otherwise indicated. One-way Analysis of Variance (ANOVA) was performed on selected data sets with $\alpha = 0.05$ using Origin software (OriginLab, MA, USA).

3. Results and discussion

3.1. Emulsion and powder structure

Emulsion and powder characteristics of all formulations are shown in Table 1. Emulsion structure can strongly impact the final powder properties as well as the oxidation stability during powder drying and storage; therefore, a thorough characterization of the starting emulsions

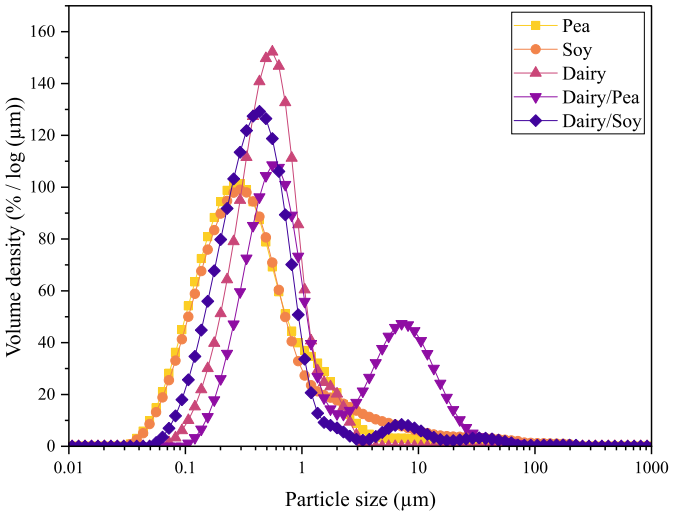

Fig. 1. Droplet size distribution of emulsions before spray drying using different proteins.

is crucial (Kurtz et al., 2025). $D_{3,2}$ values of emulsions before spray drying varied between 0.23 and 0.44 µm, while the largest sizes were found for hybrid emulsions. Because of the small particle size measured, it can be assumed that primarily single oil droplets were measured. Taking into account $d_{4,3}$ values, which mainly favor larger particles, values were in a broader range from 0.44 to 2.22 µm. These values might be related to either larger or coalesced droplets, or protein aggregates and flocculated droplets (Bernaschina et al., 2024). This was again more pronounced in hybrid and soy formulations. From the size distribution (Fig. 1), it also appears that a mixture of proteins increased droplet size, as well as droplet aggregation, as seen in the secondary peaks at around 10 µm for Dairy/Pea and Dairy/Soy formulations. This increase in droplet size might be related to competition between dairy and plant proteins at the interface during adsorption and, thus, partial coalescence of the droplets before stabilization, resulting in larger droplets. Grasberger et al. (2022) produced emulsions with pea protein isolate, whey protein isolate, and mixtures of both and also observed a large population around 10 µm in hybrid samples and a monomodal distribution of pure pea protein emulsions. The authors further measured the same emulsions in SDS solution to break up protein-bridged oil droplets and noted extensive bridging flocculation in all pea-containing systems. Kurtz et al. (2025) also reported strong flocculation in emulsions containing pea protein isolate. Both studies suggest that droplet bridging is probably caused by insoluble protein aggregates, which did not get solubilized during emulsification. Although soy protein was not included in either of these studies, it can be assumed that soy protein-containing emulsions exhibit similar characteristics.

$D_{3,2rec}$ and $d_{4,3rec}$ values increased in all reconstituted emulsions, particularly in all formulations containing plant protein. From light microscopy images (Fig. 2) it can be seen that the pea and soy emulsions exhibited strong flocculation and aggregation, while the hybrid formulations appeared to be more homogeneous. This was in agreement with the measured particle sizes. From the micrographs, it also appeared that the primary droplet size was slightly larger in hybrid samples than in single-ingredient samples, which was in accordance with observations of the droplet size before spray drying. It is possible that the dispersion of the emulsion during spray drying broke some flocculated droplet clusters, while at the same time, heat impact could also have caused protein denaturation and enhanced aggregation in the dried particle (Harshe et al., 2011).

Powder particle size varied from 22.5 to 33.1 µm. Largest particle sizes were achieved for plant-based variants (pea, soy), while there

Table 1
Emulsion and powder properties of produced formulations. Different letter represent significant differences at 0.05 level.

Emulsion		Pea	Soy	Dairy	Pea/Dairy	Soy/Dairy
$d_{3,2}$	(µm)	0.23 ±0.01	0.24 ±0.01	0.27 ±0.01	0.44 ±0.01	0.29 ±0.01
$d_{4,3}$	(µm)	0.66 ±0.02	2.22 ±0.67	0.44 ±0.01	1.11 ±0.03	1.75 ±0.65
$d_{3,2;rec}$	(µm)	1.23 ±0.31	1.01 ±0.25	0.24 ±0.01	1.19 ±0.05	0.43 ±0.01
$d_{4,3;rec}$	(µm)	6.53 ±0.58	60.13 ±5.00	1.31 ±0.19	4.1 ±0.15	8.03 ±3.69
Powder						
$d_{50,3}$	(µm)	30.3	33.1	23.7	22.5	23.8
x_{res}	(%)	1.21 $\pm0.04^{b,c}$	0.72 $\pm0.06^{d}$	1.27 $\pm0.02^{b}$	1.10 $\pm0.08^{c}$	1.47 $\pm0.02^{a}$
Free fat	(%)	10.7 $\pm1.8^{d}$	3.8 $\pm0.7^{e}$	23.8 $\pm1.8^{b}$	43.5 $\pm6.3^{a}$	15.3 $\pm1.4^{c}$
ρ_{true}	(g· cm^{-3})	1.22 ±0.01	1.21 ±0.01	1.24 ±0.01	1.25 ±0.01	1.24 ±0.01
porosity	(%)	0.67 ±0.14	0.94 ±0.22	1.99 ±0.19	0.83 ±0.15	1.17 ±0.21

were only minor differences between dairy and hybrid samples. Plant proteins such as pea and soy protein are known to increase emulsion viscosity when added in high concentrations, which can be further related to a more ineffective dispersion during atomization, leading to larger emulsion droplets and, consequently, larger particles after drying (Gharsallaoui et al., 2007).

Free fat is an important parameter for the evaluation of oxidation stability, as non-encapsulated lipids are known to oxidize much faster than encapsulated lipids (Linke et al., 2020c). Free fat ranged over a broad range from 3.8 to 43.5%, with lowest value for soy and highest value for pea/dairy. Pea is known to produce rather coarse emulsions, leading to higher free fat. Kurtz et al. (2025) and Linke et al. (2020b) both suspected that flocculated droplet clusters, as found in particularly pea-based emulsions, lead to a further increase in extractable lipids. Soy protein, on the other hand, has been shown to produce low-free-fat powders through the formation of very small droplets (Kramm et al., 2024; Yang et al., 2024). Surprisingly, the dairy powder had a markedly higher free fat content compared to the pea and soy powder, which was rather unexpected since milk proteins are known to be good emulsifiers. A possible explanation might be related to the morphological differences of the dairy powder compared to the plant-based powders (Fig. 3). Dairy powder had a strongly increased surface through collapse of the particle skin during drying, likely related to the lower viscosity of the feed. The increased surface area increased the chance of oil droplets to get exposed to the particle surface as well as the area available for lipid extraction, affecting the free fat content. The increased free fat content in hybrid samples was probably related to the larger size of primary oil droplets as observed in Fig. 2, as well as the smaller powder particle size, which increased the total surface area. These findings are consistent with data from other studies (Linke et al., 2020a; Jafari et al., 2007). From SEM micrographs (Fig. 3) it can be further seen that less fat (bright spots) was present on the particle surface of single-protein powders compared to hybrids. In a study by Roux et al. (2020), the authors also recorded an increase in free fat when substituting 50% of dairy protein for pea protein, although differences were less pronounced and overall encapsulation efficiency was still above 95%.

True density (ρ_{true}) and porosity are relevant powder properties when monitoring oxidation stability of powders during storage. Powder porosity determines how tightly packed the powder structure is at the macrosopic level and how easily oxygen can access encapsulated lipid droplets, while ρ_{true} gives an estimate of the diffusion coefficient of the wall material through molecular packing. ρ_{true} values were similar between samples, with slightly higher values for dairy-containing powders. Milk proteins are known to be significantly smaller than plant proteins, enabling them to pack more densely within the carbohydrate matrix. In combination with larger plant protein molecules, this might lead to even denser packing and thus reduced oxygen diffusion, even though the differences appear rather low (Drusch et al., 2009).

3.2. Primary lipid oxidation products

Primary oxidation products were measured in the form of hydroperoxides and free radicals. The peroxide values at the beginning of storage were in the range of 13 to 78 $mEqO_2$/kg oil, with the highest value for dairy sample (Fig. 4 a). For all single-protein samples, the peroxide value increased throughout storage. For pea and soy powders, peroxide value increased exponentially throughout the storage period, while for dairy powder, hydroperoxide concentration increased exponentially in the first 56 days of storage and continued to plateau thereafter. After 91 days of storage, hydroperoxide concentration of the single-protein powders exceeded the one of hybrid formulations more than 15 times, signifying a strong slowing of oxidation progression by combining plant and dairy proteins.

The lower increase in hydroperoxides in dairy powder was likely related to the fact that less new hydroperoxides were produced than existing hydroperoxides were degraded into secondary oxidation products, which is a sign of advanced lipid oxidation and marks a turning point (Ghnimi et al., 2017; Linke et al., 2020a). For the other samples, this point was not yet reached, and the exponential increase in hydroperoxides continued until the end of storage. Interestingly, Dairy/Soy and Dairy/Pea powders showed an initial decrease in hydroperoxides from 0 to 28 days of storage, followed by an exponential increase until the end of storage (Fig. 4 b). This kinetic has previously been observed by other authors and is related to the rapid oxidation of free lipids (Linke et al., 2020a). Because these lipids do not have any protection from environmental oxygen, they typically oxidize significantly faster than encapsulated lipids. From the peroxide value at the beginning of storage (day 0) it can be seen that already some oxidation had happened before the start of the storage experiment. After 28 days, the primary oxidation of free fat had already stagnated and hydroperoxides were degraded to secondary oxidation products. The exponential increase after 28 days was therefore most likely related to the oxidation of encapsulated lipids. This shows that encapsulated lipids in hybrid samples were clearly protected by the particle wall, which delayed oxidation and hydroperoxide formation. In comparison, single-protein powders did not exhibit a decrease in hydroperoxides, but the values increased steadily over storage. This indicates that encapsulated droplets were not as well protected as in hybrid samples and oxidation of encapsulated lipids began in parallel with oxidation of free lipids. The high oxidation stability of the Pea/Dairy sample while simultaneously having the highest free fat content, appears to be contradictory at first, since high free fat has been historically associated with a low oxidative stability. However, more recent studies have shown that the free fat content is not necessarily the main driver of the overall oxdidative stability of a system, but that the stabilization of the encapsulated lipids contributes largely to the stability of a powder (Linke et al., 2020c). A study by Drusch and Berg (2008) further suggests that a layer of free fat on the particle surface can even have a protective effect on encapsulated lipids, as it can scavenge oxygen and slow down diffusion inside the particle matrix. This further explains how peroxide and free radical concentration could be highest in the soy powder despite having the lowest free fat content.

The development of free radicals during storage was similar to the formation of hydroperoxides, although the differences were less pronounced (Fig. 5). The starting concentrations were in a narrow range from 0.15 to 0.2 µM, while concentrations after 91 days of storage

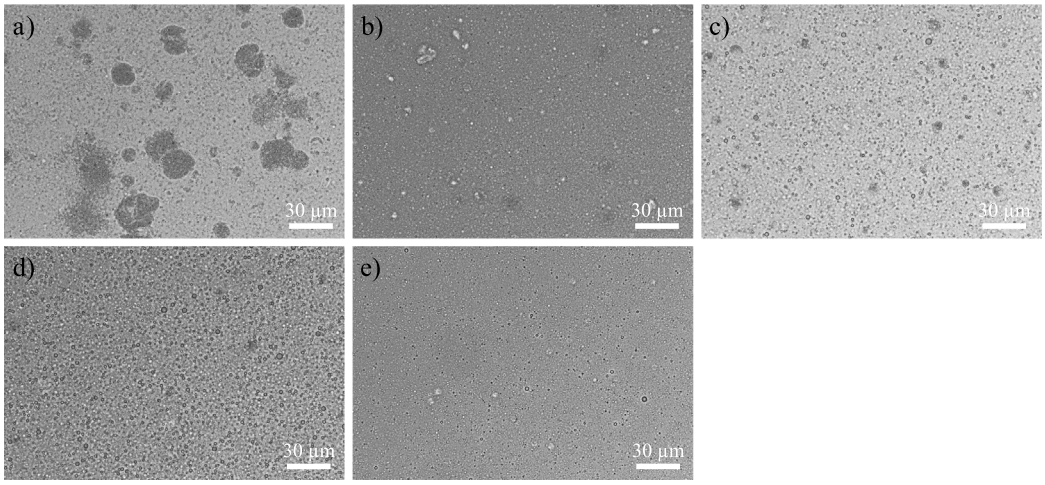

Fig. 2. Light microscopic images of rehydrated powders after spray-drying, containing pea protein (a), soy protein (b), dairy protein (c) and mixture of pea and dairy protein (d) and soy and dairy protein (e).

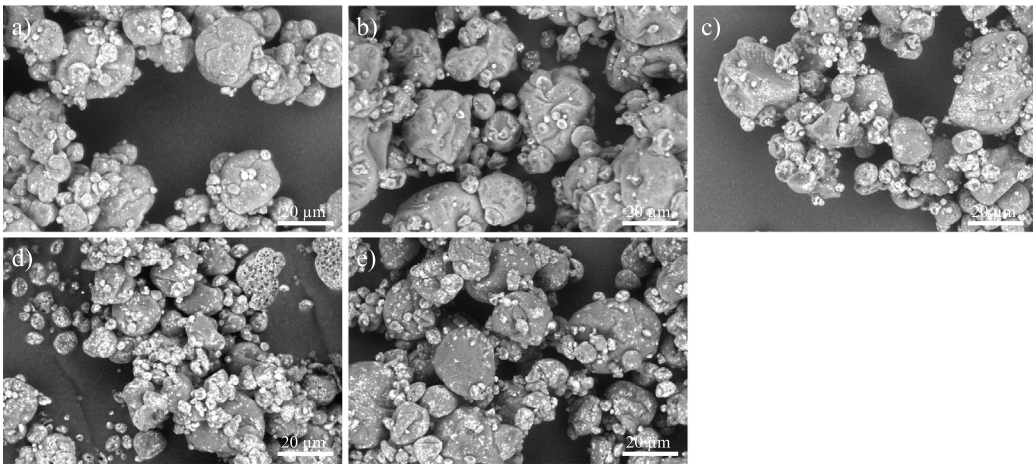

Fig. 3. SEM images of OsO_4-stained powders of powder containing pea protein (a), soy protein (b), dairy protein (c) and mixture of pea and dairy protein (d) and soy and dairy protein (e).

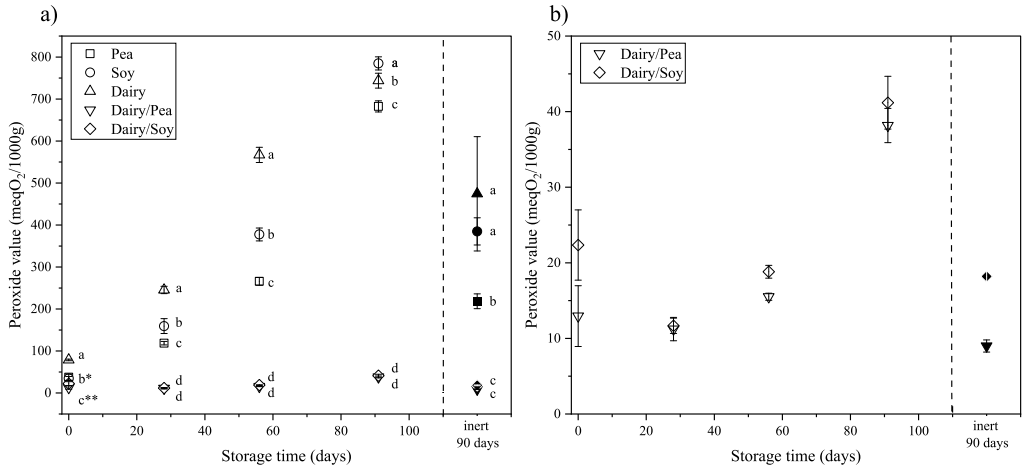

Fig. 4. Peroxide values of powders after 0, 28, 56 and 91 days of open storage (empty symbol) and after 90 days of inert storage (full symbol). ANOVA was performed individually for samples at each storage time. Different letters represent significant differences at 0.05 level. b* - Pea, Soy; c** - Dairy/Pea, Dairy/Soy.

varied significantly between 0.2 and 1.0 µM, with highest and lowest values for Soy and Dairy/Pea powders, respectively. Except for the Soy sample, free radicals increased in a close range for the first 28 days of storage. This may be related to the oxidation of non-encapsulated lipids, which progressed at a similar rate in all samples, while the soy sample had a very low quantity of free fat that could have oxidized, findings

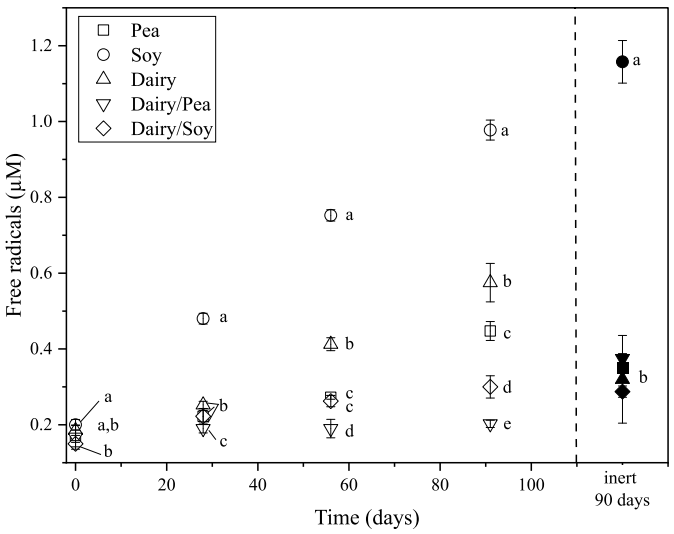

Fig. 5. Free radical values of powder at 0, 28, 56 and 90 days of open storage (empty symbol) and after 13 weeks of inert storage (full symbol). ANOVA was performed individually for samples at each storage time. Different letters represent significant differences at 0.05 level.

which are consistent with those from the hydroperoxide analysis. For the dairy powder, free radical content increased rapidly after 28 days and for pea powder after 56 days of storage, while the increase for both hybrid formulations remained low, indicating superior protection of the encapsulated lipids. At the end of storage, the slowest increase was recorded for Dairy/Pea and Dairy/Soy powders, for which starting concentrations merely increased by factor 1.2 and 2, respectively, while free radicals in the soy powder increased 4.9 times.

To understand the contribution of internal oxygen of the particles to overall oxidation, some powder was stored in hermetically closed argon-flushed vials for a total of 91 days and then were also analyzed for oxidation compounds. The peroxide values of all hermetically closed samples after 91 days of storage were lower than those of openly stored samples, which was expected, since external oxygen is a main driver of oxidation. However, internal oxygen still led to considerable oxidation, particularly for single-protein powders. Free radical content of the inert samples was in a close range for hybrid, pea, and dairy powders, partially exceeding the values of the openly stored samples, which could be due to their accumulation in closed packaging, while being lost through volatilization in open packaging. Inert soy powder showed a higher free radical content than powder stored open at the end of storage, which was unexpected. It is possible that the little internal oxygen sufficed to strongly progress oxidation while concentrating the volatile free radicals in the closed packaging. It also further supports the hypothesis that free fat might act as an oxygen scavenger and delay the oxidation of encapsulated lipids.

3.3. Secondary lipid oxidation products

In order to obtain a holistic view of the oxidative status of the powders, secondary oxidation products needed to be considered as well, particularly in regards to sensory aspects. Looking at the evolution of all volatiles over time (Fig. 6 a), there are two types of pattern that are observable. Both hybrid products (Dairy/Pea and Dairy/Soy) showed aroma formation in the first 28 days but then stabilized over the rest of the study time until 91 days of storage. In contrast, all non-hybrid products showed a continuous increase in volatiles during the whole period, with the dairy sample at highest levels, followed by soy and then pea at a clearly lower volatile load. Soy and pea powders both showed the expected exponential curve shape, the dairy product instead flattened between 56 and 91 days.

The behavior was similar when breaking the data down into compound classes. Saturated aldehydes and monounsaturated aldehydes showed a pattern similar to that of total volatiles (Fig. 6 b, c). In contrast, dienals showed a steady increase also for the hybrid products but at significantly lower formation rate compared to the non-hybrid products (Fig. 6 d). The kinetics of volatile formation showed patterns similar to those observed for the peroxide values. However, in terms of absolute values at 91 days, the dairy sample was worse than soy despite similar peroxide values at that point in time. The pea sample was further closer to the hybrid products, unlike the peroxide values would suggest for total volatiles. When comparing the 56-day peroxide values and the 91-day total aroma values, the results appear to be more similar. As peroxide formation is the primary reaction and volatile formation occurs later in the reaction cascade, a time delay between the two phenomena is somehow logical, although a 4-week delay appears pretty high. With regard to compound classes, the situation of the dienals best reflects the measurements of peroxide values. This can be explained by the fact that dienals are related to polyunsaturated fatty acids, which are known to be the most sensitive to peroxide formation. However, for the hybrid products, the stabilization of the system as seen for the peroxide values and, to a lesser extent, also for free radicals was generally well reflected in the volatile formation, which also stabilized after an initial formation period for most compounds. This initial formation probably derived from remaining surface fat that oxidized as quickly as for the non-hybrid counterparts. In fact, the results over 56 days are in line with the amount of free fat in the samples. The platforming of the volatiles, however, clearly shows that an important part of the fat was protected by hybridization of the powders and that the powder became rather stable over longer periods of storage.

In order to put this protection effect in perspective, volatiles of the hermetically closed, oxygen-free samples have been analyzed as well. For the two hybrid products, the level of aroma formation was still clearly above those of oxygen-free storage and is in agreement with the results from the peroxide value data (Fig. 7). The combination of plant and dairy proteins thus did not replace proper oxygen management to avoid oxidation over shelf-life but can be part of a protection strategy, e.g. in cases when more environmentally friendly packaging materials are used, which have lower oxygen barrier properties compared to aluminum or laminated plastic. Although no sensory evaluation could be performed, it can be assumed that the volatile formation caused deterioration of the samples and that the hybrid products can be expected to be of better quality at a given time point because there were fewer aroma compounds present.

3.4. Improved oxidation stability through combination of plant and dairy proteins

Although several studies on plant-dairy hybrid emulsions have been carried out in the past and there is certain evidence that mixtures of dairy and plant proteins might protect lipids and bioactives against oxidation, there is no current study investigating their impact in powdered systems. Yang et al. (2024) compared the oxidative stability of microcapsules prepared with pea, soy, or whey protein and found the highest and lowest stability for samples with soy and pea protein, respectively, which is not in accordance with the findings of this study. However, the authors attributed the changes in oxidative stability primarily to differences in surface fat and encapsulation efficiency and did not consider other influencing factors. Sridhar et al. (2024) prepared manuka essential oil microcapsules using mixtures of whey protein with pea or lupine protein in different protein to oil ratios. They found higher antioxidant activities for microcapsules with mixtures of proteins than for individual raw materials. However, since the results

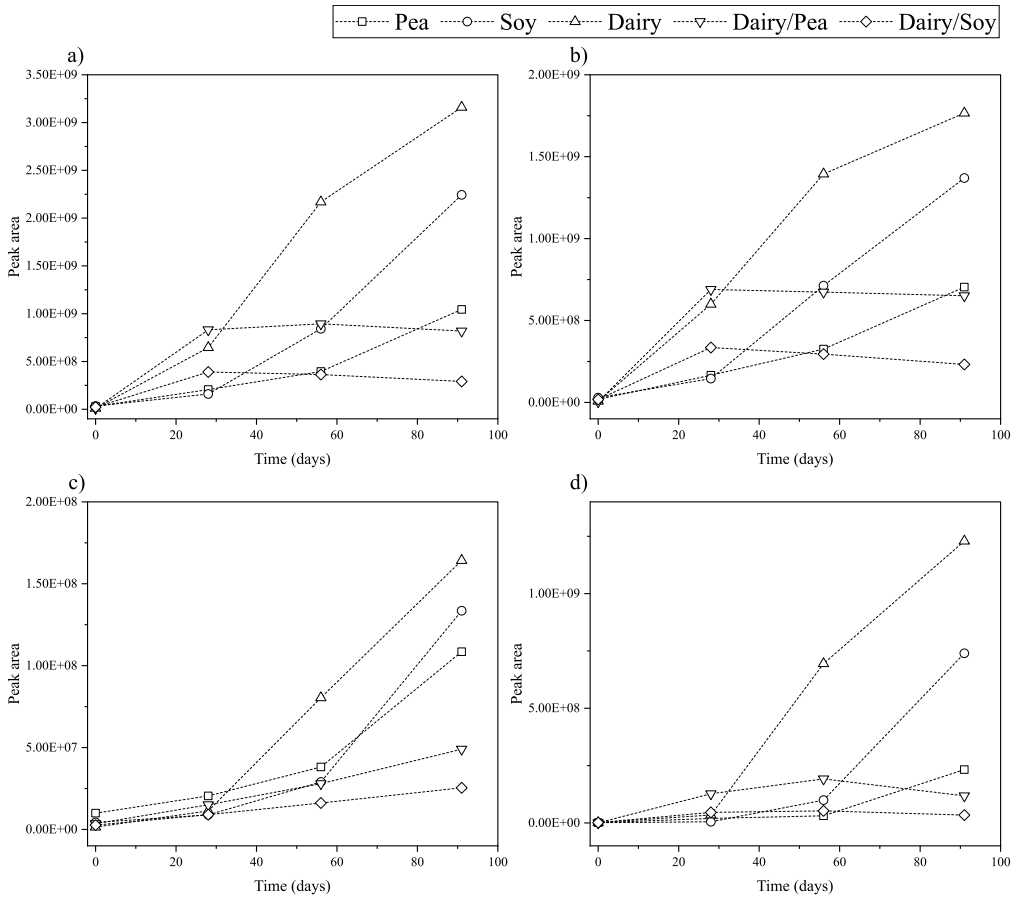

Fig. 6. Secondary lipid oxidation development of powders over the course of storage: Total volatiles (a), aldehydes (b), dienals/diedons (c), Enals (d).

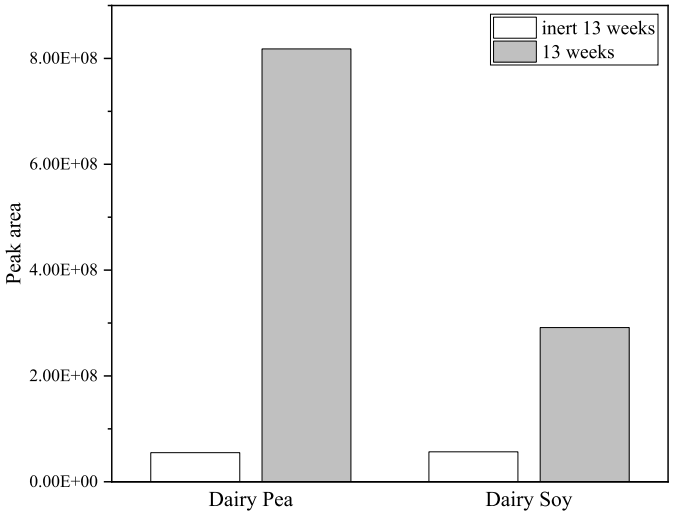

Fig. 7. Total volatiles of hybrid powders stored openly and under inert conditions after 13 weeks.

were not compared with microcapsules prepared with individual proteins as reference, the evaluation of the impact of protein combinations remains unclear.

In liquid systems, there are contradicting results about the stabilizing effect of plant-dairy mixes. Ho et al. (2018) investigated the influence of whey protein-based hybrids on the oxidative stability of lycopene-loaded emulsions during a storage period of 14 days. They found that whey and plant protein blends provided better protection against oxidation than individual proteins alone and attributed this behavior to the formation of a thick and viscoelastic interfacial layer of co-adsorbed proteins that could have superior metal chelating or radical scavenging activity. Yerramilli et al. (2018) on the other hand, observed a slightly lower retention of curcumin in emulsions, which partially substituted sodium caseinate with pea protein concentrate compared to a reference with pure sodium caseinate. Although these studies offer valuable insights into the oxidative stability of hybrid emulsions, oxidation in liquid systems is different from that of dry powders. Metal ions, for example, play a crucial role in liquid systems, while they are of low importance in dried systems (Barden & Decker, 2016). However, it is plausible that a thicker and denser interfacial layer, as formed by a mixture of different proteins, can offer improved protection of the encapsulated lipid droplets.

Another possible explanation for the improved oxidation stability of hybrids could be related to a synergy of the antioxidant properties of the different proteins. Antioxidants can be classified as primary or secondary. While the first directly neutralizes free radicals by donating a hydrogen atom or electron, effectively terminating the propagation phase of oxidation, the latter instead inhibits oxidation through other means such as metal-ion chelation, decomposition of hydroperoxides into non-radical products, or deactivation of singlet oxygen. The antioxidant activity of proteins has been reported in several studies (D'Alessio et al., 2023; Wang et al., 2017; Kim et al., 2025). For dairy proteins, such as caseins and β-lactoglobulin, radical scavenging was measured (Elias et al., 2006; Díaz & Decker, 2004), while plant proteins often contain higher levels of secondary antioxidants, such as polyphenols (Cipollone et al., 2024). Therefore, the combination of different types of antioxidants from different protein sources could lead to improved oxidation stability by increasing the oxidative resistance of the interfacial

layer and neutralizing reactive species in the bulk wall material. Khalesi and FitzGerald (2021) investigated the radical scavenging activity of milk protein concentrate, different plant proteins, and their blends. They found a higher antioxidant activity for blends of MPC and PPI, compared to pea protein isolate alone, but no changes for MPI-SPI mixtures. The authors explained this with an already high radical scavenging activity of SPI, which was comparable to that of dairy proteins. Feng et al. (2021) also reported a higher lipid oxidation inhibition of soy over whey protein isolate in oil-in-water emulsions.

Considering the different sizes of plant and dairy proteins (Kim et al., 2020), it is also possible that a mixture of proteins forms a more densely packed particle wall compared to a particle wall formed by single proteins. Drusch et al. (2009) have shown that large molecules, such as proteins, can disrupt a tightly packed glassy matrix and increase free volume elements, which are associated with a higher diffusivity of oxygen through the particle wall and towards encapsulated oil droplets (Ubbink, 2012). Combining differently sized proteins, such as plant and dairy proteins, can improve the packing density and therefore the oxidative stability. It is noteworthy that the powder with high free fat exhibited high stability, which indicates that oxygen diffusion reduction might have played a minor role. In addition, material density data (Table 1) show no major deviations between samples, which supports the hypothesis that increased stability in hybrid powders can be ascribed primarily to chemical rather than physical protection.

4. Conclusion

This study demonstrated that hybrid protein systems, which combine plant and dairy proteins, offer a clear advantage over single-protein matrices in enhancing the oxidative stability of spray-dried emulsion powders. While all formulations showed some degree of lipid oxidation during storage, hybrid systems consistently delayed the onset and slowed the progression of both primary and secondary oxidation reactions. Particularly the combination of soy and dairy proteins proved to be effective against the formation of secondary oxidation volatiles, which was likely related to their reduced free fat content (15.3%) compared to the sample with pea and dairy protein (43.5%). Even though the concrete mechanisms behind this improved oxidation stability call for further investigation, the results of this study confirm the feasibility of using hybrid proteins to stabilize lipid encapsulates against oxidation and provide the basis for further research and implementation in sustainable food powders. Future work should focus on exploring the interactions of plant and dairy proteins regarding their impact on matrix diffusivity, antioxidant properties and interfacial stabilization in powdered systems.

CRediT authorship contribution statement

T. Kurtz: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Glabasnia:** Writing – original draft, Methodology, Investigation, Data curation. **K. Haas:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **F. Giuffrida:** Writing – review & editing, Methodology. **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 the following financial interests/personal relationships which may be considered as potential competing interests: Teresa Kurtz, Klara Haas, Vincent Meunier, Francesca Giuffrida, Stefan Heinrich have patent pending to Société des Produits Nestlé S.A. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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