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Development of novel microaerogel particles from pea protein and their application as ingredient for low-saturated fat cocoa spreads

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Abstract

Pea protein aqueous dispersions were subjected to thermal gelation of a 18% (w/w) at the isoelectric pH, followed by water-to-ethanol solvent exchange and supercritical-CO_2 drying. The obtained particles presented a size in the range of 10-50 µm and showed internal surface area (142 m^2/g), density (0.28 g/cm^3) and porosity (79%) values typical of aerogels. Their SEM microstructure revealed a peculiar hierarchical structure of aggregated dried microgels. The obtained particles were thus defined pea protein microaerogel particles. One g of microaerogel particles were able to structure 1.7 g of oil, turning it in a viscoelastic material. Based on this, the microaerogel particles were used in the preparation of cocoa spreads containing sunflower oil solely as lipid phase. The spreads containing up to 2% (w/w) microaerogel particles showed rheological moduli and spreadability behaviour comparable to those of commercial spreads, along with high physical stability (oil holding capacity >96%). Despite the similar physical properties, the saturated fatty acid content of the developed spreads was 57% lower than that of commercial spreads. Obtained results highlight the possibility to obtain microaerogel particles from plant proteins and demonstrate their applicability as oil structuring ingredients, suitable for the design of spreads with physical properties similar to those of market products but with a healthier lipidic profile.

Keywords: porous ingredients; plant proteins; fat reduction; microstructure; rheological properties

1 Introduction

High levels of saturated fats in the diet are associated with an increased incidence of many non-communicable diseases, such as cardiovascular diseases, obesity and type II diabetes [1,2]. For this reason, food reformulation strategies, able to reduce the saturated fat content are highly demanded to

favour the transition to more sustainable diets with improved nutritional profile. Nevertheless, the pivotal role of saturated fats for food structure and sensory properties makes their reduction rather challenging. This is particularly critical for those foods where fat is the main ingredient, determining the final product rheological properties and consumer sensory experience [3].

Among fat-rich foods, cocoa spreads are delicious products with a rich and creamy taste. Typically, the ingredients include a considerable amount of fat (30-60%), most commonly palm oil, coconut oil and cocoa butter. Further ingredients are sugar, dairy-based powders, such as whey proteins, cocoa powder, hazelnuts, and some minor compounds such as emulsifiers and flavours [4,5].

From a physical point of view, cocoa spreads are smooth suspensions of finely ground particles, embedded in a continuous semi-solid fat crystalline network [6], preventing particle sedimentation and separation of the lipid liquid fraction, and providing the typical rheological properties [7,8].

Although the saturated fat content of cocoa spreads can be simply decreased by reducing the fat fraction in favour of the dry ingredients, this approach inevitably alters the physical and sensory attributes, eventually resulting in lower consumer acceptability [4]. Similarly, the simple replacement of the saturated fat with liquid oil, rich in unsaturated fatty acids, impairs spread structure, and increases the risk for oil separation during storage [9].

A promising strategy to overcome these issues is based on oil structuring by conversion of liquid oils into semi-solid and plastic materials (oleogelation) thanks to the addition of molecules able to form a network entrapping liquid oil [10]. The structuring ability of liposoluble molecules is traditionally exploited in oleogelation. These molecules are dissolved in oil above their melting temperature and self-assemble in a network able to entrap the liquid oil upon cooling [11]. Evidences of the application of this approach to partially replace solid fat in cocoa spreads are reported in the literature. Patel et al. [8] and Doan et al. [12] proposed a cocoa spread formulation containing liquid oil structured with shellac or beeswax wax, respectively. Fayaz et al. [13] assessed the application in cocoa spreads of monoglycerides, beeswax and propolis wax to structure pomegranate oil. As an alternative strategy, hydrocolloids can be used as oil structuring agents. For example, Bascuas et al. [14] used hydroxypropyl methylcellulose and xanthan gum to structure sunflower oil and olive oil to replace coconut butter in cocoa spreads. Nevertheless, all the oil structuring agents proposed in the literature fall into the category of food additives with specific limitations depending on the production country [15]. As a consequence, food companies are asking for reformulation strategies able to limit the use of additives, answering the increasing consumer demand for clean-label products [16].

Recently, we have demonstrated the suitability of whey protein (WP) aerogel particles as ingredients of cocoa spread containing sunflower oil solely as lipid phase instead of fats [17]. Aerogels are solid materials characterised by open porosity and very low density [18]. WP aerogel particles were prepared by grinding a three-dimensional hydrogel (i.e., a gel in which the continuous phase is represented by water or an aqueous solution), followed by ethanol solvent exchange and supercritical-CO_2-drying, leading to an aerated protein powder [19]. The latter is able to quickly absorb large quantities of oil and structure it in a network based on protein-protein interactions [19]. This strategy seems particularly promising, since it is based on the use of proteins, which are non-additive ingredients, already used in the traditional formulation of cocoa spreads.

Nevertheless, the current worries about the high environmental impact of animal proteins are strongly boosting the transition towards plant proteins. In this context, the global pea proteins market has been growing rapidly in recent years, driven by the nutritional value of its aminoacidic profile, easy digestibility and low allergenic potential [20–22]. It has recently been suggested that pea proteins (PP) could be also extracted from substandard peas, which currently represent an economic and environmental burden for food companies [23].

To our knowledge, no study reported the production of PP-based aerogels. This is mainly due to the poor gelling capacity of these proteins. Protein gelation is frequently induced by heat treatment, which causes protein chains to unfold and expose their reactive groups, driving protein networking dependently on several factors. In particular, at pH values below or above the isoelectric point (pI), proteins exhibit a net positive charge due to the functional group protonation and deprotonation, respectively. The resulting net charge of the protein molecule surface causes electrostatic repulsion between the polymer chains [24]. In these conditions, the proteins form "filamentous" structures. Conversely, at the pI, the net surface charge of the polymer chains is close to zero, resulting in a maximisation of protein-protein interactions, leading to the formation of insoluble hydrated aggregates, conventionally referred to as "microgels", since consisting of a hydrated network of proteins [25, 26]. In both cases, if the protein concentration is above a protein-specific threshold, filamentous or microgel structures may engage in surface interactions, leading to a three-dimensional network which is respectively referred to as stranded or particulate. Nevertheless, the formation of a three-dimensional network also depends on the availability of surface groups, and protein solubility. In particular, both covalent (e.g., disulfide bridges) and weak interactions (e.g., hydrophobic interactions, hydrogen bonds, and electrostatic interactions) play a role in protein networking. The presence of free sulfhydryl (-SH) groups facilitates covalent stabilization, enhancing gel strength.

Notably, PP present a lower number of -SH groups compared to animal ones, such as WP [27]. PP also shows lower solubility, since the extraction process performed to isolate the protein fraction from the vegetable matrix is known to induce structural modifications in the protein chains, further reducing gelling properties [28]. In the light of these limitations, the production of a three-dimensional PP aerogel is quite challenging, and only achieved by combining these proteins with high-gelling compounds with well-known attitude to aerogel preparation. In this sense, a recent work exploited silica and PP in the preparation of a hybrid inorganic-organic aerogel [29].

Nevertheless, PP aerogels could be obtained by exploiting the structuration of PP in microgels, independently of their ability to engage in the formation of a three-dimensional particulate network. Indeed, microgels can be regarded as hydrogel particles, which might be easily separated from the aqueous phase, since insoluble, to further undergo solvent exchange and supercritical-CO_2-drying. It must also be noted that this process would be particularly promising, since it facilitates a significant removal of the typical PP color and flavour, which commonly limits the application of PP ingredients in food application [23].

Based on these considerations, the aim of this work was to study the possibility to obtain PP aerogel particles, demonstrating their applicability as novel ingredients with oil structuring ability, suitable for the preparation of low-saturated fat cocoa spreads. To this aim, a PP isolate solution at the pI was thermally treated, followed by water-to-ethanol solvent exchange and supercritical-CO_2 drying. The obtained aerogel particles were characterised for physical properties (internal surface area, density, porosity, SEM microstructure) and oil structuring capacity. Following, they were used in the preparation of cocoa spreads containing sunflower oil solely as lipid phase. Spreads were analysed for rheological properties, spreadability, physical stability and nutritional content. Spread data were compared to those of commercial cocoa spreads to highlight the suitability of the proposed formulation strategy in the design of cocoa spreads with physical properties similar to those of market products but with a healthier lipidic profile.

2 Materials and methods

2.1 Materials

Commercial pea protein (PP) isolate (80% protein; 5.5% lipid content, carbohydrates 2.6%, fibres 4.1%, salt 1.9%) was purchased from MyProtein (The Hut Group, Manchester, England). Sunflower oil, icing sugar, cocoa powder and six commercial spread samples available on the Italian market (indicated as C1,

C2, C3, C4, C5 and C6) were purchased in a local market. HCl and NaOH were purchased from Sigma Aldrich (Milan, Italy). CO_2 (purity 99.995%,) was purchased from Sapio (Monza, Italy). P_2O_5 was purchased from Chem-Lab NV (Zedelgem, Belgium). Absolute ethanol was purchased from J.T. Baker (Griesheim, Germany).

2.2 Isoelectric point determination

PP isolate was hydrated in water at a concentration of 0.2% (w/v) and then divided into 9 aliquots, which were adjusted to a pH value of 3.0, 4.0, 4.5, 4.8, 4.9, 5.0, 5.7, 7.0 and 9.0 using HCl or NaOH 1 M. Subsequently, the ζ potential of the various aliquots was assessed using a Zetasizer (Nano Series-ZS, Malvern Instruments LTD, Worcestershire, UK). The pI was determined as the pH value at which the net surface electrical charge was zero.

2.3 Microaerogel particles

PP isolate aqueous dispersions (18%, w/w) were adjusted at the isoelectric pH (pI 4.5, as assessed by ζ potential measurement, paragraph 2.2) and stirred for 8 h. This PP isolate concentration was selected as the maximum protein amount leading to a dispersion that can be stirred. The obtained dispersion was then introduced in sealed 50 mL-plastic tubes, thermal treated at 90 °C for 15 min and cooled in an ice-water bath for 30 min, followed by 12 h storage at refrigerated temperature, to favour the spontaneous sedimentation of the obtained microgel particles. The latter were collected by centrifugation at 13,000 *g* at 4 °C (Avanti J-25, Beckman, Brea, California, USA) and dispersed in ethanol (0.1 g/mL) by 13,000 rpm homogenization for 3 min (Polytron PT-MR3000, Kinematica AG, Littau, Switzerland). The produced solvent-exchanged particles were similarly collected by centrifugation. This procedure was repeated twice to substitute water in the protein particles with ethanol completely. The ethanol was then removed using a supercritical-CO_2-drying plant at a temperature of 60 °C, and a pressure of 11 ± 1 MPa, by applying subsequent equilibrium (30 min) and extraction (5 min) stages. The obtained dried particles, defined as microaerogel particles, were stored at room temperature in a desiccator containing P_2O_5 until use.

2.4 Preparation of cocoa spreads

Based on the average composition of commercial spreads, cocoa spreads were produced as described in [17], by manually mixing protein powders (7 g/100 g spread, referring to the sum of PP isolate and microaerogel particles) with oil (30 g/100 g spread). Following, cocoa powder (10 g/100 g spread) and icing sugar (53 g/100 g spread) were incorporated by further manual mixing. The amount of microaerogel

particles in the spread varied from 0 to 7 g/100 g spread. The obtained spreads were stored in sealed sample holders at room temperature for up to 1 month.

2.5 Image acquisition

Sample images were obtained in a cabinet for image acquisition (Immagini & Computer, Bareggio, Italy) equipped with a digital camera (EOS 550D, Canon Macro Lens EF-S, Milan, Italy). The samples were illuminated by four 23 W photographic lights, placed in a position able to minimize shadows and glares. The samples were taken with a micro-spatula, placed on a slide and covered with a glass cover object.

2.6 Optical microscopy

Aqueous microgel particles (paragraph 2.3) were observed with an optical microscope (Leica DM 2000, Leica Microsystems, Heerbrugg, Switzerland). To this aim, a droplet of the aqueous dispersion containing the microgel particles was diluted 1:100 (v/v), positioned on a microscope slide and covered with a cover slip. Images were captured with magnifications of 40 and 100× using a Leica EC3 digital camera and processed with the Leica Suite Las EZ software (Leica Microsystems, Heerbrugg, Switzerland). Particle size was obtained based on the comparison with the scale bar.

2.7 Apparent density and porosity

The tap density (ρ_t, g cm^{-3}) of the microaerogel particles was determined by weighing 1 mL of dried material in a graded cylinder, after manual tapping. The density was then calculated as the ratio of the particle mass (m) and the sample volume (V).

Skeletal densities were measured using Helium pycnometry with a Micromeritics AccuPyc II 1340 device (Micromeritics, Norcross, GA, USA). Each sample was tested four times at room temperature. The overall porosity of the aerogel particles was calculated from the apparent and skeletal densities:

$$Porosity = (1 - \frac{\rho_t}{\rho_s}) \cdot 100 \quad (1)$$

2.8 Scanning electron microstructure

The microstructure of microaerogel particles was analysed using scanning electron microscopy (SEM, Zeiss Supra VP55, Jena, Germany). All samples were sputtered with a thin layer of gold (approx. 6 nm thickness) and the measurements were carried out at an accelerating voltage of 4 kV, and a working distance of 5.8-7.7 mm.

2.9 Specific surface area

The specific surface area of microaerogel particles was estimated by using a low-temperature N_2 adsorption-desorption analysis (Nova 3000e Surface Area Analyzer, Quantachrome Instruments, Boynton Beach, USA) and using the Brauner-Emmet-Teller (BET) method [30]. Before measurements, samples were degassed for 12 h at 60 °C.

2.10 Oil structuring ability

The microaerogel particles were dispersed into sunflower oil (0.1 g/mL), homogenised by using a high-speed mixer at 13,000 rpm for 3 min and collected by centrifugation as previously described [19]. This procedure was repeated twice. Control samples were produced by using PP isolate.

The oil structuring ability of microaerogel particles and PP isolate was expressed as g of oil/g PP isolate or microaerogel particles.

2.11 Rheological properties

The viscoelastic properties were assessed using an RS6000 Rheometer (Thermo Scientific RheoStress, Haake, Germany), equipped with a Peltier temperature controller. Measures were carried out at 20 °C using a parallel plate geometry with a gap of 2.0 mm. Amplitude sweep tests were performed increasing stress from 1.0×10^{-3} to 1.0×10^{3} Pa at 0.1 Hz frequency. Complex viscosity (η^*) as a function of strain % and elastic (G') moduli as a function of stress, and were obtained. Critical stress (τ_c, Pa) was identified as the shear stress value corresponding to a 10% drop in G' value.

2.12 Spreadability

A visual assessment of spreadibility was conducted by spreading the samples manually with a spoon onto a steel surace. Moreover, the spreadibility method reported by Fuhrmann et al. [31] was used, with some modifications. Specifically, a 34TM-5 Instron machine equipped with a back-extrusion food cell (S5405A, Instron), consisting of a moving head and a cup, was used. The latter was filled with 25 g of sample, which was then compressed for 5 mm by the head at 25 mm/s, upon an auto-detected force of 1 N. Yield stress was assessed using the 0.2% offset method, drawing a line parallel to the stress response starting at an offset strain of 0.2% [32].

2.13 Oil holding capacity

About 1.0 g of spread samples was weighed in Eppendorf tubes and centrifuged at 15,000*g* for 15 min at 20 °C (Mikro 20, Hettich Zentrifugen, Tuttlingen, Germany). The released oil was accurately drained

and the tubes were weighed again. The oil holding capacity (OHC) was calculated according to equation 2:

$$OHC(\%) = \frac{P_i - P_r}{P_i} \cdot 100 \quad (2)$$

where P_i (g) is the initial oil content and P_r (g) is the oil released upon centrifugation.

2.14 Lipid and saturated fatty acid content

The lipid composition of commercial spreads was determined using the information provided by the companies on their labels. In the case of aerogel spreads, the composition of ingredients used in their formulation was considered.

2.15 Data analysis

Analyses were carried out in triplicate in at least duplicate samples, and data are reported as mean values and standard deviations. Statistical analysis was performed by using R v. 2.15.0 (The R Foundation for Statistical Computing). Bartlett's test was used to check the homogeneity of variance, one-way ANOVA was carried out and Tukey test was used as a *post hoc* test to determine statistically significant differences among means ($p < 0.05$). A paired sample t-test was conducted to compare the means of two groups for statistical differences, with a significance level set at $p < 0.05$.

3 Results and discussion

3.1 Oil structuring ability of pea protein microaerogel particles

Pea proteins (PP) isolate was suspended in water, adjusted at the pI and subjected to thermal treatment. This procedure is well-known to produce microgels, i.e., hydrated protein particles of globular shape, which can engage in the formation of aggregates, depending on protein concentration [33]. Optical microscopy confirmed that this procedure produced aggregates with a rough surface and a dimension mainly in the range 10-50 μm formed by multiple spheroidal particles (Figure 1).

These particles resulted to be insoluble and were thus collected by centrifugation to be further submitted to water-to ethanol solvent exchange and ethanol removal by supercritical-CO_2 drying. The obtained powder showed an apparent density (0.28 g/cm^3) significantly lower than that of the initial isolate (0.62 ± 0.04 g cm^{-3}) ($p<0.05$), and a porosity of 79 ± 1%, associated with an internal surface area of 142 ± 33 m^2/g, suggesting that the process was able to beget highly porous particles, with physical properties within those commonly associated with aerogels [34]. To confirm this hypothesis, their SEM microstructure was captured. As shown in Figure 2A, the obtained powder was composed of particles

with irregular shapes and dimensions mainly in the range 10-50 µm, associated with a minor presence of aggregates of higher dimension. Higher magnification (Figure 2B) demonstrated these particles to be composed of multiple dried globular structures with dimensions around 2 µm which, in turn, were composed of smaller globular building blocks with dimensions lower than 200 nm (Figure 2C). The latter most likely resulted from the solvent exchange and supercritical-CO_2 drying of PP aqueous microgels. During solvent exchange and supercritical-CO_2-drying, PP aqueous microgels probably engaged in the formation of surface interactions, favoured by the high concentration of the initial dispersion and the polarity changes. Despite not leading to a continuous three-dimensional network, such interactions resulted in particles with a peculiar hierarchic architecture of interconnected dried microgels.

These results demonstrate the ability of the proposed approach to convert PP into a finely porous powder composed of dried microgel particles, which we propose to define as PP microaerogel particles.

The ability of the developed PP microaerogel particles to structure liquid oil was then assessed, according to Paragraph 2.10. Upon oil absorption, PP showed a limited oil structuring ability (0.5 ± 0.1 g oil/g powder), leading to a granular inhomogeneous material that tended to disaggregate during manipulation (Figure 3A) and was thus not further analysed. In these conditions, PP probably acted as a bulking agent, resulting in the formation of an oily powder. By contrast, PP microaerogel particles showed an oil structuring ability more than three times higher than that of the isolate (1.7 ± 0.1 g oil/g powder), begetting a semi-solid homogeneous material, whose appearance is shown in Figure 3B.

The amplitude sweep spectrum of this material (Figure 3C) showed a storage modulus (G′) higher than the loss one (G″), indicating that a visco-elastic material with a prevalence of elastic behaviour over the viscous one was obtained. Such results can be attributed to the fact that PP microaerogel particles can interact with liquid oil through mechanisms not limited to liquid phase bulking, as represented in Figure 3D, which shows an artistic representation of the oil structuring mechanisms of PP microaerogel particles. In particular, part of the oil is expected to be absorbed within the PP microaerogel particle pores (Figure 2), driven by capillary forces, as already demonstrated for whey protein aerogel particles [35]. Moreover, the changes in polarity experienced during the solvent exchange and the subsequent supercritical drying have been demonstrated to induce the exposure of hydrophobic groups originally buried in the protein core [36,37], favouring oil surface adsorption and the enrtrapment of the bulk liquid oil. Finally, the hydrophilic residues of proteins exposed onto the microaerogel particle surface can engage in the formation of a tridimensional network based on weak hydrophilic interactions, further contributing to liquid oil conversion into a structured system [38].

3.2 Low saturated fat spreads with pea protein microaerogel particles

Based on the interesting ability of PP microaerogel particles to structure liquid oil, in the second part of the study, their suitability as ingredients in the development of low-saturated fat cocoa spreads was assessed. To this aim, the microaerogel particles were used in the formulation of a cocoa spread containing, as a lipid phase, sunflower oil solely. Figure 4 reports the appearance of cocoa spreads containing increasing amounts of PP microaerogel particles before and after spreading.

The increase in the amount of PP microaerogel particles in the formulation led to a visible increase in spread structure. At a PP microaerogel particle of 3 and 4% (w/w), the spreads showed visible lumps (Figure 4) and a further increase in the PP mcroaerogel particle content, led to inhomogeneous spreads, with tendency to break into granular fragments. These samples were thus not considered for further analyses. The increase in spread structuration with the aerogel content also affected the physical stability of the spreads, as shown by the oil holding capacity (OHC) data (Figure 5).

This suggests the ability of PP microaerogel particles to stably entrap the liquid oil present in the spread. In this regard, it is important to notice that none of the samples released oil during storage at ambient conditions for up to 2 months.

To better understand the structuring effect of PP microaerogel particles, spreads were investigated through rheological analysis. Figure 6A reports the complex viscosity of the spreads containing microaerogel particles, as compared to those of six commercial spreads.

Using complex viscosity as a proxy for apparent viscosity as for Cox-Merz rule [39], all the spreads displayed pseudo-plastic (shear-thinning) behaviour since the complex viscosity was reduced increasing the strain. This behaviour can be associated with the ability of the PP particles, interconnected by weak surface interactions, to align in the flow direction [40]. The complex viscosity progressively increased with the PP microaerogel particle content in the spread, confirming visual observations (Figure 4).

Upon strain sweep within the linear viscoelastic region (LVR), all the spreads showed a solid-like nature (G' higher than the G''). As illustrated in Figure 6B, the end of LVR, also regarded as the critical stress (σ_y), causing the internal structure to disrupt [41], was found to increase with the amount of PP microaerogel particles. This suggests that the numerous particle-particle interactions established at higher microaerogel particle concentration improved the resistance of the network to deformation.

Small-amplitude rheological results alone are insufficient for evaluating the suitability of the developed samples as spreads. In particular, spreadibility is one of the most important physical properties of cocoa spreads. Figure 4 shows the developed spreads upon manual spreading onto a steel surface. This

empirical test evidenced that spreads with a PP microaerogel particle content of 3 and 4% (w/w) did not spread easily, opposing resistance to the movement imposed by the spoon during the test and forming lumps. To characterize the spread behaviour of the samples, their ability to be deformed under compression can be used. For this reason, PP aerogel spreads were subjected to spreadability analysis, which reflects the work required to spread a material under a given compressive deformation (Figure 6C). All samples showed the typical profile of a spreadable material, characterized by an initial increase of stress with compressive strain, followed by a progressive reduction of stress dependence on the strain, indicating that the sample starts flowing [42]. The increase in microaerogel particle fraction led to a progressive increase in the dependence of the stress on the tool displacement (Figure 6C). A similar trend was also observed for the maximum stress registered upon tool compression, as well as for the yielding stress (Figure 6C). The stress-strain curves of the spreads containing 3 and 4% (w/w) of PP microaerogel particles, also showed some bumps, reflecting the lumps visible upon spreading (Figure 4). Overall, these data show that the increase in PP microaerogel content decreased sample spreadability, attributable to the more intense friction between the sample spread and the walls of the spreadability cup.

The same rheological and spreadability determinations were conducted on commercial cocoa spreads. As shown in Figure 6A and B, the complex viscosity and G′ of commercial cocoa spreads were found to fall in a limited range around 1×10^4 Pa s and 1×10^4 Pa, respectively, while the spreadability curve (Figure 6C) was lower than 0.1 MPa at all the considered strains, associated to yield stress values in the range 0.011-0.039 MPa. Based on these results, it can be concluded that spreads containing sunflower oil solely as lipid phase and an amount of PP microaerogel particles lower than 2 g/100 g present structural properties similar to those of commercially-available spreads.

The microaerogel particle-spreads were also compared with commercial ones in terms of lipid content and profile, as shown in Table 1. It must be noted that all the microaerogel particle spreads showed identical nutritional composition, based on their formulation (see paragraph 2.4).

Table 1. Content of lipids and saturated fatty acids (SFA) of cocoa spreads containing pea protein microaerogel particles and of commercial spreads (samples C1-C6).

Content (g/100 g)	**Commercial spreads**						**Microaerogel**
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**particle-spreads**
Lipids	28.0	31.0	30.9	37.0	33.0	31.2	32.5
of which SFA	4.8	8.6	10.6	11.0	4.8	5.3	4.7

Despite the similar structural properties and overall lipid content, in agreement with the work aim, the spreads containing microaerogel particles showed an amount of SFA lower than the one of commercial spreads. In fact, in the microaerogel spreads, sunflower oil solely was used as lipid phase, which mainly contains unsaturated fatty acids. On the opposite commercial spreads contained different types of SFA-rich lipids, such as palm oil, cocoa butter and coconut oil.

Conclusions

Results demonstrate the possibility of obtaining micrometric aerated particles by thermal treatment of a pea protein dispersion at the isoelectric point, followed by water-to-ethanol solvent exchange and supercritical-CO_2 drying. The obtained microaerogel particles showed a peculiar hierarchical structure, based on dried, surface-interconnected microgel building blocks, thus providing a fine porosity. These microaerogel particles were also demonstrated to be suitable ingredients in the preparation of physically stable cocoa spreads by using liquid oil solely, omitting the use of solid fat. This formulation strategy for saturated fat reduction seems to be particularly promising since allowing a fine tuning of product rheological properties. Indeed, by simply changing the concentration of aerogel articles in the spreads, a wide range of rheological properties can be covered. In light of these findings, pea protein microaerogel particles could be interesting ingredients for partial or even complete replacement of solid fat with liquid oil in a number of different foods including, but not limited to cocoa spreads. In fact, when properly formulated, microaerogel particles could allow the improvement of food nutritional profile without altering mechanical properties, thus likely guaranteeing the expected sensory experience. Nevertheless, additional studies are required to deep into the sensory effect of this spread reformulation strategy.

Although the present work was focused on microaerogels prepared from pea proteins, the methodological approach here proposed could be definitely extended to a number of other plant proteins characterised by poor gelling capacity, largely expanding the range of proteins suitable for aerogelation and the availability of novel ingredients for the formulation of healthier foods. Finally, fundamental investigations to unveil the interaction mechanisms of protein aerogel particles in the presence of complex multiphasic systems containing both water and oil, typically occurring in foods, would be particularly important to expand the application of aerogel-based ingredients beyond that of anhydrous spreads.

Author Contributions

SP Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing - original draft, Writing - review and editing, Project administration, Resources, Supervision. **LDB** Data curation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review and editing; **BS** Formal analysis, Writing - review and editing; **LM** Supervision, Writing - review and editing.

Conflicts of interest

Declarations of interest: none.

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References

1. Y. Zhu, Y. Bo, Y. Liu, Dietary total fat, fatty acids intake, and risk of cardiovascular disease: a dose-response meta-analysis of cohort studies, Lipids Health Dis. 18 (2019) 1–14. doi:https://doi.org/10.1186/s12944-019-1035-2.
2. A.G. Liu, N.A. Ford, F.B. Hu, K.M. Zelman, D. Mozaffarian, P.M. Kris-Etherton, A healthy approach to dietary fats: understanding the science and taking action to reduce consumer confusion, Nutr. J. 16 (2017) 1–15. doi:10.1186/s12937-017-0271-4.
3. S. Melchior, S. Plazzotta, S. Miao, L. Manzocco, M.C. Nicoli, S. Calligaris, Design of fat alternatives using saturated monoglycerides, Food Eng. Rev. (2024) 1–16. doi:10.1007/s12393-024-09379-1.
4. L. Manzocco, S. Calligaris, M. Camerin, L. Pizzale, M.C. Nicoli, Prediction of firmness and physical stability of low-fat chocolate spreads, J. Food Eng. 126 (2014) 120–125. doi:10.1016/j.jfoodeng.2013.10.042.
5. M. Fidaleo, S. Mainardi, R. Nardi, Modeling the refining process of an anhydrous hazelnut and cocoa paste in stirred ball mills, Food Bioprod. Process. 105 (2017) 147–156. doi:10.1016/j.fbp.2017.07.004.
6. D. Rousseau, Microstructural imaging of chocolate confectionery, in: N. Sozer, Imaging Technologies and Data Processing for Food Engineers, Springer International Publishing, Switzerland, 2016, pp. 311–333. doi:10.1007/978-3-319-24735-9_10.
7. T.A. Stortz, A.K. Zetzl, S. Barbut, A. Cattaruzza, A.G. Marangoni, Edible oleogels in food products to help maximize health benefits and improve nutritional profiles, Lipid. Technol. 24 (2012) 151–154. doi:10.1002/lite.201200205.
8. A.R. Patel, P.S. Rajarethinem, A. Gręd owska, O. Turhan, A. Lesaffer, W.H. De Vos, D. Van De Walle, K. Dewettinck, Edible applications of shellac oleogels: spreads, chocolate paste and cakes, Food Funct. 5 (2014) 645–652. doi:10.1039/c4fo00034j.
9. O. Aydemir, Utilization of different oils and fats in cocoa hazelnut cream production, J. Food Process. Preserv. 43 (2019) e13930. doi:10.1111/jfpp.13930.
10. E.D. Co, A.G. Marangoni, Organogels: an alternative edible oil-structuring method, J. Am. Oil Chem. 89 (2012) 749–780. doi:10.1007/s11746-012-2049-3.
11. A.R. Patel, Alternative routes to oil structuring, Springer International Publishing, Switzerland, 2015.

12. C.D. Doan, A.R. Patel, I. Tavernier, N. De Clercq, K. Van Raemdonck, D. Van de Walle, C. Delbaere, K. Dewettinck, The feasibility of wax-based oleogel as a potential co-structurant with palm oil in low-saturated fat confectionery fillings, Eur. J. Lipid Sci. Technol. 118 (2016) 1903–1914. doi:10.1002/ejlt.201500172.
13. G. Fayaz, S.A.H. Goli, M. Kadivar, F. Valoppi, L. Barba, S. Calligaris, M.C. Nicoli, Potential application of pomegranate seed oil oleogels based on monoglycerides, beeswax and propolis wax as partial substitutes of palm oil in functional chocolate spread, LWT 86 (2017) 523–529. doi:10.1016/j.lwt.2017.08.036.
14. S. Bascuas, M. Espert, E. Llorca, A. Quiles, A. Salvador, I. Hernando, Structural and sensory studies on chocolate spreads with hydrocolloid-based oleogels as a fat alternative, LWT 135 (2021) 110228. doi:10.1016/j.lwt.2020.110228.
15. Regulation - 1333/2008 - EN - Additives - EUR-Lex Available online: https://eur-lex.europa.eu/eli/reg/2008/1333/oj (accessed on 25 July 2024).
16. S. Maruyama, N.A. Streletskaya, J. Lim, Clean label: why this ingredient but not that one?, Food Qual. Prefer. 87 (2021) 104062. doi:10.1016/j.foodqual.2020.104062.
17. S. Plazzotta, S. Calligaris, L. Manzocco, Feasibility of protein aerogel particles as food ingredient: the case of cocoa spreads, J. Food Eng. 351 (2023) 111522. doi:10.1016/j.jfoodeng.2023.111522.
18. C.A. García-González, T. Budtova, L. Durães, P. Del Gaudio, P. Gurikov, M. Koebel, F. Liebner, M. Neagu, I. Smirnova, An opinion paper on aerogels for biomedical and environmental applications, Molecules 24 (2019) 1815. doi:10.3390/molecules24091815.
19. S. Plazzotta, S. Calligaris, L. Manzocco, Structural characterization of oleogels from whey protein aerogel particles, Int. Food Res. 132 (2020) 109099. doi:10.1016/j.foodres.2020.109099.
20. Z.X. Lu, J.F. He, Y.C. Zhang, D.J. Bing, Composition, physicochemical properties of pea protein and its application in functional foods, Crit. Rev. Food Sci. Nutr. 60 (2020) 2593–2605. doi:10.1080/10408398.2019.1651248.
21. M. Barac, S. Cabrilo, M. Pesic, S. Stanojevic, S. Zilic, O. Macej, N. Ristic, Profile and functional properties of seed proteins from six pea (Pisum Sativum) genotypes, Int. J. Mol. Sci.11 (2010) 4973–4990. doi:10.3390/ijms11124973.
22. M. Barac, S. Cabrilo, S. Stanojevic, M. Pesic, M. Pavlicevic, B. Zlatkovic, M. Jankovic, Functional properties of protein hydrolysates from pea (Pisum Sativum, L) seeds, Int. J. Food Sci. Technol. 47 (2012) 1457–1467. doi:10.1111/j.1365-2621.2012.02993.x.

23. L. Manzocco, L. Barozzi, S. Plazzotta, Y. Sun, S. Miao, S. Calligaris, Feasibility of water-to-ethanol solvent exchange combined with supercritical co2 drying to turn pea waste into food powders with target technological and sensory properties, LWT 194 (2024) 115778. doi:10.1016/j.lwt.2024.115778.
24. D.I. Hitchcock, The isoelectric point of standard gelation preparation, J. Gen. Physiol. 14 (1931) 685–699. doi:10.1085/jgp.14.6.685.
25. J.M.S. Renkema, C.M.M. Lakemond, H.H.J. De Jongh, H. Gruppen, T. Van Vliet, The effect of ph on heat denaturation and gel forming properties of soy proteins, J. Biotechnol. 79 (2000) 223–230. doi:10.1016/s0168-1656(00)00239-x.
26. T. Nicolai, Formation and functionality of self-assembled whey protein microgels, Colloids Surf. B Biointerfaces. 137 (2016) 32–38. https://doi.org/10.1016/j.colsurfb.2015.05.055.
27. X. Yin, H. Cheng, Wusigale, H. Dong, W. Huang, L. Liang, Resveratrol stabilization and loss by sodium caseinate, whey and soy protein isolates: loading, antioxidant activity, oxidability, Antioxidants 11 (2022) 647. doi:10.3390/antiox11040647/s1.
28. T. Nicolai, C. Chassenieux, Heat-induced gelation of plant globulins, Curr. Opin. Food Sci. 27 (2019) 18–22. doi:10.1016/j.cofs.2019.04.005.
29. C. Yang, A. Li, T.L. Guo, J. Cheng, Z. Liu, H. Hu, J. Wang, Novel organic-inorganic composite pea protein silica food-grade aerogel materials: fabrication, mechanisms, high oil-holding property and curcumin delivery capacity, Int. J. Biol. Macromol. 273 (2024) 132832. doi:10.1016/j.ijbiomac.2024.132832.
30. S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (1938) 309–319. doi:10.1021/ja01269A023.
31. P. L. Fuhrmann, J. Powell, D. Rousseau, Structure and rheology of oil-continuous capillary suspensions containing water-swellable cellulose beads and fibres, Food Hydrocoll. 139 (2023) 108503. https://doi.org/10.1016/j.foodhyd.2023.108503.
32. G. J. Martínez-Díaz, D. Nelson, W. C. Crone, W. J. Kao, Mechanical and chemical analysis of gelatin-based hydrogel degradation, Macromol. Chem. Phys. 204 (2003) 1898–1908. https://doi.org/10.1002/macp.200350042.
33. C. Schmitt, C. Bovay, A.M. Vuilliomenet, M. Rouvet, L. Bovetto, R. Barbar, C. Sanchez, Multiscale characterization of individualized β-lactoglobulin microgels formed upon heat treatment under narrow pH range conditions, 25 (2009) 7899–7909. doi:10.1021/la900501n.

34. J. Fricke, T. Tillotson, Aerogels: production, characterization, and applications, Thin Solid Films 297 (1997) 212–223. doi:10.1016/S0040-6090(96)09441-2.
35. S. Plazzotta, I. Jung, B. Schroeter, R.P. Subrahmanyam, I. Smirnova, S. Calligaris, P. Gurikov, L. Manzocco, Conversion of whey protein aerogel particles into oleogels: effect of oil type on structural features, Polymers 13 (2021) 4063. doi:10.3390/polym13234063/s1.
36. L. Manzocco, S. Plazzotta, J. Powell, A. de Vries, D. Rousseau, S. Calligaris, Structural characterisation and sorption capability of whey protein aerogels obtained by freeze-drying or supercritical drying, Food Hydrocoll. 122 (2022) 107117. doi:10.1016/j.foodhyd.2021.107117.
37. A. De Vries, J. Hendriks, E. Van Der Linden, E. Scholten, Protein oleogels from protein hydrogels via a stepwise solvent exchange route, Langmuir 31 (2015) 13850–13859. doi:10.1021/acs.langmuir.5b03993.
38. A. De Vries, Y.L. Gomez, E. Van der Linden, E. Scholten, The effect of oil type on network formation by protein aggregates into oleogels, RSC Adv. 7 (2017) 11803–11812. doi:10.1039/c7ra00396j.
39. W. P. Cox, E. H. Merz, Correlation of dynamic and steady flow viscosities, J. Polym. Sci., 28 (1958) 619–622. https://doi.org/10.1002/pol.1958.1202811812.
40. D. Won, C. Kim, Alignment and aggregation of spherical particles in viscoelastic fluid under shear flow, J. Nonnewton. Fluid Mech. 117 (2004) 141–146. doi:10.1016/j.jnnfm.2004.01.005.
41. R. Upadhyay, J. Chen, Smoothness as a tactile percept: correlating ‘oral’ tribology with sensory measurements, Food Hydrocoll. 87 (2019) 38–47. doi:10.1016/j.foodhyd.2018.07.036.
42. I. Lončarević, B. Pajin, J. Petrović, D. Zarić, M. Sakač, A. Torbica, D.M. Lloyd, R. Omorjan, The impact of sunflower and rapeseed lecithin on the rheological properties of spreadable cocoa cream, J. Food Eng. 171 (2016) 67–77. doi:10.1016/j.jfoodeng.2015.10.001.

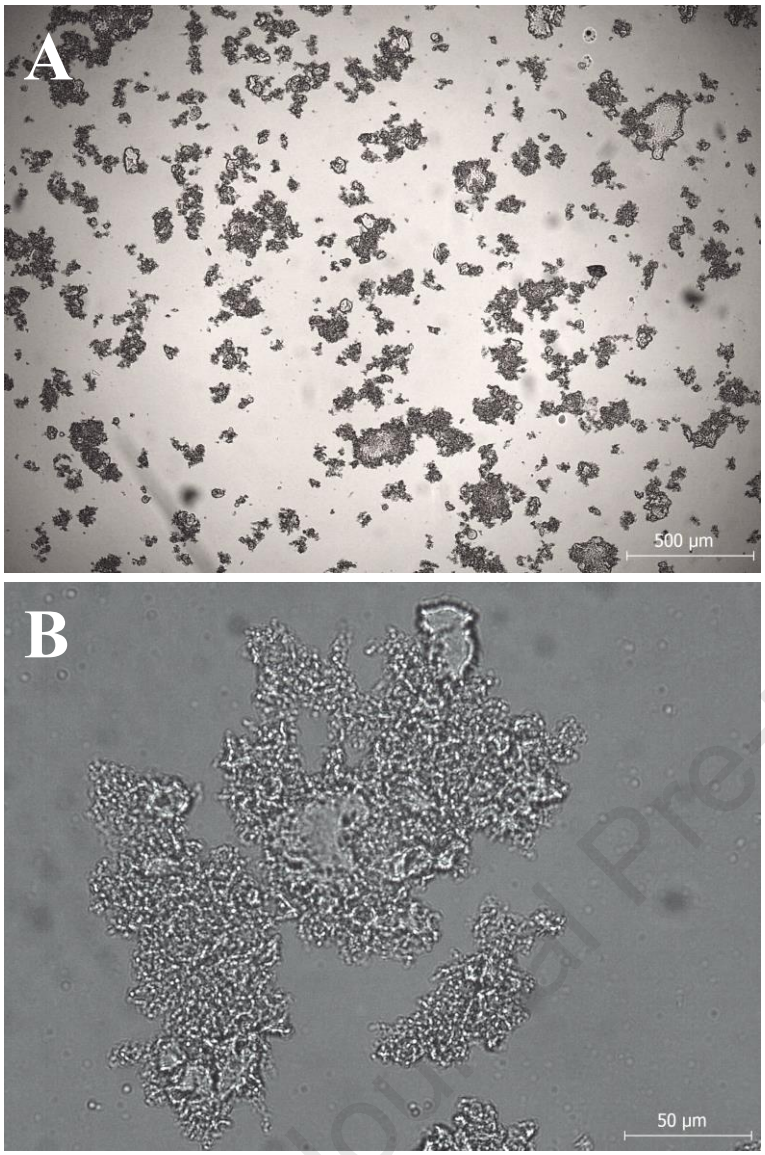

Figure 1. Microstructure obtained at the optical microscope of pea protein microgel particles at 4 (A), and 40× magnification (B).

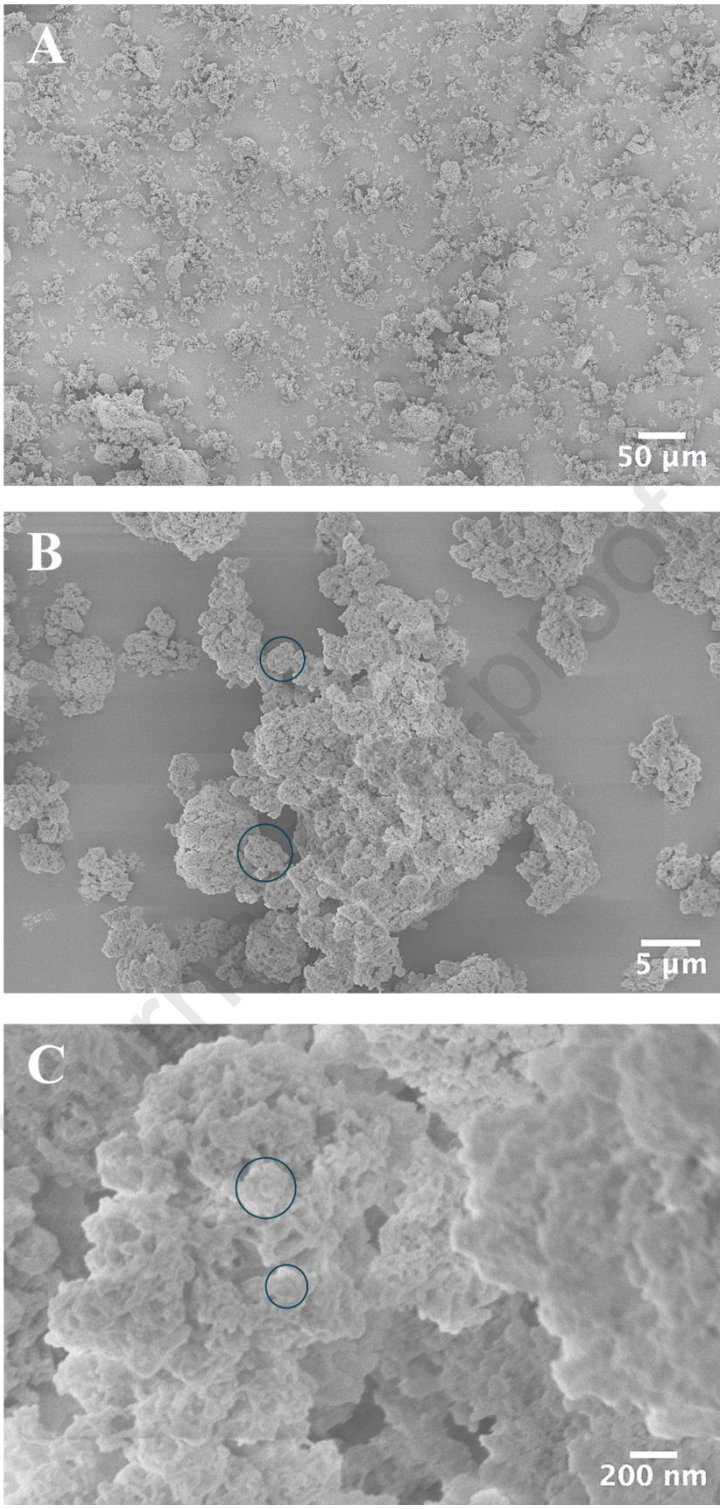

Figure 2. SEM microstructure of pea protein microaerogel particles at 500 (A), 5,000 (B) and 100,000× magnification (C).

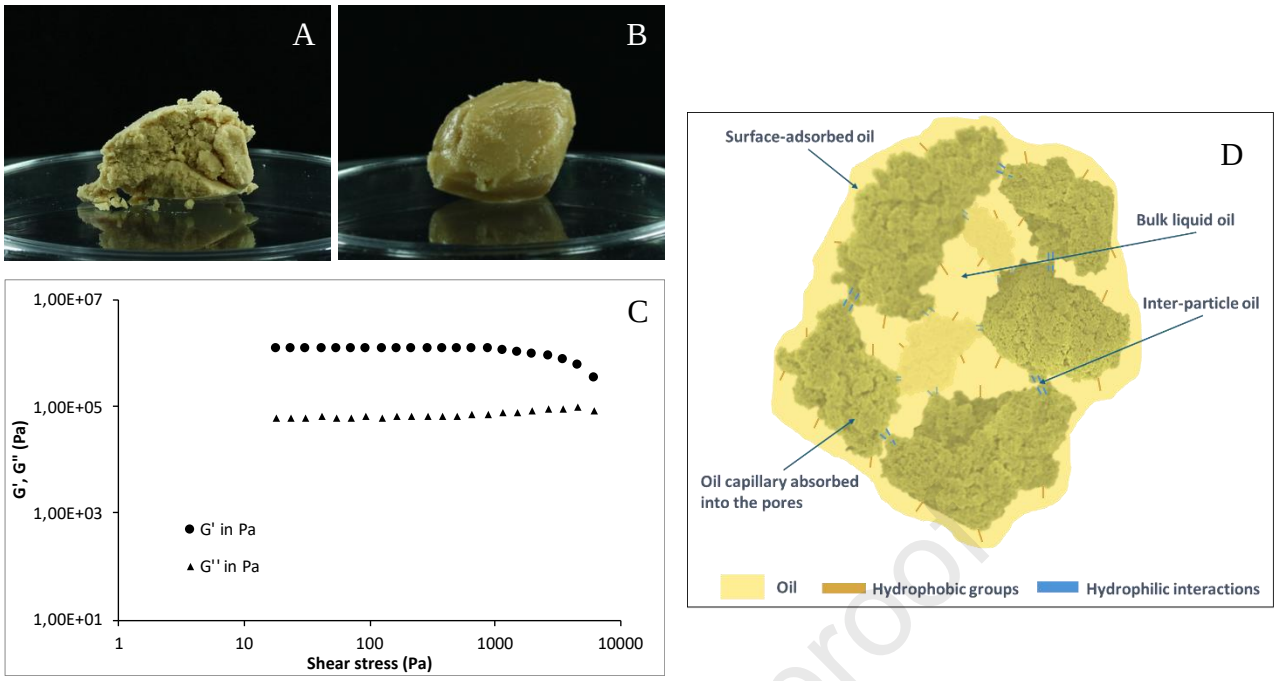

Figure 3. Appearance of the samples obtained after absorption of sunflower oil by pea protein isolate (A) and pea protein microaerogel particles (B). Figure 3C and 3D report the amplitude sweep rheogram of the sample obtained after absorption of sunflower oil by pea protein microaerogel particles and a representation of the oil structuring mechanisms, respectively.

	Aerogel amount in the spread (g/100 g spread)				
	0	1	2	3	4
Before spreading					
After spreading					

Figure 4. Appearance of cocoa spreads containing increasing amounts of pea protein microaerogel particles before and after spreading on a steel surface.

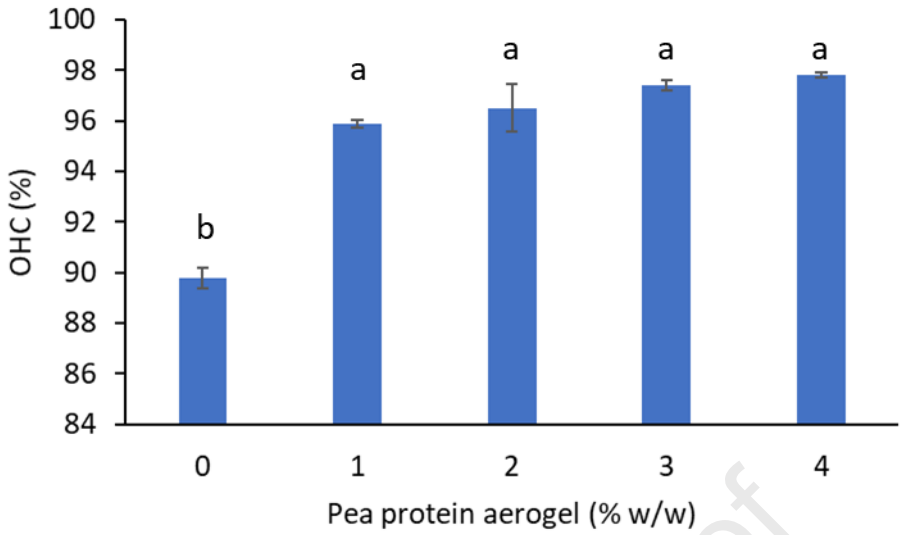

Figure 5. Oil holding capacity (OHC) of cocoa spreads containing increasing amounts of pea protein microaerogel particles. [ab] mean values indicated by different letters are significantly different ($p<0.05$).

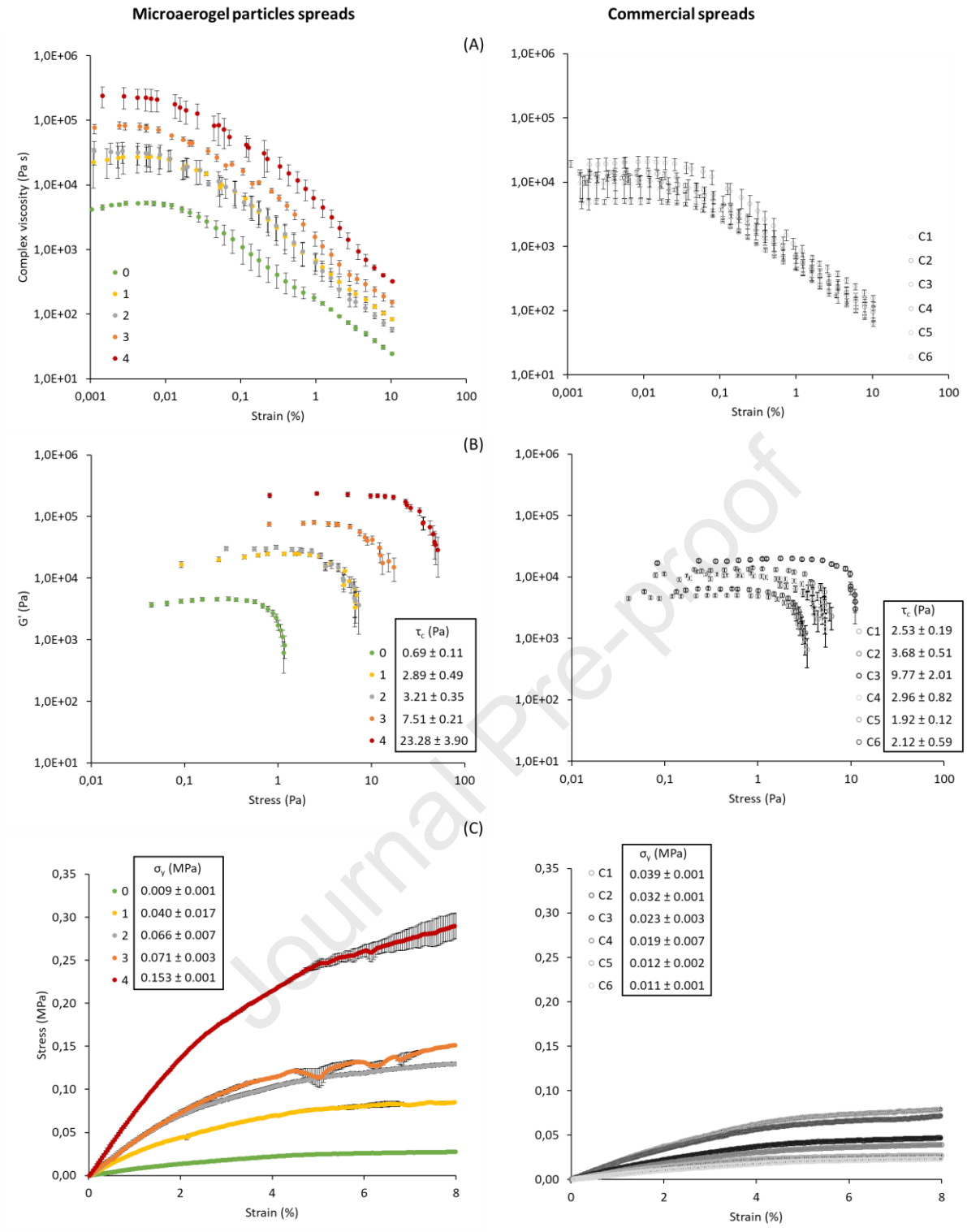

Figure 6. Complex viscosity as a function of shear rate (A), elastic modulus (G′) as a function of shear stress and critical stress (τ_c) (B), and force-displacement curves and yield stress (σ_y) (C) of cocoa spreads containing increasing amounts (g/100 g spread) of pea protein microaerogel particles and of commercial spreads (samples C1-C6).

- Pea protein microgel particles are obtained by thermal gelation at the isoelectric pH
- Microaerogel particles are produced by solvent exchange and supercritical-CO_2-drying
- Microaerogel particles show 142 m^2/g surface area, 0.28 g/cm^3 density, 79% porosity
- One g of microaerogel particles structures 1.7 g oil into a viscoelastic gel
- Microaerogel particles can be used for low-saturated fat cocoa spread formulation

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: