Feasibility of protein aerogel particles as food ingredient: The case of cocoa spreads

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Abstract:	The feasibility of aerogel particles made of whey protein isolate (WP) as food ingredient was studied for the first time. To this aim, cocoa spreads, which traditionally contain large amounts of solid fat, were prepared using sunflower oil as lipid phase instead of fats. Sunflower oil was mixed with sugar, cocoa powder and WP aerogel particles or unstructured WP (control). Two preparation methodologies were applied: (i) mixing ingredients all at once (one-step) and (ii) preliminary oil absorption into aerogel particles, and subsequent mixing with the other ingredients (two-step). WP aerogel spreads showed higher viscosity than control ones, demonstrating the peculiar functionality of porous aerogel particles in entrapping oil. However, the preparation procedure drove the inter-particle interactions among ingredients. In particular, preliminary oil absorption into aerogel particles (two-step procedure) allowed a stronger network to be obtained. Results open to the possibility of applying aerogel particles as food ingredients, highlighting the need of a dedicated process design is performed to maximise the exploitation of their functionality.
Suggested Reviewers:	Carlos García-González University of Santiago de Compostela carlos.garcia@usc.es Expert in the field of aerogels
	Miguel Cerqueira miguel.cerqueira@inl.int Expert in fat substitution

Dear Editor,

We send to your attention the research article entitled "Feasibility of protein aerogel particles as food ingredient: the case of cocoa spreads" by Stella Plazzotta, Sonia Calligaris and Lara Manzocco.

We propose as short communication because this is, in our knowledge, the first study on the possible application of aerogels in food products. Aerogels are unique materials with low density, open porosity, and high surface area. These characteristics confer unique functionalitities of these materials that could be exploited in foods. Contrary to other sectors, however, the potential of aerogels in foods remains definitively under-reported.

In this study, whey protein aerogel particles are used as food ingredient in a real food product formulation for the first time. Cocoa spreads were selected as an example of food traditionally containing large amounts of solid fat. Sunflower oil was mixed with sugar, cocoa powder and WP aerogel particles or unstructured WP (control). Two preparation methodologies were applied to understand the effect of processing on aerogel functionality.

WP aerogel spreads showed higher viscosity than control ones, demonstrating the peculiar functionality of porous aerogel particles in entrapping oil. However, the preparation procedure drove the inter-particle interactions among ingredients. Results open to the possibility of applying aerogel particles as food ingredients, highlighting the need of a dedicated process design is performed to maximise the exploitation of their functionality.

We hope that our work could meet the requirements of Journal of Food Engineering, so you might consider it for publication in this Journal.

For any further information, do not hesitate to contact me.

Best regards,

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Highlights

Whey protein aerogel particles can be used to develop cocoa spreads

Preparation procedure affects rheological behavior of spreads

Aerogel functionality depends on particle-oil interaction

Preliminary oil absorption into aerogels maximizes their functionality

Feasibility of protein aerogel particles as food ingredient: the case of cocoa spreads

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Abstract

The feasibility of aerogel particles made of whey protein isolate (WP) as food ingredient was studied for the first time. To this aim, cocoa spreads, which traditionally contain large amounts of solid fat, were prepared using sunflower oil as lipid phase instead of fats. Sunflower oil was mixed with sugar, cocoa powder and WP aerogel particles or unstructured WP (control). Two preparation methodologies were applied: (i) mixing ingredients all at once (one-step) and (ii) preliminary oil absorption into aerogel particles, and subsequent mixing with the other ingredients (two-step).

WP aerogel spreads showed higher viscosity than control ones, demonstrating the peculiar functionality of porous aerogel particles in entrapping oil. However, the preparation procedure drove the inter-particle interactions among ingredients. In particular, preliminary oil absorption into aerogel particles (two-step procedure) allowed a stronger network to be obtained. Results open to the possibility of applying aerogel particles as food ingredients, highlighting the need of a dedicated process design is performed to maximise the exploitation of their functionality.

Keywords: aerogel, oleogel, fat reduction, protein network, cocoa spreads

1. Introduction

Aerogels are solid materials with low density, open porosity, and high surface area obtained by the extraction of the solvent from hydrogels or organogels, using a method that allows the preservation of the solid network structure, typically supercritical carbon dioxide ($scCO_2$)-assisted drying being the gold-standard (Garcia-Gonzalez et al., 2019; Manzocco et al., 2021). The unique features of aerogels are being explored in many industrial fields for biomedical, environmental and cosmetic purposes (www.cost-aerogels.eu; Manzocco et al., 2021). Contrary to other sectors, however, the potential of aerogels in foods remains under-reported.

The preparation of aerogels intended for food applications is virtually possible from any bio-based material characterized by a tridimensional polymeric network. Among bioaerogels, those structured by proteins are of particular interest for food application, being proteins largely used conventional ingredients in many food formulations.

Recently, our research group demonstrated the excellent capacity of whey protein (WP) aerogel particles to form aerogels with peculiar capacity to load high quantities of oil (Manzocco et al., 2021; Plazzotta et al., 2020; Plazzotta et al., 2021, 2022). This ability is due to the establishment of a network based on protein inter-particle interactions in oil, leading to the formation of plastic materials, with rheological behaviour analogous to that of fats (Plazzotta et al., 2020; Plazzotta et al., 2021). These materials might be included in the framework of oleogels (Co & Marangoni, 2012; Patel & Dewettinck, 2016).

Based on these finding, aerogel functionality could be exploited to substitute fat in many food formulations. Since no data are available on this aspect in our knowledge, we explored the feasibility to use WP aerogel particles in the preparation of anhydrous cocoa spreads using sunflower oil instead of fats as lipid phase. Cocoa spreads were prepared by mixing sunflower oil with WP aerogel particles, sugar and cocoa powder. As control, WP aerogels were substituted with unstructured WP. The ratio among the dried ingredients was selected in the range found in cocoa spreads on the market. Two different preparation procedures were considered: (i) mixing ingredients all at once or (ii) preliminarily mixing oil with aerogel particles, followed by the addition of the other ingredients. Spreads were analysed for flow behaviour, microstructure and physical stability. Results demonstrated the critical role of processing optimization in exploiting aerogel functionality.

2. Materials and methods

2.1 Materials

Whey protein isolate (WP, 94.7% protein content; 74.6% β-lactoglobulin, 23.8% α-lactalbumin, 1.6% bovine serum albumin) was purchased from Davisco Food International Inc. (Le Sueur, MN, USA). Sunflower oil, icing sugar, cocoa powder and commercial cream samples were purchased in a local market. 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 Preparation of whey protein aerogel particles

WP aerogel particles were prepared as described elsewhere (Plazzotta et al., 2020).

2.3 Preparation of cocoa spreads

Cocoa spreads were prepared with WP aerogel particles (10% w/w), cocoa powder (7% w/w), icing sugar (33% w/w) and sunflower oil (50% w/w). The oil was added to the dry ingredients following two different procedures, characterized by a different mixing order. In the "one-step" procedure, the ingredients were manually mixed together in one step; in the "two-step" procedure, WP aerogel particles were manually mixed with oil, followed by the addition of cocoa powder and icing sugar and further manual mixing. The mechanisms involved in oil structuring by WP aerogel particles and oleogels physical features have been previously characterized (de Vries et al., 2017; Plazzotta et al., 2020; Plazzotta et al., 2021). Control spreads were produced by using WP instead of WP aerogel particles. The obtained spreads were stored in sealed sample holders at room temperature for up to 1 month.

2.4 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).

2.5 Optical microscopy

Samples were placed on a glass slide, covered with a cover slide, and observed using a Leica DM 2000 optical microscope (Leica Microsystems, Heerbrugg, Switzerland). The images were taken using standard lighting and polarized lenses at 100× magnification using a Leica EC3 digital camera and elaborated with the Leica Suite Las EZ software (LeicaMicrosystems).

2.6 Oil holding capacity

Oil holding capacity was assessed after sample centrifugation as described by Fayaz et al. (2017).

2.7 Flow behavior

Viscosity determinations were carried out using a rheometer equipped with a Peltier temperature control unit (Haake Rheostress 6000, Thermo Scientific, Karlsruhe, Germany). A plate-plate geometry (diameter 25 mm) was used and the measurements were carried out at 20 ºC. Before starting measurements, each sample was stabilized in the rheometer for 5 min. The viscosity of the spread samples was measured with a gap of 1.5 mm and in the shear rate range 0.1-50 s^{-1}. Flow curves were fitted according to Herschel-Bulkley model (Aydemir et al., 2021):

$$\sigma = \sigma_0 + K\dot{\gamma}^n \qquad (2)$$

where σ is the shear stress (Pa), σ_0 (Pa) is the yield stress, K is the consistency index (Pa s^n), $\dot{\gamma}$ is the shear rate (1/s) and n is the flow behaviour index

2.8 Data analysis

Analyses were carried out in triplicate in at least duplicate samples, and data are reported as mean values and standard deviations. Goodness of fit was evaluated through the determination coefficient (R^2). 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 ($p < 0.05$).

3 Results and discussion

Table 1 shows the appearance of cocoa spreads containing WP aerogel particles or unstructured WP (control), prepared according to two different procedures: "one-step" where ingredients were mixed all at once; “two-step” where aerogel particles were preliminary mixed with oil and then added with the remaining dry ingredients. The control spreads appeared as viscous liquids, quickly releasing oil, independently on the preparation methodology. Differently, the aerogel containing spreads resulted semisolid materials with a good capacity to entrap oil, as demonstrated by OHC values of 84.9 ± 1.2 and 86.0 ± 0.3 for the spreads prepared according to “one-step” and “two-step” methodologies, respectively. Aerogelation actually involves different processes that modify the WP structural organization, conferring them a high capacity of oil absorption can be are obtained (Manzocco et al., 2022). Different concomitant mechanisms account for the capability of aerogel particles to structure oil: (i) oil absorption into the aerogel pores, driven by capillary forces; (ii) oil adsorption onto the particle surface, driven by hydrophobic interactions; (iii) physical oil entrapment within inter-particle spaces, due to the interactions among the hydrophilic residues of WP aerogel particles (Plazzotta et al., 2020; Plazzotta et al., 2021; Selmer et al., 2019).

Spreads containing the aerogel particles presented the typical rheological behavior of “plastic” materials, which was well described by the Herschel-Bulkley model (R^2>0.89) (Figure 1). However, the procedure applied for their preparation affected their rheological behavior, as shown by model parameters.

The preliminary addition of oil to WP aerogel particles in the “two-step” procedure allowed oil to be effectively absorbed into the aerogel particles, facilitating particle-particle interactions and leading to a spread with higher K and σ_0, and lower n (Figure 1). It can be hypothesized that during the “one-step” procedure, oil-aerogel particle interactions are hindered by the presence of other ingredient leading to the formation of a weaker network among WP particles.

The microstructure of the aerogel spreads prepared with the two procedures supports this hypothesis (Figure 2). It consisted of a continuous oil phase in which particles of different nature (i.e. sugar, WP aerogel and cocoa powder) and dimension were present. Among them, protein particles of inhomogeneous shape with dimensions up to 250 μm were visible, as determined by comparison with the scale bar. Such particle conformation is in line with that of the protein aerogel particles, characterized in a previous study (Plazzotta et al., 2020). The small dark particles with spheroidal shape can be instead attributed to cocoa powder, which was evenly distributed in the spreads. Moreover, the location of sugar crystals in the system was evidenced as white areas under polarized light. In this regard, crystalline areas were identified onto the surface of the protein particles in the micrograph of the spread obtained by the "one-step" procedure. By contrast, the "two-step" preparation procedure led to a more homogeneous dispersion of sugar crystals in the spread, as indicated by the presence of smaller and evenly distributed white areas in the polarized light micrograph. These microstructural evidences confirmed the critical role of the preparation procedure in affecting the interactions among the ingredients in the spread. It can be hypothesized that when powdered ingredients are mixed all at once with oil, hydrophilic sugar-sugar and sugar-protein interactions would preferably establish to the detriment of protein-protein interactions, which are the basis of the formation of a strong protein network entrapping oil (de Vries et al., 2017; S. Plazzotta et al., 2020). In addition, sugar clusters onto the protein particle surface would reduce the accessibility of the open porosity of the aerogel surface, limiting oil absorption into the pores. By contrast, when aerogels are mixed with oil before adding the other powdered ingredients, the oil would be more efficaciously absorbed into the aerogel pores and protein-protein hydrophilic interactions would be favored. The strength of this network would be minimally affected by the following addition of sugar, which would be unable to disrupt the hydrophilic protein-protein interactions already established in oil environment.

4. Conclusions

In this work, whey protein aerogel particles were used in the formulation of chocolate spreads for the first time. Results demonstrate that this strategy allows the preparation of physically stable cocoa spreads by using liquid oil instead of solid fat. However, the preparation procedure drives the interactions among ingredients resulting in different structural organization of the system. This preliminary work opens the possibility to exploit protein aerogels as novel structuring ingredients in a number of fat-based foods and demonstrate the critical role of process design to fully exploit their potential functionality.

Author Contributions

Conceptualization SP LM, Data curation SP, Formal analysis SP, Investigation SP, Methodology SP LM, Project administration SC LM, Resources LM, Supervision SC LM, Visualization SP LM, Writing - original draft SP, Writing - review and editing All the authors.

Conflicts of interest

Declarations of interest: none.

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Figure caption

Figure 1. Shear stress as a function of shear rate of cocoa spreads containing whey proteins aerogel particles, prepared according to “one-step” or “two-step” procedures. Herschel-Bulkley rheological parameter estimates are also reported in the inset, where for each means indicated by different letters are statistically different ($p<0.05$).

Table 1. Appearance of cocoa spreads containing unstructured whey proteins or whey proteins aerogel particles, prepared according to “one-step” or “two-step” procedures. Optical microscopy images with standard and polarized light of aerogel containing spreads are also reported (Red circles cocoa powder; white circles WP aerogels).

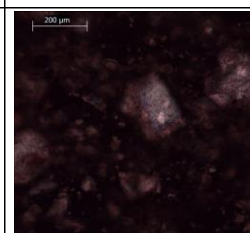
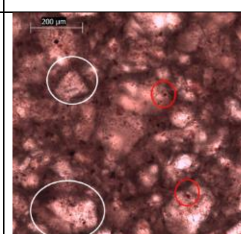
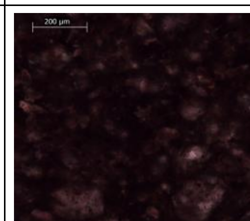
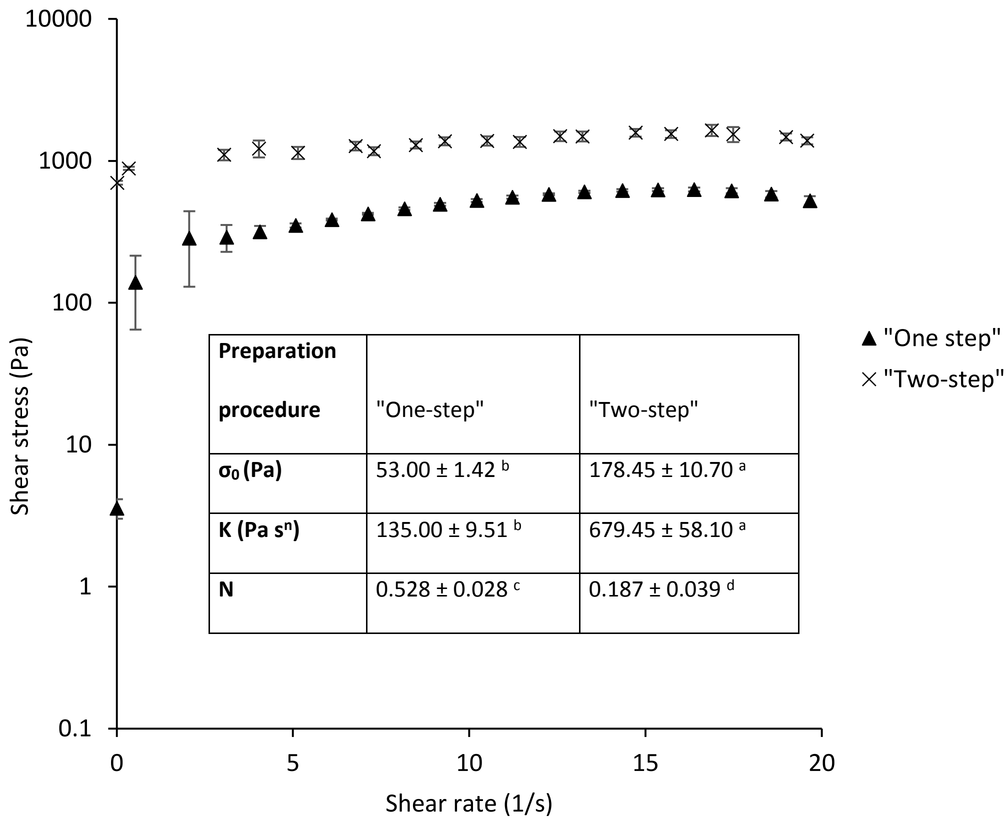
Preparatio n procedure	**Unstructured WP**	**WP aerogel particles**	**Microstructure (optical microscopy)**	**Microstructure (PLM)**
One-step				
Two-step				

Figure 1



Preparation procedure	"One-step"	"Two-step"
σ_0 (Pa)	53.00 ± 1.42 [b]	178.45 ± 10.70 [a]
K (Pa s^n)	135.00 ± 9.51 [b]	679.45 ± 58.10 [a]
N	0.528 ± 0.028 [c]	0.187 ± 0.039 [d]

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

x All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

x This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

x The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

○ The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

Author's name	Affiliation