POSTER COMMUNICATIONS

Engineering of bioaerogels as key ingredients in the development of functional foods to deliver health through diet

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The first part of this PhD project addressed the development of aerogels from protein sources and the assessment of their suitability as functional ingredients. Both animal (whey protein isolate WPI) and vegetable proteins (soy and pea protein isolate) were considered. Protein hydrogels were prepared at different pH, ground, subjected to water-to-ethanol solvent exchange and dried by means of supercritical-CO2 to obtain aerogels, which were characterised for physical properties. Liquid edible oils were then absorbed into aerogels, leading to oleogels. The latter were *in-vitro* digested to highlight the potentialities of aerogel-templated oleogels in modulating both protein and lipid digestibility.

Ingegnerizzazione di bioaerogel come ingredienti chiave nello sviluppo di alimenti funzionali per migliorare la salute attraverso la dieta

La prima parte di questo progetto di dottorato ha riguardato lo sviluppo di aerogel proteici e la valutazione della loro potenzialità come ingredienti funzionali. Sono state considerate proteine animali (isolato di siero di latte) e vegetali (isolati di soia e pisello). Gel preparati a diversi pH sono stati macinati, sottoposti ad una procedura di sostituzione acqua-etanolo ed essiccati mediante CO2 supercritica. Gli aerogel ottenuti sono stati caratterizzati in termini di proprietà fisiche. Oli alimentari sono stati fatti assorbire negli aerogel, ottenendo oleogel. Questi sono stati sottoposti a digestione *in-vitro* per evidenziarne le potenzialità nel modulare la digeribilità proteica e lipidica.

**Keywords**: animal protein, plant proteins, porous materials, fat replacement, *in vitro* digestion.

# **Introduction**

In agreement with the PhD thesis project (De Berardinis, 2022), this poster reports the main results relevant to the following activities:

1. Identification of proteins suitable as bioaerogel precursors;
2. Bioaerogel preparation and characterization;
3. Application of bioaerogels as functional ingredients able to structure oil and steer lipid digestibility.

# **Materials and Methods**

Whey protein isolate (WPI, Davisco Food International Inc., Le Sueur, MN, USA), soy and pea protein isolate (SPI and PPI, Myprotein, Manchester, England) were dispersed in water in concentration of 20, 14 and 19% w/w respectively, and adjusted at pH 7 or at the isoelectric pH (pI) adding NaOH or HCl (Sigma Aldrich, Milan, Italy). The dispersions were gelled at 85 °C for 10 min. The obtained hydrogels were then subjected to water-to-ethanol solvent-exchange until reaching a final ethanol concentration of 98% w/w. The obtained alcolgels were ground and supercritically dried at 12 MPa, 60 °C with a CO2-flow rate between 80 and 120 g/min to produce aerogel particles. The latter were characterized for density, porosity, specific surface area, SEM microstructure and ability to structure sunflower oil (SO). Aerogel particles were thus used to prepare SO oleogels (Plazzotta et al., 2020), which were characterized for rheological properties. WPI aerogel particles prepared at pI and relevant oleogels were finally selected and subjected to *in vitro* digestion (INFOGEST static digestion protocol, Brodkorb et al. 2019) and visually observed *via* confocal microscopy. The digested protein fraction of WPI oleogels was characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and bicinchoninic acid (BCA) assay, while lipid digested fraction by differential light scattering (DLS) and lipolysis degree.

# **Results and discussion**

## **Identification of proteins suitable as bioaerogel precursors**

The high solubility of WPI allowed to obtain 20% w/w protein solutions, which led to self-standing hydrogels upon thermal treatment at both pH 4.8 (pI) and pH 7.0. By contrast, the lower solubility of SPI and PPI accounted for a maximum dispersibility of 14 and 19% w/w, respectively. Upon thermal treatment, SPI and PPI dispersions were not able to form self-standing gels, independently of the pH. Rather, microgels in the form of spherical aggregates were obtained at their pI (4.5). Based on these results, WPI hydrogels at pI and pH 7, and SPI and PPI hydrogels at pI were selected to produce aerogels.

## **Bioaerogel preparation and characterization**

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| ***Table 1.*** *Physical properties of whey protein isolate (WPI) aerogel at the isoelectric point. Resulting oleogel with its oil content and elastic modulus (G′) is also shown.* | | | | | | |
| WPI aerogel microstructure | Density  (g/cm3) | Surface area (m2/g) | Oleogel  appearance | Oil content (goil/100 g) | G′  (× 105 Pa) | |
|  | 0.17 ± 0.02 | 35.6 ± 2.3 | Immagine che contiene piatto, stoviglie, Piatto, interno  Descrizione generata automaticamente | 85.2 ± 2.3 | | 3.5 ± 0.2 |

Upon conversion into aerogel particles, highly porous powders with low density were obtained. The aerogel surface area resulted higher when proteins were conditioned far from pI (up to 350 m2/g) (Jung et al. 2023), while much lower values were detected at pI (about 30 m2/g). This difference can be attributed to the minimisation of electrostatic repulsions among proteins at pI, with formation of microgels with close structure. By contrast, far from pI, a stranded gel network is obtained (De Berardinis et al., 2023). As a representative example, Table 1 reports the main physical characteristics of WPI aerogel particles at pI.

## **Application of aerogels as functional ingredients to structure oil and steer lipid digestibility**

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| ***Figure 1.*** *Confocal microscopy after gastric digestion of sunflower oil and oleogel prepared with whey protein aerogel particles at pI.* |

WPI, SPI and PPI aerogel powders quickly absorbed sunflower oil, leading to highly homogeneous plastic materials, containing 85%, 72% and 63% w/w oil, respectively. As an example, Table 1 reports the appearance of the oleogel prepared with WPI aerogel particles. In all cases, oleogels showed rheological properties (G′) similar to those of commercial fats rich in saturated fatty acids. Oil structuring through aerogel particles can be explained through different mechanisms: (i) oil absorption into the aerogel pores, driven by capillary forces; (ii) oil adsorption onto the particle surface, driven by hydrophobic interactions; (iii) entrapment of liquid oil in the spaces among the aerogel protein particles, which form a network based on weak hydrophilic interactions (Selmer et al., 2019). The potentiality of aerogel-mediated oil structuring on both lipid and protein digestibility was finally studied by *in vitro* digestion. Confocal micrographs (Figure 1) clearly demonstrated that the original WPI oleogel structure was lost at the gastric level, entrapping oil droplets which were much smaller (D32 < 10 μm) than those observed in the case of the unstructured oil (D32 > 30 μm). SDS-PAGE and BCA assay confirmed that aerogelation reduced the gastric proteolysis of WP from nearly 100% to 70%. The digestion of the oleogel led to similar gastric protein digestibility. Upon intestinal digestion, aerogel proteins resulted completely hydrolysed. The lipolysis degree of the oleogel (75%) was higher than that of the unstructured sunflower oil (66%), due to the larger surface offered by smaller oil droplets to the action of intestinal lipases. This was confirmed by dynamic light scattering, showing a shift towards smaller size in the digestive micelle distribution of oleogels at the end of the intestinal phase (Plazzotta et al., 2022).

## **Conclusions**

Whey, soy and pea protein isolates were demonstrated to be suitable precursors to produce aerogel powders with high porosity and surface area. The developed aerogel powders showed high oil structuring ability, leading to semi-solid materials resembling traditional fats. Oleogelation through aerogel-template approach was shown to steer both protein and lipid digestibility. These results support the possible role of aerogels as key ingredients in the development of foods able to deliver health through diet.

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