LWT - Food Science and Technology 213 (2024) 117078

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Hybrid aerogels of spirulin and whey proteins as novel cellular solids

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ARTICLE INFO

Keywords:
Algae
Protein transition
Meat analogues
Hydrogel
Sensory analysis

ABSTRACT

This work proposes a new strategy to obtain cellular solids of spirulin (SP) cells and whey protein (WP) using the aerogel production process. To this aim, aqueous suspensions containing 20 g/100 g of a mixture of WP and SP in different ratios, were submitted to thermal gelation. The obtained hydrogels were characterised by an intense green colour and the typical unpleasant fishy odour of SP. Hydrogels were then submitted to ethanol solvent exchange and supercritical-CO_2 drying, leading to hybrid aerogels, which were analysed for physical properties (colour, density, volume contraction, firmness, microstructure). The conversion of hydrogels into aerogels partially reduced their green colour. The increase in SP in the aerogel progressively decreased its firmness, so that the aerogels obtained from suspensions containing more than 10 g/100 g spirulin were not able to maintain the continuity of the aerogel network. The latter was demonstrated by SEM to consist of dried WP microgels organised in a tri-dimensional architecture embedding SP cells. The aerogel obtained from 10 g/100 g SP suspension did not swell or disintegrate upon absorption of both water and oil. Sensory analysis also showed this sample to have negligible SP odour. These results open new possibilities in the development of hybrid cellular solids with neutral sensory properties, posing the basis for a new approach to the engineering of food tissue analogues.

1. Introduction

The awareness that global demand for proteins cannot be satisfied by current farming practices has raised a general concern about the availability of proteins for an ever-increasing population (Henchion et al., 2017). Most animal-based foods are represented by cellular tissues (e.g., meat and fish), highly organised cellular solids based on protein networks embedding other components such as water and lipids (Ertbjerg & Puolanne, 2017). The possibility of reproducing such cellular structure by exploiting sustainable processes and relying on renewable resources stands as a key strategy for ensuring food security. In this context, cultured meat, also known as lab-grown or cell-based meat, represents a possible alternative to traditional farming (Jahir et al., 2023). According to this approach, a small sample of animal cells, typically muscle cells, is taken from a living animal and placed in a nutrient-rich culture medium allowing for cell proliferation and forming muscle tissue. Despite the high potential of this strategy, regulatory and consumer acceptance issues surrounding cultured meat are significantly limiting its adoption (Bhat et al., 2015; Bryant, 2020).

Spirulin (*Arthrospira platensis*) (SP) is a unicellular cyanobacterium, containing up to 70 g/100 g (dry matter based) of proteins rich in essential amino acids, including leucine, lysine and threonine (Becker, 2007; Lupatini et al., 2017; Mišurcová et al., 2014; Spínola et al., 2022). In addition, up to 20 g/100 g of spirulin protein fraction consists of phycocyanin (Pez Jaeschke et al., 2021), which is known for its high antioxidant activity (Chentir et al., 2018; Fernández-Rojas et al., 2014). Spirulin cultivation is well known to be amenable to large-scale production and its unique ability to fix CO_2 can be exploited in close connection to industrial plants to substantially mitigate greenhouse gas emissions of industrial processes (Moraes et al., 2016).

Despite the unique protein content of SP cells, very limited information is available on the possibility of using them as cellular units in the preparation of tissue analogues. This is probably due the planktonic nature of SP cells, which are unable to aggregate with each other or form a tridimensional gel network resembling tissue-like structures. Nevertheless, some attempts have been made by structuring SP cells in the presence of other proteins. To this regard, Grahl et al. (2018), used SP in association with soy proteins to prepare a soy-based meat analogue. In this case, extrusion was applied to obtain a matrix with an oriented protein network, but the obtained meat analogue had a black colour,

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https://doi.org/10.1016/j.lwt.2024.117078
Received 22 August 2024; Received in revised form 14 November 2024; Accepted 18 November 2024
Available online 19 November 2024

intense flavour with earthy notes and a musty odour. SP is actually characterised by a typical dark green colour and flavours associated with fish, soil, mold or wood aromas, which adversely affect consumer acceptability (Grahl et al., 2020; Niccolai et al., 2019).

The issues related to the structural and sensorial effect of SP in the development of cellular tissues could be faced by using the aerogel production process. Aerogels are solid open-porous materials characterized by low densities (as low as 0.0003 g/cm^3), high surface areas (that may exceed 3000 m^2/g), related to the presence of mesopores, and high porosities (as high as 99.8 %) (Aegerter et al., 2023). Aerogels can be derived from drying wet gels in a way that preserves their volume into the dry object (Leventis et al., 2010). For example, they might be produced by removing water from an aqueous biopolymeric gel (hydrogel) through a two-step procedure. Firstly, a water-to-ethanol solvent exchange procedure is carried out, followed by ethanol removal using a supercritical-CO_2 drying process (Manzocco et al., 2021). This approach allows for preserving the polymeric gel network, obtaining solid nano or mesoporous materials with an open-porosity. The latter accounts for the well-known capacity of certain aerogels to absorb and retain high amounts of water (Manzocco et al., 2022), as it happens in cellular tissues. Moreover, their ability to hold fluids was also exploited with reference to liquid oil in the preparation of fat analogues (Ciuffarin et al., 2023; Jung et al., 2023; Plazzotta et al., 2020).

Aerogel materials can be obtained from carbohydrate or protein hydrogels. To this regard, thanks to their high gelling capacity, whey proteins (WP) have been demonstrated to be ideal precursors able to be structured in tri-dimensional protein scaffolds (Peydayesh et al., 2024; Plazzotta et al., 2020, 2021). However, even plant tissues, such as lettuce leaves and parenchyma of parsley, celery and carrot (Gromadzińska et al., 2015; Plazzotta et al., 2018) could be turned into aerogels. In the study of Plazzotta et al. (2019), the aqueous phase of a carrageenan hydrogel was replaced with ground lettuce leaves to produce a hybrid aerogel. Plant tissues or gels containing plant cells subjected to the aerogel production process were demonstrated to be particularly beneficial since the obtained materials, besides presenting the typical features of aerogels (low density, high porosity, high water/oil absorption capacity), largely lost the colour and aroma typical of the starting plant material (Manzocco et al., 2024). This is due to the removal of molecules responsible for these sensory characteristics during aerogel production, and particularly upon water substitution with ethanol and drying with supercritical CO_2. Thanks to their neutral colour/taste along with their food-grade nature, hybrid aerogels containing cells would have the potential of being applied in several food products. Moreover, the production of a hybrid aerogel, combining a gelling polymer (e.g., WP) with plant cells, would allow to overcome the inability of plant cells to form a gel network, which is a fundamental prerequisite for aerogel production.

In light of these considerations, the production of hybrid aerogels containing SP and WP could represent a novel approach to engineering protein-rich cellular solids, while reaching multiple goals (i) structuring of planktonic cells into tissue-like food materials, (ii) development of porous cellular solids able to entrap high water amounts mimicking meat, (iii) removal of SP unpleasant sensory compounds to get neutral taste materials for wide application in the food sector. The present study aimed at developing hybrid cellular solids by subjecting SP cells and WP to the aerogel production process. To this aim, hybrid SP-WP hydrogels were prepared and subjected to ethanol solvent exchange and supercritical-CO_2 drying, leading to aerogels. The latter were analysed in terms of colour, appearance, water and oil uptake, and physical, microstructural and sensory properties. The final goal was to demonstrate that the development of a hybrid aerogel allows assembling SP into cellular solids able to entrap high amounts of food liquids but presenting lower intensity of unpleasant sensory attributes.

2. Materials and methods

2.1. Hydrogel preparation

For the preparation of hydrogels, whey proteins (WP, composition: 94.7 g/100 g protein; 74.6 g/100 g β-lactoglobulin, 23.8 g/100 g α-lactalbumin, 1.6 g/100 g albumin, Davisco Foods International Inc., Le Sueur, MN, USA) and SP (composition: 65 g/100 g protein, 10 g/100 g carbohydrates, 7.65 g/100 g fiber, 6 g/100 g fat, 2.1 g/100 g salt, NaturaleBio, International Food Europe srl, RM, Italy) were used.

Suspensions containing different ratios of WP and SP were prepared in distilled water (Table 1) and kept under agitation for 12 h at 4 °C. The total concentration of solids of the suspensions was set at 20 g/100 g.

Subsequently, the obtained colloidal suspensions were adjusted to the WP isoelectric pH value (pI, 4.8) using HCl 6 mol/L (Sigma Aldrich, Milan, Italy), distributed in 50 mL Falcon tubes with a diameter of 2.5 cm and gelled in a water bath at 90 °C for 20 min. After heat treatment, the hydrogels were cooled in an ice bath (15 min) and refrigerated at 4 °C for 24 h. The following day, the hydrogels were removed from the tubes and cut into cylinders having a height of 1.5 ± 0.1 cm and a diameter of 2.5 ± 0.1 cm. The cylinders were stored at 4 °C until conversion into aerogels.

2.2. Aerogel preparation

The hydrogels were subjected to a solvent exchange procedure to replace the water with ethanol (absolute ethanol, Carlo Erba, Milan, Italy) (Manzocco et al., 2017). For this purpose, the hydrogel cylinders were kept for 24 h in ethanol aqueous solutions at increasing ethanol concentrations (25, 50, 75 mL/100 mL, and two times in absolute ethanol) at room temperature (22 °C). Subsequently, the samples were kept for 24 h in absolute ethanol two consecutive times to remove the residual water. The solvent exchange was conducted using a sample: hydroalcoholic solution of 1:7 (g/g). The obtained alcogels were converted into aerogels by drying with CO_2 in the supercritical state, with a CO_2 pressure of 11 ± 1 N/m^2 at a temperature of 45 °C. The plant used for this purpose was developed at the Department of Agricultural, Food, Environmental and Animal Sciences of the University of Udine. The aerogels were stored in desiccators containing P_2O_5 (ERH = 0.5 %) at room temperature until use.

2.3. Image acquisition

Samples were placed on a black background and the digital camera (EOS 550D, Canon, Milan, Italy) was positioned 50 cm away from the sample enlightened by 4 × 100 W photographic reflectors, set to minimize shadow and glare. The images were acquired through a photo booth (Images & Computer, Bareggio, Italy) with the following parameters set: exposure time 1/20 s, F-stop/5, focal length 60 mm and the images were saved in jpeg format 3456 × 2304 pixels.

2.4. Colour

A tristimulus colourimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) was used, equipped with a CR-400 measuring head,

Table 1
Composition (g/100 g) of suspensions containing whey proteins (WP) and spirulin (SP).

WP:SP ratio	WP (g/100 g)	SP (g/100 g)	Water (g/100 g)
0:1	20	0	80
3:1	15	5	80
1:1	10	10	80
1:3	5	15	80
0:1	0	20	80

adopting the standard conditions of the International Commission of Enclairage (C.I.E.) with illuminating "C" (6774 K). The instrument was calibrated using a white calibration plate. The colour data was expressed in CIELAB scale as L* (brightness), a* (green-red point) and b* (blue-yellow point). The hue angle (h) was also calculated according to eq. (1):

$$h = tan^{-1}\left(\frac{b^*}{a^*}\right) \bullet 100 \quad \text{(eq. 1)}$$

2.5. Quantification of spirulin pigments

The methodology of Alotaiby et al. (2024) was followed. SP pigments were quantified in the hydroalcoholic solutions used for solvent exchange. After each solvent exchange step, the resulting solution was analysed using a UV–VIS spectrophotometer (Shimadzu UV-2501PC, Shimadzu Corporation, Kyoto, Japan) at the following wavelengths: 663, 652, 645, 620 and 470 nm. The concentration of chlorophylls, total carotenoids and phycocyanin was determined by following equations:

$$C_{chlorophyll\ a}\left(\frac{\mu g}{mL}\right) = 12.25 \times A_{663} - 2.79 \times A_{645} \quad \text{(eq. 2)}$$

$$C_{chlorophyll\ b}\left(\frac{\mu g}{mL}\right) = 21.50 \times A_{645} - 5.10 \times A_{663} \quad \text{(eq. 3)}$$

$$C_{carotenoids}\left(\frac{\mu g}{mL}\right) = \frac{1000 \times A_{470} - 1.82C_{chlorophyll\ a} - 85.2C_{chlorophyll\ b}}{198} \quad \text{(eq. 4)}$$

$$C_{phycocyanin}\left(\frac{\mu g}{mL}\right) = \frac{A_{620} - 0.474 \times A_{652}}{5.34} \times 1000 \quad \text{(eq. 5)}$$

Where A is absorbance (a.u.) at the corresponding wavelength for the sample. The concentration of each pigment is expressed as μg or mg of pigment per mL of hydroalcoholic solution.

2.6. Volume and density

Sample volume was calculated as the volume of the cylinder whose diameter and height were measured by a CD-15APXR digital caliper (Absolute AOS Digimatic, Mitutoyo Corporation, Kanagawa, Japan). Volume variation (ΔV, %) during the conversion of hydrogels to aerogels was expressed as follows (eq. (1)).

$$\Delta V(\%) = \frac{V_H - V_A}{V_H} \bullet 100 \quad \text{(eq. 6)}$$

where V_H and V_A are the volumes of the hydrogel and of the aerogel, respectively.

The bulk density was calculated as the ratio of the sample weight (m, g) to its volume (V, cm^3).

2.7. Firmness

Firmness was determined through a uniaxial compression test using an Instron 4301 (Instron LTD., High Wycombe, UK). The samples were tested with a 6.2 mm diameter cylindrical probe fixed to a 1 kN head at a speed of 25 mm/min. The firmness was expressed as the maximum force (N) required to penetrate the sample by 2 mm.

2.8. Scanning electron microscopy (SEM)

The aerogel samples were placed on aluminium supports and coated with 10 mm gold, using a Sputter Coater 108 car (Cressington Scientific Instruments, Watford, UK). The samples were then observed through the use of an electron scanning microscope (SEM, EVO 40XVP. Carl Zeiss, Milan, Italy), at room temperature (22 °C), under vacuum and using an acceleration voltage of 20 KV. The images were acquired with a magnification from 100 × to 25,000 × , using the application software SmartSEM v.5.09 (Carl Zeiss, Milan, Italy).

2.9. Water and oil absorption and holding capacity

The aerogel samples were weighed (W_i, g) and immersed in a 100 mL beaker containing 50 mL of water or sunflower oil, at room temperature (22 °C). At regular time intervals the sample was taken, dabbed with absorbent paper to remove excess water or oil and weighed. This procedure was carried out until a constant weight was obtained (W_f, g) for three consecutive measurements. The amount of absorbed solvent (A, g/$g_{aerogel}$) was calculated according to eq. (2):

$$A\left(g \Big/ g_{aerogel}\right) = \frac{(Wf - Wi)}{Wi}\ x\ 100 \quad \text{(eq. 7)}$$

The samples at the water and oil absorption plateau were centrifuged at 5000 g for 15 min at 20 °C using a microcentrifuge (Mikro 120, Hettich Zentrifugen, Andreas Hettich GmbH and Co, Tuttlingen, Germany) and the amount of released water and oil used to quantify the water and oil holding capacity, expressed as % amount of retained fluid as compared the fluid present in the aerogel before centrifugation.

2.10. Confocal microscopy

The samples obtained after water and oil absorption (Paragraph 2.9) were subjected to confocal microscopy. Aqueous solutions 0.2 g/100 mL of Fast Green (Sigma Aldrich, Milan, Italy) and Nile Red (Sigma Aldrich, Milan, Italy) were used to mark proteins and oil respectively. An aliquot of sample was manually mixed with 3 μl of each dye and placed on a slide cover (Thermo Fisher Scientific, Waltham, Massachusetts, USA), subsequently fixed on a concave slide (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The sample was observed using a 100× confocal laser scanning microscope (Leica TCS SP8 X confocal system, Leica Microsystems, Wetzlar, Germany). Images were imported in jpeg format using LasX 3.5.5 software (Leica Microsystems, Wetzlar, Germany).

2.11. Sensory analysis

A panel of 22 judges, composed of 14 women and 8 men, selected from students and employees of the University of Udine, was trained in order to recognize and quantify the smell of SP within a sample. For this purpose, two standards consisting of a WP isolate powder and a SP powder were prepared, corresponding respectively to the minimum value (no smell) and the maximum value (SP odour) of SP odour intensity. The samples were presented inside transparent, closed, plastic sample holders with a support developed to prevent that the color of the sample could be seen by the judges. The judges were asked to familiarize with the samples by interspersed sniffing between samples with a minimum time of 10 s. This time was necessary to reestablish the sense of smell. An unstructured scale of 10 cm was used to report the odour intensity perceived. The judges were informed that the WP and SP standards corresponded to the minimum (absence of odour, indicated by the term "absent") and the maximum (SP odour, indicated by the term "strong") value of odour intensity on the scale. Once the judges were trained by familiarity with the odours and the scale, they were asked to assess the intensity of the SP odour of an unknown sample on the unstructured scale. The unknown sample was represented by a powder obtained by manual grinding, using a mortar, of the WP10-SP10 hybrid aerogel. Judges were also asked to identify, if present, any perceived smell of putrid, fish, stagnant water, earthy or mold, typically associated with SP (Aguero et al., 2003; Cuellar-Bermúdez et al., 2017; Isleten Hosoglu, 2018). After the analysis, the unstructured scale length used was divided into a score from 0 to 10 and judges' scores converted in a numeric result.

The study complied with the principles established by the Declaration of Helsinki and the protocol was approved by the Institutional

Review Board of the Department of Agricultural, Food, Environmental and Animal Sciences of the University of Udine (July 22, 2024 protocol n. 0104610). Privacy rights of the participants have been observed and informed consent was obtained for experimentation.

2.12. Data analysis

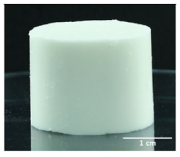
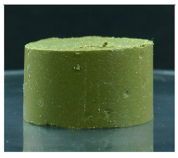
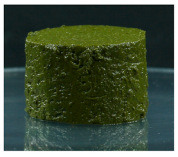
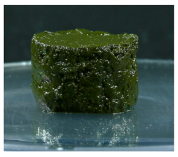
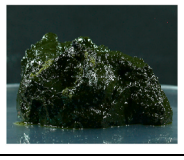
The reported data are expressed as the mean and standard deviation of at least three measurements from at least two replicates. The statistical analysis was carried out using the program R version 4.2.2 (The R Foundation for Statistical Computing, Wien, Austria). The variance was evaluated with the Bartlett test and the significance of the difference between the means was evaluated using the Tukey *post hoc* test ($p < 0.05$). 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. Hydrogel characterization

Colloidal suspensions with 20 g/100 g solid content containing increasing concentrations of SP were heat treated to induce gelation. Table 2 shows the images of the obtained hydrogels.

Table 2
Appearance, brightness (L*), hue angle (*h*), bulk density and firmness of hybrid hydrogels containing 20 g/100 g of solids whey proteins (WP) and spirulin (SP) in different ratios.

WP:SP ratio	Appearance	L*	*h* (°)	Bulk density (g/cm^3)	Firmness (N)
1:0		86 ± 1^a	97 ± 1^d	1.10 ± 0.04^a	4.1 ± 0.4^a
3:1		39 ± 1^b	134 ± 4^c	1.08 ± 0.04^a	2.2 ± 0.1^b
1:1		29 ± 1^c	136 ± 4^c	1.10 ± 0.04^a	1.5 ± 0.1^c
1:3		23 ± 1^d	236 ± 10^b	1.03 ± 0.08^a	0.3 ± 0.1^d
0:1		21 ± 1^e	294 ± 2^a	ND	0.2 ± 0.1^e

$^{a\text{-}e}$: In the same column, mean values indicated by different letters are statistically different ($p < 0.05$), n = 3; ND = not determined.

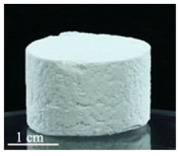
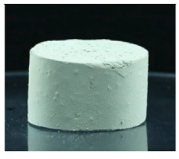
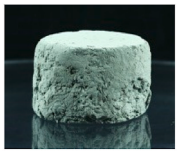
The hydrogel containing WP solely appeared white and preserved the cylindrical shape of the plastic tube used for gelling, according to the literature (Manzocco et al., 2022). As expected, with the increase in SP concentration, the hydrogels appeared progressively darker, as indicated by the significant decrease in L* values. Moreover, the samples appeared progressively greener, as confirmed by the significant increase in the *h* values. In fact, the *h* values indicated a shift of the colour form yellow (WP:SP ratio 1:0), to yellow-green (WP:SP ratio of 3:1 and 1:1), blue-green (WP:SP ratio of 1:3), and blue-purple (WP:SP ratio 0:1).

This undesired effect of SP addition on the colour has been widely demonstrated in the literature relevant to the fortification of food products with these planktonic cells (Arslan & Aksay, 2022; Lucas et al., 2018). In addition, the increase in the amount of SP led to a progressive decrease in the strength of the hydrogel network, as indicated by the loss of the original cylindrical shape and the decrease in firmness. In particular, the sample non-containing WP (WP:SP ratio 0:1) was unable to preserve its shape and was thus excluded from further experiments. This result is attributable to the progressive substitution of a strong-gelling component (*i.e.*, WP) with a less efficacious one (*i.e.*, SP) in the formulation of the hydrogel. SP indeed does not present high gelling capacity (Chronakis, 2001).

3.2. Aerogel characterization

The hydrogels shown in Table 2 were converted into aerogels by a two-step procedure based on water-to-ethanol solvent exchange and ethanol removal by supercritical-CO_2 drying (SCD). Table 3 shows the images of the obtained hybrid aerogels and their physical characteristics.

Table 3
Appearance, brightness (L*), hue angle (*h*), volume contraction, bulk density and firmness of aerogels deriving from hydrogels containing whey proteins (WP) and spirulin (SP) in different ratios.

WP:SP ratio	Appearance	L*	*h* (°)	ΔV (%)	Bulk density (g/cm^3)	Firmness (N)
1:0		98 ± 1^a	91 ± 1^d	−39 ± 4^b	0.29 ± 0.02^a	47.5 ± 0.3^a
3:1		79 ± 1^b	151 ± 4^b	−17 ± 4^a	0.24 ± 0.01^a	27.7 ± 2.9^b
1:1		72 ± 2^c	136 ± 1^c	−13 ± 4^a	0.23 ± 0.01^a	10.0 ± 0.9^c
1:3		67 ± 2^d	174 ± 3^a	ND	ND	ND

$^{a\text{-}d}$: In the same column, mean values indicated by different letters are statistically different ($p < 0.05$), n = 3; ND = not determined.

For all samples, the conversion into aerogels allowed for a significant increase in brightness, as shown by the increase in L*. Moreover, for the samples prepared with the higher SP content (WP:SP ratio 1:3), a significant reduction of the original hydrogel green colour (Table 2) was observed, as confirmed by the decrease of the *h* values. These data suggest that the SP pigments (phycocyanins, carotenoids and chlorophylls) have been partially removed, probably mainly during the water-to-ethanol exchange procedure, due to their high solubility in the used hydroalcoholic solutions (Irawati et al., 2020). Indeed, the used hydroalcoholic solutions appeared coloured after the water-to-ethanol solvent exchange. To confirm this hypothesis, the hydroalcoholic solutions remaining after the solvent exchange of the hydrogel containing SP and WP in a ratio of 1:1 were analysed spectrophotometrically to assess the concentration of extracted pigments. Data are reported in Table 4.

Data show that, as expected, both chlorophyll *b* and phycocyanins were extracted into the solutions with an ethanol content up to 75 g/100 g, due to their high hydrophilic nature (Cvitković et al., 2021). By contrast, more hydrophobic chlorophyll *a* and carotenoids were mainly extracted at higher ethanol concentrations (Cvitković et al., 2021).

On the other hand, despite the bleached appearance of the aerogels compared to the hydrogels (Table 2), colourimetric data indicate that SP pigments were not completely removed upon the aerogel production process. It can be inferred that pigment presence was masked by the intense light scattering of the porous aerogel (Table 3).

Nevertheless, by simply acting on the solvent exchange step increasing the ethanol:sample ratio and/or the exchange steps, further removal of SP pigments should be expected, leading to neutral-colour aerogels (Irawati et al., 2020). Despite the well-known bioactivity of SP pigments, they strongly impact the sensory properties of SP-containing foods, limiting consumer acceptability (Grahl et al., 2020; Niccolai et al., 2019). The production of colourless aerogels would overcome these issues, valorising the rich protein composition of SP. At the same time, SP bioactives and pigments could be easily recovered from the hydroalcoholic solutions used in the solvent exchange step, and further used.

As regards the shape of the samples, the water-to-ethanol solvent exchange and SCD of the hydrogels containing a WP:SP ratio of 1:1 allowed for obtaining cylindric aerogels well-preserving the initial hydrogel shape. Despite its lower gelling ability compared to WP, SP probably supported the WP network by acting as a bulking agent hindering collapse (Plazzotta et al., 2019). Nevertheless, the sample having higher SP concentration (WP:SP equal to 1:3) presented a rough and uneven surface with evident cracks, confirming that the strength of the gel network was impaired by the presence of SP cells both in the hydrogel (Table 2) and in the aerogel (Table 3) state. The increase in the SP amount also hindered volume contraction during hydrogel conversion into aerogel, thus resulting in aerogels with lower firmness and density (Table 3).

Based on the obtained results (Table 3), the aerogel derived from hydrogel with a WP:SP ratio of 1:1 was selected for further characterisation, since representing a reasonable compromise between the needs of (i) increasing SP concentration in the sample and (ii) preserving its overall structural integrity (Table 3). Fig. 1 reports the SEM microstructure of this sample.

From the micrographs, it can be observed that the aerogel presented a rough surface characterized by fine porosity, as previously described (De Berardinis et al., 2024). This porous structure confirmed the intense light scattering of the aerogel, which gave an opaque and whiter appearance to the samples (Table 3). Higher magnification showed that WP were present in the form of small aggregates of spherical shape, known as microgels, superficially interconnected in the formation of a three-dimensional porous network. The formation of this peculiar WP structure, typically produced at a pH close to pI (Nicolai, 2016; Plazzotta et al., 2020; Schmitt et al., 2014), was not disturbed by the presence of SP cells, recognizable by the characteristic spiral trichome shape (Benelhadj et al., 2016; Ma & Gao, 2009), which were intimately enveloped within the WP network. Accordingly, SP cells did not interact with each other, but rather acted as a filler physically entrapped in the WP continuous network, which guaranteed the robustness of the scaffold. These results demonstrate the effectiveness of the proposed strategy to fabricate protein cellular solids through the structuration of SP into aerogel scaffolds.

Table 4
Concentration of spirulin pigments in hydroalcoholic solutions remaining after water-to-ethanol solvent exchange of hydrogels containing whey proteins (WP) and spirulin (SP) in 1:1 ratio.

Hydroalcoholic solution (g/100 g)	Chlorophyll *a* (μg/mL)	Chlorophyll *b* (μg/mL)	Carotenoids (μg/mL)	Phycocyanin (μg/mL)
25	0.96 ± 0.13	1.67 ± 0.08	ND	11.26 ± 0.25
50	0.75 ± 0.14	0.89 ± 0.13	ND	4.72 ± 0.45
75	2.73 ± 0.03	1.25 ± 0.11	0.31 ± 0.05	4.93 ± 1.48
100	29.54 ± 1.15	ND	3.13 ± 0.25	ND
100	13.45 ± 0.60	ND	1.43 ± 0.82	ND

Data shown as media ±standard deviation, n = 2; ND = not detectable.

3.3. Interaction with water and oil

To have a first insight into the possible use of the developed hybrid aerogel as a food ingredient in the preparation of cellular materials, the sample obtained from a hydrogel with a WP:SP ratio of 1:1 was analysed for its ability to interact with common food liquids (*i.e.*, water and oil). Fig. 2 shows the absorption of water and oil in this aerogel.

The aerogel quickly absorbed both water and oil, reaching a plateau within a few minutes. At the plateau, the amount of water was higher than that of oil. According to the literature, this result can be traced back to the predominantly hydrophilic nature of WP and SP (Manzocco et al., 2022; Plazzotta et al., 2020). Interestingly, the amount of water absorbed matched with the water content of the original hydrogel, implying that the aerogel effectively reabsorbed all the water removed during the aerogel production process. The ability to absorb oil was similar to that of aerogels consisting of WP only (Ahmadi et al., 2016; De Berardinis et al., 2024) or egg white proteins (Kleemann et al., 2020; Selmer et al., 2019). The high oil absorption capacity of these materials is likely due to their open-porosity (Fig. 1), accounting for strong capillary forces driving fluid upload, independently on fluid polarity (Manzocco et al., 2022). Table 5 reports the physical properties of the aerogel collected after 60 min of water or oil absorption (plateau).

Independently on the absorbed fluid, the aerogels showed a green appearance. The colourimetric analysis actually showed that water and oil absorption resulted in *h* values within the blue-green and blue-purple colour intervals, respectively. These results confirm the presence of residual SP pigments upon the water-to-ethanol solvent exchange and SCD, which, upon water and oil absorption, were no more masked by the intense scattering promoted by the fine aerogel porosity. Further contributions to sample colour likely included the colour of the oil itself and the solubility of the residual pigments in water or oil.

Following the absorption of water, the aerogel sample maintained its structure and slightly increased its volume without losing integrity, even if a significant drop in firmness was observed (Table 5). Moreover, the latter was comparable to that of the initial hydrogel from which the aerogel originated (Table 2), suggesting the preservation of the protein network structure after the water-to-ethanol solvent exchange and SCD. Focusing on the confocal images (Table 5), the aerogel network appeared dense and continuous after water loading, suggesting a closer interaction among WP particles and SP cells. These interactions could be favoured by the swelling of the dried WP microgels in the presence of water, confirming the increase in volume reported in Table 5. On the contrary, upon oil absorption, a slight volume decrease was observed,

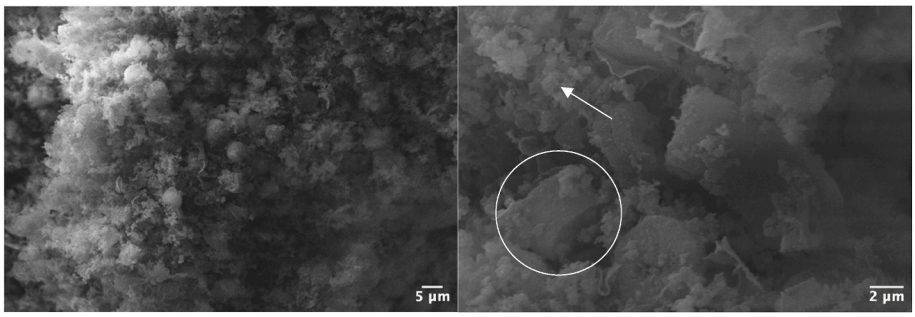


Fig. 1. SEM images of the internal section of the hybrid aerogel produced from a hydrogel containing 20 g/100 g of solids whey proteins (WP) and spirulin (SP) in a ratio of 1:1. The arrow indicates microgels, the circle spirulin cells.

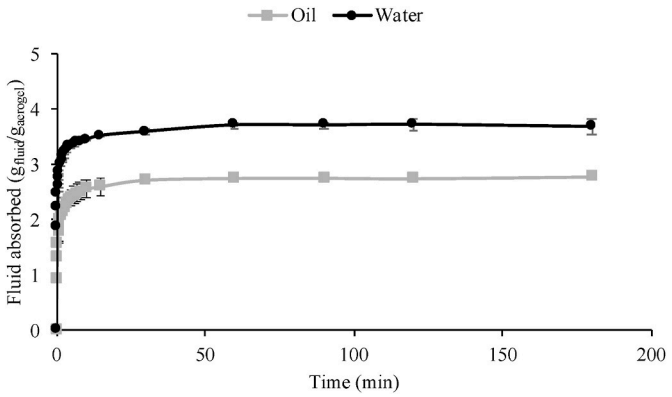


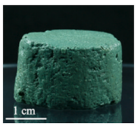
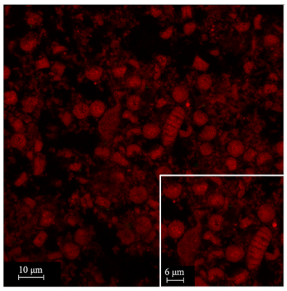
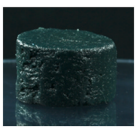
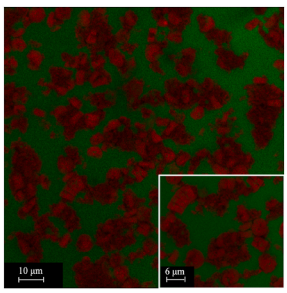
Fig. 2. Water and oil absorbed in hybrid aerogels prepared from hydrogels containing 20 g/100 g of solids whey proteins (WP) and spirulin (SP) in a ratio of 1:1.

with no significant changes in firmness (Tables 3 and 5). This suggests that the oil was simply physically absorbed into the aerogel pores, filling the voids without affecting the strength of the aerogel skeleton, in agreement with previous works (De Berardinis et al., 2024; Manzocco et al., 2022). This result was confirmed by the confocal image analysis (Table 5), which suggests a limited interaction among hydrophilic WP particles in oil. Both water- and oil-loaded aerogels did not show any fluid release upon centrifugation (water and oil holding capacity of 100%), highlighting a high physical stability.

3.4. Sensory profile analysis

In the last part of the study, a sensory analysis was carried out to verify the effect of the proposed approach based on the aerogel production technology on the intensity of the SP odour, which largely limits its application in foods, being perceived as unpleasant, similar to hints of fish, earth, mould or wood (Aguero et al., 2003; Kurt et al., 2023). The results of the sensory analysis showed that the aerogel derived from the hydrogel containing 20 g/100 g of WPs and SP in a ratio of 1:1 had an odour intensity of 1.2 ± 1.0 on a scale from 0 (no odour) to 10 (maximum SP odour intensity). In addition, only 2 out of 22 judges perceived an unpleasant odour, which they described as “earthy”. This

Table 5
Appearance, confocal images, brightness (L*), hue value (*h*), changes in volume and firmness of hybrid aerogels produced from hydrogels containing 20 g/100 g of solids whey proteins (WP) and spirulin (SP) in a ratio of 1:1 after 60 min absorption of water or oil. Red = proteins, green = lipids.

Absorbed fluid	Appearance	Confocal images	L*	*h* (°)	ΔV (%)	Firmness (N)
Water			44 ± 1[a]	192 ± 1[b]	4 ± 3[a]	1.0 ± 0.1[b]
Oil			30 ± 1[b]	278 ± 2[a]	−4 ± 2[b]	9.7 ± 3.3[a]

[a-b]: In the same column, mean values indicated by different letters are statistically different ($p < 0.05$), n = 3.

result shows that the proposed approach allowed a significant reduction of SP odour intensity.

4. Conclusions

In this work, a dried cellular solid based on hybrid hydrogels containing a gelling biopolymer and cells subjected to the aerogel production process was developed for the first time, using whey proteins and spirulin. The developed approach allowed for incorporating pre-existing planktonic cells in a continuous gel protein network, which was then subjected to solvent exchange and supercritical-CO_2 drying. This process was demonstrated to efficaciously reduce the undesired colour and flavour associated with spirulin, which are the main limitations associated with its food application.

The peculiar structure of the obtained aerogel, associated with its remarkable water and oil absorption capacity, offers promising prospects for the development of cellular-based tissue-like structures. Indeed, the physical and nutritional features of these materials could be customized by acting on multiple formulations (polymer type and concentration, cell type, absorbed fluid) and processing parameters (gelling procedure, solvent exchange).

CRediT authorship contribution statement

Lorenzo De Berardinis: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Stella Plazzotta:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Conceptualization. **Michele Magnan:** Formal analysis. **Lara Manzocco:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Fundings

This work was supported by the University of Udine in the framework of the collaborative research project UNITED (DM737).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Mrs Rebecca Comelli for helping with sample preparation and analysis.

Data availability

Data will be made available on request.

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