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Optimising soy and pea protein gelation to obtain hydrogels intended as precursors of food-grade dried porous materials

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Abstract: Dried porous materials based on plant proteins are attracting large attention thanks to their potential use as sustainable food ingredients. Nevertheless, plant proteins present lower gelling properties than animal ones. Plant protein gelling could be improved by optimizing gelation conditions by acting on protein concentration, pH, and ionic strength. This work aimed at systematically studying the effect of these factors on the gelation behaviour of soy and pea protein isolates. Protein suspensions having different concentrations (10, 15, and 20% w/w), pH (3.0, 4.5, 7.0) and ionic strength (IS, 0.0, 0.6, 1.5 M) were heat treated (95 °C for 15 min) and characterized for rheological properties and physical stability. Strong hydrogels having elastic modulus (G') higher than 103 Pa and able to retain more than 90% water were only obtained from suspensions containing at least 15% soy protein far from isoelectric point and at IS above 0.6 M. By contrast, pea protein gelation was achieved only at high concentration (20%) and always resulted in weak gels, which show increasing G' with the increase in pH and IS. Results were rationalized into a map identifying the gelation conditions to modulate the rheological properties of soy and pea proteins hydrogels, for their subsequent conversion into xerogels, cryogels and aerogels.

Keywords: plant proteins; heat gelation; gelling behaviour; structure; pH

1. Introduction

Xerogels, cryogels, and aerogels indicate dry porous materials produced by removing the solvent from a gel. Most studies have been carried out on the development of inorganic dried porous materials (e.g., silica and carbon-based) [1–3] to be used in a wide variety of applications, from catalysis, environmental remediation, energy storage, and insulation [4–7]. Nevertheless, in recent years, growing interest has been focused on the development of biopolymeric-based dried porous templates, due to their biocompatibility, and non-toxic profile. Thanks to these characteristics, their application has been successfully extended to life-science fields, including biomedical and pharmaceutical sectors [8–10]. The potentialities of dried porous materials in the food sector are nowadays attracting large attention, due to their unique physico-chemical properties and techno-functionalities. Both cryogels and aerogels have been suggested as innovative delivery systems to protect bioactives and flavours during processing, storage and digestion [11–16]. In addition, their capacity to absorb large amounts of food solvents has been identified as a key-feature to modulate food structural properties [17,18]. For instance, they have been suggested as templates for oil structuring, leading to fat replacers with improved nutritional properties [16,19–21]. By contrast, as concerns xerogels, to the best of our knowledge, no applications in the food sector are nowadays reported, despite the high potentialities of these materials have been demonstrated in other life-science sectors.

To produce food-grade dried porous material, an aqueous gel is first produced by inducing the networking of the selected biopolymer in water, leading to a hydrogel [22].

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Subsequently, water is removed from the network by evaporative drying. The latter can be also performed by evaporating ethanol after substitution of hydrogel water with ethanol [23,24]. The evaporative drying usually induces capillary forces during solvent removal, leading to xerogels with low porosity [25]. Cryogels are instead obtained through freeze-drying, and thus by water sublimation [18]. This reduces the capillary forces, leading to materials with large pores and channels, left upon sublimation of water crystals grown during freezing [26]. Finally, aerogels are obtained by replacing the water contained in the starting gel with ethanol, followed by ethanol removal with a flow of CO₂ in the supercritical state [27]. This technique preserves the structure of the material, characterized by low density and high internal surface area due to the presence of micro and macropores [28].

Food-grade xerogels, cryogels and aerogels can be prepared either from polysaccharides or proteins. As concerns proteins, most literature studies focus on animal ones (e.g. whey, egg white, caseine, gelatine) [29,30], while studies on the development of dried porous templates from plant proteins are limited to a few works exploiting silk fibroin, patatins and soy proteins [31–38]. The interest for plant-based products is constantly growing due to their lower environmental impact, low cost and possibility of being obtained from food industry wastes, in a circular economy perspective [39–41]. For these reasons, plant proteins represent ideal candidates for the development of sustainable dried porous materials intended as innovative ingredients for the food sector. However, the production of plant-based xerogels, cryogels and aerogels is rather challenging. This is mainly due to the poor gelling properties of vegetable proteins as compared to their animal counterpart. Protein gelation is commonly induced by heat treatment, during which the protein chains unfold, exposing their reactive groups, which subsequently drive protein reassembling in a three-dimensional network. Although both covalent (i.e., S-S bridges) and weak interactions (i.e., hydrophobic interactions, hydrogen bonds, and electrostatic interactions) play an important role in the formation and stabilization of protein gels [42], the availability of free -SH groups available for covalent stabilization is known to lead to stronger gels. The possibility to obtain strong hydrogels is pivotal in determining their suitability in the conversion into dried porous material, since the stronger the gel, the higher its capacity to structurally withstand the subsequent drying steps. In this regard, plant proteins present a lower number of -SH groups as compared to animal ones [43]. Moreover, the extraction process performed to isolate the protein fraction from the vegetable matrix, where it is intimately embedded in fiber-protein complexes, is known to induce structural modifications in the protein chains, further reducing gelling properties [44]. Nevertheless, several factors, including protein concentration, pH, and ionic strength, can be properly modulated to improve plant protein gelling capacity. To this regard, the increase in protein concentration usually leads to a denser protein network, accounting for the formation of firmer gels that better maintain the original volume upon water removal [45]. When gelation occurs at pH approaching the isoelectric point (pI), globular and strongly aggregated protein structures are formed, mostly driven by hydrophobic interactions [46,47]. At pH far above or below the pI, instead, proteins form a fine-stranded network, as a result of the presence of surface charges which prevent intimate protein aggregation [48]. For example, aerogels derived from gels prepared near protein pI have been shown to present higher structural stability during drying, associated with lower density and higher pore sizes as compared to aerogels prepared far from the pI [28,49]. Gelation properties are also affected by ionic strength. For instance, the increase in ionic strength reduces electrostatic repulsive forces among protein chains, favouring the formation of a stronger network. The elastic modulus of pea protein gels was increased by 12 times by adding 0.3 M NaCl [50]. However, beyond a salt threshold specific for each protein (usually > 2.0 M), a weakening of the hydrogel structure is commonly observed, due to salt-induced stabilization of protein structure, which suppresses protein unfolding during gelation [51,52].


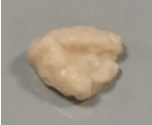




This work aimed at systematically studying the effect of gelation conditions on the physical properties of plant protein-based hydrogels, with the final aim of identifying the conditions leading to hydrogels suitable for the development of dried porous materials. To this purpose, soy and pea proteins were selected as protein sources widely used as alternatives to animal proteins. Aqueous suspensions containing increasing amounts of soy and pea proteins at different pH (3.0, 4.5, 7.0) and ionic strength (0.0, 0.6, 1.5 M) were heat treated to induce gelation. The obtained hydrogels were characterized for rheological properties and physical stability, and the results were rationalized into gelation map.

2. Results and Discussion

2.1. Effect of protein type and concentration

SPI and PPI solutions were prepared at increasing concentrations from 10 to 20% (w/w) at pH 7.0, and thermally treated. Table 1 reports the appearance of the obtained SPI and PPI samples.

Table 1. Appearance, elastic (G'), loss modulus (G''), loss tangent ($\tan \delta$), and water holding capacity (WHC) of soy (SPI) and pea (PPI) systems obtained after heat treatment of protein solutions at 10, 15, and 20% w/w, at pH 7.0 and 0.0 ionic strength.

Protein	Concentration (%, w/w)	Appearance	$G' \times 10^2$ (Pa)	$G'' \times 10^2$ (Pa)	Tan δ	WHC
SPI	10		N.D.	N.D.	N.D.	N.D.
	15		3.94 ± 0.24	0.77 ± 0.04	0.19 ± 0.01	89.51 ± 3.56
	20		47.57 ± 1.61	6.60 ± 0.24	0.14 ± 0.01	99.60 ± 0.44
PPI	10		N.D.	N.D.	N.D.	N.D.
	15		N.D.	N.D.	N.D.	N.D.
	20		13.64 ± 0.24	3.13 ± 0.05	0.23 ± 0.01	75.19 ± 0.03

N.D. Not determined, since the system did not gel

As expected, for both SPI and PPI the increase in protein concentration resulted in a visible increase in system structuring [53,54]. At a given protein concentration, SPI always led to more structured system as compared to PPI, so that a minimum protein concentration of 15 and 20% (w/w) was required to form a semi-solid system by using SPI and PPI, respectively (Table 1). This difference was also confirmed by rheological analysis.

Supplementary Figure S1 reports the frequency sweep test results for SPI and PPI hydrogels obtained from 20% (w/w) protein solutions.





For both proteins, G' higher than G'' and parallel to G'' was obtained, indicating the formation of gel systems [55]. The moduli of the PPI gel showed a higher frequency dependence (higher slope) than those of the SPI gels; **the latter showed negligible frequency dependence, indicating that a strong gel structure was obtained; SPI gels also** presented rheological moduli higher than those of the PPI gel and a lower loss tangent ($\tan \delta$) (Table 1). These results confirm the higher gelling ability of SPI as compared to PPI. In agreement with the literature [56,57], this difference between SPI and PPI gelation properties can be attributed to the different composition of the globulin fraction of the considered proteins. Soybean globulins are mainly represented by glycinin (11S) and β -conglycinin (7S), which present higher solubility than pea ones (legumin 11S and vicilin 7S). As a result, a higher protein fraction would remain homogeneously suspended during gelation of soy proteins [44,56]. Moreover, soybean globulins have been previously demonstrated to present a threshold gelling concentration lower than pea ones [58].

The higher strength of the gel obtained with SPI rather than PPI was also related to an improvement in gel stability, as shown by higher WHC values (Table 1). The increased density network obtained increasing protein concentration is actually able to retain more water, due to the better distribution of the solvent in the 3-D structure, as well as to the higher number of protein residues available for interaction with water [59].

2.2. Effect of pH

The precursor protein solutions were adjusted to pH 3.0, 4.5 and 7.0 and thermally treated. Independently on pH, self-standing gelled systems were only obtained at 15 and 20% (w/w) SPI concentration and at 20% (w/w) PPI concentration. As representative examples, Table 2 reports the appearance and the rheological parameters of the hydrogels obtained from SPI and PPI solutions at 20% (w/w) protein concentration and adjusted at the different pH.

Table 2. Appearance, storage modulus (G'), loss modulus (G''), loss tangent ($\tan \delta$), and water holding capacity (WHC) of soy (SPI) and pea (PPI) hydrogels at 20% protein concentration at pH 3.0 and 4.5, and 0.0 ionic strength.

Protein	pH	Appearance	$G' \times 10^2$ (Pa)	$G'' \times 10^2$ (Pa)	Tan δ	WHC
SPI	3.0		30.14 ± 2.78	4.10 ± 0.46	0.14 ± 0.01	99.80 ± 0.09
	4.5		24.32 ± 0.66	3.36 ± 0.85	0.14 ± 0.01	99.68 ± 0.07
PPI	3.0		8.52 ± 1.26	2.38 ± 0.31	0.28 ± 0.01	67.07 ± 0.73
	4.5		7.89 ± 0.59	1.99 ± 0.73	0.25 ± 0.01	75.00 ± 2.99

Similar to data achieved at pH 7 (Table 1), also at pH 3.0 and 4.5, SPI led to higher system structuration as compared to PPI. At pH 4.5, which is close to protein pI, a particulate gel, otherwise known as microgel, was obtained with both proteins [60,61]. Proteins

actually show a higher tendency towards aggregation in the isoelectric region, where the net charge is low, and thus protein-protein interactions are promoted, with the formation of spherical particles, which, at high protein concentration, can randomly associate into larger self-supporting hydrogels [61]. By contrast, at pH values away from the pI, where strong electrostatic repulsions are present, the gels present a fine-stranded structure.

For both proteins, the decrease of pH from 7.0 (Table 1) to 3.0 (Table 2) caused a significant decrease in system structuration, as evidenced by the rheological parameters. In fact, not only both moduli resulted lower for gels prepared at pH 3.0 as compared to those obtained at neutral pH, but they also presented higher frequency dependence. In this regard, Supplementary Figure S2 shows the effect of pH change on the frequency sweep results of SPI gels prepared at 20% (w/w) protein concentration. This can be attributed to the lower solubility of the proteins near their pI [52,56,62]. A significant decrease in gel strength was instead observed upon adjusting the protein solution at pH 4.5 (Supplementary Figure S2, Table 1 and Table 2).

This can be attributed to the different microstructure of the hydrogels obtained at different pHs. In particular, microgelled systems obtained near the pI are stabilized by weak surface interactions among spherical protein aggregates, which can easily flow one on the other [63]. By contrast, at pHs far from the pI (pH 3.0 and 7.0), stranded gel structures are obtained, stabilized by numerous disulphide bridges and weak-interaction entanglement regions, thus accounting for higher resistance to mechanical perturbation [61]. Moreover, in the isoelectric region, protein solubility is minimized, resulting in a significant decrease of well-solubilized protein fractions able to efficaciously interlink in a 3-D gel network [56].

For both SPI and PPI, pH had a negligible effect on gel stability, as indicated by the comparable WHC values (Table 1 and 2). This is probably due to the counterbalancing effect of the high protein concentration on the effect of pH. In other words, the effect of the different gel architectures induced by pH would be made negligible in the presence of a high protein concentration, which would increase the network density allowing a high solvent retention [56].

2.2. Effect of ionic strength








The precursor protein solutions were added with different NaCl amounts to modulate the ionic strength of the system. As representative examples of the effect of this parameter at low protein concentrations, Table 3 shows the appearance of systems obtained upon thermal treatment of 10% (w/w) SPI and 15% (w/w) PPI solutions, at pH 7.0 and having 0.6 and 1.5 M ionic strength.

Although the final system showed evident phase separation, as compared to the system with no salt added (Table 1), which showed a liquid-like structure, the increase in IS resulted in a local gelling effect with the formation of a microgel-like structure. This effect can be traced back to the shielding effect of salt ions of protein surface charge, favouring protein aggregation [64]. The positive effect of IS increase on SPI and PPI gelling properties was also observed at higher protein concentration. In this regard, Table 3 reports the appearance and the rheological parameters of the hydrogels obtained from 20% (w/w) SPI and PPI solutions at pH 7.0, at 0.6 and 1.5 M ionic strength. As compared to the gels obtained without salt addition (Table 1), the increase in IS resulted into particulate gels, well-evident in the case of PPI-based systems (Table 3). This is due to the changes induced by the increase in IS in gel microstructure, which shifted from a fine-stranded structure (low IS) to a particulate structure (high IS) [22]. NaCl concentration increase also caused a considerable increase in both SPI and PPI gel strength, as indicated by the increase in G' values (Table 1 and 3), as well as by the reduction in frequency-dependence of the rheological moduli, as shown in Supplementary Figure S3 for SPI gels at 20% (w/w) protein concentration at 0.0, 0.6 and 1.5 M. The presence of Na^+ ions actually promotes protein-protein interactions during gelation, due to the reduction of the repulsive electrostatic interactions

between protein chains [54]. Moreover, the increase in IS is known to promote the so-defined “salting-in” effect, i.e, the increase in the solubility of globulins, which are the main protein fraction of both SPI and PPI [65]. Higher IS thus result in a higher availability of well-hydrated proteins available for networking during gelation [54,66].

Ionic strength also affected stability. In the case of SPI gels, WHC decreased with IS, despite the higher gel strength (Table 3). Similar results were found for gels from both soy [67,68] and egg white proteins [69–72] and can be attributed to the microstructural changes induced by the presence of ions. In this regard, Munialo et al. [73] have demonstrated that a gel with an evenly distributed fine-stranded network, obtained at low IS, generally presents higher WHC as compared to particulate gels, where water is less tightly trapped. Likewise, Maltais et al. [74] and Urbonaite, et al. [75,76] reported an inverse correlation between aggregate size and WHC, with larger aggregates resulting in lower WHC. On the contrary, in the case of PPI hydrogels, the increase in salt content promoted the increase in WHC. It can be inferred that, in this case, the increased gel structural properties obtained upon NaCl addition (Table 1 and 3) prevailed over the microstructural changes induced by IS increase.

Table 3. Appearance, storage modulus (G'), loss modulus (G''), loss tangent ($\tan \delta$), and water holding capacity (WHC) of soy (SPI) and pea (PPI) hydrogels at 10, 15 or 20% (w/w) protein concentrations at 0.6 and 1.5 M ionic strength.

Protein	Concentration (% w/w)	Ionic strength (M)	Appearance	$G' \times 10^2$ (Pa)	$G'' \times 10^2$ (Pa)	Tan δ	WHC
SPI	10	0.6		N.D.	N.D.	N.D.	N.D.
		1.5		N.D.	N.D.	N.D.	N.D.
	20	0.6		58.21 ± 7.10	9.97 ± 1.19	0.17 ± 0.01	97.95 ± 0.70
		1.5		115.51 ± 46.08	22.40 ± 8.52	0.19 ± 0.01	89.24 ± 3.17
PPI	15	0.6		N.D.	N.D.	N.D.	N.D.
		1.5		N.D.	N.D.	N.D.	N.D.
	20	0.6		48.48 ± 0.29	11.52 ± 0.07	0.24 ± 0.01	84.76 ± 3.56

1.5		51.57 ± 6.38	1256 ± 1.64	0.24 ± 0.01	91.96 ± 0.02
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N.D. Not determined, since the system did not gel

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2.2. Gelation map

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Collected data were further elaborated and rationalized in order to obtain a gelation map (Figure 2), useful to have an immediate view of the gelation performances of SPI and PPI under the considered conditions.

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The obtained map clearly highlights the complex effect of protein type, pH, IS and their combination on sample structure. For example, the higher gelation propensity of SPI as compared to PPI is immediately visible, as well as the higher structuration obtained far away from protein isoelectric region pH or increasing the ionic strength. This map represents a useful tool to identify optimal conditions leading to SPI and PPI gels presenting the desired physical properties. In particular, the conditions allowing for the preparation of hydrogels presenting a network strong enough to withstand the conversion into xerogels, cryogels, and aerogels can be identified. Moreover, additional considerations can be drawn, with the aim of optimizing the production process of these dried porous materials. For example, at pH 3.0 or 7.0, in view of minimizing the consumption of SPI, and thus raw material costs, while also maintaining a strong gel structure, the possibility to reduce SPI concentration from 20 to 15% (w/w) while increasing the ionic strength can be identified. Similarly, in the case of PPI, it is immediately evident how only weak gels can only be obtained at 20% concentration.

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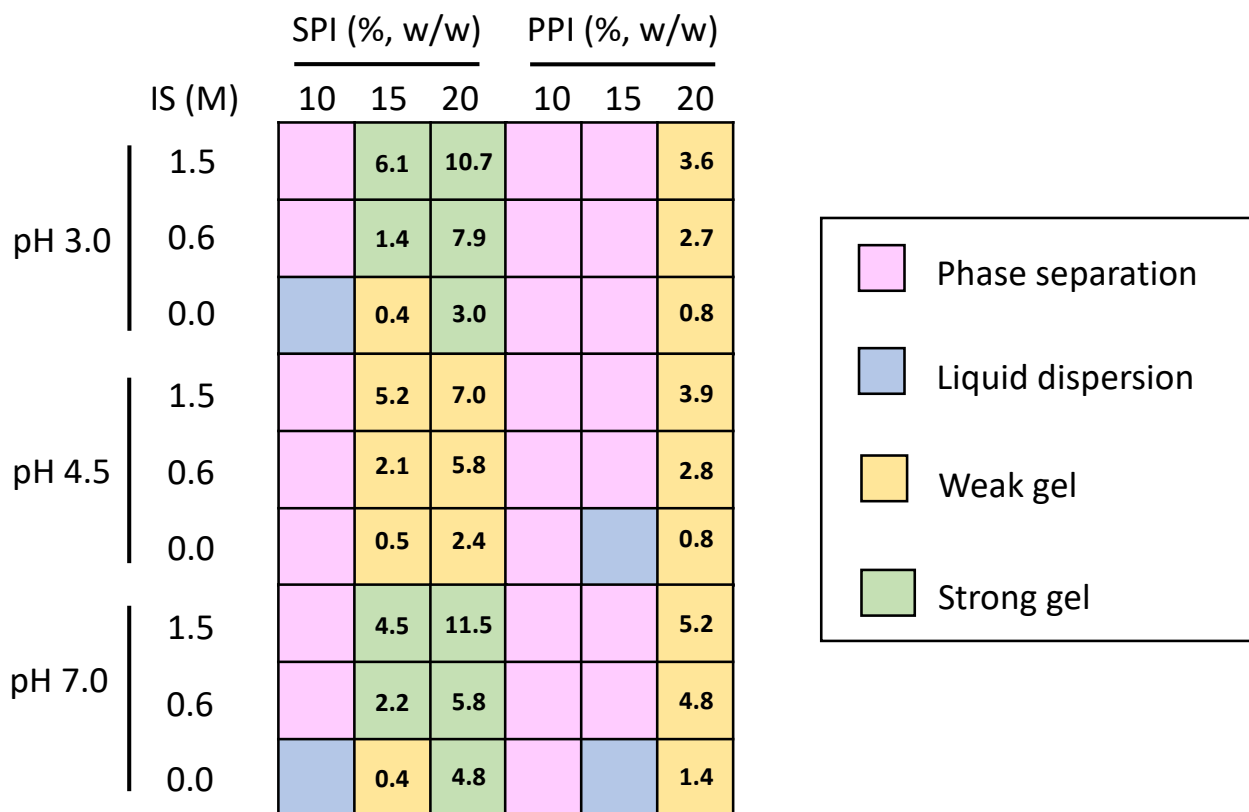
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Figure 1. Gelation map of soy (SPI) and pea protein isolate (PPI) at increasing protein concentration (% w/w), pH and ionic strength (IS). The mean values of elastic modulus ($G' \times 10^3$ Pa) of the gelled systems are also reported within cells.

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3. Conclusions

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The results collected in this study show that the gelling behaviour of vegetable proteins is highly dependent on both protein nature and formulation parameters (protein concentration, pH, ionic strength). In particular, hydrogel strength can be enhanced choosing soy proteins over pea ones, as well as avoiding the isoelectric region and increasing ionic strength. The obtained gelation map can be considered a useful tool to identify the optimal conditions to produce soy and pea protein hydrogels with physical properties suitable for the subsequent conversion into xerogels, cryogels and aerogels.

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The results obtained in this research, although relevant to soy and pea protein isolates solely, clearly indicate the potential of plant proteins as interesting precursors for the production of food-grade and plant protein-based dried porous materials. Further studies are therefore required to investigate the correlation between the physical and technological properties of the precursor hydrogel and the resulting dried materials. **In this regard, different drying processes such as evaporative drying, freeze drying, supercritical drying can be applied to convert the obtained hydrogels into xerogels, cryogels and aerogels, respectively. At the same time, a comprehensive characterization of the dried templates obtained thereof could be performed. The latter should include the physical characterization of the materials (e.g., SEM microstructure, BET surface area, porosity) but also their interaction properties with food fluids (oil, water) to get a first insight into their applicability as innovative food ingredients.**

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4. Materials and Methods

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4.1. Soy and pea protein solution preparation

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Aqueous solutions presenting different ionic strength (IS), 0.6 and 1.5 M, were prepared by adding NaCl (Sigma Aldrich, Milan, Italy) in deionized water (System advantage A10®, Millipore S.A.S, Molsheim, France). Deionised water without the addition of NaCl was considered to have IS equal to 0.0 M. Aqueous solutions were added with 10, 15 or 20% (w/w) of soy (SPI) or pea (PPI) protein isolates (Myprotein, Manchester, England). The suspensions were subjected to high shear mixing at $1,120 \times g$ for 1 min (Polytron PT-MR3000, Kinematica AG, Littau, Switzerland) and pH was adjusted to 3.0, 4.5 and 7.0 by adding 1 M NaOH or HCl.

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4.2. Heat treatment

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To induce gelation, soy and pea protein suspensions were transferred in 50 mL-sealed falcon tubes and subjected to thermal treatment in a water bath (95 °C for 15 min), followed by cooling in an ice bath (0 °C for 15 min). The hydrogels were then stored at 4 °C for 48 h, until analysis.

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4.3. Image acquisition

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Images were captured with a digital camera (EOS 550D, Canon, Milano, Italy) in an image acquisition cabinet (Immagini & Computer, Bareggio, Italy). The digital camera was positioned in an adjustable stand positioned at 45 cm from the samples, and

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enlightened by 4 × 100W frosted photographic floodlights, in a position allowing minimum shadow and glare.

4.4. Rheological properties

Hydrogel rheological properties were tested using an RS6000 Rheometer (Thermo Scientific RheoStress, Haake, Germany), equipped with a Peltier system for temperature control. The analysis was performed with a parallel plate geometry, with a gap of 2.0 mm at 20 °C. Hydrogels were cut into cylinders with 2 mm of height and 20 mm of diameter. The linear viscoelastic region (LVR) was determined using an oscillatory sweep test (0.01 to 1,000 Pa at 1 Hz frequency). Critical Stress (Pa) was determined as the stress value related to a 10% drop in the storage modulus. The frequency sweep tests were carried out increasing the frequency from 0.1 to 20 Hz, at stress values selected in the LVR.

4.5. Physical stability

The physical stability of hydrogels was evaluated based on their water holding capacity (WHC). Hydrogels were accurately weighed (W_1) and transferred into 1.5 mL-Eppendorf microcentrifuge tubes, and then centrifugated at $15,000 \times g$ for 15 min at 4 °C (D3024, DLAB, Scientific Europe S.A.S, Schiltigheim, France). The supernatant was then removed, and the samples were weighed again (W_2). The WHC was determined according to eq. 1.

$$WHC = \frac{W_1 - (W_1 - W_2)}{W_1} \cdot 100 \quad (1)$$

4.6. Data analysis

Data are expressed as the mean ± standard deviation of at least three measurements resulting from two replicates. The statistical analysis was performed using the program R version 4.1.2 (The R Foundation for Statistical Computing). The homogeneity of the variance was evaluated with Bartlett tests, one-way ANOVA was applied, and the difference between the averages was assessed by the post-hoc Tukey test ($p < 0.05$).

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Elastic (G') and viscous (G'') modulus of soy (SPI) and pea (PPI) hydrogels obtained from 20% (w/w) protein solutions.

Author Contributions: Lorenzo De Berardinis: Investigation, Formal analysis, Visualization, Writing - Original Draft; Stella Plazzotta: Conceptualization, Methodology, Writing - Review & Editing, Supervision; Lara Manzocco: Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition.

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References

1. Khoshnevis, H.; Mint, S.M.; Yedinak, E.; Tran, T.Q.; Zadhoush, A.; Youssefi, M.; Pasquali, M.; Duong, H.M. Super High-Rate Fabrication of High-Purity Carbon Nanotube Aerogels from Floating Catalyst Method for Oil Spill Cleaning. *Chem. Phys. Lett.* **2018**, *693*, 146–151, doi:10.1016/j.cplett.2018.01.001. 340–342
2. Di Luigi, M.; Guo, Z.; An, L.; Armstrong, J.N.; Zhou, C.; Ren, S. Manufacturing Silica Aerogel and Cryogel through Ambient Pressure and Freeze Drying. *RSC Adv.* **2022**, *12*, 21213–21222, doi:10.1039/d2ra03325a. 343–344
3. Ptaszowska-Koniarz, M.; Goscianska, J.; Bazan-Wozniak, A.; Pietrzak, R. Amine-Modified Carbon Xerogels as Effective Carbon-Based Adsorbents of Anionic Dye from Aqueous Solutions. *Materials* **2022**, *15*, 5736, doi:10.3390/ma15165736. 345–346
4. Li, Z.; Yang, L.; Cao, H.; Chang, Y.; Tang, K.; Cao, Z.; Chang, J.; Cao, Y.; Wang, W.; Gao, M.; et al. Carbon Materials Derived from Chitosan/Cellulose Cryogel-Supported Zeolite Imidazole Frameworks for Potential Supercapacitor Application. *Carbohydr. Polym.* **2017**, *175*, 223–230, doi:10.1016/j.carbpol.2017.07.089. 347–349
5. Sakuma, W.; Yamasaki, S.; Fujisawa, S.; Kodama, T.; Shiomu, J.; Kanamori, K.; Saito, T. Mechanically Strong, Scalable, Mesoporous Xerogels of Nanocellulose Featuring Light Permeability, Thermal Insulation, and Flame Self-Extinction. *ACS Nano* **2021**, *15*, 1436–1444, doi:10.1021/acsnano.0c08769. 350–352
6. Kudaibergenov, S.E. Physicochemical, Complexation and Catalytic Properties of Polyampholyte Cryogels. *Gels* **2019**, *5*, 8, doi:10.3390/gels5010008. 353–354
7. Pham, T.H.; Jung, S.H.; Kim, Y.J.; Kim, T.Y. Adsorptive Removal and Recovery of Organic Pollutants from Wastewater Using Waste Paper-Derived Carbon-Based Aerogel. *Chemosphere* **2021**, *268*, 129319, doi:10.1016/j.chemosphere.2020.129319. 355–357
8. Križman, K.; Novak, S.; Kristl, J.; Majdič, G.; Drnovšek, N. Long-Acting Silk Fibroin Xerogel Delivery Systems for Controlled Release of Estradiol. *J. Drug. Deliv. Sci. Technol.* **2021**, *65*, 102701, doi:10.1016/j.jddst.2021.102701. 358–359
9. Sharma, M.; Tavares, A.P.M.; Nunes, J.C.F.; Singh, N.; Mondal, D.; Neves, M.C.; Prasad, K.; Freire, M.G. Hybrid Alginate-Protein Cryogel Beads: Efficient and Sustainable Bio-Based Materials to Purify Immunoglobulin G Antibodies. *Green Chem.* **2020**, *22*, 2225–2233, doi:10.1039/c9gc04449c. 360–362
10. Goimil, L.; Santos-Rosales, V.; Delgado, A.; Évora, C.; Reyes, R.; Lozano-Pérez, A.A.; Aznar-Cervantes, S.D.; Cenis, J.L.; Gómez-Amoza, J.L.; Concheiro, A.; et al. ScCO₂-Foamed Silk Fibroin Aerogel/Poly(ϵ -Caprolactone) Scaffolds Containing Dexamethasone for Bone Regeneration. *J. CO₂ Util.* **2019**, *31*, 51–64, doi:10.1016/j.jcou.2019.02.016. 363–365
11. Raschip, I.E.; Fifere, N.; Varganici, C.D.; Dinu, M.V. Development of Antioxidant and Antimicrobial Xanthan-Based Cryogels with Tuned Porous Morphology and Controlled Swelling Features. *Int. J. Biol. Macromol.* **2020**, *156*, 608–620, doi:10.1016/j.ijbiomac.2020.04.086. 366–368
12. Kleemann, C.; Schuster, R.; Rosenecker, E.; Selmer, I.; Smirnova, I.; Kulozik, U. In-Vitro-Digestion and Swelling Kinetics of Whey Protein, Egg White Protein and Sodium Caseinate Aerogels. *Food Hydrocoll.* **2020**, *101*, 105534, doi:10.1016/j.foodhyd.2019.105534. 369–371
13. Selmer, I.; Karnetzke, J.; Kleemann, C.; Lehtonen, M.; Mikkonen, K.S.; Kulozik, U.; Smirnova, I. Encapsulation of Fish Oil in Protein Aerogel Micro-Particles. *J. Food Eng.* **2019**, *260*, 1–11, doi:10.1016/j.jfoodeng.2019.04.016. 372–373
14. Schroeter, B.; Yonkova, V.P.; Goslinska, M.; Orth, M.; Pietsch, S.; Gurikov, P.; Smirnova, I.; Heinrich, S. Spray Coating of Cellulose Aerogel Particles in a Miniaturized Spouted Bed. *Cellulose* **2021**, *28*, 7795–7812, doi:10.1007/s10570-021-04032-0. 374–375
15. Koshy, S.T.; Zhang, D.K.Y.; Grolman, J.M.; Stafford, A.G.; Mooney, D.J. Injectable Nanocomposite Cryogels for Versatile Protein Drug Delivery. *Acta Biomater.* **2018**, *65*, 36–43, doi:10.1016/j.actbio.2017.11.024. 376–377
16. Plazzotta, S.; Alongi, M.; De Berardinis, L.; Melchior, S.; Calligaris, S.; Manzocco, L. Steering Protein and Lipid Digestibility by Oleogelation with Protein Aerogels. *Food Funct.* **2022**, *13*, 10601–10609, doi:10.1039/d2fo01257j. 378–379
17. Mallepally, R.R.; Bernard, I.; Marin, M.A.; Ward, K.R.; McHugh, M.A. Superabsorbent Alginate Aerogels. *J. Supercrit. Fluids* **2013**, *79*, 202–208, doi:10.1016/j.supflu.2012.11.024. 380–381
18. Manzocco, L.; Plazzotta, S.; Powell, J.; de Vries, A.; Rousseau, D.; Calligaris, S. Structural Characterisation and Sorption Capability of Whey Protein Aerogels Obtained by Freeze-Drying or Supercritical Drying. *Food Hydrocoll.* **2022**, *122*, 107117, doi:10.1016/j.foodhyd.2021.107117. 382–384
19. Plazzotta, S.; Calligaris, S.; Manzocco, L. Structure of Oleogels from κ -Carrageenan Templates as Affected by Supercritical-CO₂-Drying, Freeze-Drying and Lettuce-Filler Addition. *Food Hydrocoll.* **2019**, *96*, 1–10, doi:10.1016/j.foodhyd.2019.05.008. 385–386
20. Plazzotta, S.; Jung, I.; Schroeter, B.; Subrahmanyam, R.P.; Smirnova, I.; Calligaris, S.; Gurikov, P.; Manzocco, L. Conversion of Whey Protein Aerogel Particles into Oleogels: Effect of Oil Type on Structural Features. *Polymers* **2021**, *13*, 4063, doi:10.3390/polym13234063. 387–389
21. Plazzotta, S.; Calligaris, S.; Manzocco, L. Structural Characterization of Oleogels from Whey Protein Aerogel Particles. *Int. Food Res. J.* **2020**, *132*, 109099, doi:10.1016/j.foodres.2020.109099. 390–391
22. Brodkorb, A.; Croguennec, T.; Bouhallab, S.; Kehoe, J.J. Heat-Induced Denaturation, Aggregation and Gelation of Whey Proteins. *Advanced Dairy Chemistry: Volume 1B: Proteins: Applied Aspects: Fourth Edition* **2016**, 155–178, doi:10.1007/978-1-4939-2800-2_6. 392–394
23. Manzocco, L.; Valoppi, F.; Calligaris, S.; Andreatta, F.; Spilimbergo, S.; Nicoli, M.C. Exploitation of κ -Carrageenan Aerogels as Template for Edible Oleogel Preparation. *Food Hydrocoll.* **2017**, *71*, 68–75, doi:10.1016/j.foodhyd.2017.04.021. 395–396

24. Yamasaki, S.; Sakuma, W.; Yasui, H.; Daicho, K.; Saito, T.; Fujisawa, S.; Isogai, A.; Kanamori, K. Nanocellulose Xerogels with High Porosities and Large Specific Surface Areas. *Front. Chem.* **2019**, *7*, 316, doi:10.3389/fchem.2019.00316. 397
398
25. Buchtová, N.; Budtova, T. Cellulose Aero-, Cryo- and Xerogels: Towards Understanding of Morphology Control. *Cellulose* **2016**, *23*, 2585–2595, doi:10.1007/s10570-016-0960-8. 399
400
26. Baudron, V.; Gurikov, P.; Smirnova, I.; Whitehouse, S. Porous Starch Materials via Supercritical- and Freeze-Drying. *Gels* **2019**, *5*, 12, doi:10.3390/gels5010012. 401
402
27. García-González, C.A.; Alnaief, M.; Smirnova, I. Polysaccharide-Based Aerogels – Promising Biodegradable Carriers for Drug Delivery Systems. *Carbohydr. Polym.* **2011**, *86*, 1425–1438, doi:10.1016/j.carbpol.2011.06.066. 403
404
28. Betz, M.; García-González, C.A.; Subrahmanyam, R.P.; Smirnova, I.; Kulozik, U. Preparation of Novel Whey Protein-Based Aerogels as Drug Carriers for Life Science Applications. *J. Supercrit. Fluids* **2012**, *72*, 111–119, doi:10.1016/j.supflu.2012.08.019. 405
406
407
29. Selmer, I.; Kleemann, C.; Kulozik, U.; Heinrich, S.; Smirnova, I. Development of Egg White Protein Aerogels as New Matrix Material for Microencapsulation in Food. *J. Supercrit. Fluids* **2015**, *106*, 42–49, doi:10.1016/j.supflu.2015.05.023. 408
409
30. Kleemann, C.; Selmer, I.; Smirnova, I.; Kulozik, U. Tailor Made Protein Based Aerogel Particles from Egg White Protein, Whey Protein Isolate and Sodium Caseinate: Influence of the Preceding Hydrogel Characteristics. *Food Hydrocoll.* **2018**, *83*, 365–374, doi:10.1016/j.foodhyd.2018.05.021. 410
411
412
31. Arboleda, J.C.; Hughes, M.; Lucia, L.A.; Laine, J.; Ekman, K.; Rojas, O.J. Soy Protein-Nanocellulose Composite Aerogels. *Cellulose* **2013**, *20*, 2417–2426, doi:10.1007/s10570-013-9993-4. 413
414
32. Andlinger, D.J.; Bornkeßel, A.C.; Jung, I.; Schröter, B.; Smirnova, I.; Kulozik, U. Microstructures of Potato Protein Hydrogels and Aerogels Produced by Thermal Crosslinking and Supercritical Drying. *Food Hydrocoll.* **2021**, *112*, 106305, doi:10.1016/j.foodhyd.2020.106305. 415
416
417
33. Yetiskin, B.; Okay, O. High-Strength and Self-Recoverable Silk Fibroin Cryogels with Anisotropic Swelling and Mechanical Properties. *Int. J. Biol. Macromol.* **2019**, *122*, 1279–1289, doi:10.1016/j.ijbiomac.2018.09.087. 418
419
34. Križman, K.; Novak, S.; Kristl, J.; Majdič, G.; Drnovšek, N. Long-Acting Silk Fibroin Xerogel Delivery Systems for Controlled Release of Estradiol. *J. Drug. Deliv. Sci. Technol.* **2021**, *65*, 102701, doi:10.1016/j.jddst.2021.102701. 420
421
35. Marin, M.A.; Mallepally, R.R.; McHugh, M.A. Silk Fibroin Aerogels for Drug Delivery Applications. *J. Supercrit. Fluids* **2014**, *91*, 84–89, doi:10.1016/j.supflu.2014.04.014. 422
423
36. Li, Y.; Wang, S.; Zhang, G.; Liu, X.; Liu, H.; He, Y.; Zhu, D. Morphological and Structural Changes in Thermally-Induced Soybean Protein Isolate Xerogels Modulated by Soybean Polysaccharide Concentration. *Food Hydrocoll.* **2022**, *133*, 107967, doi:10.1016/j.foodhyd.2022.107967. 424
425
426
37. Andlinger, D.J.; Schlemmer, L.; Jung, I.; Schroeter, B.; Smirnova, I.; Kulozik, U. Hydro- and Aerogels from Ethanolic Potato and Whey Protein Solutions: Influence of Temperature and Ethanol Concentration on Viscoelastic Properties, Protein Interactions, and Microstructure. *Food Hydrocoll.* **2022**, *125*, 107424, doi:10.1016/j.foodhyd.2021.107424. 427
428
429
38. Cheng, K.; Tao, X.; Qi, Z.; Yin, Z.; Kundu, S.C.; Lu, S. Highly Absorbent Silk Fibroin Protein Xerogel. *ACS Biomater. Sci. Eng.* **2021**, *7*, 3594–3607, doi:10.1021/acsbiomaterials.1c00467. 430
431
39. de Boer, J.; Aiking, H. On the Merits of Plant-Based Proteins for Global Food Security: Marrying Macro and Micro Perspectives. *Ecol. Econ.* **2011**, *70*, 1259–1265, doi:10.1016/j.ecolecon.2011.03.001. 432
433
40. Prag, A.A.; Henriksen, C.B. Transition from Animal-Based to Plant-Based Food Production to Reduce Greenhouse Gas Emissions from Agriculture – The Case of Denmark. *Sustainability* **2020**, *12*, 8228, doi:10.3390/su12198228. 434
435
41. Machovina, B.; Feeley, K.J.; Ripple, W.J. Biodiversity Conservation: The Key Is Reducing Meat Consumption. *Sci. Total Environ.* **2015**, *536*, 419–431, doi:10.1016/j.scitotenv.2015.07.022. 436
437
42. Sun, X.D.; Arntfield, S.D. Gelation Properties of Salt-Extracted Pea Protein Induced by Heat Treatment. *Int. Food Res. J.* **2010**, *43*, 509–515, doi:10.1016/j.foodres.2009.09.039. 438
439
43. Yin, X.; Cheng, H.; Wusigale, Dong, H.; Huang, W.; Liang, L. Resveratrol Stabilization and Loss by Sodium Caseinate, Whey and Soy Protein Isolates: Loading, Antioxidant Activity, Oxidability. *Antioxidants* **2022**, *11*, 647, doi:10.3390/antiox11040647/s1. 440
441
442
44. Nicolai, T.; Chassenieux, C. Heat-Induced Gelation of Plant Globulins. *Curr. Opin. Food Sci.* **2019**, *27*, 18–22, doi:10.1016/j.cofs.2019.04.005. 443
444
45. Sun, X.D.; Arntfield, S.D. Gelation Properties of Salt-Extracted Pea Protein Induced by Heat Treatment. *Int. Food Res. J.* **2010**, *43*, 509–515, doi:10.1016/j.foodres.2009.09.039. 445
446
46. Chen, N.; Lin, L.; Sun, W.; Zhao, M. Stable and pH-Sensitive Protein Nanogels Made by Self-Assembly of Heat Denatured Soy Protein. *J. Agric. Food Chem.* **2014**, *62*, 9553–9561, doi:10.1021/jf502572d. 447
448
47. Ako, K.; Nicolai, T.; Durand, D.; Brotons, G. Micro-Phase Separation Explains the Abrupt Structural Change of Denatured Globular Protein Gels on Varying the Ionic Strength or the pH. *Soft Matter* **2009**, *5*, 4033–4041, doi:10.1039/b906860k. 449
450
48. Renkema, J.M.S.; Gruppen, H.; van Vliet, T. Influence of pH and Ionic Strength on Heat-Induced Formation and Rheological Properties of Soy Protein Gels in Relation to Denaturation and Their Protein Compositions. *J. Agric. Food Chem.* **2002**, *50*, 6064–6071, doi:10.1021/jf020061b. 451
452
453

49. Fitzpatrick, S.E.; Staiger, M.P.; Deb-Choudhury, S.; Ranford, S. Protein-Based Aerogels: Processing and Morphology. *RSC Green Chem.* **2018**, *201*, 67–102, doi:10.1039/9781782629979-00067. 454
455
50. Sun, X.D.; Arntfield, S.D. Gelation Properties of Salt-Extracted Pea Protein Induced by Heat Treatment. *Int. Food Res. J.* **2010**, *43*, 509–515, doi:10.1016/j.foodres.2009.09.039. 456
457
51. Hermansson, A.M. Aggregation and Denaturation Involved In Gel Formation. *ACS Symposium Series* **1979**, *5*, 81–103, doi:10.1021/bk-1979-0092.ch005. 458
459
52. Hermansson, A.M. Soy Protein Gelation. *J. Am. Oil Chem. Soc.* **1986**, *63*, 658–666, doi:10.1007/bf02638232. 460
53. Kang, J.; Matsumura, Y.; Mori, T. Characterization of Texture and Mechanical Properties of Heat-Induced Soy Protein Gels. *J. Am. Oil Chem. Soc.* **1991**, *68*, 339–345, doi:10.1007/bf02657690. 461
462
54. Chen, N.; Zhao, M.; Chassenieux, C.; Nicolai, T. The Effect of Adding NaCl on Thermal Aggregation and Gelation of Soy Protein Isolate. *Food Hydrocoll.* **2017**, *70*, 88–95, doi:10.1016/j.foodhyd.2017.03.024. 463
464
55. Osen, R.; Toelstede, S.; Wild, F.; Eisner, P.; Schweiggert-Weisz, U. High Moisture Extrusion Cooking of Pea Protein Isolates: Raw Material Characteristics, Extruder Responses, and Texture Properties. *J. Food Eng.* **2014**, *127*, 67–74, doi:10.1016/j.jfoodeng.2013.11.023. 465
466
467
56. Shand, P.J.; Ya, H.; Pietrasik, Z.; Wanasundara, P.K.J.P.D. Physicochemical and Textural Properties of Heat-Induced Pea Protein Isolate Gels. *Food Chem.* **2007**, *102*, 1119–1130, doi:10.1016/j.foodchem.2006.06.060. 468
469
57. Hua, Y.; Cui, S.W.; Wang, Q.; Mine, Y.; Poysa, V. Heat Induced Gelling Properties of Soy Protein Isolates Prepared from Different Defatted Soybean Flours. *Int. Food Res. J.* **2005**, *38*, 377–385, doi:10.1016/j.foodres.2004.10.006. 470
471
58. O’Kane, F.E.; Happe, R.P.; Vereijken, J.M.; Gruppen, H.; van Boekel, M.A.J.S. Heat-Induced Gelation of Pea Legumin: Comparison with Soybean Glycinin. *J. Agric. Food Chem.* **2004**, *52*, 5071–5078, doi:10.1021/jf035215h. 472
473
59. Kuhn, K.R.; Cavallieri, A.L.F.; da Cunha, R.L. Cold-Set Whey Protein Gels Induced by Calcium or Sodium Salt Addition. *Int. J. Food Sci. Technol.* **2010**, *45*, 348–357, doi:10.1111/j.1365-2621.2009.02145.x. 474
475
60. Nicolai, T. Formation and Functionality of Self-Assembled Whey Protein Microgels. *Colloids Surf. B.* **2016**, *137*, 32–38, doi:10.1016/j.colsurfb.2015.05.055. 476
477
61. Nicolai, T.; Durand, D. Controlled Food Protein Aggregation for New Functionality. *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 249–256, doi:10.1016/j.cocis.2013.03.001. 478
479
62. Tanger, C.; Müller, M.; Andlinger, D.; Kulozik, U. Influence of pH and Ionic Strength on the Thermal Gelation Behaviour of Pea Protein. *Food Hydrocoll.* **2022**, *123*, 106903, doi:10.1016/j.foodhyd.2021.106903. 480
481
63. van der Linden, E.; Venema, P. Self-Assembly and Aggregation of Proteins. *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 158–165, doi:10.1016/j.cocis.2007.07.010. 482
483
64. Chen, N.; Zhao, M.; Chassenieux, C.; Nicolai, T. Structure of Self-Assembled Native Soy Globulin in Aqueous Solution as a Function of the Concentration and the pH. *Food Hydrocoll.* **2016**, *56*, 417–424, doi:10.1016/j.foodhyd.2015.12.028. 484
485
65. Kumar, S.; Nussinov, R. Relationship between Ion Pair Geometries and Electrostatic Strengths in Proteins. *Biophys. J.* **2002**, *83*, 1595, doi:10.1016/s0006-3495(02)73929-5. 486
487
66. Jiang, J.; Xiong, Y.L.; Chen, J. pH Shifting Alters Solubility Characteristics and Thermal Stability of Soy Protein Isolate and Its Globulin Fractions in Different pH, Salt Concentration, and Temperature Conditions. *J. Agric. Food Chem.* **2010**, *58*, 8035–8042, doi:10.1021/jf101045b. 488
489
490
67. Puppo, M.C.; Lupano, C.E.; Añón, M.C. Gelation of Soybean Protein Isolates in Acidic Conditions. Effect of pH and Protein Concentration. *J. Agric. Food Chem.* **1995**, *43*, 2356–2361, doi:10.1021/jf00057a008. 491
492
68. Puppo, M.C.; Añón, M.C. Structural Properties of Heat-Induced Soy Protein Gels As Affected by Ionic Strength and pH. *J. Agric. Food Chem.* **1998**, *46*, 3583–3589, doi:10.1021/jf980006w. 493
494
69. Li, J.; Li, X.; Wang, C.; Zhang, M.; Xu, Y.; Zhou, B.; Su, Y.; Yang, Y. Characteristics of Gelling and Water Holding Properties of Hen Egg White/Yolk Gel with NaCl Addition. *Food Hydrocoll.* **2018**, *77*, 887–893, doi:10.1016/j.foodhyd.2017.11.034. 495
496
70. Li, J.; Wang, C.; Zhang, M.; Zhai, Y.; Zhou, B.; Su, Y.; Yang, Y. Effects of Selected Phosphate Salts on Gelling Properties and Water State of Whole Egg Gel. *Food Hydrocoll.* **2018**, *77*, 1–7, doi:10.1016/j.foodhyd.2017.08.030. 497
498
71. Croguennec, T.; Nau, F.; Brulé, G. Influence of pH and Salts on Egg White Gelation. *J. Food Sci.* **2002**, *67*, 608–614, doi:10.1111/j.1365-2621.2002.tb10646.x. 499
500
72. Khemakhem, M.; Attia, H.; Ayadi, M.A. The Effect of pH, Sucrose, Salt and Hydrocolloid Gums on the Gelling Properties and Water Holding Capacity of Egg White Gel. *Food Hydrocoll.* **2019**, *87*, 11–19, doi:10.1016/j.foodhyd.2018.07.041. 501
502
73. Munialo, C.D.; van der Linden, E.; de Jongh, H.H.J. The Ability to Store Energy in Pea Protein Gels Is Set by Network Dimensions Smaller than 50nm. *Food Res. Int.* **2014**, *64*, 482–491, doi:10.1016/j.foodres.2014.07.038. 503
504
74. Maltais, A.; Remondetto, G.E.; Gonzalez, R.; Subirade, M. Formation of Soy Protein Isolate Cold-Set Gels: Protein and Salt Effects. *J. Food Sci.* **2005**, *70*, C67–C73, doi:10.1111/j.1365-2621.2005.tb09023.x. 505
506