Gelling Properties of Pea Protein Isolate in Combination with Protein from Wild Harvested *Ulva* SPP

Marianne Hammershøj[1], Ana J. Valnion[1], Louise J. Pedersen[1], Signe H. Nissen[1], Marie-Luise A. Olsen[1] and Trine K. Dalsgaard[1]

[1]Aarhus University, Department of Food Science, DK-8200 Aarhus N, Denmark

ABSTRACT

Plant-based proteins as food functional ingredients are increasing in demand due to lower climate impact and consumer interests in vegetarian and vegan diets. Furthermore, marine macro- and microalgae are also considered as food protein sources for the same reasons. The macroalgae *Ulva* spp. (common name sea lettuce) is a highly nutritious and fast-growing seaweed species that is of interest to extract protein from as it grows in Danish fjords. Pea protein isolates are available commercially, but with varying functional properties as gelling agents in foods. The study aim was to assess the functional properties of Ulva protein compared to a commercial pea protein isolate (PPI). The gelling properties of *Ulva* spp. protein and PPI were analyzed at food relevant pH and with/without NaCl addition. Gelling properties of *Ulva* spp. protein were assessed, but showed poor gelling ability alone, while PPI forms heat induced soft gel depending on protein concentration. Combinations with 10% and 30% relative substitutions with *Ulva* spp. protein in PPI gels were analysed. Rheological small amplitude oscillation measurements revealed synergistic effects of the two protein sources combined with significantly increased gel strength when *Ulva* spp. protein inclusion was increased. Based on the results, *Ulva* spp. protein may have potential as functionality ingredient in combined plant-based foods.

INTRODUCTION

Plant based products, such as pulses, nuts and seeds can offer a sustainable alternative to animal-based proteins, as they have less impact on the environment if cultured sustainably[1]. Peas (*Pisum sativum L.*) are a protein source which has a protein content dependent on environmental factors and cultivar and varies between 18 – 30%. *Ulva lactuca,* common name sea lettuce, is a species of the family *Ulvacae* belonging to the group of green macroalgae, which develops into sheet-like blades[2]. The protein content and nutritional profile of Ulva depends on environmental conditions and nutrient availability and may vary between 3.7 – 22% [3]. Because it is an edible algae, *Ulva* spp. is suggested used in food products, but this is still object to active research throughout the world.

Food gels are matrices which contain a continuous and well-defined network that has been assembled from particles or polymers (solid phase) and is embedded in a water-based solvent (liquid phase)[4]. Gels are of great importance as providers of texture to food products.

Pea protein isolates have the ability to form gels above a protein concentration of 17% [w/v] but they are less stable, when compared to e.g. soy protein isolate[5].

Proteins from *Ulva* spp. have not yet been assessed thoroughly, only one publication was found, where foaming properties and emulsifying properties were assessed[6], while no studies on gelling properties are reported.

The aim of the current study is to assess the gelling properties of pea protein isolate and *Ulva* spp. protein alone and in combination.

MATERIALS AND METHODS

Protein material

Commercially available pea protein isolate (PPI) named Pisane® was provided by COSUCRA (Warcoing, Belgium). The protein content of the dried PPI powder was 81.7 g/100g. A protein fraction from wild harvested *Ulva* spp. was provided by the Department of Food Science, Aarhus University. The protein fraction contained the whole seaweed which was juiced and then subjected to acidic protein precipitation. The *Ulva* spp. protein was freeze dried and had a protein content of 26.5 g/100g (own data, not published).

Gelation properties

To assess the lowest gelation concentration (LGC) for PPI, protein solutions of 1, 4, 8, 12, 15, 17, 20, and 23 % [w/v], respectively, were prepared with 100 mM bis tris pH 7 buffer. The LGC of *Ulva* spp. protein fraction was determined using 12, 15, 17, and 20 % [w/v] due to sample availability restrictions. All samples were incubated in 50 mL tubes in a water bath at 95°C for 1 hour and then cooled on ice for 1 hour. The LGC was determined as the concentration, at which the sample had gelled and held its shape, when turned upside down[5].

To study the effect of *Ulva* spp. substitution on PPI gelling properties, two levels of relative protein substitutions (10% and 30%) with *Ulva* spp. in gel systems with a final total protein concentration of 20% [w/v] were prepared in 100 mM bis tris base pH 7 with and without the addition of 0.075 M NaCl to study the effect of salt addition on gelling behaviour.

Dynamic rheology by small oscillatory measurements

The gel strength and gel stability were measured within a rheometer (HR20, TA instruments, New Castle, DE, USA).To define the linear viscoelastic region (LVR) of samples, stress sweep and frequency sweeps were conducted. Stress sweep tests with samples were conducted using a plate-to-plate geometry (40 mm), where 3 mL of protein sample were exposed to a strain sweep from 0.1-110 % with a constant frequency of 0.2 Hz at 25 °C. Frequency sweep tests with each sample were also conducted, with exposure to a frequency sweep from 0.1-120 Hz with a constant strain of 1 % at 25°C.

Small oscillatory shear measurements were conducted on 14, 18, and 20 % [w/v] pea protein samples, and on 20 % [w/v] PPI samples with and without the substitution (10% or 30%) with *Ulva* spp. protein. The heat induced gels were prepared while measuring the viscoelastic properties given as storage (G') and loss modulus (G'') during heating and cooling in the rheometer using oscillatory mode at a frequency of 0.2 Hz and strain of 1.5%. The thermal program started with 5 min equilibration at 25°C, heating to 90°C (3°C/min), holding at 90°C for 10 min, cooling down to 25°C (3°C/min), and finally holding at 25°C for 8 minutes. It was decided to use a slightly lower temperature compared to the LGC experiment, i.e. 90 °C instead of 95°C, as the protein gelation was observed at 90°C and to minimize evaporation and limit any trapped air bubble expansion during gelation. Each sample was tested in duplicates.

RESULTS

Lowest gelation concentration

The lowest concentration for gelation of PPI was 17 % at pH 7, while a protein concentration of 15 % led to no gelation. As gels with 17 % seemed very soft, higher protein concentrations were investigated, and in conclusion a concentration of 20% PPI was chosen for further experiments as they presented self-standing gels (**Fig. 1**). Salt addition led to softer gels, when compared to gels without salt addition. The *Ulva* spp. protein alone did not at any of the studied concentrations of 12, 15, nor 20 % [w/v] result in a gel structure (**Fig. 1**). Hence, no further gelation experiments with *Ulva* spp. protein as only protein source were conducted.

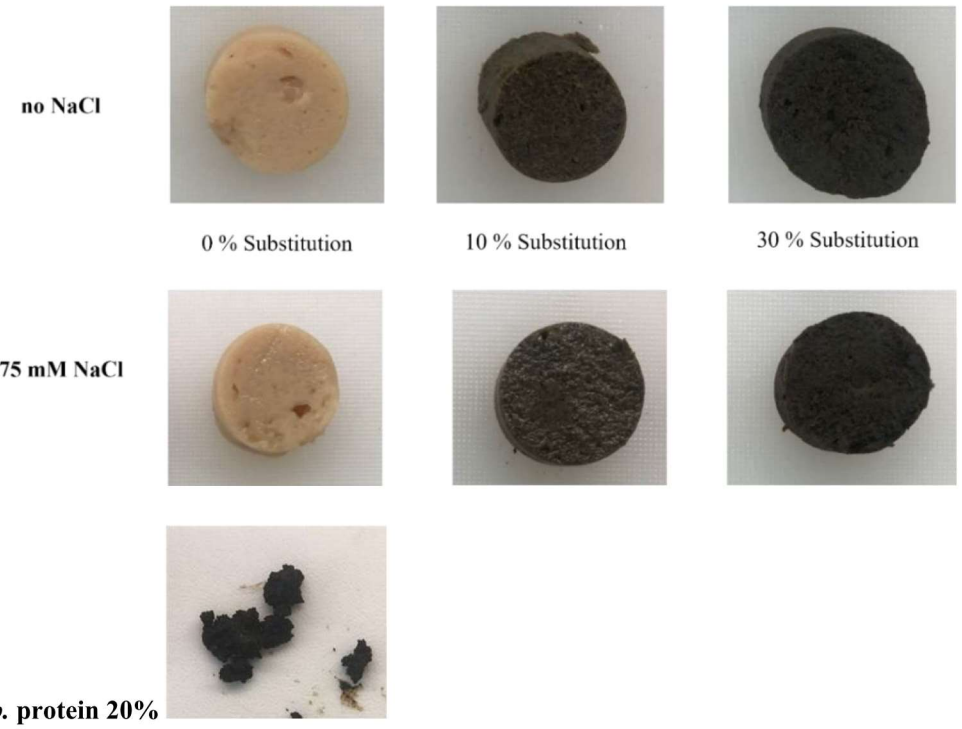

FIGURE 1: Appearance of 20% protein gels with 20% PPI alone (0% substitution), 18% PPI and 2% *Ulva* spp. protein (10% substitution) or 14% PPI and 6% *Ulva* spp. protein (30% substitution) in 100 mM pH 7 bis-tris buffer without (upper row) or with (second row) addition of 0.075 M NaCl. Third row shows the structure of 20% *Ulva* spp. protein alone after gelling treatment.

Protein substitution with *Ulva* spp. in pea protein isolate gels

The composition of the samples where partial substitution of PPI with *Ulva* spp. protein is given in **Table 1.** Both substitutions of 10% and 30% were able to form gels under the tested conditions. As seen in **Fig. 1,** the colour of the gels was dominated by the *Ulva* spp. protein fraction, leading to grey gels for the 10% substitution and dark grey colour for 30% substitution. Furthermore, both substitutions were able to prevent the formation of air bubbles, which occurred in the 0% substitution PPI gels.

TABLE 1 Sample composition of *Ulva* spp. protein substitution of pea protein isolate (PPI) gels with a total protein concentration of 20 % [w/v].

Substitution with *Ulva* spp. protein fraction [%]	*Ulva* spp. protein [%]	PPI protein [%]	Total protein [%]
0	-	20	20
10	2	18	20
30	6	14	20

Gel strength and gelling behaviour

As seen in **Fig. 2A**, storage modulus of PPI gels increased with increasing protein concentration. Highest final storage modulus, G', was measured with 20% gels, differing significantly from the lowest G', which was measured for the 14 % protein sample (P<0.05). Salt addition had no significant effect on G' but showed a tendency to decrease the storage modulus for 18 % gels and 20 % gels and to increase G' in the 14 % sample (**Fig. 2B**). Furthermore, gelling behaviour differed between 14% samples and gels with a concentration of 18% or higher. The G' of the 14% PPI sample started to increase shortly after the start of the heating step. The G' of 18% and 20% gels, first decreased during heating and holding and increased as soon as the cooling began.

Fig. 3 depicts the effect of *Ulva* spp. protein substitution on gel strength of PPI gels. The 10% substitution led to a 3-fold increase in the final gel strength (P<0.05), when compared to the 20% PPI gel, and furthermore, the 30% substitution led to a 9-fold increase in gel strength in comparison (P<0.05). Regarding gelling behaviour, substituted samples showed the same trend during heating and cooling as 20% PPI gels. They showed a decreasing G' during heating and holding and increasing G' during the cooling process. Addition of 0.075 M salt led to no significant differences in gel strength and gelling behaviour (**Fig. 3B**).

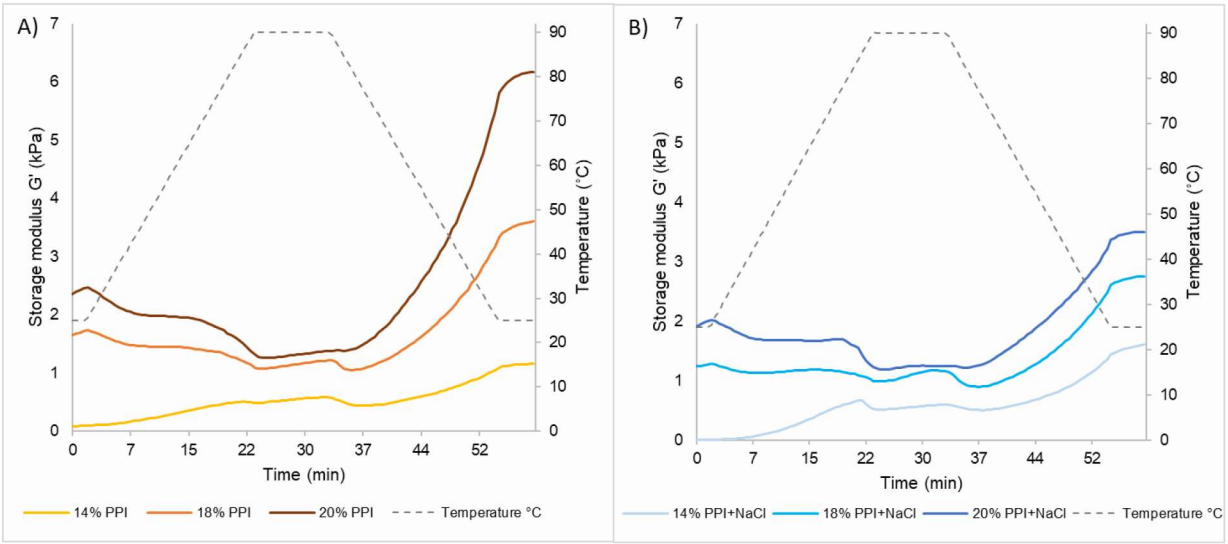

FIGURE 2: Storage moduli (G') of pea protein isolate (PPI) gels as function of temperature with different protein concentrations in 100 mM bis-tris pH 7 buffer without (A) and with (B) addition of 0.075 M NaCl. (n=2).

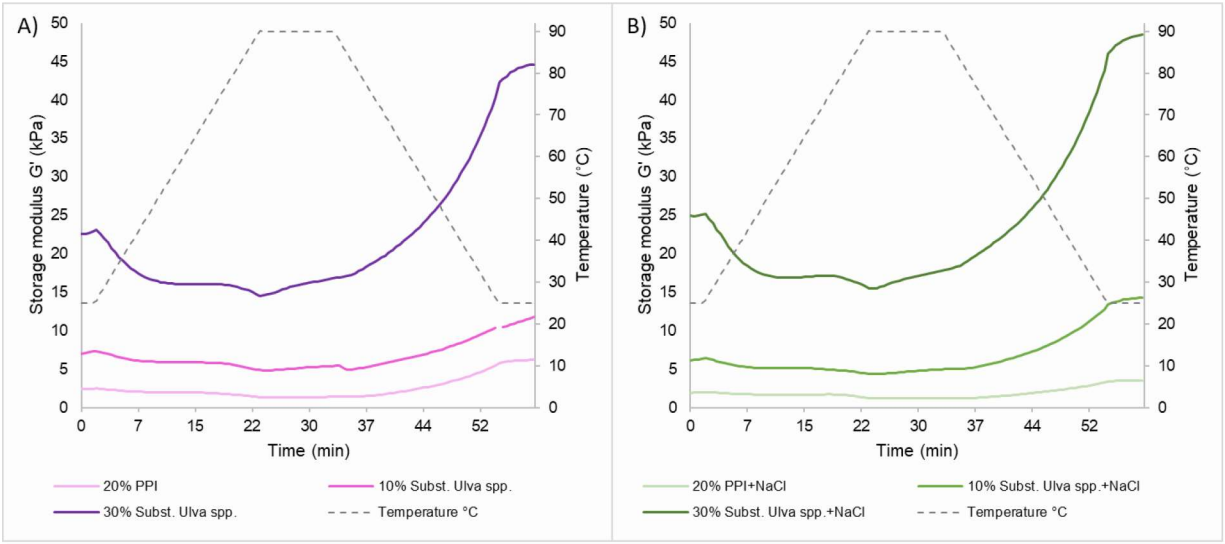

FIGURE 3: Storage moduli (G') of 20% protein gels pea of protein isolate (PPI) with *Ulva* spp. protein substitutions of 0, 10 and 30%, respectively as function of temperature in 100 mM bis-tris pH 7 buffer without (A) and with (B) addition of 0.075 M NaCl. (n=2).

DISCUSSION

Small oscillatory measurements were used to gain understanding on the gelling behaviour and gel strength of PPI gels and gels with *Ulva* spp. protein substitution of PPI. The gelling mechanism of globular proteins can usually be described by heat induced gelation, were hydrophobic or covalent interactions lead to a fast increase in G'[7]. However, the present data show that a fast increase in G' during the heating step can only be seen in 14% PPI gels.

For 18% and 20% gels we see a fast increase in G' during the cooling step, which is characteristic for cold set gels, where hydrogen bonds are the primary driving force of gel strength development during cooling[8]. Even though hydrogen bonds are weak bonds they may be many by number and can contribute significantly to protein gel texture increase of 3-5 fold upon cooling, e.g. of whey and egg albumen gels[9].

Gel strength increased with increasing PPI concentration, which is in line with the results from the LGC experiment and the literature[10]. This also supports the suggestion of hydrogen bonds being the major driver for gelation, as a higher protein concentration leads to closer proximity between protein side chains increasing the chance of H-H interactions.

The effect of salt addition on gel properties was also assessed. For 14% PPI gels, salt addition tended to have a positive effect on gel properties, while for 18% gels and 20% gels a decrease in G' was observed. This may be caused by disruptions in the gel network through salt ions, which lead to a weaker gel network and therefore a decrease in gel strength[8].

The *Ulva* spp. protein substitution affected PPI gelling behaviour depending on the percentage of substitution. The 10% *Ulva* spp. protein substituted gels show a positive effect in gel properties, indicated by a significantly higher gel strength (P<0.05). The 30% *Ulva* spp. protein substitution showed an even higher effect on gel strength. One explanation for that is, that *Ulva* spp. protein served as a filler substance and depending on the concentration affected gel properties. Katz, et al., 2021[11] conducted a study investigating the effect of filler particles on rheological properties of meat protein gels. They reported a positive effect on material performance, which was caused by a void filling effect, meaning that the particles served as

filler in the molecular structure and therefore increased material performance[11]. Although the study was conducted in a different system (namely sausages), it still shows similar effects then what we observed here. Since the *Ulva* spp. protein itself was unable to form a gel, this observation supports a filler mechanism in the PPI gels.

Fig. 1 shows a more homogeneous macrostructure for the 10% *Ulva* spp. protein substitution when compared to the 20% PPI gels. This could also be an indicator for *Ulva* protein acting as a filler and therefore improving the gel properties.

Finally, it is noted that the 10% and 30% *Ulva* spp. protein substituted gels have a dark grey colour, which could be problematic from the sensory perspective. It is not clear, whether the colour was caused by changes during processing, such as chinone oxidation or if the colour of the used biomass was responsible hereof.

CONCLUSION

The gelling properties of PPI and *Ulva* spp. protein were assessed. Least gelation experiments revealed a minimum of 17% PPI for gelling, but no gelling properties for pure *Ulva* spp. protein. Gelling properties of PPI in combination with *Ulva* spp. protein were assessed. Substitution of protein in PPI gels in low (10%) and moderate concentration (30%) revealed that *Ulva* spp. protein apparently served as inert filler and led to mixed effects depending on its concentration. A positive effect on gel strength evaluated as storage modulus G' was observed for 10% substitution, and even higher for 30% substitution.

ACKNOWLEDGEMENT

Data were generated through accessing research infrastructure at Aarhus University, including FOODHAY (Food and Health Open Innovation Laboratory, Danish Roadmap for Research Infrastructure). This work was funded by the Green Development and Demonstration Programme (GUDP) of the Ministry of Food, Agriculture and Fisheries of Denmark (SEASUS project).

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