



Plant protein adsorption at oil–water interfaces: A mapping review using alternate subphase tensiometry

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Plant proteins are rapidly emerging as functional food ingredients as a more sustainable and ethical alternative to established animal proteins, e.g., for the interfacial stabilization of emulsions and foams. To date, there is a large knowledge gap between plant and animal proteins, and it is often difficult to predict the interfacial performance of novel plant proteins, their individual fractions, and behavior as a function of processing conditions. Here, we introduce alternate subphase tensiometry (AST) as a novel method to map literature data on interfacial tension reduction of novel protein sources. We collected, compiled, and reanalyzed interfacial tension literature data from pea, soy, chickpea, lentil, faba bean, wheat, and microalgae protein. AST allows standardized plotting of literature data in uniform graphs, providing a rapid overview of the present data and pinpointing current gaps. Furthermore, grouped data can be readily singled out to identify promising protein fractions and effects of processing conditions such as pH, ionic strength, or pretreatments. Hence, AST is a powerful tool to summarize the existing data landscape, identify research gaps and particularly promising protein fractions, and ultimately predict the interfacial performance of plant proteins for interfacial stabilization of emulsions and foams.

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Introduction

The consumption of animal products is increasingly questioned due to environmental, health, and ethical concerns [1–4]. Despite a trend towards more animal-free food products [5,6], the food industry as well as home cooking still rely heavily on animal-based products as functional food ingredients. Particularly proteins from egg or dairy are widely employed as thickening or gelling agents (e.g., in cheese, yoghurts, omelets, or cakes) or for the stabilization of emulsions and foams (e.g., in sauces, mayonnaise, whipped cream, or meringues) [7–10]. Despite a surge in academic and industrial interest in alternative proteins from plant or unicellular sources [11–21], replacing animal protein functionality often proves difficult due to the large knowledge gap between animal and plant protein composition, structure, and colloidal behavior [7]. Hence, there is a pressing need for methods that facilitate the prediction of plant protein functionality and comparison to established animal proteins.

In context of interfacial stabilization, the ability of proteins to adsorb at the oil–water (o/w) or air–water (a/w) interface and reduce the interfacial tension is one of the key criteria to evaluate their potential for the stabilization of emulsions and foams. Emulsions and foams are metastable multiphase systems of immiscible fluids, and a reduction of the interfacial tension is crucial to increase their stability [22]. The adsorption behavior of proteins at fluid–fluid interfaces depends on several factors, including their structural stability [23–25] as charge and solubility [26,27]. For o/w interfaces, protein adsorption further depends on the hydrophobicity (inverse polarity) of the used oil phase, and protein surface tension reduction directly correlates with oil hydrophobicity [25,28,29]. This facilitates the comparison of protein adsorption data across literature by alternate subphase tensiometry (AST), i.e., the measurement or

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collection of existing interfacial tension data at various oil subphases [25]. Here, we establish AST as a novel approach to map and compare available data of plant protein adsorption at o/w interfaces. We find that AST facilitates the mapping of available literature data in uniform plots, providing a rapid overview of the current data, as well as allowing identification of particularly promising protein fractions, conclusions on their interfacial performance under specific conditions, and ultimately predicting and comparing their performance to established animal proteins.

Mapping protein adsorption by alternate subphase tensiometry

Protein adsorption at oil-water interfaces

Emulsions are characterized by a high interfacial contact area of oil and water. This contact area is energetically unfavorable, giving rise to o/w interfacial tension γ_{ow} which drives emulsion instabilities like oil droplet coalescence. Surface active species such as proteins can adsorb from the aqueous bulk at the o/w interface and reduce γ_{ow} , thereby promoting emulsion stability [22]. The adsorption behavior and γ_{ow} reduction by proteins at o/w interfaces is dictated by the structural stability of proteins, the aqueous solution conditions, and the hydrophobicity of the oil phase. Unstructured (random coil) proteins typically adsorb fastest and trigger the highest γ_{ow} reduction. For more structured (globular) proteins, the adsorption speed and γ_{ow} reduction are limited by the proteins' structural stability that hinders structural rearrangements upon adsorption (Fig. 1a) [23–25,29]. The aqueous phase conditions affect protein adsorption by altering their solubility and surface charge. Proteins typically are most surface active near their isoelectric point or at increased ionic strength due to a decreased aqueous solubility and reduced electrostatic repulsion at the interface. However, they may precipitate out of solution and lose surface activity if their net charge gets too low [26,27]. Regarding the oil phase, the adsorption kinetics and γ_{ow} reduction of proteins are governed by the hydrophobicity, i.e., inverse polarity, of the oil phase. Proteins adsorb faster and reduce γ_{ow} to a higher extent at more hydrophobic oils, as the hydrophobic interface provides an increased net energy gain upon protein adsorption [25,28,30,31]. Particularly for globular proteins, hydrophobic oils trigger increased protein restructuring due to higher lateral surface stresses while for polar oils the proteins immerse deeper in the oil phase with limited rearrangements (Fig. 1b) [29,31–34]. Based on this interrelationship of oil hydrophobicity and protein adsorption, Bergfreund et al. [25] found that there is a universal correlation of oil hydrophobicity and γ_{ow} reduction of proteins, allowing the mapping of literature data and prediction of γ_{ow} reduction for any oil by AST.

Principles of alternate subphase tensiometry

AST exploits the correlation of oil hydrophobicity and interfacial tension reduction of surface active species at respective interfaces [30]. The γ_{ow} reduction by proteins is most commonly determined by the pendant drop technique [34], the Wilhelmy-plate [24] or Du Nouy ring technique [35]. In the following, we provide a brief guide on choosing suitable oils and process data to correlate γ_{ow} reduction and oil hydrophobicity, in order to map and potentially predict the interfacial tension reduction of a protein at any oil using AST.

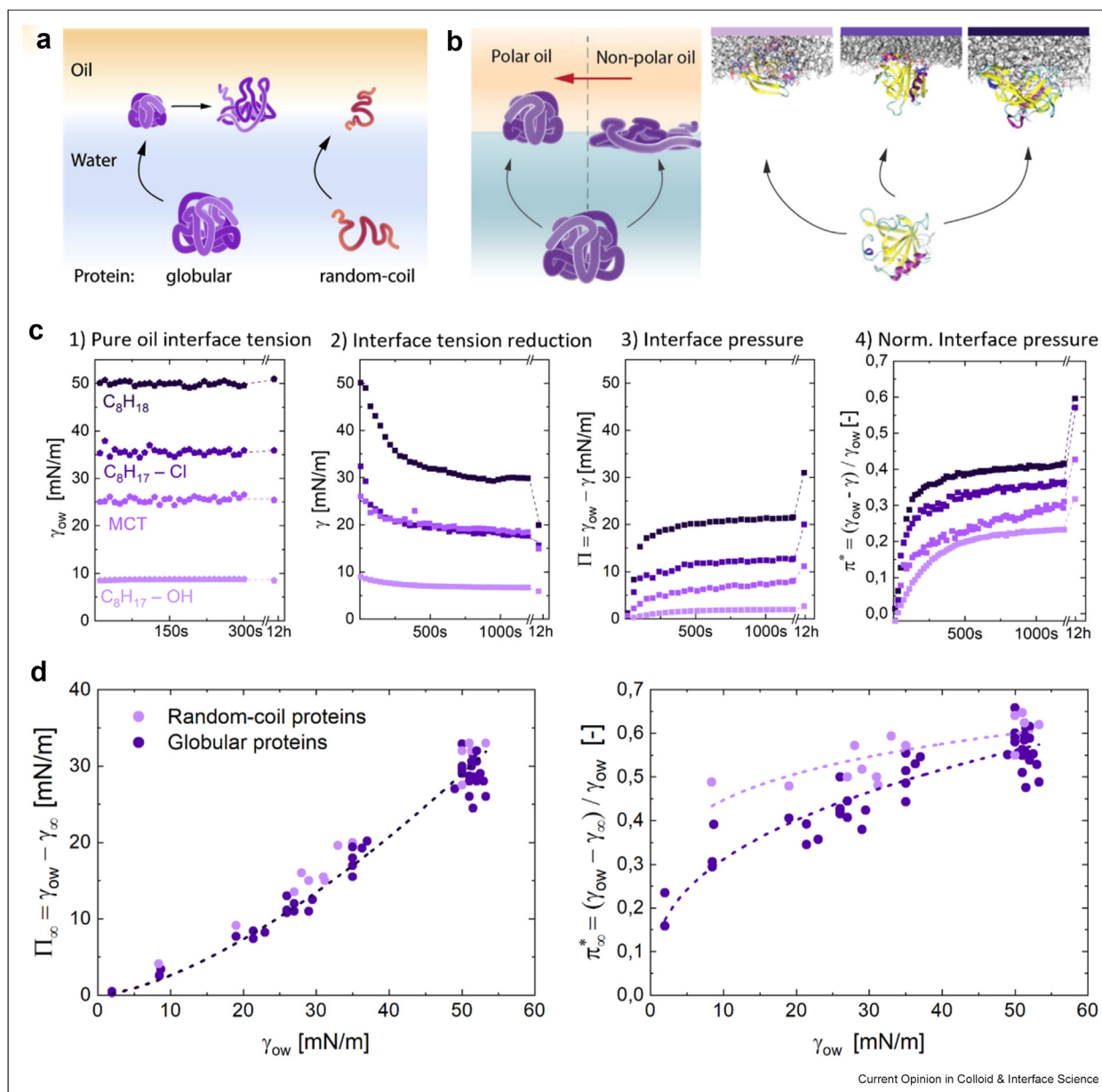
1) Choice of oils and measurement of clean o/w interfacial tension γ_{ow}

The interfacial tension of the clean o/w interfacial tension γ_{ow} is a good measure for the hydrophobicity of oils [30]. Fig. 1c₁ shows γ_{ow} of oils with comparable C₈ hydrocarbon backbone structure but altering headgroup polarity. Octane, like other n-alkanes, is amongst the most hydrophobic oils with $\gamma_{ow} \approx 52$ mN/m. The introduction of an increasingly polar headgroup results in a lower γ_{ow} , e.g., ≈ 36 mN/m for 1-chlorooctane and ≈ 8.5 mN/m for octanol. Medium chain triglyceride (MCT) oil containing triglycerides with C₈ and C₁₀ fatty acids is a well-suited model triglyceride for food applications with an intermediate $\gamma_{ow} \approx 25$ mN/m. Measurements employing those four oils are suitable for AST as they cover a broad spectrum of γ_{ow} . Interfacial tension measurements at the clean o/w interface as shown in Fig. 1c₁ are crucial to confirm that γ_{ow} is stable over time and no surface active contaminants are present in the water or oil phase before adding protein. Table S1 shows a compilation of other common oils used in interfacial tension measurements and their γ_{ow} at the clean o/w interface. More detailed guidelines for choosing the right oil or cleaning oil from surface active contaminants for interfacial measurements were provided by Bergfreund et al. [30].

2) Measurement of protein o/w interfacial tension reduction

Following the selection of suitable oils and determination of γ_{ow} of the clean o/w interface, the dynamic interfacial tension γ upon protein adsorption may be determined (Fig. 1c₂). The adsorption of proteins from the bulk at the o/w interface results in a steady decrease in γ . Note that a protein concentration above the minimal monolayer coverage concentration should be chosen in order to facilitate comparison to literature values. This concentration varies for different proteins but typically lies between 1 and 10 mg/L and may be experimentally determined by increasing protein concentration until no further decrease in γ is found [30,34].

Figure 1



a) Schematic of globular and random-coil protein adsorption at oil-water interfaces. b) Schematic and molecular dynamics simulation of globular protein adsorption at non-polar and polar oils. c) Methodology of alternate subphase tensiometry (AST) to obtain protein interfacial pressure data at oils with varying polarity: 1) Measurement of clean o/w interface to obtain initial interfacial tension γ_{ow} and confirm oil purity, 2) measurement of γ_{ow} reduction by protein adsorption, 3) plotting as interfacial pressure Π and 4) normalized interfacial pressure π^* for better comparison of protein adsorption at oils with different polarity. d) Example of AST-plots showing the correlation of final interfacial pressure Π_∞ or π_∞^* as a function of initial interfacial tension γ_{ow} for animal globular and random-coil proteins. Adapted with permission from [25] (a, c, d), Copyright Elsevier 2021 [29], (b, schematic), The Royal Society of Chemistry, and [33] (b, simulation), Copyright 2016 American Chemical Society. Data for d) was reused from a published dataset [36]. Data of animal protein adsorption [30,36]. We thus speculate that AST is a powerful method for novel plant proteins in order to collect, compare, reanalyze, and potentially predict their performance in terms of interfacial stabilization.

3) Determination of interfacial pressure

For better visualization and comparison of interfacial tension reduction at oils with altering γ_{ow} , it is beneficial to express γ as the net interfacial tension reduction, also known as interfacial pressure $\Pi = \gamma_{ow} - \gamma$ (Fig. 1c₃).

4) Normalization of interfacial pressure by γ_{ow}

For better comparison of interfacial tension reduction at oils with a wide range of γ_{ow} , Π can be normalized by the oils' individual γ_{ow} to obtain the normalized interfacial pressure $\pi^* = (\gamma_{ow} - \gamma)/\gamma_{ow}$ (Fig. 1c₄).

5) Mapping data in AST-plots

Ultimately, the final surface pressures Π_∞ or π_∞^* can be plotted as a function of the oils' initial γ_{ow} in AST-plots to visualize the correlation of protein interfacial tension reduction and oil hydrophobicity (Fig. 1d). Note that it typically takes several hours particularly in case of stable globular proteins to reach equilibrium Π_∞ , and it is advisable to run experiments at least a few hours. The final interfacial pressure of proteins and other surface-active species as a function of oil hydrophobicity follows a power law according to $\Pi_\infty \propto \gamma_{ow}^a$, where a is a fitting parameter that typically is 1.25–1.50 for proteins depending on their structural stability [25,30]. Due to this universal correlation, it is possible to complement AST-plots with literature data reporting interfacial tension reduction of proteins at different oils. Fig. 1d depicts literature data for fractionated animal proteins typically used in interface science or food industry, i.e., globular lysozyme, β -lactoglobulin, and bovine serum albumin or random coil β -casein. Hence, AST allows the holistic visualization and fitting of literature.

Data collection and inclusion criteria

Literature reporting the adsorption of plant proteins at oil-water interfaces was screened for interfacial tension data at various oil subphases. Surface pressure data was extracted and plotted in AST-plots as a function of oil hydrophobicity (Fig. 1d). For successful extraction of universally comparable data, the following inclusion criteria had to be met:

- Use of protein concentrate or isolate and aqueous concentration ≥ 10 mg/L (≥ 0.001 % (w/v))
- Physicochemical boundary conditions clearly reported (pH, ionic strength, temperature)
- Final surface pressure Π_∞ or dynamic interfacial tension γ were reported or could be unambiguously extracted from graphs using PlotDigitizer
- Initial oil-water interfacial tension γ_{ow} was reported and in agreement with literature data, or oil was obtained *reagent grade*, or oil was purified before use [30].

Following screening of literature and extraction of surface pressure data, sufficient data for AST-plots were obtained for pea (55), soy (36), chickpea (9), lentil (14), faba bean (18), wheat (5), and microalgae (26) protein. All collected data is compiled as [Supplementary Material](#) and is made available in editable form via Zenodo [37].

Results and discussion

Pea (*Pisum sativum*)

Pea protein was found to be the most extensively studied plant protein for interfacial stabilization with the most literature reporting adsorption at o/w interfaces. Studies that investigated pea protein adsorption at various o/w interfaces are compiled in Table 1. Most studies used common food oils such as sunflower oil, canola/rapeseed oil, corn oil, or MCT oil with medium interfacial tensions, while few studies investigated adsorption at high γ_{ow} oils like vaseline oil. Plotting the normalized interfacial pressure π_∞^* as a function of initial oil interfacial tension γ_{ow} allows the visualization of all available literature data in combined AST-plots, as shown in Fig. 2. The power law fit follows the same trend of higher γ_{ow} reduction at more hydrophobic oils known from other proteins (Fig. 1). Furthermore, distinct coloration allows easy identification of different pea protein fractions and their individual surface activity or effects of measuring conditions such as pH. For example, the compiled AST-plots for pea protein adsorption promptly reveal that individual pea protein fractions are more surface active compared to whole pea protein dispersions (Fig. 2a). Furthermore, pea proteins are more surface active at neutral or basic pH compared to acidic conditions (Fig. 2b). Hence, compiling interfacial tension data in AST-plots can rapidly reveal the trends across all available literature data.

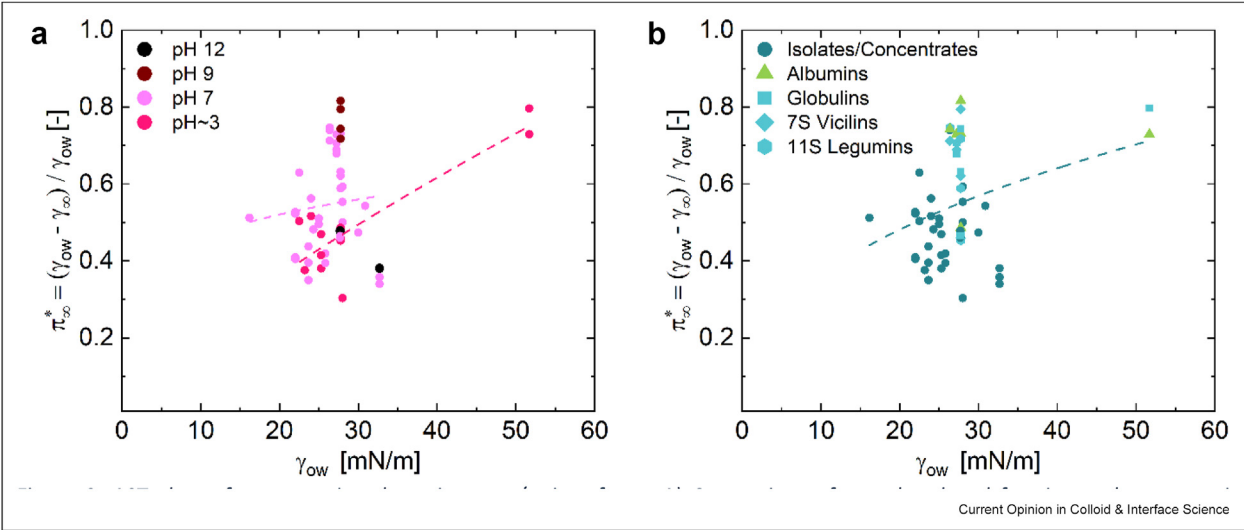
Effect of fractionation

Fig. 2a shows an AST-plot of pea protein adsorbed at different o/w interfaces, distinguishing whole pea protein isolates as well as individual fractions. Pea protein isolate consists of 65–80 % globulins (salt-soluble) and 10–20 % albumins (water-soluble) [38]. Globulins are storage proteins including 11S legumin, 7S vicilin, and 7S convicillin fractions, characterized by respective sedimentation coefficients and number of subunits [39]. 11S legumins are hexamers with a molecular weight of 300–400 kDa. 7S Vicilins are trimeric glycoproteins that are ≈ 150 kDa [40,41]. 7S Convicilins are trimeric and have 80 % sequence homology with vicilin, differing in one cysteine residue and a highly charged N-terminus [42]. From distinctive coloration in AST-plots (Fig. 2a) it is apparent that whole pea protein isolates are less surface active compared to their individual fractions. A comparison between globulins, legumins and vicilins adsorption at the corn oil-water interface was made by Chang et al.

Table 1
Compilations of studies measuring pea protein adsorption at o/w interfaces. PP: pea protein, PPI: pea protein isolate, PPC: pea protein concentrate.

Oil Phase	Protein Description	pH	Remarks	Ref.
Sunflower oil	1 % commercial PPI, Commercial PPI followed by high pressure homogenization, Mildly extracted PPI	7		[67]
Corn oil	0.1 % PP globulin fraction	3,7,9	Ionic strength variations	[43]
Corn oil	0.1 % PP legumin fraction	3,7,9	20 mM, 100 mM NaCl at pH	[43]
Corn oil	0.1 % PP vicilin fraction	3,7,9	7	[43]
Corn oil	0.1 % PP albumin fraction	3,7,9		[43]
MCT oil (Miglycol 812)	0.04 % PPI (95 % globulin fraction)	2.4, 7	5 mM imidazole + acetate buffer	[68]
Rapeseed oil	0.01 % PPI molecule solution	3		[84]
Vaseline oil	1 % PP albumin fraction	3		[56]
Vaseline oil	1 % PP globulin fraction	2.6		[56]
Sunflower oil	1 % PPI, ultrasonicated PPI	7	100 mM phosphate buffer	[77]
Canola oil	0.25 % untreated PPI, heat-treated PPI, 5 % degrees of hydrolyzed PPI, 10 % degrees of hydrolyzed PPI	7	Heat treatment to 85 °C, enzymatic hydrolysis with trypsin	[76]
Canola oil	2 % PPI	3, 7		[64]
Sunflower oil	0.01 % PPI	3		[85]
Sunflower oil	1 % PPC	7		[15]
Canola oil	0.25 % PPI	7		[86]
Sunflower oil	PPI	7		[87]
MCT oil (Miglycol 812)	0.05 % PPI	7		[88]
Rapeseed oil	0.002 % PPC	7		[89]
Canola oil	2 % PPC, de-flavored PPC, PPI	2,7		[90]
MCT oil (Miglycol 812)	0.01 % PPC	7		[91]
Corn oil (triglyceride)	1.1 % native PPI, pH shifted PPI, Ultrasonicated PPI	7,12	300 mM NaCl, regular corn oil	[73]
Corn oil (60 % diglyceride)	0.1 % native PPI, pH shifted PPI, Ultrasonicated PPI	7,12	300 mM NaCl, more polar corn oil	[73]

Figure 2



AST-plots of pea protein adsorption at o/w interfaces. a) Comparison of completed and fractionated pea protein solutions. b) Comparison of pea proteins at pH ≤ 3, pH 7, pH 9 and pH 12. Both graphs represent the same set of literature data compiled in [Table 1](#).

[43]. Across all pH and ionic strengths, legumin attained lower interfacial pressures compared to mixed globulin or vicilin fractions. Generally, 7S globulins are more surface active than 11S globulins due to their smaller size. Vicilins adsorb faster at the interface compared to legumins, likely due to their higher solubility at pH 7 caused by the absence of disulfide bridges and higher structural flexibility [38,40,44,45]. Legumins exhibit a more rigid and compact structure [46] and higher surface hydrophobicity [44,47] compared to vicilin. Moreover, legumins have more sulfur-containing amino acids, are larger and more structurally stable [40], which results in slower unfolding and reduced emulsifying properties compared to vicilin [48]. However, legumin was found to have a higher emulsifying capacity than vicilin at pH 7 [44]. This indicates that other factors than γ_{ow} reduction alone such as the formation of viscoelastic interfacial layers are important for interfacial stabilization, as discussed further below. Legumin/vicilin ratios in pea protein isolate can vary from 1.2 to 4.8 [49] depending on cultivars, which thus impacts the adsorption behavior of pea protein isolates at the o/w interface [50].

Albumins are water soluble metabolic proteins that comprise PA1 (pea albumin 1), PA2 (pea albumin 2), protease inhibitors, amylase inhibitors and lectins [40]. Pea albumins have a lower molecular weight (4–26 kDa), are present as monomers or dimers, and contain more sulfur-containing amino acids such as cysteine and methionine compared to globulins [51,52]. Albumins are more soluble compared to other pea protein fractions due to their smaller size [39] and abundance of hydrophilic amino acids [43,53]. In our AST-plot (Fig. 2a), albumins are more surface active compared to globulin fractions. Chang et al. [43] found that albumin fractions showed a higher γ_{ow} reduction than globulin fractions across all pHs and ionic strengths. This could be attributed to albumins having a less stable structure. On the other hand, Shen et al. [54] found that fractionated globulins, i.e., vicilins and legumins, are more efficient at reducing γ_{ow} compared to albumins. Kornet et al. [55] confirmed that the thermal denaturation enthalpy of albumins is significantly lower compared to globulins. Albumins require less energy to unfold despite a higher number of cysteine residues and presence of disulfide bonds. On the contrary, Ducel et al. [56] found that globulins were more surface active than albumins at the Vaseline oil-water interface. However, measurements were carried out at pH 3, which potentially interfered with the conformation of legumins. The 11S hexameric structure of legumins is only maintained at pH 7–9, whereas they completely dissociate into a mixture of monomers/dimers/trimers resulting in reduction in size and hydrophobicity [56–58]. The pH effect on pea protein interfacial activity will be discussed in detail below.

Although albumins diffuse faster and are preferentially adsorbed compared to globulins [59], their lower structural stability results in less viscoelastic interfacial layers [54,55,60]. Lu et al. [61] found that PA2 plays a more dominant role in stabilization of o/w interfaces compared to PA1. PA2 contains more low molecular weight hydrophobic units that adsorb at the interface more efficiently and form more elastic interfacial layers. However, PA2 only contains one covalent disulfide bond [62], which makes the conformation easily variable, resulting in emulsions or foams that destabilize faster [61]. Interfacial rheological tests also confirm that interfaces with albumins have generally the lowest dilatational [43,54,59] and shear [63] moduli. In summary, albumins have a distinct amphiphilic structure, smaller molecular weight, higher solubility, lower surface hydrophobicity, lower denaturation enthalpy, as well as a lower net charge. These traits favor fast adsorption and high interfacial coverage at the o/w interface, but do not allow the formation of stable viscoelastic interfacial layers [55,63]. Furthermore, albumin surface properties are also pH dependent as discussed below [59]. In conclusion, the behavior of albumins at the o/w interface is multifactorial, potentially explaining the scattered results in literature [54,56,59,64].

The higher attained normalized surface pressure of fractionated pea protein compared to whole pea dispersions could stem from higher purity since other substances such as soluble carbohydrates (e.g., pea raffinose and stachyose) are removed during the extraction processes [55,65]. The most common isolation method is alkaline extraction followed by isoelectric point precipitation. This process involves solubilizing legumes at high pH followed by isoelectric precipitation around pH 4–5 where proteins aggregate, followed by centrifugation and collection [21,40]. At the isoelectric point, mostly globulin fractions aggregate and are present in the pellet after centrifugation, while soluble albumins are in the supernatant and often discarded. Many pea protein isolates mainly contain globulins, whereas albumins are often overlooked [21]. Also, irreversible aggregation of globulins might occur, resulting in lower protein solubility which can impair their surface activity [21,66]. Size exclusion chromatography and SDS-PAGE of pea protein isolates purified by alkaline extraction and isoelectric point precipitation have shown much lower albumin (PA1 and PA2) content compared to isolates obtained by diafiltration [66]. The extraction and processing methods can also affect the state of aggregation and denaturation of the protein isolates [38]. Grasberger et al. [67] demonstrated that mildly extracted pea protein via alkaline extraction–isoelectric precipitation has slower rearrangement times but forms the stiffest interface compared to commercial pea protein isolates. On the other hand, commercial pea protein isolates show higher adsorption rates, stemming from its more homogenous

composition and higher degree of unfolded proteins which makes it easier for the hydrophobic patches to rearrange and expose at the interface. TEM images from Shen et al. [54] showed that 2S albumins have a more aggregated morphology, compared to 7S vicilins and 11S legumins. Hence, pea protein isolates may differ considerably in composition depending on supplier or extraction techniques. To obtain a more comprehensive composition of plant protein fractions, other alternative purification approaches such as membrane filtration, diafiltration and lowering extraction pH were also suggested [21,40].

Effect of pH

Figure 2b demonstrates the π_{∞}^* of pea protein at different pH values. The mean isoelectric point of pea globulins is 4.5 [38], while albumins have an average isoelectric point of 6 [50]. Notable differences in interfacial activity between neutral and acidic conditions were observed, with pea protein isolates at pH 3 being the least surface active. Chang et al. [43] compared pH 3, 7, and 9 of all pea protein fractions at the corn oil-water interface, finding lower final interfacial tensions at higher pH. Gharsallaoui et al. [68] compared the adsorption of pea protein globulins at the MCT oil-water interface at pH 2.4 and 7. Under acidic conditions, the dynamic interfacial tension decreased at a slower rate despite pea protein dissociation into 3S forms, whereas faster initial adsorption was found for globulins in their larger form (11S legumin and 7S vicilin) at pH 7. It was hypothesized that at neutral pH 7S vicilins adsorb quickly at the interface forming an initial layer, followed by subsequent adsorption of 11S legumins. However, the viscoelastic moduli of pea proteins at pH 2.4 is significantly higher compared to neutral conditions. Relatively rigid viscoelastic films at pH 2.4 were possibly caused by a more densely packed interfacial structure, whereas at pH 7 larger protein fractions are more loosely packed. The counterintuitive outcome that dissociated pea proteins are less surface active was assumed to be a result of repulsive interactions between adsorbed and bulk proteins. Chang et al. [64] measured dynamic interfacial tension of pea, soy, and lentil protein isolates at the corn oil-water interface at pH 3 and 7. All protein isolates exhibited lower final interfacial tension at pH 7 than at pH 3. Moreover, viscoelastic moduli under acidic conditions were significantly higher than at neutral pH, in agreement with work from Gharsallaoui et al. [68]. Emulsion studies from Olsmats and Rennie [69] showed that acidic conditions result in net positive zeta potential as well as smaller droplets. On the other hand, neutral pH conditions resulted in larger droplets as well as considerable polydispersity. These findings are in accordance with the dissociation of pea protein fractions under different pH. Under alkaline extraction conditions, protein-phenol complexation that reduces pea protein

surface activity can be induced [21,70–72]. Data for pH 12 from Dong et al. [73] exhibited lower interfacial activity. SDS-PAGE confirmed that pea protein samples at alkaline pH lacked legumin AB subunits and at more polar interfaces the AB subunit band was completely absent. Extraction at pH 11 or above may further induce starch swelling which possibly interferes with the purity of protein isolates [40,74].

Effects of oil polarity, pretreatment, and ionic strength

Proteins generally adsorb faster and undergo more rearrangements at more hydrophobic oils, while they tend to immerse deeper without major rearrangement at polar oils [25,28–30]. Grasberger et al. [67] investigated the behavior of pea proteins at hydrophobic n-octane and polar octanol interfaces. Pea proteins formed less viscoelastic interfaces at polar octanol interfaces compared to hydrophobic n-octane, in line with previous findings for animal proteins [28,30].

Pretreatments can also affect protein adsorption behavior [75]. High pressure homogenized pea protein isolate samples from Grasberger et al. [67] displayed no significant difference in interfacial viscoelastic moduli at different oil polarities. Hence, such pretreated proteins are already partially unfolded and differences in oil polarity no longer affect their degree of unfolding upon adsorption. Proteins that underwent high pressure homogenization had substantially lower interfacial viscoelasticity, which was attributed to a reduction in aggregate size and lack of structural flexibility as well as protein–protein interactions. Sareen et al. [76] compared untreated pea protein isolate with heat-treated and enzymatically hydrolyzed samples. Heat-treated samples did not significantly differ in γ_{ow} reduction at the canola oil-water interface. Conversely, enzymatically hydrolyzed samples were able to reduce γ_{ow} substantially more than heat-treated and control samples. SDS-PAGE showed that enzymatically hydrolyzed pea protein size is reduced to around 15 kDa. Hence, treatments like enzymatic hydrolysis that reduce protein size can enhance their surface activity, while heat treatment that can induce protein aggregation might decrease their surface activity. Sha et al. [77] found that ultrasonicated pea proteins decreased γ_{ow} faster during the initial adsorption phase, however, eventually native pea proteins attained higher interfacial pressure at the sunflower oil-water interface. It was assumed that the adsorption slowed down due to smaller aggregates crowding the interface, supported by reduced penetration rates and rearrangement rates in ultrasonicated samples. Contrary results from Dong et al. [73] showed that ultrasonicated pea protein exhibit higher surface activity than native samples.

Several studies used buffers with different ionic strengths for pea protein adsorption experiments [43,68,73,77–79]. Chang et al. [43] investigated the

effect of ionic strength at pH 7. The pea globulins, legumins and vicilins, were more soluble at 20 mM compared to 100 mM NaCl. Those proteins showed faster initial adsorption and attained higher surface pressures at 100 mM, possibly caused by their lower solubility and electrostatic repulsion which reduced the adsorption energy barrier [80–82]. Kornet et al. [79] found that pea proteins aggregated at 50 mM NaCl (salting-out) but were dispersed with substantially less coacervates above 200 mM NaCl (salting-in). Bogahawatha et al. [78] found that pea protein solubility was decreased at 100 mM NaCl but increased at 200 mM NaCl. The complex non-linear relationship between protein solubility and salt concentration is possibly caused by reversible protein unfolding under ionic conditions that alter protein conformation as well as solubility [43,78,83].

In summary, the compilation of literature data in AST-plots provides a concise overview of the current status of pea protein interfacial adsorption data. Most studies were performed at oils with medium γ_{ow} with limited data for both polar and hydrophobic oils. Fractionated pea globulins are more surface active compared to less purified pea protein isolates. Pea albumins appear to be the most surface active fraction, although limited data on fractionated albumins was found. Furthermore, albumins alone form weak interfacial layers and may fail to form stable emulsions. Pea proteins are generally more surface active at neutral or basic pH due to protein dissociation at low pH. In turn, there are indications that pea protein layers are more viscoelastic at acidic pH and form more stable emulsions.

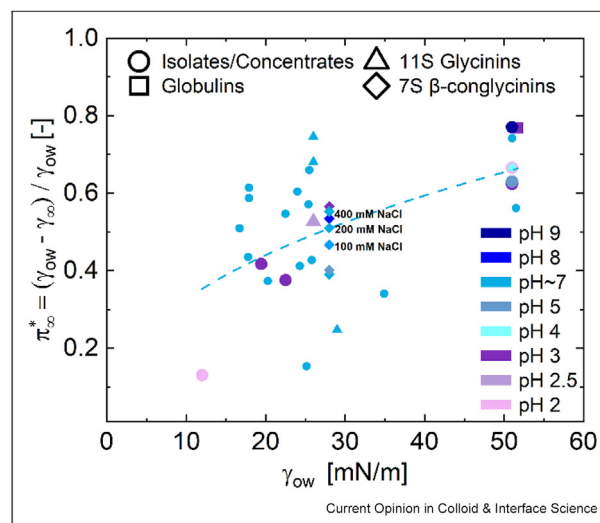
Soybean (*Glycine max*)

Soy protein is one of the most widely cultivated and utilized food crops globally, and numerous studies have investigated the adsorption of soy proteins at o/w interfaces. The AST plot for soy compiled from literature data (Figure 3) shows the power-law correlation of π^* and γ_{ow} . Most studies used common food oils, while high interfacial tension alkanes such as tetradecane and dodecane were also investigated. A wide range of pH was investigated, e.g., by Zhang et al. [92], however, overall data points at different pH are scattered and not sufficient to draw a correlation between pH and soy protein adsorption.

Effect of fractionation

Major soy globulin fractions include 11S glycinin and 7S β -conglycinin [93]. Glycinin are hexamers with several 3S AB subunits that contain one disulfide bond that connects acidic and basic polypeptides [94]. β -conglycinin are trimeric glycoproteins with subunits that are randomly bound by non-covalent interactions [95,96]. Our AST plot in Figure 3 shows that glycinin fractions were only tested at oils with similar γ_{ow} , while β -

Figure 3



AST-plots of soy protein adsorption at o/w interfaces at different pH and ionic strength. Based on literature data compiled in Table 2.

conglycinin data was only found for the MCT oil-water interface ($\gamma_{ow} = 28$ mN/m). Ducel et al. [56] compared soy protein globulins with pea protein globulins, albumins and wheat gliadins at the vaseline oil-water interface. It was found that soy globulins reduce the interfacial tension less than pea globulins and wheat gliadins. Isolated glycinin fractions were investigated by Abirached et al. [97], comparing native glycinin with its acid-treated counterpart at the 95 % sunflower + 5 % maize oil-water interface. Native glycinin reduced the interfacial tension the most, but also had the lowest adsorption rate, surface dilatational moduli, and interfacial protein concentration. These findings were attributed to the rigid conformation and unfolding energy of glycinin [98]. Bos et al. [99] compared the adsorption of β -casein, wheat gliadin and soy glycinin at the air/water and sunflower oil-water interface. Soy glycinin was the least surface active at both interfaces, possibly due to its larger Mw (≈ 360 kDa) and its compact globular structure. β -conglycinin was found to reduce interfacial tension slightly more than glycinin at the soybean oil-water interface within 5 min, however dynamic interfacial tension requires longer measuring time to generate a more definite comparison [100]. Interfacial pressure measurements of purified β -conglycinin showed significantly higher diffusion rates at 0.05 % than at 0.1 %, 0.5 % and 5 % (w/v) [80]. The phenomena could be attributed to less steric hindrance and electrostatic repulsions, enabling rapid initial adsorption at the interface. Studies on emulsions have demonstrated that 7S β -conglycinin presented better emulsifying capacities than 11S glycinin across pH 2–10

Table 2

Compilations of studies measuring soy protein adsorption at o/w interfaces. SP: soy protein, SPI: soy protein isolate, SPC: soy protein concentrate.

Oil Phase	Protein Description	pH	Remarks	Ref.
Vaseline oil	1 % SP globulin fractions	3		[56]
Canola oil	0.1 % SPI	7		[118]
Canola oil	2 % SPI	3,7		[64]
Sunflower oil	1 % SPI nanoparticles	7	SPI nanoparticles prepared by dissolving soy protein isolate, followed by stirring for 3h and 80 °C incubation	[115]
Sunflower oil	0.1 % SPI solution	3,7		[105]
95 % sunflower oil+ 5 % maize oil	0.1 % SP glycinin fraction (isoelectric precipitation)	7	Soy glycinin fraction purified from soy flour	[97]
95 % sunflower oil+ 5 % maize oil	0.1 % SP glycinin fraction (acid treated and lyophilized after isoelectric precipitation)	2.5, 7		[97]
Corn oil	1 % SPI	7		[119]
Soybean oil	2 % SP nanoparticles (prepared from 92.07 % SPI)	7	Nanoparticles fabricated by adding 300 mM NaCl to SPI solution, followed by 95 °C incubation for 20 min, then cooled immediately to 25 °C [114]	[116]
Soybean oil	4 % native SPI	2		[120]
Sunflower oil	1 % SPI	7	98 % water soluble denatured soy protein isolate, γ measured at 37 °C	[121]
Soy oil	4 % SPI	7		[122]
Soybean oil	0.3 % SPI, heat denatured SPI	7	Heat treatment: 95 °C for 30min	[117]
Maize oil	0.1 % SPI	7.2		[123]
Canola oil	0.25 % SPI	7		[86]
Sunflower oil	0.01 % SP glycinin fraction	6.7		[99]
n-tetradecane	0.01 % SPI	7.4	γ_{ow} value used for π^* calculation from [28]	[124]
MCT oil (Miglyol 812)	0.01 % SPC	7	γ_{ow} value used for π^* calculation from [88]	[91]
Dodecane	0.1 % SPI	2, 3, 4, 5, 7, 9	γ_{ow} value used for π^* calculation from [28]	[92]
MCT oil	1 % SP β -conglycinin fraction	3,5, 7,8	γ_{ow} value used for π^* calculation from [125]	[80]
MCT oil	1 % SP β -conglycinin fraction 100 mM, 200 mM, 400 mM	7	γ_{ow} value used for π^* calculation from [125]	[80]

[101,102]. This can potentially be attributed to their smaller size and non-covalent linkage that accelerates diffusion to the interface [80,103]. However, β -conglycinin is susceptible to conformational changes that are strongly dependent on ionic strength, pH and protein concentration, resulting in inconsistent adsorption behavior [80,103,104].

Effect of pH

Chang et al. [64] evaluated soy protein isolates at pH 3 and 7 at the canola oil-water interface and found that interfacial tension decreased more at neutral pH. Yang et al. [105] reported similar results, attributing the phenomenon to the more open and loose structure of soy proteins. Zhang et al. [92] investigated soy protein at the dodecane–water interface at pH 2–5, 7 and 9. Surface pressure at pH 7 and 9 was higher than at pH 2–5. Additionally, soy dispersions at pH 9 showed the

highest rearrangement constant and diffusion rate. Abirached et al. [97] examined glycinin dispersions at pH 2.5 and 7, utilizing glycinin that had been precipitated via isoelectric point precipitation followed by acid treatment and lyophilization. The equilibrium interfacial tension at the 95 % sunflower +5 % maize oil-water interface at pH 7 was lower than at pH 2.5. Conversely, initial adsorption rates and dilatational viscoelastic moduli at pH 2.5 were higher than at pH 7.

The isoelectric points for 11S and 7S globulins are 6.4 and 4.8, respectively [106]. Glycinin is known to dissociate from hexameric to trimeric form at pH 3.8 [107]. In AST-plots in Fig. 3, glycinin (triangular symbols) shows slightly higher attained π_{∞}^* . β -conglycinin (rhombic symbols) exhibits higher attained π_{∞}^* at pH 3 than at pH 5, 7, and 8. However, data points are around the same γ_{ow} and are not sufficient to draw a definite

conclusion. An emulsion study by Yamauchi et al. [101] showed pH-turbidity curves of 7S emulsions sharply decreased only at the isoelectric point (pH 4.8), whereas 11S emulsions demonstrated a concave curve around pH 6. Jiang et al. [108,109] studied soy protein isolates and fractions by extreme acidic and alkaline pH-shifting, followed by refolding the proteins at pH 7. SDS-PAGE and circular dichroism analyses showed that at pH 1.5 and pH 12, 11S glycinin AB subunits dissociated, while 7S β -conglycinin retained its structure. This indicates that 11S glycinin could not refold after extreme pH exposure, indicating its structural breakdown, while 7S β -conglycinin demonstrated greater structural integrity by successfully refolding. The authors suggested that extreme pH treatments induce glycinin to adopt an intermediate, partially unfolded ‘molten globule’ state, which may enhance its emulsifying properties. Emulsion activity and stability indices showed a notable increase in pH-shifted samples, with acid-treated samples exhibiting the most improvement in emulsifying ability.

Effect of ionic strength

Soy protein has been proven to be sensitive towards changes in salt concentration [93,98,110]. Foams made from soy protein isolate (SPI) show improved stability up to an ionic strength of 0.1 M NaCl [110]. Glycinin dissociates from 11S to the intermediate 7S and 3S at ionic strengths lower than 0.01M. Subsequent ionic strength increase reverts 7S to 11S, however, the 3S does not revert to the 11S structure [93]. β -conglycinin is known to dimerize when ionic strength is changed from 0.5 to 1.0 M, showing a conversion from 7S to 9S at pH 7.6 [93,111,112]. In the AST-plot (Fig. 3), data points from Tian et al. [80] of β -conglycinin demonstrate that increasing ionic concentration from 100 to 400 mM can result in higher attained π_{∞}^* . However, the viscoelastic moduli and net zeta potential decreased when ionic strength increased, possibly due to electrostatic screening [113]. Alternatively, the salting-out effect that contributes to protein aggregation might also result in a decrease in viscoelastic moduli [83].

Effect of heat treatment

Soy protein was shown to aggregate into soy protein particles when heat-treated that can be utilized as Pickering-stabilizers [114]. These particles are 100–200 nm and result from thermal aggregation of glycinin. Our AST-plots include heat-treated SPI [115,116], as well as heat-denatured SPI [117]. Data points of 1 % SPI from Wen et al. [115] and 0.3 % heat-treated SPI from Cui et al. [117] demonstrated higher attained π_{∞}^* (0.57 and 0.6, respectively) than native SPI and its fractions. Wen et al. [115] reported increased initial adsorption rates and γ_{ow} reduction with increasing SPI nanoparticle concentrations from 0.2 % to 3.0 % (w/v). However, rearrangement rates reduced with sample concentration above 0.6 %, which was attributed to a crowded interface. Cui et al. [117] compared native SPI and heat-denatured SPI. Heat-denatured samples that underwent 30 min of 95 °C heat treatment displayed higher γ_{ow} reduction. Our AST-plots also show that heat-denatured SPI attain a higher π_{∞}^* than native SPI. However, 2 % SPI nanoparticles from Yan et al. [116] showed lower attained π_{∞}^* of 0.34.

Chickpea (*Cicer arietinum*)

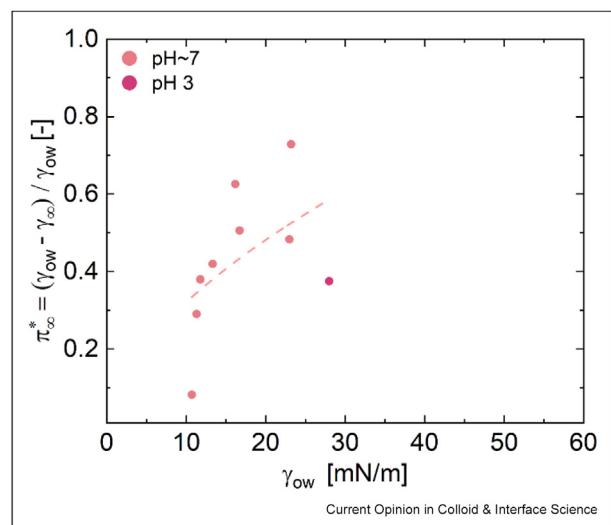
Chickpea is one of the most widely grown legumes in the Mediterranean region and is gaining popularity globally. Chickpea protein isolate has an isoelectric point of 4.3 and contains 60 % globulins and 15 % albumins [126]. Globulins include 11S legumin and 7S vicilin. Chickpea albumins comprise chickpea albumin PA1 and PA2 [127]. Chickpea PA2 has a high degree of homology to the amino acid sequence of pea albumin PA2. Chickpea albumins exhibit limited digestibility due to its high cysteine content and presence of trypsin inhibitors [128]. Literature data for chickpea protein adsorption at o/w interfaces is compiled in Table 3 and visualized in an AST-plot in Figure 4. Data points are mainly scattered at low γ_{ow} at common food oil-water interfaces. Overall, Shen et al. [129] reported the highest π_{∞}^* value (0.73) of chickpea protein extracts at the stripped rapeseed oil-water interface.

Table 3

Compilations of studies measuring chickpea protein adsorption at o/w interfaces. CPI: chickpea protein isolate. CP: chickpea protein.

Oil Phase	Protein Description	pH	Remarks	Ref.
Canola oil	0.1 % CPI solution	7		[118]
Sunflower oil	0.01 % CPI solution	3		[85]
Canola oil	0.25 % CPI solution	7.8		[137]
Sunflower oil	1 % CPI solution	7		[87]
Corn oil	0.01 % CPI (isoelectric precipitation), 0.01 % CPI (ultrafiltration)	6.5		[130]
Corn oil	0.01 % CPI albumin fraction	6.5	Chickpea protein isolate obtained by ultrafiltration	[130]
Corn oil	0.01 % CPI globulin fraction	6.5		[130]
Rapeseed oil	0.1 % CP extract	7	Protein content from extracts: 78.90 %, 20 mM phosphate buffer, γ_{ow} used for π^* calculation from [84]	[129]

Figure 4



AST-plots of chickpea protein adsorption at o/w interfaces at different pH. Based on literature data compiled in Table 3.

Effect of fractionation

Papalamprou et al. [130] compared the surface activity of globulin and albumin fractions of ultrafiltrated chickpea protein isolate. The π_{∞}^* value of globulins (0.38) was significantly higher than albumins (0.08) despite albumins showing rapid initial adsorption. The higher surface hydrophobicity of globulins compared to albumins is the main hypothesized reason. Although globulins adsorb slowly to the interface because of the larger size of ≈ 300 kDa, they have more hydrophobic patches to expose to the oil subphase resulting in a higher reduction of interfacial tension. Conversely, the elastic moduli of albumin were higher than globulin at the o/w interface during initial adsorption. This phenomenon is possibly linked to higher cysteine content and intramolecular disulfide bonds in albumins than in globulins [131,132]. Protein extraction conditions can influence the yield and globulin/albumin ratio of chickpea proteins [127,130,133], which will be discussed below. Peng et al. [134] investigated the adsorption kinetics of pulse protein fractions at the a/w interface. Authors demonstrated that chickpea albumins adsorb more rapidly than globulins. This behavior was attributed to the higher surface hydrophobicity of chickpea albumins compared to chickpea globulins, lentil albumins, and faba bean albumin fractions.

Effect of pH

Most studies were conducted at neutral pH, except for one study from Ladjal Ettoumi et al. [85] with pH 3. Therefore, conclusions on pH effects cannot be

thoroughly discussed. Chickpea protein showed the fastest initial adsorption and lowest final interfacial tension, followed by whey, pea and lentil proteins. Literature also suggests that alkaline treatment can enhance the emulsifying properties of chickpea protein [135,136]. However, more interfacial tension measurements at varying pH are needed to draw more concrete conclusions.

Effect of protein isolation method

Papalamprou et al. [130,132] compared chickpea proteins that were isolated via isoelectric point precipitation and ultrafiltration. Isoelectric point precipitated samples exhibited higher π_{∞}^* (0.42) compared to ultrafiltrated samples (0.29). This discrepancy was ascribed to the ultrafiltrated samples containing albumins whereas the isoelectric precipitated samples did not. Centrifugation and supernatant removal during isoelectric precipitation yield a globulin-rich protein isolate [21]. Compared to albumins, globulins are more hydrophobic and surface active (see above). Liu et al. [127] extracted chickpea protein with K_2SO_4 , NaCl and deionized water finding differences in albumin concentrations. NaCl extraction yielded ≈ 60 % albumin (out of total protein), K_2SO_4 extraction yielded globulin-rich isolates with the highest protein recovery rate. In conclusion, protein extraction procedures cause variations in chickpea protein fractions and their ratios, influencing the adsorption behavior of chickpea isolates.

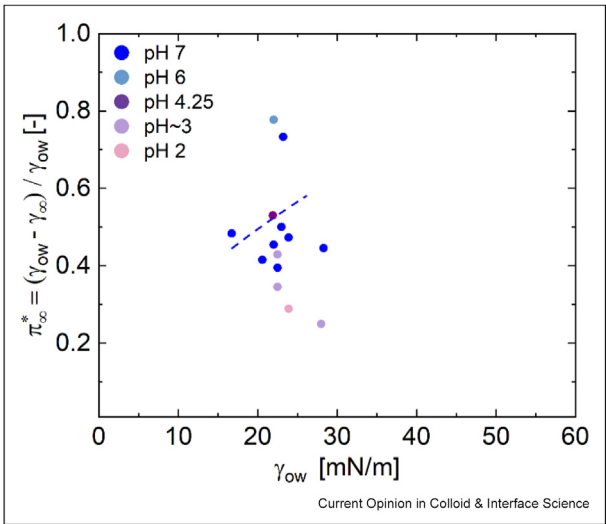
Lentil (*Lens culinaris*)

Lentil has grown to be a popular staple food around the world. Nutritional benefits include high fiber, high protein and low fat [138,139]. Lentil proteins show minimal solubility at pH 4–6, with precipitation typically at pH 4.5 [138]. They comprise ≈ 70 % globulins (11S legumins and 7S vicilins), and ≈ 16 % albumins (lentil albumin 1 and 2). The isoelectric points of globulins, legumins, and vicilins are 4.6, 5.2, and 4.2, respectively [139]. Our AST-plot in Fig. 5 shows o/w interfacial tension measurements in literature from Table 4. The majority of studies utilized oils that are around the same γ_{ow} with scattered data points.

Effect of fractionation

Currently there are no studies that have measured o/w interfacial tension with fractionated lentil proteins. Chang et al. [139] investigated individual lentil fractions and compared their emulsifying and foaming capacity, stability indices, as well as a/w surface tension at pH 3, 7 and 9. Albumins were the most efficient in reducing a/w surface tension and had a higher foaming capacity. However, albumins had the lowest emulsifying capacity and stability. This phenomenon was attributed to their high solubility, low molecular weight, and flexible conformation, which facilitate rapid adsorption at

Figure 5



AST-plots of lentil protein adsorption at o/w interfaces at different pH. Based on literature data compiled in Table 4.

the a/w interface. Nevertheless, these traits, combined with low net charge reduce their capacity to effectively stabilize oil droplets. Vicilins showed the highest emulsifying capacity within the fractions, possibly due to its higher surface hydrophobicity and less rigid structure compared to legumins [45]. Surface pressure measurements from Shen et al. [134] also confirmed that lentil albumins exhibit higher surface pressure than lentil globulins at the a/w interface. Additionally, lentil globulins adsorbed more rapidly at the interface than chickpea and faba bean globulins. In conclusion, lentil protein fractions potentially behave very differently at the a/w compared to o/w interfaces. However, limited data is currently available on lentil protein adsorption at o/w interfaces, particularly for individual fractions.

Effect of pH

Lentil protein adsorption measured at varying pH was colorized individually in the compiled AST-plots (Figure 5). Attained π_{∞}^* values at pH 6–7 are higher than acidic pH and around the isoelectric point (4.5). Studies comparing lentil protein isolates at acidic (2, 3, 3.5) and neutral pH (6, 7) conditions found a lower γ_{ow} reduction at acidic pH [64,140,141]. Interfacial rheological tests of Gadkari et al. [141] show that the linear viscoelastic regime was shorter at pH 2 than at pH 7, indicating a more brittle interfacial layer at pH 2. Measurement at pH 4.25 from Wang et al. [142] demonstrated higher attained π_{∞}^* than at acidic pH. This result might be counterintuitive as 4.25 is close to the isoelectric point, although the limited solubility close to the isoelectric point might favor adsorption. At neutral pH, lentil proteins generally attain higher π_{∞}^* . A study on emulsification and foaming using lentil proteins confirmed better performance at neutral pH [139]. This is attributed to higher net charge under neutral and slightly alkaline conditions, resulting in electrostatic repulsion and stabilization at the interface [64,152].

Faba bean (*Vicia faba*)

Faba bean, also known as fava bean, is an emerging source of alternative protein. They are cold season annual crops that are globally grown as food and feed. Like other pulses, faba bean possesses high amounts of essential amino acids [145]. Faba beans contain 69.5–78.1 % globulins (including 11S legumin and 7S vicilin), and about 20 % albumins [146]. The isoelectric point of faba bean protein isolate varies between 4.5 and 4.9 across cultivars, with precipitation typically performed at pH 4.5 [86].

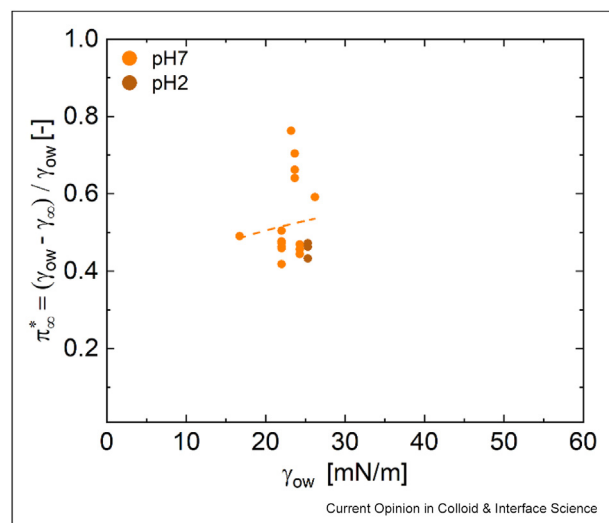
In our faba bean protein AST-plot (Figure 6) all current data are at the canola/rapeseed oil-water interface. Data points are around the same γ_{ow} , with π_{∞}^* ranging from 0.42 to 0.76. Shi and Nickerson [86] compared interfacial

Table 4

Compilations of studies measuring lentil protein adsorption at o/w interfaces. LPI: lentil protein isolate, LP: lentil protein.

Oil Phase	Protein Description	pH	Remarks	Ref.
Canola oil	0.25 % LPI solution	7.9		[137]
Sunflower oil	0.01 % LPI solution	3		[85]
Canola oil	2 % LPI solution	3,7		[64]
Canola oil	0.1 % LPI solution	7		[118]
Canola oil	0.5 % LPI solution	2,7		[141]
Olive oil	1 % LPI solution	7		[143]
MCT oil (MCT 60X)	0.01 % LPI solution	7		[125]
Canola oil	1 % LPI solution	3		[144]
Canola oil	1 % LPI solution	3.5, 6		[140]
Canola oil	1 % LPI solution	4.25		[142]
Rapeseed oil	0.1 % LP extract	7	Protein content from extracts: 85.20 %, 20 mM phosphate buffer, γ_{ow} value used for π^* calculation from [84]	[129]

Figure 6



AST-plots of faba bean protein adsorption at o/w interfaces at different pH. Based on literature data compiled in Table 5.

activity of pea, soy and faba bean protein isolates. Initial o/w interfacial tension reduction of faba bean and pea protein was faster than for soy protein. Similarly, Sareen et al. [76] reported comparable final interfacial tension values for pea and faba bean proteins at neutral pH. In contrast, Keivaninahr et al. [90] observed similar γ_{ow} at pH 2 but noted higher interfacial tension reduction by faba bean proteins than pea proteins at pH 7.

Effect of fractionation

Although studies on o/w interfacial adsorption of fractionated faba bean protein are lacking, Shen et al. [134] examined the behavior of their globulins and albumins at the a/w interface. Faba bean globulins exhibited higher surface pressure after 3h adsorption compared to lentil and chickpea globulins. Nonetheless, faba bean globulin and albumin fractions exhibit substantially lower dilatational viscoelastic moduli compared to those of lentil and chickpea. Shi and Nickerson [86] found a correlation between legumin/vicilin ratio and final interfacial tension. Higher ratios result in higher final interfacial tension, which is consistent with other pulse legumin and vicilin properties mentioned in this paper.

Effect of pH

Keivaninahr et al. [90] studied emulsifying properties of faba beans and pea proteins at the canola oil-water interface at pH 2 and 7. In the AST-plot, low pH results in lower attained π_{∞}^* , consistent with the higher net zeta potential measured at pH 2 compared to pH 7. This was attributed to the higher mineral (ash) content in faba bean protein isolates compared to pea protein isolates as acidic environments increase mineral solubility and charge screening.

Effects of cultivar and enzymatic hydrolysis

Variations in growing conditions, cultivar and genotype can affect fraction ratios and interfacial behavior of faba bean proteins [86,146,147]. Interfacial tension values did not differ substantially between faba bean protein isolate and concentrate [90]. Pretreatments such as heat treatment and enzymatic hydrolysis resulted in a decrease in final interfacial tension, and an increase in

Table 5

Compilations of studies measuring faba bean protein adsorption at o/w interfaces. FBPI: faba bean protein isolate, FBPC: faba bean protein concentrate, FB: faba bean.

Oil Phase	Protein Description	pH	Remarks	Ref.
Canola oil	0.1 % FBPI solution	7		[118]
Canola oil	0.25 % untreated, heat-treated, 5 % degrees of hydrolysis, 10 % degrees of hydrolysis, 15 % degrees of hydrolysis, 20 % degrees of hydrolysis FBPI solution	7	Heat treatment to 85 °C, enzymatic hydrolysis with trypsin	[76]
Canola oil	0.25 % FBPI solutions	7	Data average from 7 genotypes grown in year 2011 and 2012	[147]
Canola oil	0.25 % FBPI solution	7		[86]
Canola oil	Fabelle, Malik, Snowbird cultivar			
Canola oil	2 % FBPC	2,7		[90]
Canola oil	2 % de-flavored FBPC	2,7		[90]
Canola oil	2 % FBPI solution	2,7		[90]
Rapeseed oil	0.1 % FB extract	7	Protein content from extracts: 66.50 %, 20 mM phosphate buffer, γ_{ow} value used for π^* calculation from [84]	[129]

surface net charge and hydrophobicity [76]. Whether enzymatic hydrolysis and heat aggregation alters surface activity of faba bean proteins is not fully clear at this point. There is a general lack of data on faba bean protein interfacial behavior. Effects of polarity of oil sub-phases, pH and fractions on faba bean protein surface activity remain to be investigated.

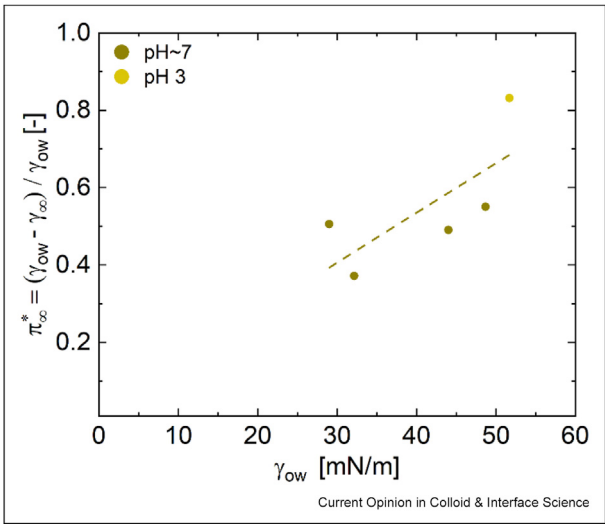
Wheat (*Triticum aestivum*)

Wheat is the most grown cereal worldwide [148]. The viscoelasticity of gluten proteins are commonly exploited in bread making and the baking industry. Wheat proteins consists of $\approx 80\%$ gluten and $\approx 20\%$ non-gluten proteins. Gluten proteins include α -gliadin and glutenin [149]. The isoelectric point of native gluten is 6.5 and 4.5 when deaminated, respectively [150]. α -gliadin is a heterogeneous group of monomeric gluten proteins (α -, γ -, and ω -types). In contrast, glutenins are polymeric and are constituted of low Mw and high Mw glutenin subunits [149]. Gliadins form intermolecular disulfide linked particles, whilst glutenin form intra-molecular disulfide linked networks. Therefore, α -gliadin is related to dough viscosity and glutenin is associated with dough elasticity [151]. Non-gluten proteins include triticin (globulin-like) and enzymes such as β -amylase. Triticin is closely related to 11S legumins found in pulses but constitutes only about 5% of wheat's storage proteins [149]. Interfacial tension measurements of wheat proteins with various oils with a wide range of γ_{ow} are presented in Table 6. AST-plot data (Figure 7) shows the power-law of π_{∞}^* increasing with γ_{ow} , although π_{∞}^* is lower compared to the other plant proteins discussed.

Effect of fractionation

π_{∞}^* values for wheat gliadins show a wide discrepancy from 0.37 to 0.83 [56,99]. Ducelet et al. [56] reported that at the vaseline-oil water interface wheat gliadin reduced γ_{ow} most efficiently compared to pea globulin, albumin and soy globulin. Subsequent rheological tests in the same study showed that gliadin forms the most viscoelastic layer. Bos et al. [99] measured soy glycinin, beta casein and gliadin at the sunflower oil-water interface. Wheat gliadin exhibited the highest surface pressure

Figure 7



AST-plots of wheat protein adsorption at o/w interfaces at different pH. Based on literature data compiled in Table 6.

after 7000 s, followed by β -casein and soy glycinin, consistent with measurements at the a/w interface.

The whole gluten dispersion measurement from van der Schaaf et al. [152] had a high π_{∞}^* value of 0.81. SDS-PAGE results of gluten fraction adsorbed at the interface show significant bands of α -, γ -gliadins and low molecular weight glutenin subunits. Native wheat solutions exhibited a π_{∞}^* value of 0.55 at the dodecane–water interface, showing comparable interfacial tension reduction with β -lactoglobulin and bovine serum albumin [153].

Li et al. [154] examined wheat germ proteins at the soybean oil-water interface with a π_{∞}^* value of 0.37. The wheat germ is the embryo of wheat grains, they consist of mainly non-gluten proteins, i.e., 30% albumins and 15% globulins. Wheat germ proteins are byproducts of flour production and are of high nutritional value due to abundant essential amino acids, high

Table 6

Compilations of studies measuring wheat protein adsorption at o/w interfaces.

Oil Phase	Protein Description	pH	Remarks	Ref.
Vaseline oil	1 % wheat alpha gliadin solution	3		[56]
Sunflower oil	0.01 % wheat gliadin fraction	6.7		[99]
Dodecane	0.01 % wheat protein solution	–	Fraction no. 4	[153]
Soybean oil	1 % wheat germ protein solution	7		[154]
D-limonene	1 % gluten dispersion	–		[152]

fiber and mineral content. They are typically low Mw and are less suitable for emulsification [155], which could be a probable reason why their π_{∞}^* value is lower compared to gluten proteins. In conclusion, various wheat compositions or fractions can result in very different interfacial adsorption. More systematic o/w interfacial measurements with isolated wheat protein fractions, gluten and non-gluten proteins, wheat proteins and wheat germ proteins are required to make more direct comparisons and allow more insight into how wheat proteins behave at o/w interfaces.

Other effects

Measurements at different pH values of wheat protein are lacking. van der Schaaf et al. [152] found that while gluten protein solubility varies with pH, its emulsifying properties are largely unaffected by pH and depend more on gluten concentration. For example, 0.5 % (w/v) gluten at pH 3 and 1.0 % (w/v) gluten at pH 6 showed

similar and stable emulsification. Additionally, studies have also suggested that deaminated and enzymatically hydrolyzed wheat gluten have increased emulsifying capacity [150,156].

Microalgae

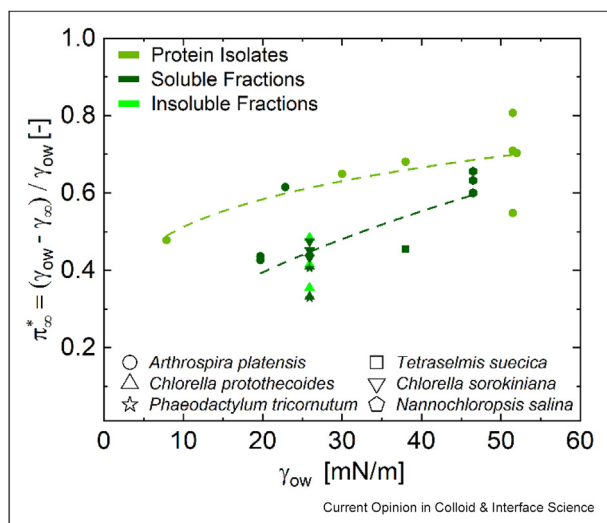
Microalgae are protein-rich unicellular microorganisms and hold great potential as an alternative protein source, mostly due to their ability to be cultivated on non-arable land. Great diversity exists in strains, cultivation conditions, purification and fractionation, and composition [157]. Studies that utilize microalgae at o/w interfaces are listed in Table 7 and compiled in AST-plots in Figure 8. Note that there might be considerable variations in composition and protein content and structure between microalgae strains. Nevertheless, we plotted all current microalgae interfacial adsorption data in one AST-plot to provide an overview of the current research landscape. Presently, there are no established methods

Table 7

Compilations of studies measuring microalgae protein adsorption at o/w interfaces.

Oil Phase	Protein Description	pH	Remarks	Ref.
Sunflower oil	1 % <i>Arthrospira platensis</i> supernatant fraction	6,8	γ_{ow} measured with chlorhexidine gluconate (0.07 g/L) phosphate buffer	[163]
Soybean oil	0.1 % <i>Arthrospira platensis</i> protein	7		[164]
n-tetradecane	0.01 %, 0.001 %, 0.0001 % <i>Arthrospira platensis</i> protein isolate solution	7	20 mM phosphate buffer	[160]
n-Octane	0.001 % <i>Arthrospira platensis</i> protein isolate solution	7	20 mM phosphate buffer	[160]
1-Chlorooctane	0.001 % <i>Arthrospira platensis</i> protein isolate solution	7	20 mM phosphate buffer	[160]
MCT oil (Myritol 318)	0.001 % <i>Arthrospira platensis</i> protein isolate solution	7	20 mM phosphate buffer	[160]
1-Octanol	0.001 % <i>Arthrospira platensis</i> protein isolate solution	7	20 mM phosphate buffer	[160]
Hexadecane	0.1 % <i>Tetraselmis suecica</i> soluble fraction	7	Crude soluble extract after centrifugation	[161]
MCT oil (Miglyol 812)	0.04 % <i>Chlorella protothecoides</i> water soluble extract	3,5,7	dry water-soluble extract obtained by vacuum filtering supernatant after centrifugation then lyophilized	[165]
MCT oil (Miglyol 812)	0.1 % <i>Chlorella protothecoides</i> insoluble microalgae protein fraction IMPF	6.7	IMPF production: Biomass protein powder dispersed, followed by homogenization, centrifugation, pellet purification and drying at low humidity. γ_{ow} used for π^* calculation from [165]	[166]
MCT oil (Miglyol 812)	0.1 % <i>Chlorella protothecoides</i> insoluble microalgae protein fraction hydrolyzed at 65 °C, 85 °C	6.7	Hydrolyzed IMPF with 0.5M HCl for 4h at 65 °C or 85 °C. γ_{ow} value used for π^* calculation from [165]	[166]
MCT oil (Miglyol 812)	0.04 % <i>Chlorella sorokiniana</i> , <i>Phaeodactylum tricornutum</i> soluble lyophilized protein extract	3,5,7	γ_{ow} used for π^* calculation from [165]	[167]
n-Hexane	5 % <i>Nannochloropsis salina</i> serum (supernatant after centrifugation),	—	Consists of soluble intracellular components particularly protein. γ measured at n-hexane/sea water interface.	[159]
n-Hexane	5 % <i>Nannochloropsis salina</i> cell debris (pellet washed with artificial sea water)	—	Consists of ruptured/intact cells + some lipids. γ measured at n-hexane/sea water interface.	[159]
n-Hexane	5 % <i>Nannochloropsis salina</i> delipidated debris (pellet after hexane extraction and centrifugation)	—	Consists of ruptured/intact cells and nonpolar components collected by hexane extraction and sea water washes. γ measured at n-hexane/sea water interface.	[159]

Figure 8



AST-plots of microalgae protein adsorption at o/w interfaces showing different fractions and microalgae strains. Based on literature data compiled in Table 7.

for microalgae protein fractionation and therefore adsorption data for different fractions is not available. Instead, we focus our discussion on the level of protein purification.

Effect of protein purification

Böcker et al. [158] systematically investigated the effect of *A. platensis* (*Spirulina*) purification, i.e., crude extract, soluble extract, isolate, and diafiltrated isolate, on adsorption behavior and emulsification efficiency. The *A. platensis* isolate adsorbed faster and formed more viscoelastic interfacial layers compared to the soluble extract, while further diafiltration had no further effect. Our AST-plot (Fig. 8) clearly supports this trend that microalgae protein isolates are more effectively reducing γ_{ow} . Surprisingly, diluting the soluble isolate increased its surface activity and interfacial viscoelasticity, suggesting that other surface active species are still present in the soluble extract that compete with protein adsorption. Similarly, Law et al. [159] studied various purification degrees of *N. salina* at the hexane-sea water interface. Even when the protein and lipids were all removed, the delipidated debris could still lower the interfacial tension significantly, confirming that the cell wall fragments also contain surface active components that compete with protein adsorption. The authors further confirmed that an existing interfacial protein layer breaks upon addition of the extracted lipids, underlining the importance of sufficient protein purification. The isolated *A. platensis* proteins were reported to reduce γ_{ow} more than most established animal proteins [160]. Suarez Garcia et al. [161] compared soluble fractions of *T. suecica* with whey protein isolate at hexane/water and air/water interfaces,

finding *T. suecica* to exhibit higher surface activity in both cases. This phenomenon was attributed to the presence of glycoproteins and charged carbohydrates [162].

Effect of pH, ionic strength, oil polarity, and pretreatments

Shimada et al. [163] investigated *A. platensis* (*Spirulina*) supernatant fractions of dried biomass dissolved in water at pH 6 and 8. The two pH levels exhibited similar π_{∞}^* values of approximately 0.43, with minimal differences likely due to the short 900 s measurement duration. Bertsch et al. [160] studied the interfacial pressure and interfacial elasticity of *A. platensis* protein isolate with pH, ionic strength and oil polarity variation. The isolate adsorbed similarly at pH 7 and 5 but showed a reduced adsorption at the isoelectric point (pH = 3.5). A steady increase in ionic strength from 20 to 300 mM resulted in a continuously increased π_{∞}^* . Limited adsorption at the characteristically low isoelectric point and resistance to high ionic strength has been observed for several microalgae strains [14]. Oil polarity gradients were conducted at octane ($\pi_{\infty}^* = 0.70$), 1-chlorooctane ($\pi_{\infty}^* = 0.68$), MCT oil ($\pi_{\infty}^* = 0.65$), and 1-octanol ($\pi_{\infty}^* = 0.48$) interfaces, i.e., decreased protein adsorption at more polar oils. A viscoelastic interfacial layer was formed at all oils except the most polar 1-octanol interface. Guo et al. [164] measured adsorption of protein Pickering nanoparticles obtained from *A. platensis* protein isolate by high pressure homogenization at the soybean oil interface. After 3000 s of dynamic interfacial tension measurement, the samples showed a relatively high π_{∞}^* value of 0.62. Weiss and colleagues measured adsorption of *C. protothecoides* [165,166], *C. sorokiniana* [167], and *P. tricornutum* [167] at the MCT oil-water interface. *C. protothecoides* was studied for both its insoluble fractions with hydrolysis [166] and its soluble fractions at pH 3, 5, and 7 [165]. Within the insoluble fractions, proteins hydrolyzed at 85 °C demonstrated the highest attained π_{∞}^* value of 0.48 compared to samples that were hydrolyzed at 65 °C ($\pi_{\infty}^* = 0.42$) or unhydrolyzed ($\pi_{\infty}^* = 0.33$). Soluble fractions obtained the highest attained π_{∞}^* value of 0.44 at pH 5 in comparison with pH 3 ($\pi_{\infty}^* = 0.41$) and pH 7 ($\pi_{\infty}^* = 0.35$). At pH 7 without hydrolysis, insoluble and soluble fractions show no significant differences in surface activity at the MCT oil-water interface. However, acid hydrolyzed insoluble fractions and soluble fractions under acidic conditions result in increased π_{∞}^* values. Ebert et al. [167] measured soluble fractions of *C. sorokiniana* and *P. tricornutum* at pH 3, 5 and 7. *C. sorokiniana* showed no substantial difference in final interfacial tension at different pH. On the other hand, *P. tricornutum* reduced γ_{ow} more at pH 3 and 5 compared to pH 7. The authors proposed that soluble microalgae proteins are covalently bound to carbohydrates as glycoproteins. These glycoproteins are less sensitive to pH variations due to their consistent charge density [168].

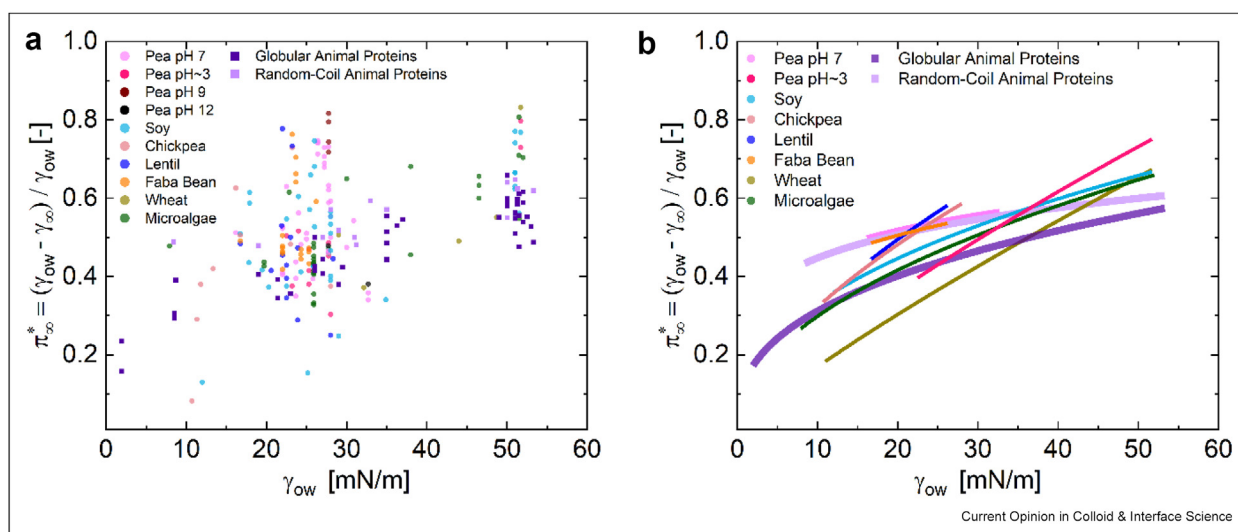
Thoroughly understanding the adsorption of microalgae proteins is important to assess how these proteins can be utilized as alternative emulsifiers. However, the exact composition and structure of microalgae proteins remains mostly unknown. Limited interfacial adsorption data is currently available, while fractionation protocols and respective adsorption studies are lacking completely.

Comparison within protein sources

Ultimately, AST facilitates the combined plotting and comparison of protein adsorption data from different plant sources and established animal proteins (Figure 9). It is apparent that interfacial pressure data from all protein sources follow the established power law behavior as a function of oil polarity, $\Pi_{\infty} \propto \gamma_{ow}^a$. This confirms that there is a universal correlation of protein adsorption behavior and oil polarity, which we previously postulated for proteins and other surface active species [30]. From the currently available data, it appears that proteins from pea, soy, chickpea, lentil, and faba bean can induce an γ_{ow} reduction comparable to animal-based proteins and mostly exceed the surface activity of common globular animal proteins. On the other hand, proteins from wheat are generally less surface active than other plant and animal proteins. It is important to note that fits for some plant proteins are currently based on limited number of data points or range of oil polarity, limiting the conclusiveness of fits and comparison within protein sources. However, we found that AST can readily pinpoint particularly promising processing conditions for specific protein sources, particularly in terms of pH, where optimum conditions were found to vary considerably for different protein sources.

The power-law fits in Figure 9b are shown for all adsorption data including isolates/concentrates and individual fractions. Hence, it is not surprising that plant protein fits exceed the surface activity of globular animal proteins whose interfacial adsorption is limited by their structural stability and rearrangements upon adsorption [29]. A general limitation of AST is that final interfacial pressure is only one aspect of interfacial stabilization alongside adsorption kinetics, interfacial viscoelasticity, as well as bulk properties of final emulsions or foams [169]. AST is based on equilibrated interfacial tension values, and does not reflect on the adsorption kinetics of proteins that might be of importance at the much shorter timescales of emulsification or foaming processes. Sagis and Yang [170] have recently addressed the interfacial viscoelasticity of plant compared to animal proteins and found that animal-based globular proteins form more viscoelastic interfacial layers compared to plant proteins. In the course of screening plant protein literature, we also observed that several plant proteins were reported to be more surface active than established animal proteins but often formed less viscoelastic interfacial layers. This is particularly pronounced for fractionated plant albumins, which often were most surface active but reported to form weaker interfacial layers and emulsions. Hence, although our mapping analysis of plant protein interfacial adsorption indicates that more purified and fractionated proteins might be more surface active, they might ultimately not be the most suitable for interfacial stabilization. This is particularly important to keep in mind as increased processing efforts for plant proteins negatively affect their price, and plant proteins are still struggling to

Figure 9



Overall AST graph of all plant proteins compared to animal protein data from Bergfreund *et al.* [28] a) Data points scattering plot. b) Power law fits of all proteins.

compete with animal-based proteins from an economic perspective to date [171–173].

Conclusions

The food industry is undergoing a transition aiming to replace animal-based protein with novel plant-based alternatives. Besides nutritional aspects, it is also a challenge to replace animal proteins as functional ingredients, such as in the interfacial stabilization of emulsions and foams. A plethora of alternative protein sources, fractionation techniques, and processing conditions are currently investigated, and it is often difficult to directly compare experimental findings between different studies or plant protein sources. Here, we demonstrated that alternate subphase tensiometry (AST) allows the mapping of interfacial tension data from 57 studies investigating the adsorption of various plant proteins at o/w interfaces. This compilation of data from different studies provides a rapid overview of the current data landscape and identification of particularly promising protein sources, fractions, and processing conditions.

The most data are currently available for pea protein isolates and its fractions. The compilation of available adsorption data reveals that fractionated pea globulins and albumins are more surface active than whole isolates, and pea proteins reduce γ_{ow} more at neutral compared to acidic pH. The example of pea protein shows the full potential of AST when sufficient interfacial tension data is available for a given protein and its fractions. Less adsorption data is available for soy protein and its fractions, but still an overall trend to higher surface activity at neutral pH was observed. Limited protein adsorption data is available for chickpea, lentil, faba bean, wheat, and individual microalgae strains to draw generalized conclusions, but the same trends that individual protein fractions are more surface active and certain pH values favor their adsorption were observed.

AST further allows the compilation of all present plant protein adsorption data and comparison within different protein sources and to established animal proteins. Based on the current data, it appears that proteins from pea, soy, chickpea, lentil, and faba bean are effective at reducing interfacial tension and partially exceed the surface activity of fractionated animal-based proteins, particularly globular animal proteins. On the other hand, proteins isolated from wheat seem less surface active than other plant and animal proteins. A limitation of AST is that interfacial tension reduction is only one aspect of interfacial stabilization. We found several examples where the most surface active fractions, i.e., fractionated albumins, were reported to form the weakest interfacial layers in interfacial rheology experiments and formed the least stable emulsions. Complementary interfacial rheology

or emulsification experiments are thus required to conclusively identify promising protein sources for interfacial stabilization.

AST exploits the universal adsorption behavior of proteins at oil-water interfaces as a function of oil polarity. We expect that AST can be a powerful tool to collect, visualize, and compare interfacial tension data from different research groups and draw more holistic conclusions than possible from individual datasets. However, an important requisite is that interfacial tension data is properly acquired and unambiguously reported. Several studies had to be excluded from our analysis, mostly because the initial or final interfacial tension data was not unambiguously reported, the used oil was not sufficiently pure or cleaned from contaminations, or experiments were not performed sufficiently long to reach equilibrium interfacial tension. To improve the comparability and universal use of interfacial tension data, we recommend obtaining reagent grade oils or strip oil from contaminants as described in an earlier review [30]. Furthermore, to facilitate comparison between protein sources and research groups, it is pivotal to use protein concentrations beyond the monolayer coverage concentration, let the interfacial tension equilibrate for minimum a few hours, and report final equilibrium surface tension. For AST the use of properly equilibrated values is particularly important for polar oils, as normalization by their low initial interfacial tension can potentially augment small deviations. In general, there is currently not a vivid culture of data sharing in experimental interface science. To improve the reusability of interface science data, we advocate preparing data according to open science, i.e., FAIR [174] principles and share all relevant information and data in manuscripts, supporting information, or data repositories.

In summary, AST is a promising new tool to collect and reanalyze existing interfacial tension data, observe trends across literature data, and detect existing gaps or particularly promising approaches. For instance, in the present case of novel plant proteins AST will allow to more rapidly detect promising protein sources, fractions, and optimum processing conditions. An important requisite to improve the usability of AST will be to share interface science data in a more open and reusable manner.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cocis.2025.101920>.

Data availability

Plant protein adsorption at oil-water interfaces: Collection of literature interfacial pressure data <https://zenodo.org/records/15100906>

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- * of special interest
- ** of outstanding interest

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