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Protein-stabilized interfaces in multiphase food: comparing structure-function relations of plant-based and animal-based proteins

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Proteins are an important category of stabilizers for multiphase food systems such as foams and emulsions. In recent years, a growing interest can be observed in replacing animal-based by plant-based proteins to stabilize such products. Often, these plant-based proteins have inferior functionality compared to animal-based proteins. In this review, we will discuss recent insights into possible reasons for the differences in behavior between animal-based and plant-based proteins, and present an overview of strategies to improve the performance of plant-based extracts. Improving plant-protein functionality may ultimately allow us to engineer interfaces with properties tailored to specific applications.

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Introduction

When developing novel multiphase food products, the stability of their air–water and/or oil–water interfaces is an important design parameter. Common stabilizers used in such products are low molecular weight surfactants (e.g. lecithin, monoglycerides and diglycerides, spans, polysorbates), polysaccharides, and proteins. Recently, colloidal particles (modified starch granules, lipid-based or protein-based particles) have also gained interest [1]. For products stabilized by proteins, animal-based proteins are still the most commonly used proteins, in view of their excellent stabilizing properties. They are highly soluble in aqueous solutions, and adsorb readily at both air–water and oil–water interfaces. Globular animal-based proteins tend to form stiff interfacial films, which provide mechanical stabilization against droplet or bubble coalescence. Away from the isoelectric point of the proteins, and at relatively low ionic strength, they also provide stabilization by inducing electrostatic repulsion between interfaces.

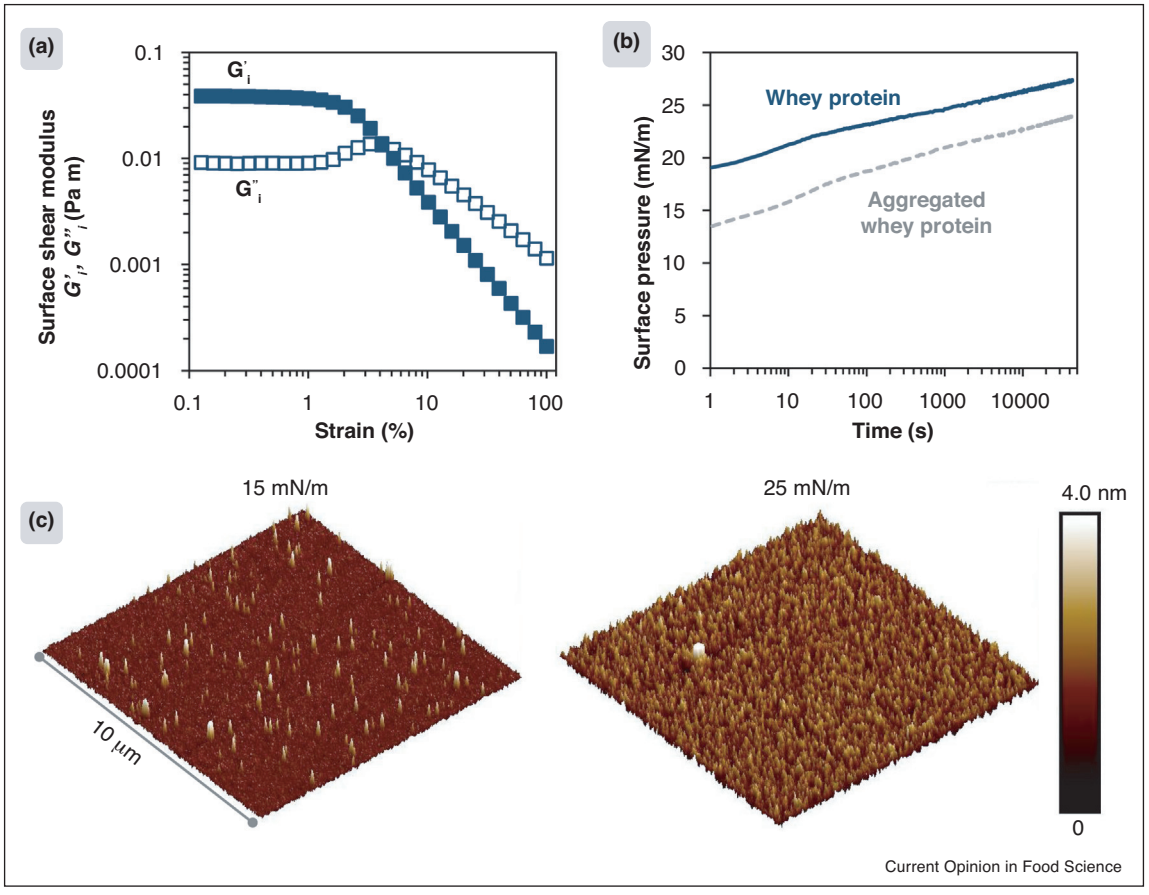
In recent years, a clear trend can be observed to replace animal-based proteins by proteins extracted from plant sources (e.g. soy, pea, lentil, fava bean, seeds) [2]. The former have a much more significant ecological footprint, and concerns about climate change, a growing need for more protein to accommodate population growth, and health concerns are driving this trend [3,4]. Replacement by protein from plant-based sources, either complete or partial, has proven to be difficult, since the functionality of plant protein extracts is often not as good as that of the animal-based proteins they are supposed to replace. Commercial plant-based protein extracts are often less soluble and less surface active. They tend to be complex mixtures of multiple proteins, present in various states (peptides, native protein, aggregates), and can contain additional non-protein components, such as polyphenols, lipids, or (poly)saccharides [5–7]. This complexity can affect the solubility, adsorption behavior, and interfacial properties of the extract.

In this review, we will discuss recent insights into why plant proteins do not perform as well as animal-based ones, with respect to the stabilization of multiphase systems. We start with a discussion of the current views on the state and properties of interfaces stabilized by animal-based proteins, and then present an overview of recent results on the microstructure and properties of interfaces stabilized by several plant-based proteins. These insights provide possible strategies to improve plant-protein functionality with respect to foam and emulsion stabilization, several of which will be discussed. A better understanding of the behavior of proteins at interfaces will facilitate the design of multiphase food products, with more natural and more sustainably produced ingredients.

Microstructure and properties of protein-stabilized interfaces

In multiphase food products, based on foams and emulsions, the surface rheological properties often play an

Figure 1



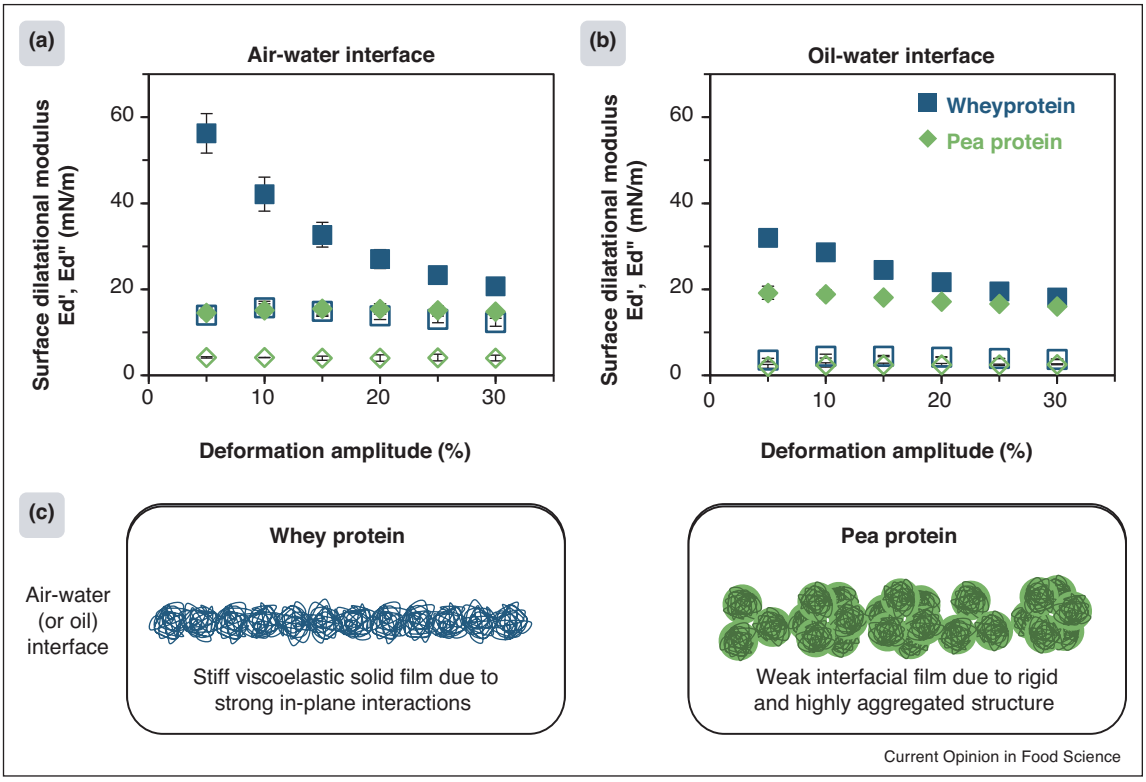
(a) Surface shear rheology on a whey protein-stabilized air–water interfacial film studied using a rheometer with a double wall ring geometry. **(b)** Surface pressure isotherms of whey protein and aggregated whey protein at an air–water interface studied using drop tensiometry. **(c)** Atomic force microscopy (AFM) images of whey protein-stabilized interfaces at various surface pressures, created using Langmuir-Blodgett deposition. The results were obtained and adapted from Ref. [9].

important role in the stability of the product. Proteins, and in particular globular proteins, tend to form stiff interfacial films at the air–water and oil–water interface. A considerable fraction of the research literature on protein-stabilized multiphase systems is for that reason focused on quantifying and understanding these rheological properties.

The surface rheological properties of interfaces stabilized by globular proteins can be probed in either shear or dilatational mode. In the former the shape of surface elements is changed at constant surface area, while in the latter, the area of the interface is changed at constant shape [8]. The most relevant properties to describe the behavior of interfaces in shear are the surface shear viscosity, surface shear storage modulus, surface shear loss modulus, and surface shear relaxation time(s). Relevant dilatational properties are the dilatational viscosity, dilatational storage modulus and dilatational loss modulus. The dilatational moduli are, in essence, the two-dimensional equivalent of the three-dimensional bulk modulus. Typical techniques to measure these properties can be found in recent reviews [8]. Most studies probe surface properties in the linear viscoelastic regime (or at least assume data was obtained in that regime); but in recent years, more and more studies are also focusing on properties in the nonlinear viscoelastic regime [8–11], since this regime is highly relevant for food products.

For globular proteins, whey protein isolate (WPI) and more specifically its major component, beta-lactoglobulin (BLG), can be considered a benchmark. It is an excellent stabilizer for foam and emulsions. BLG is a relatively small protein with a molecular weight of about 18−36 kDa, depending on its quaternary structure (i.e. whether it is in its mono form or dimer form) [12]. Its secondary structure has been well-characterized, and consists of about 15% α-helix, 50% β-sheet, and 15–20% reverse turn [13,14]. After adsorption at the interface, it forms films with viscoelastic solid characteristics: the storage

Figure 2



Surface dilatational rheology on a whey protein-stabilized and pea protein-stabilized air–water **(a)** and oil–water **(b)** interfacial film using a drop tensiometer (closed symbols are Ed′, open symbols are Ed″). **(c)** A schematic representation of a whey and pea protein interfacial film. The results were obtained and adapted from Ref. [28•].

moduli are significantly higher than the loss moduli, in both shear and dilatation (Figure 1a), with loss tangents ~ 0.1 at low deformations in dilatation (Figure 2a,b). Dilatational moduli also show a power-law dependence on frequency ($\sim f^n$), with low n-values (~0.1), and in strain sweeps, the interface displays softening in extension and hardening in compression [9]. The softening in expansion points to a gradual disruption of the solid microstructure with increasing deformation. In step-expansion/step-compression tests, the surface pressure relaxation shows stretch-exponential behavior ($\sim\exp(t^{\beta})$), with stretch exponent β equal to 0.5–0.6 in extension. This points to a phenomenon referred to as dynamic heterogeneity, which implies local variations in the relaxation kinetics on the interface [15]. These characteristics together suggest that the microstructure of the BLG stabilized interface is in a disordered solid state. There is no clear consensus yet whether the interface is in a (quasi) two-dimensional gelled state or a soft glassy state. The surface density of BLG is much higher than in the bulk, and at high density, these two states are hard to distinguish from each other [16]. They are however substantially different: the gelled state is generally assumed to be formed by a first-order type transition, and hence is similar to (arrested) spinodal phase separation, leading to inhomogeneous far-from-equilibrium local structures [16]. Glass formation is a continuous transition, leading to more homogeneous and locally equilibrated structures [16].

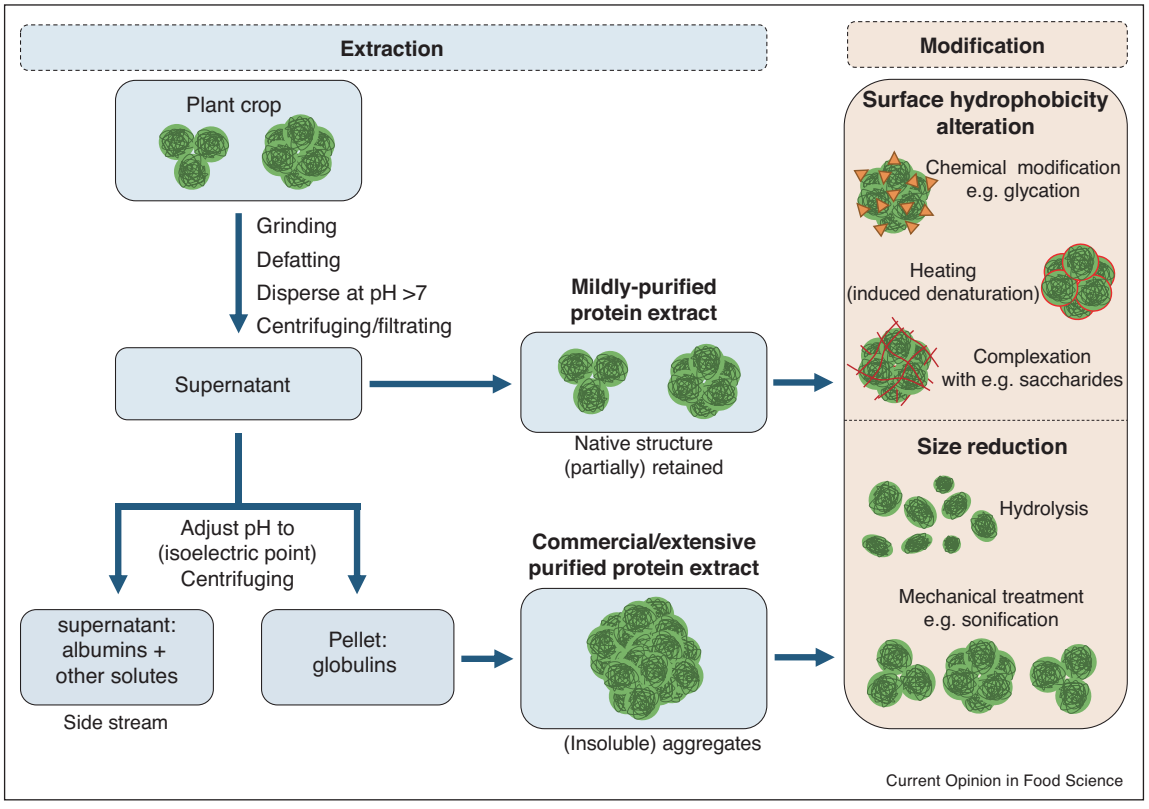
Imaging of the interfacial microstructure with atomic force microscopy revealed that BLG and other proteins form heterogeneous structures after adsorption, with clearly visible clusters (see example in Figure 1c) [17–19], which appears to favor the gelled state, although this is not completely conclusive. In the bulk, these two types of disordered systems can be distinguished by their relaxation behavior, where beyond the liquid–solid transition, the relaxation modulus displays power-law behavior for both, but with exponents of opposite sign (positive for glasses, negative for gels) [20]. But for protein-stabilized interfaces relaxation modulus data is generally not available.

On a macroscopic level, the adsorption process and subsequent formation of the interfacial structure is typically studied by monitoring the surface pressure as a function

of time. In general, three distinct phases can be observed: a lag phase in which the protein diffuses towards the interface and rearranges before adsorption, an adsorption phase in which the surface pressure changes rapidly, followed by a final phase where the surface pressure changes very slowly (sometimes over several hours (Figure 1b) [9,21•]. The latter is again an indicator that in this final stage we are looking at an interface that is in a kinetically arrested state. These long time tails are sometimes attributed to continuous denaturation or intra-molecular rearrangements of the proteins. But they also occur in interfaces stabilized by protein aggregates (i.e. proteins denatured before adsorption (Figure 1b) [9,12]. Moreover, proteins also show this type of slow aging in 3D gels, at room temperature. All this makes kinetic arrest a more plausible explanation for the long time tales in the surface pressure.

Upon adsorption at an interface, BLG and several other animal-based proteins do at least partially unfold and lose some of their tertiary and secondary structure, but the degree to which this happens depends on the nature of the interface (air–water or oil–water), and for oil–water interfaces even on the polarity of the oil phase [22••]. At the air–water interface, it appears BLG loses only about 10% of its β-sheet structure [23,24], but at the oil–water interface the loss of β-sheet structure appears to be more significant (±44%), and a doubling of the α-helix content can be observed [25]. Interestingly, the dilatational storage modulus of BLG-stabilized interfaces is highest (~100 mN/m at 1–2% amplitude) at the air–water interface, and substantially lower for oil–water interfaces. For oil–water interfaces, the surface shear storage modulus appears to decrease with increased oil polarity [22••]. In a recent study, Garcia-Moreno *et al.* [26•] studied a range of peptides obtained by enzymatic cleaving of potato proteins using synchrotron radiation techniques. There were distinct differences in the surface rheology of these peptides at the oil–water interface, with peptides that had a significant content of β-sheets having higher dilatational moduli than those with a structure dominated by α-helix. This indicates that the in-plane interactions responsible for the formation of the disordered solid microstructure of the interface could be highly specific (e.g. intermolecular β-sheet formation or β-sheet stacking). But for the moment this remains speculative, and more structural evidence has to be obtained to confirm or disprove this.

Figure 3



Schematic overview of plant protein extraction processes and its impact on the protein structure. The scheme includes a brief overview of commonly applied protein modification techniques.

Plant-based versus animal-based proteins

In comparison to animal-based proteins, plant-based ones tend to have lower foamability and emulsifiability, and also give lower foam and emulsion stability. With respect to interfacial properties, plant proteins tend to adsorb slower at both air–water and oil–water interfaces, and in general create interfaces with lower moduli, both in dilatation and shear [27,28•,29••,30]. At least in dilatation, the storage moduli of interfaces stabilized by most plant proteins also show a lower strain dependence, and specifically a less pronounced softening behavior (Figure 2a and b).

The reasons for this lower functionality can be found both in the structure of the proteins themselves, but also in the process used to obtain plant protein extracts. Most purified commercial extracts are obtained using a process consisting of grinding and oil removal, followed by dissolution of the protein from the resulting meal in a (saline) alkali solution, and subsequent precipitation of the protein in acidic conditions (near the pI of the protein) (Figure 3) [31]. This method specifically extracts a fraction of proteins referred to as globulins. These storage proteins, which based on their sedimentation coefficient are often categorized as 7S and 11S proteins, tend to have molecular weights in the range of 150−380 kDa, and are typically in a trimeric or hexameric conformation, with most of the hydrophobic residues buried in the interior of that structure [32,33]. A larger size and lower exposed hydrophobicity of 7S and 11S plant storage proteins can result in slower adsorption at interfaces, compared to a protein such as BLG, which is much smaller and can more easily unfold. This could explain the lower foamability and emulsifiability [34]. If the structure of a protein remains more rigid after adsorption, the ability to interact with neighboring proteins may be reduced, leading to weaker in-plane interactions, and a reduction in stiffness of the interfacial film (Figure 2c). That would explain the reduction in modulus when replacing BLG with plant proteins, resulting in poorer foam and emulsion stability when (partially) replacing WPI with pea proteins [34].

Another factor resulting in lower functionality is that the aforementioned protein extraction process can cause considerable aggregation of the globulins (Figure 3), resulting in reduced solubility. Several steps can be responsible for irreversible protein aggregation, and two important ones are: (1) protein precipitation at the pI of the globulins, and (2) phenol–protein interactions in the alkali solubilization step. Several works already demonstrated increased protein aggregation after the precipitation step, leading to lower solubility, weaker protein-stabilized oil–water interfacial films, and poorer emulsifying properties [29••,35,36]. Also, phenols might oxidize during the alkali extraction step, which is sometimes performed with pH values up to 13. Oxidized phenols can form highly reactive quinones that can covalently bind to proteins, leading to large aggregates [37–39].

Even when the aggregates are small enough to remain dispersed in the solution, their contribution to interfacial stabilization is lower than soluble native proteins [5,40]. In the acid precipitation step, the albumins (2S proteins) which were co-extracted in the alkali-dissolution step, remain soluble, and are typically discarded with the supernatant after the globulin precipitation step [41]. These much smaller proteins adsorb much more readily at interfaces, and recent work has shown that some of these have very high dilatational moduli at the air–water interface (in the same range as animal-based proteins) and can be excellent foam stabilizers (unpublished results). Their functionality in emulsion stabilization is often not as good as that of globulins, in view of the lower surface charge they impart on the oil–water interface (leading to flocculation of droplets) [42•].

Methods to improve plant-based protein functionality

The observations from the previous section provide us with some handles to improve the functionality of plant-based extracts. One obvious step is of course the optimization of the extraction step, to improve the solubility of the globulin fraction, and retain the albumins (either as a separate stream or in a mixture with globulins). Co-extraction of albumins and globulins is achievable by excluding the protein precipitation step (Figure 3) [29••,43]. This protein mixture can be further purified using, for instance, diafiltration to remove small non-protein components, such as phenols, sugars and minerals [43]. Such a method is known as a mild extraction method, and another upcoming method is dry fractionation in combination with air-classification, which yields protein-rich extracts with their native protein structure largely intact [44]. Another great advantage of mild purification is the requirement of fewer resources and higher protein yields [45].

Retaining the native protein structure as much as possible is crucial to obtain functional protein, with respect to interface, foam and emulsion stabilization. Process-related protein aggregation can be avoided by avoiding (extreme) alkaline pH to prevent phenols from oxidizing, and by excluding the protein precipitation step. Of course, specific aggregate inducing processing steps might be inevitable, such as microbial-related heat-treatments and drying. The exact impact of these potentially aggregate-inducing steps should be carefully examined for plant-based proteins.

Obtaining (partly) native proteins could be an essential step in obtaining functional plant-based proteins. However, several plant proteins, such as globulins, seem to

lack the molecular properties to stabilize interfaces effectively. As a result, there has been a substantial effort to improve their interface stabilizing properties by protein modification [46]. Many types of modification methods exist, such as physical, chemical, or biological treatments (Figure 3). The treatments focus on improving the molecular properties, such as the surface hydrophobicity, which can be chemically modified by attaching saccharides, or controlled heat treatments [47]. Heating might also induce protein structural alterations, and the formation of heat-induced peptides, which might dominate the interfacial properties [48]. Protein surface properties can also be improved by complexation; an example is the complexation of insoluble gliadin protein with polysaccharides, leading to surface-active particles and effective foam stabilizers [49]. Other treatments focus on size reduction by breaking down aggregates using ultrasound or high-pressure homogenization [50]. Proteins can also be hydrolyzed chemically or by enzymes to obtain peptides, which can be more surface-active due to their smaller size and higher surface hydrophobicity after exposing previously buried hydrophobic regions [26•,51]. The formation of stiffer interfacial layers is not always guaranteed by this approach, as the peptides might be too small for effective interactions at the interface, or perhaps be lacking specific secondary structures [23].

Summary and outlook

In this review, we have summarized current insights into which differences in protein structure between animal-based and plant-based globular proteins might be responsible for the lower functionality of the latter with respect to interface stabilization in foams and emulsions. Whereas animal-based proteins appear to form stiff viscoelastic solid layers after adsorption at an air–water or oil–water interface, the layers formed by most plant-based proteins appear to be significantly weaker, either as a result of their intrinsic structure, or structural changes induced by the extraction process. Plant-protein functionality can be improved either by switching to milder extraction methods, or by physicochemical modification of the proteins. At the moment, the exact mechanism by which animal-based proteins form these stiff layers is not yet fully understood. The protein structure at the interface seems to largely determine the interfacial properties, which can be particularly studied by combining techniques, such as interfacial rheology and synchrotron radiation techniques. Atomistic molecular dynamics simulations could also shed more light on the details of the interfacial structure, but the simulation of large numbers of protein molecules adsorbed at a fluid–fluid interface is currently still not possible [52,53]. Perhaps coarse-grained simulations of simpler structures (such as small peptides rich in either α-helix or β-sheet structure) could already provide useful information on the interfacial microstructure. Simpler molecules, like block-copolymers [15], were simulated in this way, and this revealed a rich in-plane phase behavior depending on molecular details and strength of interactions between the molecules.

More research into the relation between protein microstructure at the interface and interfacial (mechanical) properties would lead us to find the optimal modification path for plant-based proteins, and allow us to engineer interfaces with specific functional properties.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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