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Review

# Enzymatic hydrolysis of legume proteins: lessons on surface property outcomes

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There is burgeoning research interest in utilising legume proteins as eco-friendly and nutritionally relevant plant-based alternatives to animal proteins. However, legume proteins exhibit technofunctional limitations that impede their widespread food applications. Herein, we review how enzymatic hydrolysis of legume proteins can be exploited to alter physical properties, improving their surface-related functionalities based on current literature. The choice of enzyme, particularly the selectivity, plays a significant role in obtaining different emulsifying and interfacial properties of legume protein hydrolysates. Several physicochemical characteristics synergistically affect the surface functional performance of legume proteins. Outlining future directions, this review highlights the transformative potential of enzymatic hydrolysis in legume proteins for enhancing the textural (lubrication) aspects. Additionally, precise measurements of structures of hydrolysates are a necessary undertaking in the future.

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## Introduction

In recent decades, the need to explore alternative protein ingredients has become imperative due to growing consumer veganism base and concerns associated with the sustainability for the provision of animal-derived proteins. Among various sources of proteins, legumes, such as soybeans, chickpeas and lentils, have been recognised for their significant contribution to achieve agricultural sustainability and also sources of high protein contents (20–40% dry mass) [1]. Due to their nutritional advantages and functional benefits, legume proteins have recently attracted considerable research attention for their utilisation in foods. The predominant proteins in legume seeds are storage proteins, with salt-soluble globulin (> 50%) and water-soluble albumin (< 20%) being the major fractions. However, the transition from conventional animal-based proteins to legume proteins is often not straightforward, given their distinct protein composition and intrinsic structure. The limited solubility, high surface hydrophobicity leading to pronounced aggregation, as well as tendency for proteins to aggregate during extraction and food processing have been shown to hinder the widespread application of legume proteins in foods [2]. These structural limitations are detrimental to enrich protein content and utilise legume proteins as effective molecular emulsifiers in emulsions. Additionally, from the mouthfeel aspects, protein aggregation may increase oral friction, potentially leading to undesirable astringent perception [3].

Hence, it is imperative to explore structural modification strategies on legume proteins to reduce such aggregation and its undesirable consequences, thus improving their technofunctional performance for their widespread utilisation. Enzymatic hydrolysis, that is, a protein degradation process catalysed by biological catalysts enzymes, is one of the widely researched approaches in food science, noted for its mild processing conditions, yet with high efficiency, safety, and generally a 'clean' label connotation [4]. This method is effective in altering the physicochemical attributes of proteins, such as reducing molecular weight (Mw), increasing the exposure of both hydrophobic and hydrophilic residues/patches and releasing relevant ionisable groups. Recent enzymatic hydrolysis studies on oat, pea, and rice proteins have shown that hydrolysis leads to significant improvement in technofunctionality, such as solubility, gelling, emulsifying, and foaming properties [5,6].

In this review, we aim to create a concise understanding of what is known so far on how enzymatic hydrolysis can alter the structure, physical properties, and surface adsorption of legume proteins purely focusing on molecularly adsorbed scenario (i.e. particulate-based Pickering properties of legume protein particles and aggregates are outside the scope of this discussion) and explore the structure–surface property relationship. We attempt to consider all surface properties in this article, including interfacial behaviour at liquid/liquid interfaces and emulsifying attributes, as well

as lubrication properties on soft hydrophobic, solid surfaces. However, it is important to note that research in this latter area is currently far more limited than former ones, and hence, the section on lubrication is more speculative and opinion-based compared to the other two sections. Other aspects of protein hydrolysates, like bioactive performance and sensory properties, are beyond the scope of this review. Following a short discussion of several critical parameters of enzymatic hydrolysis, we examine the effects of hydrolysis (at various degrees of hydrolysis [%DH]) on interfacial and emulsifying properties of legume proteins based on revisiting the literature data that have been reported in the last 5 years. We then pinpoint the effects of hydrolysis on physicochemical attributes of legume proteins (e.g. structures, Mw, size, surface hydrophobicity, and charge), elucidating their relationship with interfacial and emulsifying performance. Finally, we identify knowledge gaps in the oral lubrication aspect and offer speculations on the role of hydrolysis in the lubrication properties of legume proteins. We also highlight the potential of enzyme engineering for legume protein hydrolysates as a strategy to improve their mouthfeel performance, which has attracted little attention to date and may revolutionise the acceptance of next-generation, enzymatically modified legume-based products.

## Critical parameters of enzymatic hydrolysis

Enzymatic hydrolysis of proteins involves the cleavage of peptide bonds by specific enzymes, which recognise specific sequences and structures within protein molecules, resulting in smaller peptides or amino acids. Two types of binding mechanisms adopted by enzymes have been covered in a recent review by Gouseti et al. [4]. However, in general, the active sites of the given enzyme bind to specific regions of the substrate (i.e. proteins) where digestion is required. Therefore, the reaction is considerably dependent on factors such as enzyme type, enzyme-to-substrate ratio, and environmental conditions. These factors are crucial for determining the extent of the reaction and can subsequently lead to different technofunctional outcomes for hydrolysed proteins. The degree of hydrolysis (%DH) is a valuable index for evaluating the extent of the protein breakdown process, expressed as the proportion of cleaved peptide bonds relative to the total number of peptide bonds in the protein. Several researchers have confirmed a strong association between low %DH and better functionalities of resultant protein fragments [7], emphasising the importance of its precise control. In this section, we briefly discuss the choice of enzymes, pH and temperature to understand how the enzymatic engineering can be modulated.

### Type of enzymes

The selection of enzymes significantly impacts the cleavage points on the protein backbone during hydrolysis. Enzymes can broadly or specifically target covalent peptide bonds and initiate cleavage either at chain ends or within the chain, resulting in (poly)peptides with varying size distributions. The types of proteases commonly used for protein hydrolysis have been comprehensively summarised in two previous reviews [4,8].

With respect to achieving better functionalities, limited or moderate proteolysis is generally advocated irrespective of the protein type, and the use of specific enzyme is thought to be conducive to this purpose. Ding et al. [9] proposed that enzymes such as trypsin, with a greater degree of specificity (i.e. cleaving bonds simply on the carboxyl side of arginine and lysine [4]), have to diffuse further into the interior regions of proteins and take longer time to achieve the same %DH, compared to enzymes with limited specificity (ELS) such as Alcalase, which is capable of cleaving the C-terminal side peptide bond of up to 10 amino acids [4]. Therefore, at a similar, relatively low %DH, such an enzyme with high specificity can disrupt the dense structure of proteins more efficiently. This can in particular be beneficial in relation to compacted and aggregated plant proteins, such as legumins, which are largely globulins. In contrast, ELS are likely to find and break the required number of bonds located on the surface of proteins to achieve the required DH, with only limited effects on the core protein molecules buried in the interior of the aggregates.

Apart from enzyme specificity, enzyme selectivity (or preference) is another crucial feature, often defined as the relative hydrolysis rate at an individual cleavage site compared to the total hydrolysis rate of all cleavage sites in the protein. This selectivity can vary due to changes in the charge state of amino acids either at or neighbouring the cleavage sites, which are influenced by the environmental pH [10]. Therefore, it is essential to carefully control the environmental pH throughout the reaction to ensure consistent hydrolysis products between batches, especially since it is well known that the new release of carboxyl and amino groups would change the system pH during reaction.

### Reacting pH and temperature

Generally, each enzyme has an optimum pH and temperature, at which it exhibits the maximal activity. Deviations from these optimal conditions can lead to partial denaturation of the enzyme and reduced enzyme activity, thereby hydrolysis is typically conducted within the range of optimum pH and temperature. However, an interesting finding from a recent study [11] suggested that non-optimised pH condition is plausible to make the given ELS (i.e. pepsin) somewhat more specific, producing relatively large fragments with good emulsifying properties. In particular, the authors demonstrated that pepsin functioned suboptimally, though more selectively, in hydrolysing $\beta$-conglycinin away from the optimal pH of 2.1. At a pH of 4.7,

a dominant fraction derived from $\beta$-conglycinin with an Mw of 25 kDa was specifically identified among the mixture of generated polypeptides. Theoretical self-consistent field calculations predicted a rather superior surface properties for this hydrolysis product. Indeed, hydrolysates formed at this suboptimal pH were found experimentally to be much better emulsifiers than those formed at optimal pH of 2.1 for pepsin action [11]. Additionally, pH and temperature may be also used as effective enzyme engineering variables to influence the structural conformation and susceptibility of proteins, which are crucial to substrate binding at the active site of the enzyme. Under specific conditions, this can lead to 'selective hydrolysis', where certain fragments are generated exclusively from the targeted protein composition. In the work of Li et al. [12], soy protein was treated by selective hydrolysis that preserved the intact $\beta$-conglycinin while digesting glycinin. This hydrolysed soy protein exhibited the ability to form interfacial layers with high elastic and viscous moduli, as measured by interfacial shear rheology. Under optimum conditions, such hydrolysates could display emulsifying properties comparable to animal-derived sodium caseinate.

## Effects of degrees of hydrolysis on emulsifying and interfacial properties of legume proteins

Before considering the specific structure–function relationships, it is useful to first understand how emulsifying and interfacial properties of legume proteins can be modulated by altering the %DH. To this end, we have compiled data sets from recent literature of legume protein hydrolysates summarising how %DH can affect their emulsifying and interfacial performance. It is worth noting that comparisons between studies should be made with caution due to the different methods possibly used to determine %DH. However, within an individual study, comparison across a range of %DH are still valuable. Generally, emulsifying properties include emulsifying activity and emulsifying stability, which are commonly represented by emulsifying activity index (EAI) and emulsifying stability index (ESI). Other methods for evaluating emulsifying properties, for example, determining emulsifying capacity and monitoring the size of emulsion droplets during storage, are also important and feasible and have been employed in some studies. However, in this section, we only use EAI and ESI as comparison indices. EAI is defined as the area of the oil/water interface stabilised per unit weight of protein, whilst ESI is expressed as the time required for the turbidity of the emulsion to decrease to half of its original value. The evolution of EAI and ESI of legume proteins, such as pea, soybean, and chickpea protein, as a function of %DH is displayed in Figure 1a1,a2. As expected, in most cases, EAI increased posthydrolysis (Figure 1a1), but this increase was reversed once %DH exceeded a certain threshold. It produced very small peptides that were unable to provide emulsification abilities. In contrast, changes in ESI (Figure 1a2) were more variable and highly dependent on the unmodified parent protein. Therefore, it can be concluded that enzymatic hydrolysis can indeed enhance the emulsifying performance of legume proteins within a limited and often relatively low value range of %DH.

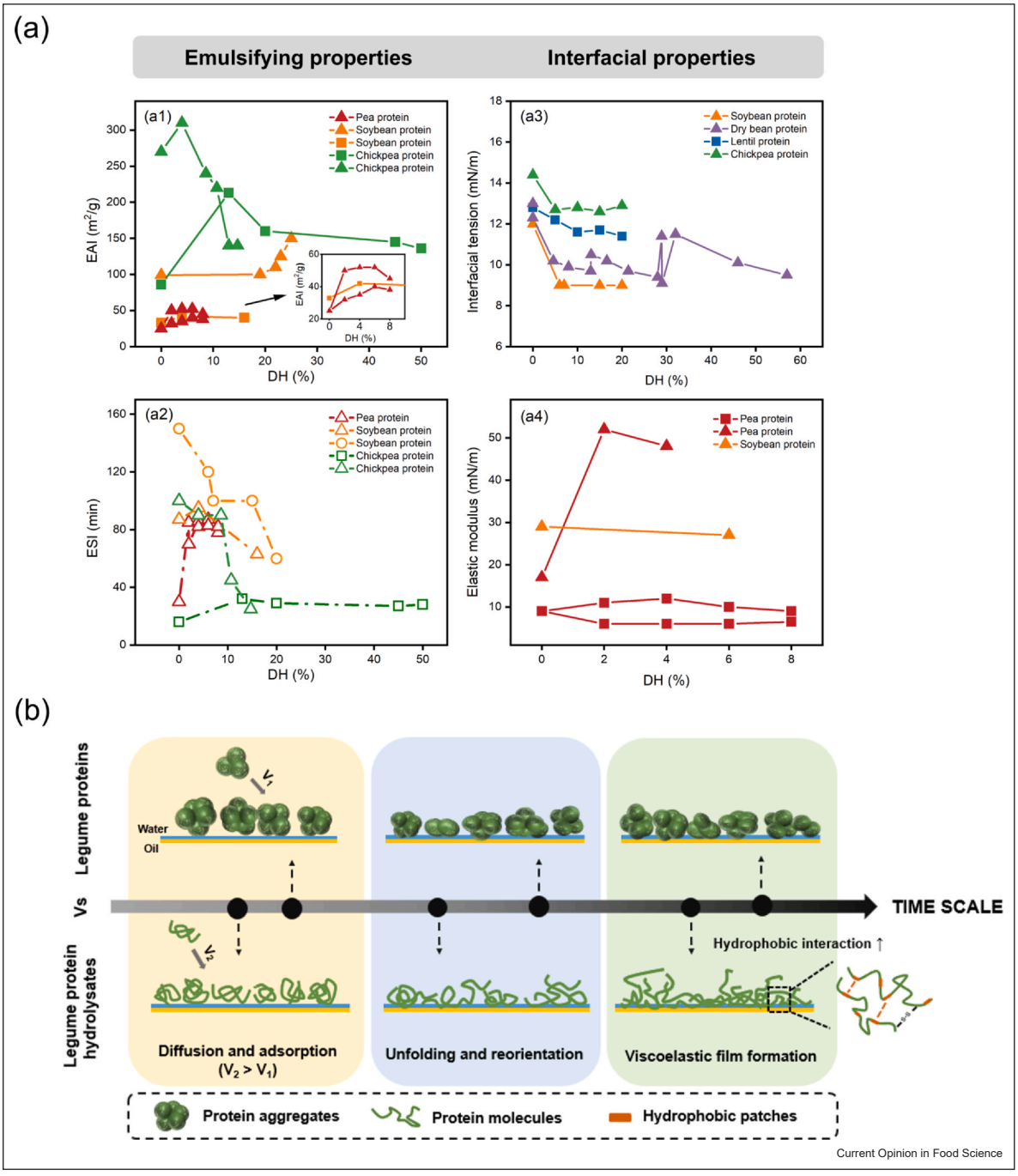
With the aim at better understanding the protein emulsification, increasing attention has been given to their interfacial behaviours, for example, the capacity to reduce interfacial tension ($\gamma$) and also the mechanical properties, that is, dilatational elastic modulus ($E'$) of the formed interfacial protein layer at the oil–water interface.

Given that the lowering of $\gamma$ and increase of $E'$ are important factors that may influence EAI and ESI, respectively, we collated data on the effects of varying % DH on $\gamma$ and E′, as depicted in Figure 1a3,a4, respectively. Under specific hydrolysis conditions, a lower $\gamma$, one of the indicative parameters of a superior interfacial performance, was a fairly common feature of the hydrolysates. Such low values of $\gamma$ were not present for the parent legume proteins (Figure 1a3). As for $E'$ (Figure 1a4), the trends were less obvious except for pea protein in one of the studies, where the $E'$ increased more than twofold upon hydrolysis (4% DH) [13]. This highlights how enzymatic hydrolysis not only can impart higher surface activity, with more compact alignment of proteins at the oil–water interface, but also results in a more cohesive, elastic film at oil–water interface, as schematically shown in Figure 1b. Besides $E'$, the shear elastic modulus ($G'$) obtained from interfacial shear rheology can also be useful in providing insights into the viscoelastic properties of the interfacial film against shear deformation. However, extra caution is required when applying findings from two-dimensional rheology, that is, in-plane protein behaviour, to real emulsions that consist of curved interfaces, where fluid dynamics and stress forces are quite different. In addition, emulsions can be significantly influenced by interdroplet interactions. In the work of Chutinara et al. [14], clear evidence of bridging flocculation was observed in emulsions stabilised by lentil protein hydrolysates (with 1.5% and 4.5% DH) compared to the unhydrolysed protein isolate. This finding was particularly contradictory with the higher $E'$ of the interface formed by the 1.5%DH hydrolysates.

## Surface properties influenced by hydrolysis of legume proteins: physicochemical aspects

Several physicochemical characteristics of proteins upon enzymatic hydrolysis are discussed separately in this section, providing detailed information on the structure–surface property relationships. Although the structural properties of proteins (e.g. hierarchy structures,

**Figure 1**




Effects of %DH on emulsifying and interfacial properties of legume proteins **(a)**, including EAI (a1) [15–18], ESI (a2) [15,17–19], interfacial tension at oil–water interfaces (a3) [19–21] and elastic modulus of the interfacial film (a4) during dilatational rheology measurements [19,13,22]. All the graphs are plotted using the data from the aforementioned literature. It is important to note that numerical comparisons in **(a)** are only meaningful within the same study across different DH levels, as variations in protein concentrations and measurement methods from different studies could considerately affect the outcomes. **(b)** Schematic illustration of interfacial behaviour of legume proteins postenzymatic hydrolysis at oil–water interfaces compared with that of unhydrolysed counterparts, with interfacial diffusion rate ($V_1$) being lower than that of the former, that is, the hydrolysates ($V_2$). The illustrated aggregates (upper) and molecules (bottom) are not drawn on real scale.

Mw, surface hydrophobicity, and charge) are discussed separately, the synergetic effects of these features on overall protein behaviours should be considered.

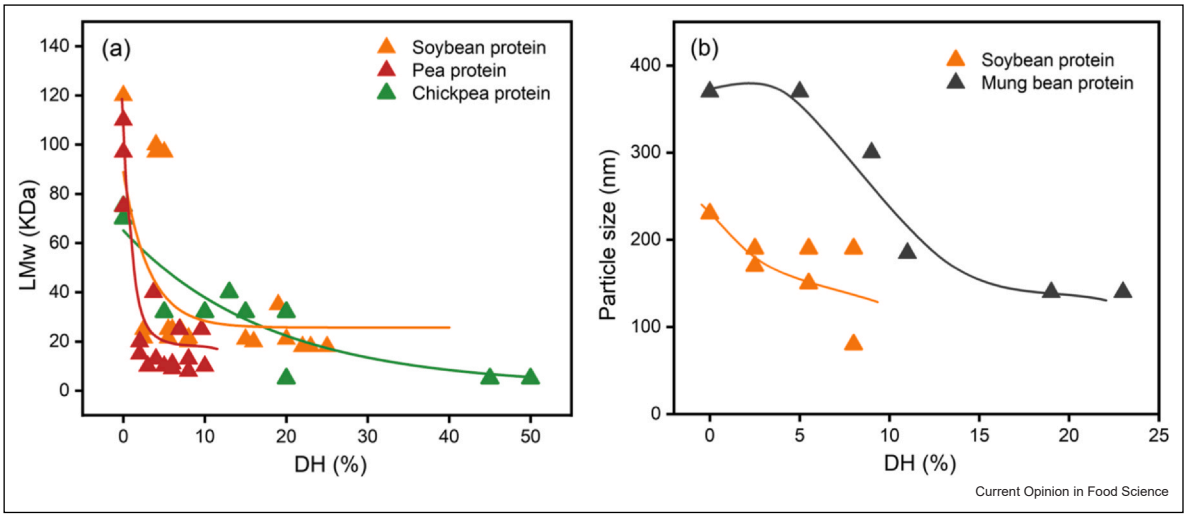
### Molecular weight

It is an obvious fact that enzymatic hydrolysis should result in a reduction in the Mw of generated polypeptides. Herein, we collated the Mw data of the largest major band (LMw) from the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) image reported in the literature for soybean, pea, and chickpea proteins. The relationship between this LMw value versus %DH is plotted in Figure 2a. Of course, there is no guarantee that the LMw band represented protein will be the only, or the most dominant, fragment on the surface if applied to emulsion systems or real surface contacts. At the early stage of hydrolysis with %DH < 10%, which typically refers to a limited hydrolysis regime, there is a significant drop in LMw. This is followed by a rather slower decrease, almost plateauing off with further progressive hydrolysis. Despite some discrepancies among the various legume proteins studied, this experimentally observed trend is in line with previous theoretical data on average Mw as a function of %DH [11]. Thus, it is clear that at low %DH level, a very good control of %DH is extremely important, but practically quite difficult to achieve. Even within the standard error range for %DH that is usually reported in scientific studies, Mw distributions may exhibit substantial variation when DH is low. Consequently, this is an experimental challenge for researchers and an issue to be considered in realising viable approaches to achieve highly precise control of the reaction and resulting %DH.

Following the above discussion of Mw changes with the level of hydrolysis, let us now consider the relationship between Mw and the surface properties of the hydrolysed legume proteins. In real laboratory experiments, the molecular mass of protein is known to govern the gradient-driven bulk diffusion of proteins to the oil–water interface. Smaller molecule sizes, resulting from hydrolysis, are able to diffuse more rapidly to oil–water interfaces, thus reducing the adsorption time [14] (Figure 1b).

Give the considerable differences in time scales, the fast diffusion of surface active material to the freshly created interfaces, can prevent the recoalescence of droplets during and shortly after homogenisation, mainly through Gibbs-Marangoni effect [28]. Nevertheless, excessively small protein sizes could also be disadvantageous in relation to both emulsifying activity and longer term stability, as discussed previously (Figure 1b3). Eckert et al. [29] reported a pronounced increase in EAI from 35 to 151.2 $m^2/g$ for pepsin hydrolysed fraction of fava bean protein with Mw > 10 kDa, whilst noted poor emulsifying performance for low Mw fractions (< 5 kDa), with low EAI and ESI. This might be attributed to the small peptides lacking sufficient hydrophobic groups failing to effectively adsorb and cover the oil–water interface [30]. As a result, it is also more likely that a thin and loosely interconnected protein layer forms, leading to weaker steric-type repulsion interactions, and hence inferior colloidal stability [28]. Moreover, the competitive displacement of larger, more desirable Mw fractions by small polypeptides in hydrolysate mixtures would further compromise emulsion stability. These results highlight the potential benefits of filtering and removing

**Figure 2**




Variation of largest Mw (LMw) peptides characterised using SDS-PAGE [9,16,17,19,22–26] **(a)** particle size [9,27] **(b)** of typical legume proteins at around neutral pH conditions as a function of %DH. Lines are used as guide to eye.

small peptides out of hydrolysate mixtures in future research to achieve better emulsifying-related functionalities. This aspect of hydrolysates has attracted limited attention in the literature to date.

## Protein structures

### *Secondary structure*

The secondary structure ($\alpha$-helix, $\beta$-sheets, and random coils) of proteins/peptides refers to the spatial arrangement of the protein chain and is usually determined by circular dichroism (CD). The interpretation can be conducted directly from the CD spectra or quantitatively from the fitted percentage content of structural units. Work by Shuai et al. [15] suggested that with an increasing %DH (from 2% to 8%), the percentage of $\alpha$-helix structure increased, while that of $\beta$-sheets decreased, regardless of the enzyme applied. Among the enzymes tested, trypsin-hydrolysed pea protein exhibited the lowest content of $\beta$-sheets, weakening the compact structure of pea protein. Unexpectedly, with further hydrolysis to 30% DH, the authors reported a comparable content of ordered and disordered structures (with 10% random coils) to that of unhydrolysed protein. Thus, hydrolysis can lead to the structural transition from ordered to disordered states; however, there are no consistent trends with varying %DH. This can be attributable to the specific location of cleavage sites and the original protein structure.

Conformations of hydrolysates are known to contribute to their functional performance, but linking these structures to surface-related properties is not often straightforward. Generally, in CD analysis, the secondary structure of hydrolysates is studied when dispersed in an aqueous phase. It is well known that, for emulsifying oil droplets, a conformational reorientation of these hydrolysates occurs once they approach and absorb to the oil/water interface [31]. For the emulsion stability, in practice, the surface equilibrium structure of proteins and the resulting equilibrium properties of adsorbed film are determinant. However, given the long-time scale of proteins to achieve this equilibrium status, many of interfacial and emulsifying properties of hydrolysates observed in experimental studies are influenced more directly by their initial surface configuration when absorbed at the interface. Importantly, the ability of hydrolysates to undergo initial structural rearrangement at the interface is closely linked to their structural compactness in the aqueous bulk. Hence, the rearrangement of hydrolysates upon absorption needs to be studied. Recent studies have proposed a method to measure the CD spectra of surface-absorbed hydrolysates in an emulsion [32,33]. For instance, whey hydrolysates displayed a conformational shift from a disordered state to α-helix upon absorption [33]. In contrast, a gain in $\beta$-sheet structure was reported for hydrolysed sunflower and olive proteins after absorption to the oil/water interface, which is thought to explain the formation of a more elastic interfacial protein film with a complex dilatational modulus ($E$) of 30 mN/m at pH 7 [32]. A similar response was previously revealed by García-Moreno et al. [34] for synthetic potato peptides, where a high percentage of $\beta$-strand structure (63–65%) was correlated with strong interpeptide interactions, subsequently leading to the formation of stiff and solid interfaces.

### *Tertiary and quaternary structure*

Understanding peptide/protein or peptide/protein aggregations after enzymatic hydrolysis is crucial for studying the functional surface properties of the resulting hydrolysates. This subsection only aims to show readers the potential of aggregation and highlights the need for appropriate hydrolysis conditions. Any discussion on aggregate-based Pickering emulsification is not covered, as it is outside the scope of this review.

Peptides generated from enzymatic hydrolysis often coexist with intact protein (i.e. unhydrolysed protein) in the system. The coexistence increases the likelihood of new cluster formations driven by hydrophobic interactions between the generated peptides and the remaining unhydrolysed protein. As reported in a previous work, the binding of whey peptides to the central hydrophobic region of the intact whey protein occurred at pH 6.8 and 8 [35]. Although specific information on legume proteins is lacking, their general higher hydrophobicity compared to animal proteins suggests that a similar binding behaviour is likely to occur. In addition to peptide/protein interactions, aggregation between generated peptides that display high hydrophobicity or opposite charges is also possible. This is especially true at high %DHs, which produce smaller peptides with lower steric repulsions. Environmental parameters, such as pH and temperature, can further influence these interactions. In particular, enzyme inactivation, a common final step in hydrolysis, typically involves heat treatment, which can significantly alter the properties of hydrolysates, potentially leading to further aggregation. Similarly, a shift in pH from the reaction to analysis levels may also cause peptide/peptide aggregation due to changes in the charge state of the resulting peptides and the balance between hydrophobic interactions and electrostatic repulsions.

These formed aggregates can negatively impact the stability of the system and hinder the molecularly-based interfacial adsorption of the hydrolysates; hence, proper reaction parameters are required. The particle size of proteins can be a useful index for monitoring the level of

occurred aggregation mentioned in this section and is further discussed in a following section below.

### Surface hydrophobicity and surface charge

Surface hydrophobicity ($S_0$) is a structure-dependant factor playing an important role in the functionalities of proteins, particularly for plant proteins, as they are rich in hydrophobic groups and display significant hydrophobic interactions leading to protein–protein aggregation impeding their functionalities [36]. Several methods for characterising $S_0$ have been employed, with the fluorescence probe method being predominantly used. The value of $S_0$ obtained by this method is usually determined by the slope of the detected fluorescence intensity plotted against a series of protein concentrations where a higher $S_0$ indicates an increased presence of hydrophobic groups on the surface of the protein molecule.

After enzymatic treatments, proteins are partially denatured, leading to the exposure of interior buried hydrophobic residues. However, there has been no consensus regarding the changes in $S_0$ of legume proteins after enzymatic hydrolysis. The $S_0$ of legume protein with varying %DH is summarised in Table 1. This table demonstrates that the values of $S_0$ for enzymatically treated samples can range widely from 20 to 100. Moreover, with the progressive increase in %DH, fluctuations in $S_0$ values were spotted in the case of black bean hydrolysates [37]. The phenomenon of an initial increase followed by a decrease in $S_0$ is usually explained by the greater exposure of hydrophobic groups and the subsequent reburying of smaller (poly)peptides into new clusters via hydrophobic interactions between them post hydrolysis [20]. Similar to $S_0$, the change of surface charge (quantified experimentally by measuring zeta potential) following hydrolysis also varied significantly across different studies (Table 1). These unpredictable changes in $S_0$ and surface electric potential account for the variability in amino acid composition over different legume proteins and also specific cleavage sites for the employed enzymes. For instance, enzymes such as Alcalase preferentially expose the hydrophobic residues, being much more likely to impart a higher level of $S_0$, compared to other enzymes [38].

**Table 1**

**Examples of surface hydrophobicity and absolute magnitude of zeta potential of legume protein hydrolysates, with the former measured by 8-anilino-1-naphthalenesulfonic acid fluorescence method.**

| Sources of hydrolysates | DH (%) | Surface hydrophobicity (AU) | Absolute zeta potential (mV) |
|---|---|---|---|
| Chickpea protein [39] | 0 | 24 | 17 |
| | < 10 | 38–51 | 18–21 |
| | < 20 | 40–54 | 20–21 |
| Soybean protein [23] | 0 | 200 | – |
| | < 20 | 85 | – |
| | < 25 | 30–70 | |
| Pea [13] | 0 | – | 25 |
| | < 5 | – | 28–29 |
| Faba bean protein [29] | 0 | 1370 | 25 |
| | < 10 | 100 | 28–32 |
| Mung bean protein [27] | 0 | – | 22 |
| | < 10 | – | 18–20 |
| | < 25 | – | 18–33 |
| Dry bean protein [20] | 0 | 65 | – |
| | < 20 | 40–90 | – |
| | > 20 | 30–52 | – |
| Dry bean protein [20] | 0 | 75 | – |
| | < 20 | 20–70 | – |
| | > 20 | 20–90 | – |

Regarding surface functionalities, the abundance of hydrophobic groups may impart good affinity of the hydrolysed legume proteins to the oil–water interface and thus improve their interfacial behaviours [40]. Hence, some level of hydrophobicity is indeed essential for proteins to be surface active. Furthermore, as displayed in Figure 1c, enhanced intramolecular hydrophobic interactions after hydrolysis tend to promote the protein interactions among adsorbed molecules. This leads to the formation of stronger and more stable protein films. However, high charges of protein may hinder this closely pack interfacial layer formation as a result of higher interprotein electrostatic repulsion. This highlights the crucial role of environmental salt concentration, which significantly influences the electrostatic repulsion and therefore the balance between attractive hydrophobic and electrostatic interactions. A study on pea hydrolysates demonstrated the negative effects of increasing ionic strength from 0 to 0.4 M on $E'$ and $G'$ values of the interfacial layer [41]. Specifically, at a dilatational frequency of 0.4 Hz, the resulting tryptic hydrolysate with 4%DH showed a decrease in $E'$ (from 14.6 to 11.1 mN/m) at salt concentration of 0.1 and 0.2 M. However, at even higher salt concentration of 0.4 M, there was a slight increase in E′ to 12.5 mN/m, though this value still remained below that of the control sample with no added salt. This salt dependency was attributed to altered electrostatic interactions between adsorbed peptides. However, this still requires further in-depth understanding.

It is worth emphasising that whilst normal $S_0$ and charge measurements provide useful insights into the proportion of hydrophobic and charged groups, their distribution along the protein chain is also of considerable importance, particularly for the former. A previous theoretical study has provided evidence for this, indicating that a biopolymer with localised hydrophobic patches is more desirable as a stabiliser compared to one with uniformly distributed hydrophobic groups [42]. This theory has been further confirmed by a recent practical study on synthetic plant protein peptides by García-Moreno et al. [43]. The distribution of hydrophobic residues significantly influences the adsorption energies,

configuration of the absorbed proteins lying on the surface and consequently, the thickness of the resulting interfacial films [28,43]. Nevertheless, a more systematic study on legume proteins, undergoing enzymatic hydrolysis, is needed to better elucidate the variation of the conformation adopted by hydrolysates and the relation to their surface properties. In particular, a comparison of the behaviour of fragments with hydrophobic patches intermittently distributed along the protein backbone versus those possessing hydrophobic patches that reside close to the terminus ends should prove very interesting in a future research study in this area.

### Size distribution

The tendency of legume proteins to aggregate is a key feature constraining their use in molecularly adsorbed emulsification studies. Due to the peptide bond breakage, hydrolysis is believed to be effective in breaking up the aggregated legume protein particles so that the hydrolysate behaves as a rather molecularly adsorbed type emulsifier (or close to it), instead of a particulate (Pickering) emulsifier (Figure 1b). Studies on soybean and mung bean proteins have all demonstrated a dramatic size reduction after hydrolysis as shown in Figure 2b. Additionally, in the work of Wang et al. [44], soy protein, after 1% DH, exhibited a unimodal size distribution below 10 nm. However, the efficacy of different selective enzymes varied. Trypsin being a specific enzyme showed a superior capacity to decrease the size of soy protein (up to a 65% decrease to 80 nm at 8% DH) when compared to Alcalase with its limited specificity (a 15% decrease to 190 nm) [9]. Nevertheless, this size reduction may reverse after reaching a certain threshold of %DH. For instance, Hao et al. [45] observed a reduction in the population of particles larger than 10 μm and an increase in those smaller than 1 μm at 1–5% DH. However, this trend reversed at 6% DH where a greater number of larger particles was detected. However, such aggregation is unfavourable in terms of using such hydrolysed legume proteins as molecular emulsifiers. Thus, the size measurement with microstructural analysis may provide important information for distinguishing molecularly adsorbed versus Pickering emulsifiers. If the hydrolysed protein reaggregates and behaves as a particle to form Pickering-type emulsions, then the use of enzymatic hydrolysis might not necessarily be the ideal processing approach to improve the emulsifying properties of the given legume protein. Regarding particles, surface chemistry and resulting contact angle play the most important role in regulating their surface behaviour, regardless of the interior structure, whether it consists of either intact protein or small hydrolysed peptides. So, for example, the influence of Mw on molecular diffusion becomes totally irrelevant when considering such particulate emulsifiers.

For emulsifying properties of molecularly adsorbed hydrolysates, the greater the decrease in particle size, the more pronounced was the enhancement in EAI. Similarly, better emulsion stability and submicron-sized fine droplets were observed respectively, following an appropriate reduction in particle size, in the two studies reproduced in Figure 2b. In summary, reducing particle size of legume protein through enzymatic hydrolysis is beneficial to allow molecular adsorption of legume hydrolysates on surfaces. However, the possible reaggregation behaviour and resulting increase in particle size of formed protein fragments still require further investigation, particularly with various types of legume proteins under different reaction conditions.

## Effects of hydrolysis on lubrication properties: an opinion

We have discussed the effects of enzymatic hydrolysis on several physicochemical properties and subsequent emulsifying and interfacial behaviours of legume hydrolysates on oil/water interface. We now aim to extend this knowledge to another type of surface, which is soft solid surface that is weakly polar, more representative of oral surfaces. Such surface behaviour is relevant to oral lubrication properties of legume proteins and can be measured by multiscale tribology. Oral lubrication has been recognised as a meaningful *in vitro* parameter for assessing friction-related mouthfeels of foods, such as creaminess, smoothness, and even astringency [3]. Previous tribological studies have demonstrated high coefficient of friction between soft hydrophobic surfaces for several plant proteins, including pea, lupin, and soybean protein [46,47]. The proposed explanation for this phenomenon was lubrication failure caused by plant proteins that tend to form aggregates. More specifically, the accumulation of aggregated protein particles leads to jamming in the contact zone, which hinders the flow of proteinaceous lubricants hindering sliding [46,48]. In addition, the irregular morphology and large size of protein aggregates have also been identified as creating asperities contributing to poor lubrication. To date, there has been only one notable study investigating the effects of hydrolysis on lubrication. In this study, hydrolysates derived from soy proteins were used to form hydrogel systems that exhibited lower oral frictions and better lubricity compared to the less satisfactory lubrication performance of nonhydrolysed parent soy protein [49].

Related to the changes in physical and surface adsorption properties induced by hydrolysis, we hypothesise that moderate enzymatic hydrolysis could improve the oral lubrication performance of aqueous legume proteins through the following potential mechanisms:

1. Reduced particle size: An appropriate %DH can reduce legume protein aggregation. The size of the

resulting hydrolysate particles could be within the scale of the contact gap between the oral surfaces, allowing these hydrolysates to be entrained unlike the jamming effects often reported in parent legume proteins.

2. Altered surface hydrophobicity: Hydrolysis may increase the exposure of buried hydrophobic region and/ or can also increase the concentration of hydrophilic moieties. Such increased surface hydrophobicity can enhance adsorption strength and entrainment, whilst the hydrophilic groups may provide hydration lubrication. Importantly, the subtle balance between the hydrophobic and the hydrophilic groups is critical to determine the ultimate lubrication performance, which requires careful control via enzymatic hydrolysis and further investigation. Therefore, more research attention is worth being given to this area to better understand the effects of enzymatic hydrolysis and explore novel strategies for addressing the undesirable mouthfeel of legume proteins.

## Conclusions and future perspectives

This review has summarised the physicochemical and surface-related functional properties of legume proteins after enzymatic hydrolysis. Hydrolysis has been demonstrated to be an effective tool for inducing structural changes based on various reaction conditions applied. The %DH remains the most commonly used parameter for evaluating the extent of reaction. The type and specificity of enzyme is one of the most critical factors for producing hydrolysates with desired functional properties. According to the current literature, enzymatic hydrolysis can create smaller hydrolysate fragments, conferring faster surface adsorption, more disordered structures, and smaller particle sizes. These physicochemical alterations work synergistically to potentially enhance the interfacial and emulsifying properties of legume proteins, particularly when the original properties are inferior. Furthermore, changes in surface hydrophobicity and charge are challenging to predict as they largely depend on the cleavage sites and the original protein sequence but play a crucial role in the functional performance of legume proteins.

In the future, considering the conformational rearrangement of proteins upon adsorption at oil/water interface, the secondary structure of hydrolysates when present at the emulsion droplet surface would provide valuable information on how structure is influencing the interfacial properties. Precise measurements of this feature are currently lacking on legume proteins in the literature, thus further investigations would be valuable. In addition, more attention should be given to the interfacial shear rheology of legume protein hydrolysates, which has yet to be thoroughly studied, to provide insights into how the interfacial film responds under shear deformation. Knowledge gap has been identified in the oral lubrication performance of legume proteins post hydrolysis, both with and without the presence of human saliva. Such mechanistic knowledge on tribological performance is crucial before confirming that enzymatic hydrolysis is a useful processing strategy to reduce astringency of legume proteins, and needs validation by sensory studies.

## Data Availability

No data were used for the research described in the article.

## Declaration of Competing Interest

None.

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