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Mclauchlan, J., Tyler, A., Chakrabarti, B. et al. (2 more authors) (2024) Oat protein: Review of structure-function synergies with other plant proteins. Food Hydrocolloids, 154. 110139. ISSN 0268-005X

<https://doi.org/10.1016/j.foodhyd.2024.110139>

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Oat protein: Review of structure-function synergies with other plant proteins

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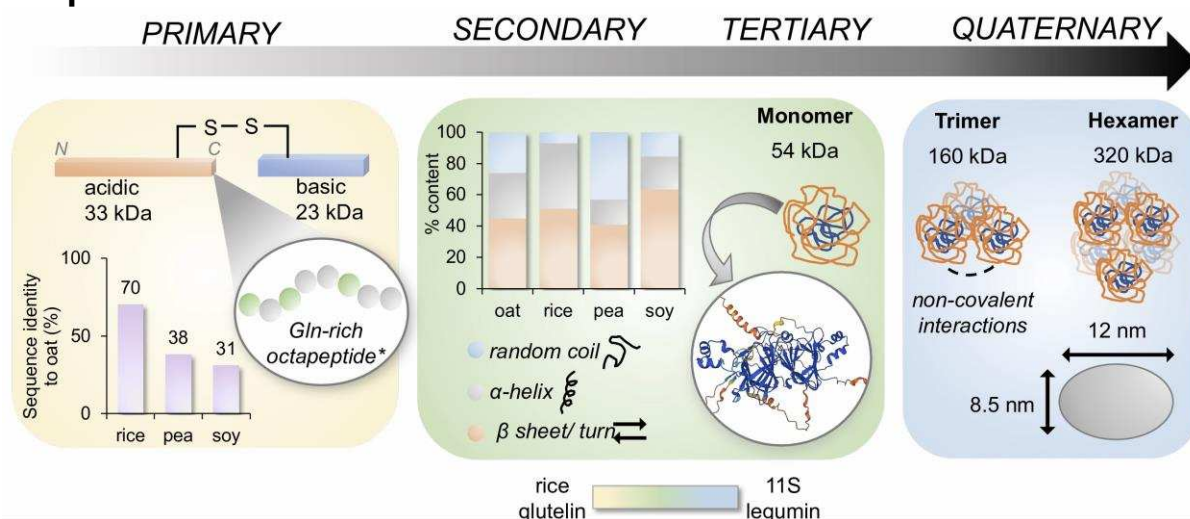
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21 Graphical abstract



22 Highlights

- 23 Self-assembly of oat 12S globulin is covered at multiple length scales
- 24 Oat globulin structure is compared to rice glutelin, pea legumin and soy
- 25 glycinin
- 26 Aqueous, interfacial and gelation behaviour of oat protein isolate are
- 27 discussed
- 28 Learnings from rice, pea and soy are used to complement oat protein data
- 29 We identify a lack of fundamental studies on oat protein structure-functionality
- 30
- 31

ABSTRACT

Widespread adoption of plant-based alternatives over animal derived proteins provides a sustainable path to ensure food security for an increasingly growing population. Oat proteins, in particular, have generated noteworthy interests due to their high nutritional value, low environmental footprint and lack of allergenicity compared to other plant proteins. However, limited aqueous solubility at neutral pH can impair the techno-functional performance of oat proteins. Therefore, this review aims to provide a comprehensive understanding of structure-function relationships of oat proteins. In particular, we focus on current structural knowledge of the major oat protein fraction, 12S globulin, at three different length scales: (i) primary structure and amino acid composition, (ii) secondary structure and (iii) tertiary and quaternary protein structure. We then proceed to discuss (i) their properties in solution, *i.e.*, solubility, surface hydrophobicity and surface charge in aqueous systems, (ii) their interfacial behaviour at air-water and oil-water interfaces, and (iii) their gelation and simple coacervation behaviour. We identify gaps in structural information and functional properties of oat proteins throughout and, where possible, complement these with parallel knowledge drawn from the cereal (rice) and legume family (pea, soy). Whilst oat proteins share stronger genetic similarities to rice based on amino acid sequence, their folded structure and overall functionality are surprisingly closer to legumes. Finally, we also emphasise the need for further structural and interfacial characterisation of oat proteins, in addition to an evaluation of their mouthfeel performance to increase their applications in sustainable plant protein-based food design.

Keywords: Globulin; solubility; surface hydrophobicity; interfacial tension; gelation; coacervation

59 Table 1. List of abbreviations used in this article.

OPI	Oat protein isolate
RPC	Rice protein concentrate
SPI	Soy protein isolate
PPI	Pea protein isolate
pI	Isoelectric point
M _w	Molecular weight
XRD	X-ray diffraction
RMSD	Root mean square deviation
DLS	Dynamic light scattering
SAXS	Small angle X-ray scattering
T _d	Thermal denaturation temperature
AFM	Atomic force microscopy
AF4	Asymmetric flow field flow fractionation
H ₀	Surface hydrophobicity
ANS	8-anilinonapthalene-1-sulphonic acid
γ ₀	Equilibrium surface tension
SDS-PAGE	Sodium dodecyl-sulphate polyacrylamide gel electrophoresis
DTT	Dithiothreitol
GDL	Glucono-δ-lactone
I	Ionic strength
κ ⁻¹	Debye screening length
QCM-D	Quartz crystal microbalance with dissipation
G'	Storage modulus
G''	Elastic modulus
MGC	Minimum gelation concentration

61 1. INTRODUCTION

62 A paramount challenge for the food industry is the development of sustainable and
63 healthy alternative products to maintain a growing global population. Food production
64 alone is responsible for approximately one third of global greenhouse emissions, with
65 animal derived products accounting for 57% of food emissions (Crippa et al., 2021; Xu
66 et al., 2021). Despite the recent popularity of the vegetarian and vegan movements
67 leading to larger availability of new plant-based products, plant proteins suffer from
68 poor techno-functionality, *i.e.*, high surface hydrophobicity, increased aggregation and

limited aqueous solubility at neutral pH, which restricts their widespread food applications. Sensorial aspects are also a key limitation since alternative proteins can be associated with astringency, off-flavours and poor oral lubrication performance (Kew et al., 2023; Kew, Holmes, Stieger, & Sarkar, 2021; Liamas, Connell, & Sarkar, 2023; Tanger et al., 2022b; Vlădescu et al., 2023).

Among plant proteins, wheat proteins and legumes, in particular soy proteins, are extensively studied in the literature and used by the food industry to date. However, significant potential lies in the adoption of oat proteins due to their relatively higher protein content (typically 10-20% in oat groats compared to 7-12% in other cereals) sustainable production, low cost, and low allergen content compared to other alternative protein sources including legumes and wheat (Dhanjal, Sharma, & Prakash, 2016; Mäkinen, Sozer, Ercili-Cura, & Poutanen, 2017; Spaen & Silva, 2021). However, underutilisation of oat proteins is often traced back to their limited solubility at neutral and mildly acidic pH conditions (Ma, 1983).

Importantly, both protein solubility and extractability are reported to be influenced by heat treatment or kilning of oats used to prevent rancidity via lipase action (Runyon, Sunilkumar, Nilsson, Rascon, & Bergenståhl, 2015; Wouters & Nicolai, 2024). The location of the protein within the oat grain may also have an effect on its extractability and functional properties. Oat storage proteins including 12S globulins are typically found in protein bodies within cells of the starchy endosperm (Klose & Arendt, 2012). The aleurone layer also contains protein bodies in the form of globoids and carbohydrate-protein bodies. Finally, the bran contains different types of proteins, often associated with the cell wall. This indicates that protein structure and composition vary depending on their location in the grain, and their functionality may vary due to associations with other components, e.g. binding to polysaccharides. Therefore, it is important to delve into structure-function relationships of oat proteins in order to effectively overcome techno-functional limitations and improve widespread acceptability.

Remarkably, oat protein shares more similarities with legumes over other cereals based on Osborne classification. Osborne divided cereal proteins into four fractions according to their relative solubilities, namely water-soluble albumins, saline-soluble globulins, alkaline-soluble glutelins and alcohol-soluble prolamins (Osborne, 1907). As shown in Table 2, albumin and prolamins contribute 1-12% and 4-15% of total oat protein respectively, whilst <10% is made up of glutelin fractions.

Importantly, the major Osborne fraction in oat is not prolamin or glutelin, as observed in most other cereals, but globulins which are similarly predominant in legumes. The oat globulin fraction can also be further separated into 2S, 7S and 12S fractions based on their sedimentation coefficient. The latter dominates the oat globulin fraction and thus will be the focus of this review. Although the major fraction in rice is glutelin (Table 2), genomic organisation, sequence characteristics and expression patterns of oat storage proteins were all found to be closer to rice and other dicotyledonous plants than, *e.g.*, wheat (Kamal et al., 2022). Further understanding of oat protein structure and what aspects are important for functionality may aid in systematic oat protein modification to improve its properties.

Table 2. Approximate protein content and Osborne classification of select cereals and legumes (Boukid, 2021; Fukushima, 1991).

Plant family	Crop	Protein content (g/100g)	Osborne Classification (% of total protein)			
			Albumin	Globulin	Prolamin	Glutelin
Cereals	Oat	10-20	1-12	70-80	4-15	<10
	Rice	7-10	Trace	2-8	1-5	80-90
	Barley	10-16	3-4	10-20	35-45	35-45
	Wheat	10-15	3-5	6-10	40-50	30-40
Legumes	Soybean	36-40	10	90	N/A	N/A
	Pea	23-31	21	66	N/A	12
	Broad bean	23	20	50	N/A	15

Therefore, aim of this review is to understand current structural knowledge of the 12S globulin oat protein fraction and attempt to link its structural properties to protein functionality. Other recent reviews on oat protein have highlighted its potential as an emerging ingredient and covered important aspects such as an overview of oat protein structure and composition, the effect of extraction methods and processing on protein functional properties as well as improvements *via* enzymatic and chemical treatment (Boukid, 2021; Kumar, Sehwat, & Kong, 2021; Mel & Malalgoda, 2021; Spaen et al., 2021).

Herein, we explore the structural aspects further by methodically providing a description of the major oat globulin fraction (12S globulin) across length scales, from amino acid composition to folded protein structure, highlighting key similarities and differences to other plant proteins (rice, pea and soy). We then use these structural findings to understand (i) the behaviour of oat protein molecules in solution (aqueous phase) including solubility, surface hydrophobicity, and surface charge, (ii) oat protein

behaviour at air-water and oil-water interfaces and (iii) oat protein gelation and coacervation properties. Whilst previous reviews have covered an excellent overview of oat protein techno-functionality by comparing structure to functional properties such as emulsification activity, foaming capacity and water/fat binding content, we focus on fundamental colloidal science across length scales, *e.g.*, by discussing interfacial tension rather than emulsifying activity and by attempting to understand the structural interactions involved in gelation. Additionally, we take a different approach, whereby, gaps found in the literature in oat globulins are addressed via predictions and comparisons with other plant proteins based on similarities in structural features using complementary studies. Since legumes analogously contain globulins as a principal storage protein component (Table 2), these are included in the review. Specifically, soy and pea are selected as well-studied representatives of legume protein behaviour. Rice protein is also explored since it has been reported as the cereal which most closely resemble oat protein structure (Robert, Nozzolillo, & Altosaar, 1985b; Shotwell, Afonso, Davies, Chesnut, & Larkins, 1988a). Importantly, studies on rice, pea and soy are only used in this review where their findings may help understand oat protein structure and function. Any plant protein studies beyond this are considered out of scope.

2. MULTISCALE STRUCTURE OF OAT 12S GLOBULIN

Oat protein consists of approximately 70-80% salt-soluble globulins according to the Osborne classification (Table 2). These are typically heterogeneous and can be further categorised according to their sedimentation constant, *S*, into 3S, 7S and 12S (Peterson, 1978). Whilst glycosylated 3S and 7S globulins have been identified in oats *via* sugar density ultrafiltration, Burgess *et al.* determined that 12S globulin is the most prevalent fraction and relatively low quantities of 3S and 7S were present (Burgess, Shewry, Matlashewski, Altosaar, & Mifflin, 1983). For the reasons outlined, 12S globulin will be the focus of this section.

Briefly, oat 12S globulin consists of two polypeptide chains, α and β , which are linked via a disulphide bond, as shown in the structural illustration across multiple length scales (Figure 1). Similar to legumes, α and β subunits are synthesised as a single polypeptide (proglobulin) which subsequently undergoes post-translational cleavage by asparaginyl endopeptidase between a conserved Asp at position 317 and Gly at position 318 (Robert, Nozzolillo, & Altosaar, 1985a; Shotwell *et al.*, 1988a;

Tandang-Silvas et al., 2010). The α polypeptide has a higher molecular weight (M_w) of ~33 kDa and is characterised by its higher hydrophilicity and acidity than the β polypeptide, *i.e.*, an isoelectric point (pI) of between 4 and 5 (Burgess et al., 1983; Klose et al., 2012; Li & Xiong, 2021b; Peterson, 1978; Shotwell, 1999). In contrast, the β polypeptide has a smaller M_w of ~23 kDa and a more basic pI range of 7-8.

In the following sections, current knowledge of how native oat 12S globulin folds and assembles into hexameric complexes is covered. Particularly, we examine how it compares to major storage proteins from rice (rice glutelin) and legumes (pea legumin and soy glycinin) with the aim of gaining insights on structural features which may influence protein techno-functionality.

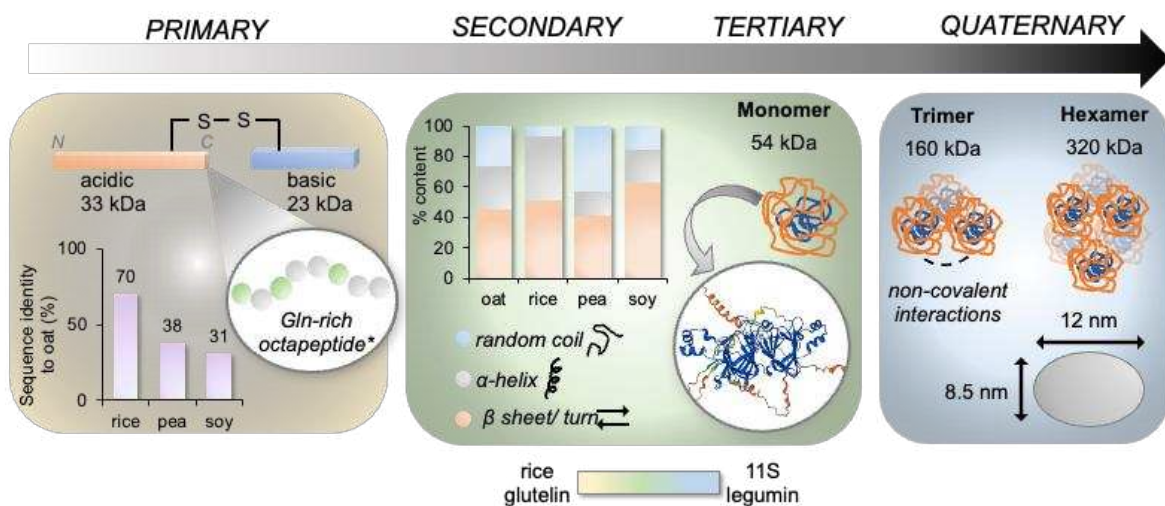


Figure 1. Summary of current understanding of oat 12S globulin self-assembly from its primary structure (left) to its native quaternary folded states (right) (Jiang et al., 2015b; Li et al., 2021b; Liu et al., 2011; Shotwell et al., 1988a; Subirade, Gueguen, & Schwenke, 1992; Zhang et al., 2022). Values for sequence identity from alignment of oat globulin to rice glutelin, pea legumin and soy glycinin were reported by Shotwell *et al.* (Shotwell et al., 1988a). Similarities to rice glutelin and 11S legumins are highlighted here at different length scales via colour coding (yellow: stronger structural resemblance to rice glutelin; blue: stronger structural resemblance to 11S legumins; green: exhibits properties of both rice glutelin and 11S legumins). The Gln-rich octapeptide (*) at the C-terminus of the acidic (α) polypeptide is highlighted as a previously identified unique structural feature of oat proteins. Due to a lack of experimentally defined structure, the predicted folded 3D monomeric structure of oat 12S globulin prior to post-translational modification was obtained using AlphaFold (Jumper et al., 2021). Dimensions of the hexameric structure were obtained from a dynamic light scattering study of extracted oat globulin in solution (Zhao, Mine, & Ma, 2004).

2.1 Primary structure

Primary sequence is the blueprint for protein folding. From the late 1970s onwards, separation of seed storage proteins from cereals and legumes using sodium dodecyl-

189 sulphate polyacrylamide gel electrophoresis (SDS-PAGE) led to their successful
190 isolation and characterisation (Gwiazda, Schwenke, & Rutkowski, 1980; Kitamura &
191 Shibasaki, 2014; Peterson, 1978; Shotwell, 1999; Villareal & Juliano, 1978; Wen &
192 Luthe, 1985). These typically included amino acid composition analysis (in mol%) via
193 an acid hydrolysis method. More recently, the use of genome sequencing has
194 accelerated understanding of plant protein structures, for example via the publication
195 of the oat genome in 2022 (Kamal et al., 2022). In this section, amino acid composition
196 analysis followed by sequence alignment similarities are used to compare the structure
197 of oat 12S globulin with rice glutelin and 11S legumins (pea legumin, soy glycinin).
198 Note that whilst 11S and 12S globulins are hexameric proteins which have been
199 modified by post-translational cleavage, the sequences used for alignment and amino
200 acid comparisons are of individual monomers containing α and β subunits prior to post-
201 translational cleavage. Care must be taken with interpretation of amino acid
202 compositions here since reported values are relative to the chosen extraction methods
203 and crop varieties used in each study (Amagliani, O'Regan, Kelly, & O'Mahony, 2017).

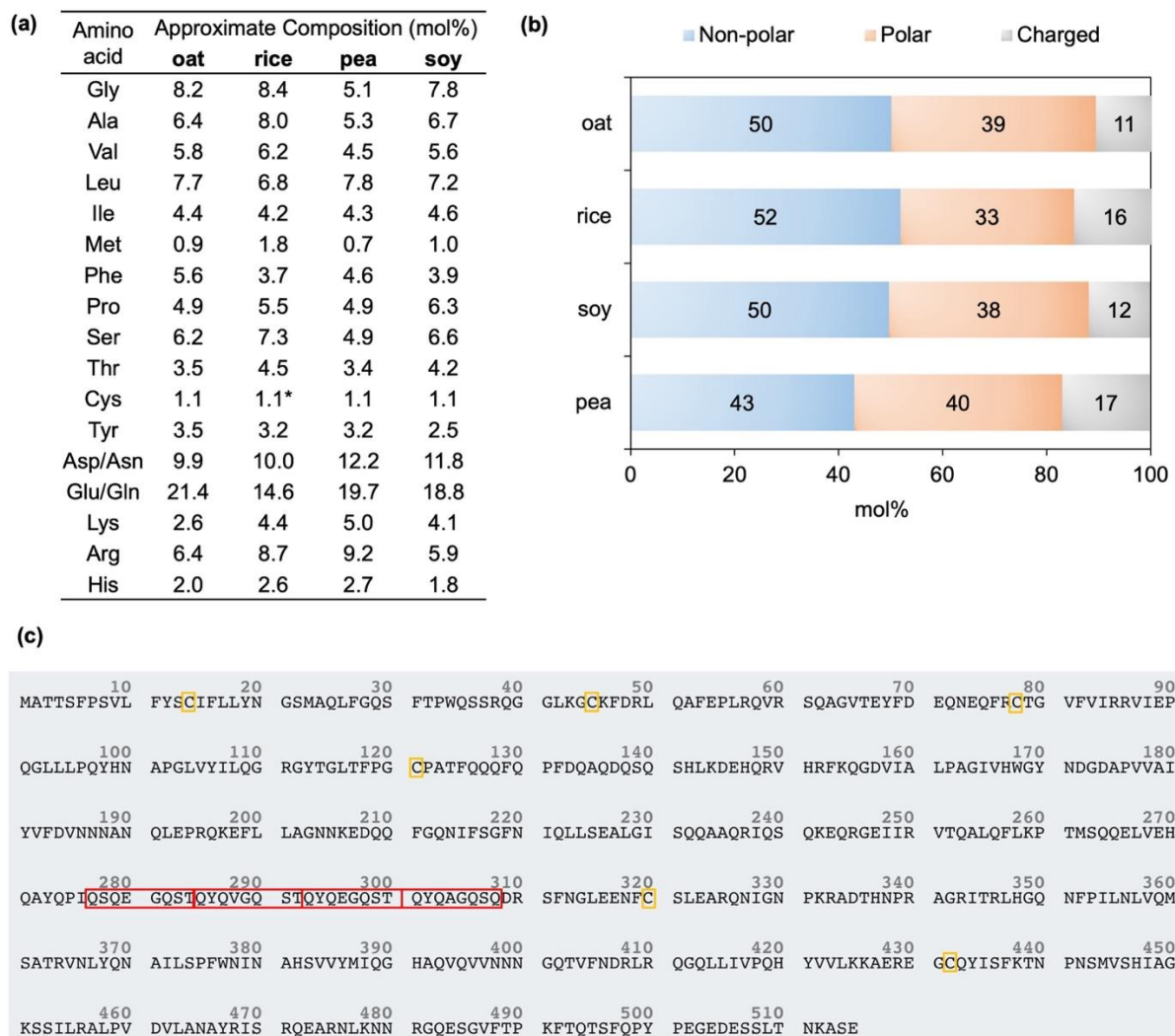


Figure 2. Amino acid analysis and amino acid sequence of oat 12S globulin monomers compared to cereal (rice glutelin) and selected legume (11S globulins, pea and soy) monomers. (a) Amino acid composition values shown here were reported for rice glutelin and 11/12S globulin fractions in oat, pea and soy (Gwiazda et al., 1980; Kitamura et al., 2014; Peterson, 1978; Shotwell, 1999; Wen et al., 1985). Cys value for rice glutelin (*) was obtained from a separate source (Villareal et al., 1978). No values for Trp were identified since it degrades under standard acidic conditions used for amino acid analysis (Friedman, 2018). During acid hydrolysis, Asn and Gln are converted into Asp and Glu, so isolated values were not distinguished using this method (Holt, Milligan, & Roxburgh, 1971). Figure (b) shows the relative mol% of polar, non-polar and charged amino acids calculated based on values in (a). Figure (c) is the amino acid sequence for a monomer of 12S oat globulin prior to post-translational cleavage taken from UniProt database: O49258. Cysteine residues are highlighted in yellow whilst the Gln-rich region containing 4 octapeptides is highlighted in red.

Figure 2a shows the amino acid composition for a combination of α and β polypeptides in the major fractions of oat, rice, pea and soy proteins. Values highlight a key property of seed storage proteins: a high content (>24%) of amidic building blocks Gln/Glu and Asn/Asp. This corresponds to their biological role as a source of

carbon and nitrogen to aid seedling growth after germination (Derbyshire, Wright, & Boulter, 1976; Fukushima, 1991). Note that composition values of Gln and Asp are shown together with Glu and Asp respectively, as these cannot be distinguished by the acid hydrolysis method (Holt et al., 1971). However, analysis of cDNA clones which encode oat seed storage globulin suggest that uncharged Gln and Asn are more abundant than their charged counterparts in the native oat protein state, which may have implications in solubility. For example, Shotwell *et al.* report a ratio of 2.5:1 of Gln:Glu and 2.3:1 of Asn:Asp, leading to predicted mol% values of 15.2% Gln, 6.1% Glu, 6.9% Asn and 3.9% Asp (Shotwell, 1999; Shotwell, Afonso, Davies, Chesnut, & Larkins, 1988b).

Another similarity between oat, pea and soy globulins shown here is their sulphur content, since Cys and Met make up approximately 2 mol% (Shotwell, 1999; Shotwell et al., 1988a). Cys residues are structurally important due to their role in disulphide bond formation between α and β polypeptides. However, it is worth noting that the Lys and Phe content is different in oat globulins compared to the other proteins, with Lys being at lower (2.6 mol%) and Phe at higher concentrations (5.6 mol%).

In Figure 2b, the amino acid analysis was separated into 3 categories: polar, non-polar and charged residues. Note that a hydrophobic scale categorisation was not used, as their accuracy is debated within the literature due to variability with environmental conditions and starting materials used (Moelbert, Emberly, & Tang, 2004; Simm, Einloft, Mirus, & Schleiff, 2016). Charged amino acids are shown to be less abundant (11-17%) in all proteins investigated compared to non-polar residues (43-52%). Oat contained the lowest percentage of charged residues (11%), with values closest to soy (12%), whilst pea legumin exhibits the overall lowest composition of non-polar residues (43%). However, these values can vary between source types and only subtle differences between the proteins investigated are observed.

To further distinguish oat from the other plant proteins investigated, we next outline the sequence alignment of monomers prior to post-translational cleavage conducted by Shotwell *et al.* which provides information on residue locations within the peptide chain (Shotwell et al., 1988a). This delivers additional insight into residues predicted to be exposed at the surface, which often defines functionality. In this study, the authors conducted genome sequencing of oat globulin and subsequent alignment to rice glutelin, pea legumin and soy glycinin. As we summarise in Figure 1, high

sequence identity was found with rice glutelin (70%) over pea (38%) and soy (31%) legumins.

We confirmed the high sequence identity to rice reported by Shotwell *et al.* by running a BLAST function in the National Center for Biotechnology Information database with a separate 12S oat globulin monomer sequence published by Schubert *et al.* (Altschul *et al.*, 1997; Schubert, Bäumlein, Czihal, & Wobus, 1990). The BLAST function gave similar values of 60-67% sequence identity depending on the rice variety selected, consistent with previous reports of a closer evolutionary relationship of oat proteins to rice (Robert *et al.*, 1985b). Similar to variation in protein content and composition in different rice varieties, variation is also observed between oat varieties (Mel *et al.*, 2024). To test the effect of this variation on our sequence analysis, we selected another oat sequence in the genomic database and repeated the BLAST analysis. Similar results were obtained confirming high level of sequence identity amongst oat proteins. Therefore, oat globulin was confirmed to resemble rice glutelin structure more closely than soy and pea legumins at the amino acid sequence scale.

Despite the high similarities reported thus far, Shotwell *et al.* previously highlighted 3 major differences between oat globulin and rice glutelin monomers following sequence alignment (Shotwell, 1999; Shotwell *et al.*, 1988a). These include (i) an extra 7 amino acid insertion in the middle of the acidic polypeptide of the rice sequence, and (ii) additional residues at the C-terminus of the basic polypeptide in oat. The third difference appears to be a unique property of oat globulins alone, *i.e.*, the presence of highly conserved 4 to 5 repeats of a Gln-rich octapeptide in the C-terminus of the acidic α polypeptide (Figure 2c). Whilst in rice glutelin the hypervariable region is also rich in Gln, these are not organised into the repeats observed in oat 12S globulin. Since the Gln-rich octapeptide is less hydrophilic than the same region in soy 11S globulin and is proposed to reside at the surface of the protein, *i.e.*, in contact with solvent, authors have also suggested it may influence differences in solubility between oat versus other plant globulins (Shotwell *et al.*, 1988a). Whilst this is often mentioned as a potential reason for discrepancies compared to legume 11S globulins, there is no simulation-based evidence to date which confirms the effect of this octapeptide on functionality despite its potential implications.

2.2 Secondary and tertiary structure

The next section explores amino acid chain folding at the secondary and tertiary structure level. Briefly, amino acid chains can typically fold into α helices, β -sheets (parallel and antiparallel) or random coils via a range of non-covalent interactions, most notably, *via* hydrogen bonding. Based on studies which conducted secondary structure determination using circular dichroism spectroscopy and/or Fourier-transform infrared spectroscopy (FT-IR) presented in Figure 1, all proteins investigated were determined to contain a high number (>40%) of β -sheets and turns, with soy showing the highest value at 63%. The oat protein isolate (OPI) sample showed similar content of α helices (29%) and random coils (26%) to soy. Notably, a higher α helix content was observed in rice glutelin (42%), whilst this value was lower in pea (16%). Since β -sheet conformations predominated in all 4 protein structures, oat globulin secondary structure was determined to share properties with seed proteins from both legumes and rice.

Whilst secondary structure analysis of oat globulin is readily available, there is a distinct lack of X-ray diffraction (XRD) structural data for oat globulins and rice glutelin. Therefore, structure prediction based on their primary amino acid sequences using AlphaFold was used to compare tertiary structures with a previously obtained 12S globulin monomer sequence containing both α and β subunits prior to post-translational cleavage (Jumper et al., 2021; Schubert et al., 1990). The folding algorithm predicts a conserved jelly roll β -barrel for both proteins at levels of very high confidence. For oat globulin, this is circled in the monomer structure in Figure 3. However, less can be inferred from the terminal sequence at the protein surface due to lower model confidence.

Despite the lack of information on oat, recent structural investigations on legume proteins, including pea and soy, have been conducted, likely as a result of their popularity as meat substitutes. X-ray diffraction (XRD) patterns of crystallised legumins and prolegumins have identified two extended helical domains in the 11S monomer and two highly conserved jelly roll β -barrel, composed of 8 β -strands in 4 antiparallel sheets (Adachi, Takenaka, Gidamis, Mikami, & Utsumi, 2001; Tandang-Silvas et al., 2010). These results match the AlphaFold prediction for oat and rice, with all proteins belonging to the cuprin superfamily characterised by their highly conserved barrel region in the protein core. The predicted locations of these regions in oat 12S

globulin are highlighted in Figure 3ai. Therefore, all proteins investigated here were deemed to share secondary and tertiary structure features.

It should also be noted that the hydrogen bonded network of the β -strands in the jelly roll barrel regions is integral for protein stability, resulting in resistance to degradation by temperature, pH and non-specific proteolytic enzymes (Dunwell, Gibbings, Mahmood, & Saqlan Naqvi, 2008). Increased stability accounts for the conservation of the amino acids involved in β -polypeptide (core) formation over the α -polypeptide (surface) across oat varieties (Moreira, Hermodson, Larkins, & Nielsen, 1979; Wen et al., 1985). It has also been reported that variation is larger in the number of helices than β -strands among 11S globulins, once again confirming the conservation of the structurally important jelly roll barrel across oat species (Tandang-Silvas et al., 2010).

To validate the AlphaFold model of oat 12S globulin monomer, in Figure 3c a soy 11S legumin monomer structure obtained via XRD was overlaid with the predicted structure of the same amino acid sequence (PDB Identifier: P04776). The structure alignment was conducted in ChimeraX software and gave a root mean square deviation (RMSD) value of 0.991 Å across all residue pairs which suggests a good model fit. An RMSD value of 0 Å corresponds to identical structures, whilst a RMSD of 2.0 Å and below is commonly accepted to determine that two protein structures are similar (Yusuf, Davis, Kleywegt, & Schmitt, 2008). The tertiary structure sections shown in green (Figure 3ci) correspond to areas in which there were significant differences between the crystalline structure and the predicted AlphaFold structure. In particular, it was determined that the predicted β subunit structure, corresponding to the core of the protein (jelly roll β -barrel and extended helical section) matches the XRD structure almost perfectly. The area of highest discrepancies between the two structures were at the α subunit terminals, with particularly significant differences in the C-terminus. Therefore, we conclude that AlphaFold provides useful insight into the core (β subunit) of the oat 12S globulin monomer. However, without XRD data, less can be inferred from the predicted α subunit tertiary structure at this stage, which is likely exposed at the protein surface.

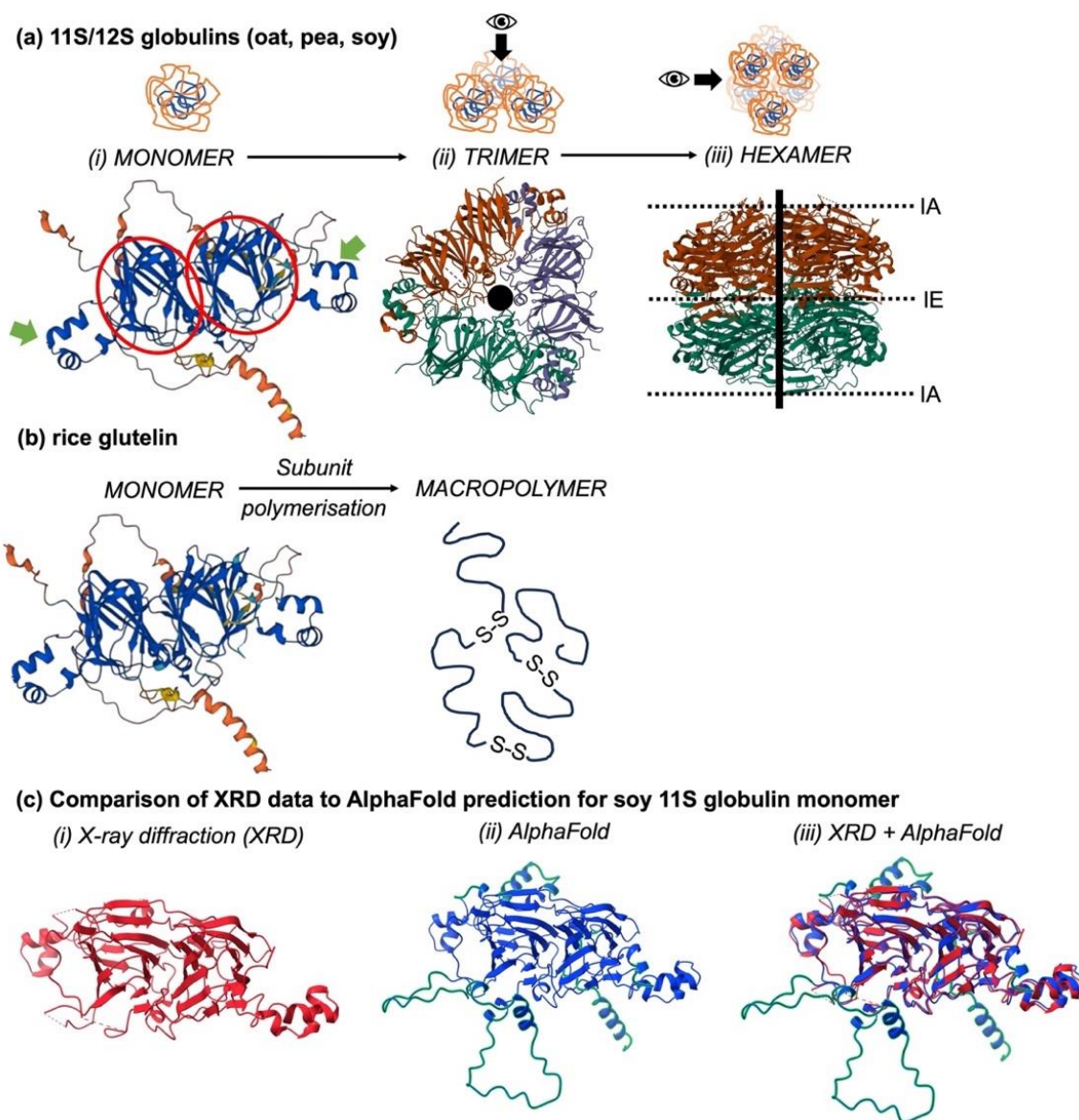


Figure 3. Tertiary and quaternary structure development of (a) 11S/12S globulins and (b) rice glutelin alongside a comparison of (c) X-ray diffraction (XRD) data overlaid with predicted AlphaFold structure for soy 11S globulin monomer. In (a), images of the predicted oat globulin monomer structure (i) were obtained by running the oat 12S globulin sequence in Figure 1c in AlphaFold (Jumper et al., 2021). A conserved jelly roll region in oat 12S monomer is circled in red whilst green arrows point to the extended helical domains. Since XRD data is unavailable for oat globulin, pea trimer (ii) and soy hexamer data (iii) are shown with PDB identifiers 3KSC and 1OD5. The viewing angle of the trimer and hexamer is depicted using the cartoon above each image. The three-fold axis is shown with a black circle/ bold line depending on the viewing angle. IA and IE faces are labelled in (iii) using dashed lines (Adachi et al., 2003). In (b), no hexamerisation takes place as rice glutelin exists as a macropolymer which we depict schematically. The macropolymer is formed *via* intermolecular disulphide linkages between α - and β -subunits (Amagliani et al., 2017). In (ci), XRD data was obtained from PDB identifier P04776. AlphaFold structure prediction shown in (cii) was performed on the same sequence whilst both structures were

overlaid in (ciii). Areas of the AlphaFold structure which differ in tertiary structure from XRD data are outlined in green.

2.3 Quaternary structure

In this section, the folding of oat 12S globulin individual protein monomers into their folded state in solution is explored. A model for oat 12S globulin self-assembly was first proposed by M_w analysis of 12S globulin and its subunits using SDS-PAGE (Peterson, 1978). In 11S legumin fashion, two subunits (α and β) were reported to be present in equimolar amounts within 12S globulin, likely forming a compact hexameric structure held together by non-covalent interactions. In 11S soy globulin, the ratio of α and β subunits has been found to vary slightly according to the variety of soybean used in the study, which may also be the case with oat (Peng, Quass, Dayton, & Allen, 1984).

Oat globulins have not yet been characterised using crystallography, so only the predicted AlphaFold structure for its monomeric form is shown. As previously mentioned, the AlphaFold monomer shows poorer model confidence in the α subunit C-terminal sequence predicted to be at the protein surface. Additionally, the predicted structure is based on a monomeric sequence prior to post-translational cleavage, which has been reported to be a key step when inducing trimer and hexamer stacking (Dickinson, Hussein, & Nielsen, 1989; Jung et al., 1998; Kumamaru et al., 2010; Scott, Jung, Muntz, & Nielsen, 1992; Wakasa, Yang, Hirose, & Takaiwa, 2009). For the reasons outlined, simulations to predict how monomers stack to form the hexameric oat 12S globulin is challenging. We therefore resort to legume trimer and hexamer XRD data as a basis for understanding the mechanism of oat hexamer formation.

Figure 3 shows a model for 11S/12S globulin folding determined by XRD whereby in the first instance, 3 monomeric subunits interact to form a trimer via electrostatic and hydrophobic interactions (Adachi et al., 2001; Plietz, Drescher, & Damaschun, 1988). Legumin trimers are characterised as having two faces, IE and IA (Figure 3), which contain different hydrophilicities and curvatures (Adachi et al., 2003). Within the trimer, the C-terminus of each monomer is located at the surface whilst the N-terminus is near the hollow centre of the trimer (Tandang-Silvas et al., 2010). An XRD study of soy glycinin reported that trimer formation involves stacking of the extended helical regions on adjacent monomer units and also highlighted the importance of non-specific hydrophobic interactions between two IE faces during the association of two trimers (Adachi et al., 2001).

The second stage of self-assembly involves two trimers stacking on top of each other. Stacking of oat globulin is predicted to occur at an offset angle of 60° to form an oblate cylinder, with dimensions of 12 x 8.5 nm (Figure 1) obtained in a laser light scattering study (Shotwell, 1999; Zhao et al., 2004). Similar dimensions were obtained using atomic force microscopy (AFM), which described the OPI hexamer as ellipsoidal with a diameter of 12.1 nm (Liu et al., 2009). These values match the range of dimensions of legume proteins obtained via small angle x-ray scattering (SAXS) and electron microscopy with diameters of 10.4-12.6 nm and heights of 7.5-9 nm (Badley et al., 1975; Plietz, Damaschun, Müller, & Schwenke, 1983; Plietz, Zirwer, Schlesier, Gast, & Damaschun, 1984; Reichelt, Schwenke, König, Pähitz, & Wangermann, 1980; Zhao et al., 2004). In legumes, trimer dimensions indicate that two identical faces stack preferentially to opposite faces, *i.e.*, IA faces do not interact with IE faces upon hexamerisation and vice versa. Specifically, work conducted on soy glycinin revealed that when two trimers associate, their IE faces form stronger interactions due to their lower curvature and higher exposure of hydrophobic residues on the surface (Adachi et al., 2001). This results in IA faces being exposed to solvent as shown in Figure 3a.ii. Whilst IE stacking is speculated to be mirrored in oat globulins, this requires experimental confirmation.

Importantly, 11S hexamer formation in legumes results in stronger thermal stability compared to its trimer precursor since more energy is required to overcome the additional interactions. With soy glycinin, hexamerisation resulted in an increase of approximately 10°C in the thermal denaturation temperature compared to the trimer alone (Tandang-Silvas et al., 2010). Ma and Harwalkar also showed that pre-heating oat globulin (110°C for 60 mins) increased the thermal denaturation temperature (T_d) by up to 3°C (Ma & Harwalkar, 1988a). Authors suggested that the increase in stability is due to re-arrangement of the protein to assume a more compact conformation. Therefore, hexamer packing properties are likely to influence the gelation mechanism of 12S globulin.

A study by Tandang-Silvas *et al.* compared crystal structures of various 11S globulins from different legume sources and related differences to changes in hydrophobicity and thermal stability (Tandang-Silvas et al., 2010). In particular, the authors defined 5 regions of variability within the globulin structure that can lead to different physicochemical properties. The poor thermal stability of pea prolegumin, for

example, was attributed to differences in cavity size, lower number of intramonomer hydrogen bonds, a longer loop length and less Pro residues than proteins from other legumes. Importantly, the cavity size, *i.e.*, the space in the centre of trimers that is inaccessible to the bulk solvent, was found to be larger in pea prolegumin (4492.8 Å³) compared to soy prolegumin A1aB1b (4086.1 Å³). Authors suggested this decreases the hexamer packing efficiency and reduces the thermal stability of the globulin. However, cavity size information is not currently available for oat proteins, and it is unclear if the same parameters are important for functionality.

As shown in Figure 3, the quaternary structure marks the divergence between oat globulin and rice glutelin. In rice glutelin, the monomers do not form the hexameric structure observed in oat, pea and soy globulins. Instead, the monomeric subunits (containing α and β polypeptides) polymerise via intermolecular disulphide bonds and hydrophobic interactions to form a water insoluble macropolymer, as schematically depicted in Figure 3b (Amagliani et al., 2017; Buggenhout, Brijs, & Delcour, 2013; Katsube-Tanaka et al., 2004; Sugimoto, Tanaka, & Kasai, 1986; Van Der Borght et al., 2006). Importantly, the lack of functional properties of rice glutelin compared to 11/12S globulins is attributed to the formation of a significantly higher M_w structure (Katsube-Tanaka et al., 2004).

Although little is currently known about the specific mechanisms that lead to the discrepancies in quaternary structure between oat and rice, differences after the post-translational cleavage of seed storage protein, which occurs in both species, appears to be a critical step in determining protein folding and assembly (Dickinson, Hussein, & Nielsen, 1989; Jung et al., 1998; Kumamaru et al., 2010; Scott, Jung, Muntz, & Nielsen, 1992; Wakasa, Yang, Hirose, & Takaiwa, 2009). For example, Katsube-Tanaka *et al.* hypothesise that in rice glutelin precursors, the exposure of Cys residues at the surface after cleavage possibly drives the polymerisation process (Katsube-Tanaka et al., 2004).

We determined here that oat 12S globulin structure shares properties with both rice glutelin and 11S legumins at different length scales. Similarity to rice is more prevalent at the amino acid sequence level, but clear differences arise in the quaternary structure due to high order polymerisation in rice. Strikingly, quaternary folding of 12S globulin has been suggested to mimic that of legume storage proteins over other cereals which is seldom reported in literature (Burgess et al., 1983; Klose

462 et al., 2012; Peterson, 1978). However, it is clear that information on oat 12S globulin
463 quaternary structure is limited compared to legumes. Although dynamic light scattering
464 (DLS) is commonly used to investigate particle size, oat 12S globulin's ellipsoidal
465 shape and tendency to aggregate increase complexity when trying to characterise
466 individual proteins (Liu et al., 2009). There is also a distinct lack of fundamental
467 crystallography studies and small angle X-ray/neutron scattering, with the former being
468 presumably due to issues with solubility and sample heterogeneity disrupting crystal
469 formation (Adachi et al., 2001; Shigeru, Chan-Shick, Toshiro, & Makoto, 1988).
470 Without precise structural information, it becomes a challenge to precisely determine
471 which features of the quaternary structure affect techno-functionality.

3. FUNCTIONALITY OF OAT PROTEIN ISOLATE

The following section of the review covers current knowledge on oat protein isolate (OPI) functionality. Where possible, oat globulin data is preferentially shown since it is the most prominent fraction. Since specific studies on oat globulin are limited, OPI data is more commonly cited here with the assumption that functionality will likely be dominated by the major 12S globulin fraction. Where data on oats is lacking altogether, structuring properties are derived and complemented with studies conducted on other plant proteins (rice, pea and soy).

Whilst different effects (protein concentration, temperature, pH *etc.*) are decoupled and their effects on protein functionality are discussed separately, it is worth emphasising the complexity of the topic, since all these variables are in reality interconnected. In addition, different extraction methods and starting protein (isolate/concentrate) materials will naturally affect the reported functionality data discussed here. Whilst oat protein studies typically use lab-scale extraction that differ in methodology, soy and pea data is often reported from both commercial protein isolates and lab-scale extracts. Any pre-treatments, e.g. heating, which is common in oat groats to prevent rancidity development, will also likely impact functionality such as interfacial properties or gelation kinetics (Runyon et al., 2015; Wouters et al., 2024). Since the impact of extraction method on functionality is a vast but noteworthy topic, we will not attempt to cover this in detail but instead refer to another review which focuses on oat protein extraction (Spaen et al., 2021). We use such extraction data available where relevant to discuss general trends observed from a compilation of studies.

We begin this section by focusing on properties of OPI in solution, including solubility and surface hydrophobicity in aqueous systems, whilst outlining internal and external factors affecting these. Stage two covers multiple phase properties of OPI, focusing on fundamental understanding of behaviour at both the air-water and oil-water interface. The final section uncovers current knowledge of OPI gelation and coacervation properties.

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Table 3. Physicochemical properties of cereal (oat, rice) and legume (soy, pea) seed storage proteins.

Plant source	Range of isoelectric point (pI)	Relative aqueous solubility (%) at pH 7.0 ^a	Surface hydrophobicity (H ₀ -ANS) ^b	Hydrodynamic radius (nm) at pH 7.0	ζ- potential (mV) at pH 7.0 ^a	Interfacial tension (mN/m) ^c		Thermal denaturation temperature (T _d , °C) ^d	References
						Air-water	Oil-water		
Oat	4.5-7.5	15-40	50-450	50-620	-20 to -30	45-60	N/A	110	(Ercili-Cura et al., 2015; He, Wang, & Hu, 2021a; Immonen et al., 2022; Jiang et al., 2015b; Li & Xiong, 2021a; Li et al., 2021b; Li & Xiong, 2023a; Nieto-Nieto, Wang, Ozimek, & Chen, 2016; Nivala, Makinen, Kruus, Nordlund, & Ercili-Cura, 2017; Zhao et al., 2017)
Rice	3.0-8.0	<10	110-1200	490-2,200	-20 to -30	40-45	10-18*	82	(Amagliani et al., 2017; Amagliani, O'Regan, Schmitt, Kelly, & O'Mahony, 2019; Dai et al., 2022; Felix, Romero, & Guerrero, 2016; Ju, Hettiarachchy, & Rath, 2001; Mileti et al., 2022; Omura et al., 2021; Romero et al., 2012; Xu et al., 2016; Yang, Dai, Sun, McClements, & Xu, 2022c; Zhao, Shen, Wu, Zhang, & Xu, 2020; Zhao et al., 2012)
Soy	4.0-5.0	23.6-97	50-400	21-300	-20 to -30	40-50	25-45	81.2 (11S) 65.7 (7S)	(Karaca, Low, & Nickerson, 2011; Liu & Tang, 2016; Mileti et al., 2022; Omura et al., 2021; Ruiz-Henestrosa, Martinez, Patino, & Pilosof, 2012; Ruiz-Henestrosa et al., 2007; Santiago et al., 2008; Zhao et al., 2020)
Pea	4.0-5.2	11-80	77-400	244-2,000	-18.5 to -30	35-45	13-43	80.8 (11S) 71.7 (7S)	(Cui et al., 2020; Karaca et al., 2011; Kew et al., 2021; Ma et al., 2022a; Omura et al., 2021; Sha & Xiong, 2022; Shen et al., 2023; Shen et al., 2022; Yang et al., 2022b; Zhao et al., 2020)

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*Value of 18 obtained at a degree of hydrolysis of 2%.

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^a Values cited were for protein isolates at low ionic strength (<20 mM) and measurements conducted at room temperature (between 20 and 25 °C). Solubility is defined as protein content in aqueous solution determined via a range of methods including the industry standard (Kjeldahl) and common spectrophotometric assays (Biuret, Lowry, and Bradford).

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^b Surface hydrophobicity (H₀) was determined using a fluorescence probe anilinonaphthalene-8-sulfonate (ANS) in aqueous conditions. Slope of linear regression of fluorescence intensity against sample concentration against a known standard gives H₀ value.

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^c Interfacial tension of protein isolates and concentrates were conducted using the du Nouy ring method, Wilhelmy plate method and/or an oscillating drop tensiometer.

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^d Major Osborne fraction values of T_d shown *i.e.*, pea, soy and oat (globulin) and rice (glutelin). Where possible, 7S and 11S globulin values are distinguished.

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3.1 Behaviour in aqueous solution

3.1.1 Solubility

Protein solubility is an important thermodynamic parameter that can affect the processing conditions needed for a defined application, such as extraction method and heating time (Grossmann & McClements, 2023). Good aqueous solubility can improve functional properties including emulsification, foaming and gelation which in turn affects food product storage quality, mouthfeel, digestibility and other metabolic responses (Deng, Mars, Van Der Sman, Smeets, & Janssen, 2020; Grossmann et al., 2023; Jiang et al., 2015b; Yousefi & Abbasi, 2022). However, most plant proteins have an aggregated state and limited aqueous solubility compared to soluble animal-based proteins, *e.g.*, whey protein, thus limiting their techno-functionality (Kew et al., 2021).

Notably, a few complications in measuring and comparing data on plant protein solubility are that i) fractionation, purification and dehydration processes alter molecular structure and aggregation state ii) other natural components present after extraction can affect solubility including starch, dietary fibres, minerals and lipids iii) cultivar and their environmental growth conditions result in variable solubility values when different cultivars within the same species are used and iv) nitrogen based solubility measurements are highly dependent on protein concentration and an accurate nitrogen-to-protein conversion factor (Amagliani et al., 2017; Grossmann, 2023; Grossmann et al., 2023).

To exemplify this, Table 3 shows a range of solubility values recorded for four types of plant protein isolates at neutral pH. Although it is clear that rice protein appears to be the least soluble, the large ranges reported in the literature make it difficult to conclude a definite hierarchy between oat, pea and soy. However, in comparative studies which exhibit less variation in methodology, pea isolates typically shows lower solubility than soy isolates under a given set of conditions (Karaca et al., 2011; Zhao et al., 2020). Additionally, OPI is frequently referred to as being less soluble than isolates from legume sources, despite their similarities in tertiary and quaternary structure (Nivala et al., 2017; Shotwell, 1999). Therefore, a comparative study between oat and legume proteins, from sources that use comparable extraction/isolation methods, is necessary to further validate these findings.

Whilst factors affecting solubility in oat proteins have been covered in previous reviews (Kumar et al., 2021; Mel et al., 2021), these will be summarised here briefly due to the importance of solubility in influencing other aspects of plant protein

functionality discussed in this review, *e.g.* gelation properties. Note that typically such factors are classified as internal or external, whereby the former is primarily dictated by the type of amino acids inherently present within the protein at its surface, whilst the latter includes variable conditions such as pH, ionic strength, temperature and any additives present in solution that affect the protein functionality (Kramer, Shende, Motl, Pace, & Scholtz, 2012; Yousefi et al., 2022).

3.1.1.1 Surface charge

A key internal factor which influences solubility is protein surface charge. High charge reduces protein-protein interactions by increasing electrostatic repulsion, leading to improved aqueous solubility. A study investigating seven animal-derived proteins found that solubility had a higher correlation with a negative surface charge than changes in molecular weight (Kramer et al., 2012). Authors explain that carboxylate containing residues, *i.e.*, Asp and Glu, have a high charge density and thus promote protein hydration. In oat, rice and legume storage proteins, high quantities of amidic side chains (Figure 2a) result in negative zeta potential values between -18.5 and -30 mV at pH 7.0 (Table 3). Whilst a negative surface charge of these storage proteins results in a higher solubility at pH 7.0 than at their *pI* values, in practice solubility under these conditions typically remains below 40% (Figure 4a). Further modifications to increase net surface charge at neutral pH are also used to enhance the plant protein solubility, for example, by enzymatically converting Gln and Asn side chains into charged carboxylates, Glu and Asp (Jiang et al., 2015b).

3.1.1.2 Molecular flexibility

Although surface charge is often considered the most important internal factor, molecular flexibility is also crucial. Recently, Jiang *et al.* found that OPI solubility was influenced by its secondary structure (Jiang et al., 2015b). Authors suggested that α -helices, β -turns and random coils can be more flexible and dynamic than pleated β -sheet folds. Therefore, a reduction of β -sheet content *via* enzymatic deamidation with protein glutaminase improved OPI flexibility. This was suggested to have contributed to the observed increase solubility compared to the untreated OPI, alongside an increase in net protein charge. Another factor which can affect molecular flexibility is native protein aggregation. Plant storage proteins are often found in a self-aggregated quaternary state for efficient space storage inside vacuoles which increases structural rigidity (Vitale & Hinz, 2005). To counteract this, the mechanical break up of insoluble

aggregates can be used to improve solubility *e.g.*, via ultrasound treatment (Hu et al., 2013; Li et al., 2021b; Li et al., 2016; Sha et al., 2022).

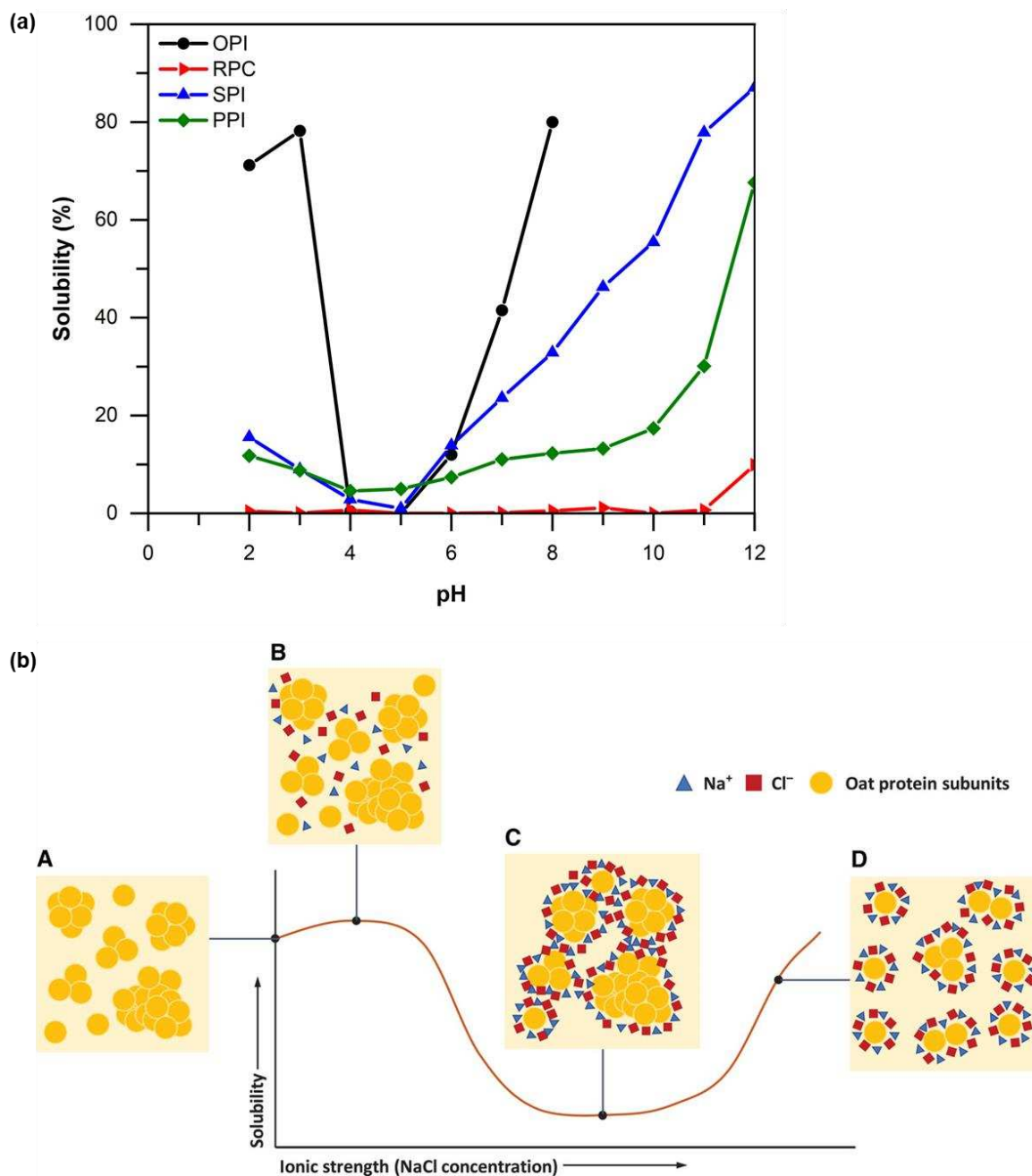


Figure 4. The effect of (a) pH and (b) ionic strength on aqueous plant protein solubility. In (a), soybean protein isolate (SPI), pea protein isolate (PPI), and rice protein concentrate (RPC) powders were obtained commercially, and their solubility was reported using a nitrogen analyzer (Zhao et al., 2020). Oat data in (a) was obtained from a separate study whereby oat protein isolate was extracted from whole oat groats by defatting, followed by alkaline extraction isoelectric precipitation (Li et al., 2021a). The Biuret solubility method was used to determine protein content and experiments were performed at room temperature (21°C) and residual ionic strength (<20 mM). Image (b) is a schematic obtained

with permission from Li and Xiong (Li et al., 2021a). It represents aggregation of OPI as a result of increasing ionic strength.

3.1.1.3 pH

The solubility of OPI also largely depends on external factors such as pH which modifies protein structure and surface charge. In general, plant protein solubility varies with pH to form a recognisable “U-shaped” curve, exhibiting low solubility at the pI and higher solubility at values well above or below the pI (Figure 4a). Low electrostatic repulsion between proteins at the pI is due to positively charged amino acid side chains being equal in magnitude to negatively charged residues, encouraging protein-protein interactions (Grossmann et al., 2023; Ma et al., 2022a). When $pH < pI$, amino acid residues become ionised such that amino groups get protonated. Conversely, when $pH > pI$, the carboxylic acid groups become deprotonated. Therefore, the protein experiences a stronger electrostatic repulsion and increased solubility as you move away from the pI .

In Figure 4a, we gathered data from two sources to observe differences in solubility of storage proteins from oat, rice, pea and soy (Li et al., 2021a; Zhao et al., 2020). As predicted, all proteins showed minimum solubility in the pH range 4-6, corresponding to their pI range (Table 3), which is in line with most dairy proteins such as caseins and whey proteins. The data importantly highlights that rice protein concentrate (RPC) stood out as having extremely low solubility ($<10\%$) at all measured pH values compared to PPI and SPI (Felix et al., 2016; Romero et al., 2012). This results from its major Osborne fraction being glutelin (prolamin family) which is largely alcohol soluble, whilst oat, pea and soy proteins predominantly consist of salt-soluble globulins. However, a key limitation with this comparison is that oat data was obtained from a separate source. As solubility is highly variable between different sources and extraction methods, this may explain the narrower shape of the OPI curve compared to soy protein isolate (SPI) and pea protein isolate (PPI). Whilst some information can be gathered from existing data, a direct comparison of oat to pea, soy and rice under similar conditions is needed to confirm differences as a function of pH.

3.1.1.4 Ionic strength

Another key external factor when comparing solubility is the ionic strength of the solvent or buffer used. Li and Xiong recently outlined how ionic strength (I) affects OPI solubility (Figure 4b) at pH 7.0 (Li et al., 2021a). Changes in solubility upon addition of salt (e.g., NaCl) are attributed to electrostatic screening of the protein

surface charge by ions. Typically, at low salt content, the resulting electrostatic repulsion can be explained using the Debye-Hückel theory, whereby the Debye screening length (κ^{-1}) can be approximated as $\kappa^{-1} = 0.304 / \sqrt{I}$ for an aqueous system at room temperature (Grossmann et al., 2023). When I was <0.005 , a negligible effect on solubility was observed since the protein surface already carries a strong negative charge at pH 7.0 (Table 3). The strong electrostatic repulsion is shown by a high value of κ^{-1} (9.6 nm) at $I = 0.001$. Above $I = 0.005$, electrostatic repulsion is weakened as κ^{-1} decreases below 4.3 nm, resulting in a loss of protein solubility. At this point, the protein's negative surface charge is being screened by cationic Na^+ ions, leading to protein aggregation (Grossmann et al., 2023). The solubility reaches a minimum (0.86%) at $0.03 < I < 0.1$. At this ionic strength, proteins are 'salted out' of solution, as shown by the κ^{-1} values of below 1.75 nm. When increasing the salt concentration even further (> 0.1 M), the Debye-Hückel theory begins to break down (Hyde et al., 2017). At this point, a 'salting-in' effect occurs, whereby the anionic Cl^- counterions preferentially bind to the positively charged protein residues and break up protein aggregates by increasing electrostatic interactions. Importantly, maximum solubility was attained at $I = 1$ (1.0 M NaCl) in Li and Xiong's study. Note that the same hill-valley-hill-valley profile has also been observed with other plant-based globulins (soy, pea, etc.) and the same profile continues as the ionic strength increases beyond 1 (Grossmann et al., 2023).

A key difference between legume and oat globulins is that the former only require 0.4 M NaCl to reach maximum solubility whilst a higher salt concentration (1.0 M) is needed for oat (Shotwell et al., 1988a). Such differences are likely accounted for by internal factors since the external factors are being kept constant. It has previously been suggested by Shotwell *et al.* that the lower solubility of oat may be influenced by the Gln-rich octapeptide region which is located at end of the α polypeptide and is predicted to be exposed to solvent (Figure 2c). Similar regions in pea and soy 11S legumin were reported as containing more hydrophilic amino acids than that in oat 12S globulin, therefore previous authors have suggested this as a reason for discrepancies in solubility with oat globulin (Shotwell et al., 1988a).

3.1.1.5 Temperature

Temperature is an important factor in solubility measurements. Elevated temperature regimes, for example, can encourage protein aggregation and lead to decreased solubility. Whilst the temperature at which the solubility measurements are conducted is key when interpreting results, it is also important to highlight whether the protein was subjected to elevated temperatures during or prior to extraction. For oats in particular, which typically undergo heat treatment to prevent rancidity development, such considerations are essential and are often not explicitly reported in the methodology section of different studies which may lead to erroneous interpretations.

Runyon *et al.* used asymmetric flow field flow fractionation (AF4) coupled to investigate oat protein and polysaccharide fractions before and after heat treatment of oat groats (Runyon, Nilsson, Alftren, & Bergenstahl, 2013; Runyon *et al.*, 2015). In particular, three peaks were identified corresponding to (i) soluble individual proteins, *i.e.* albumins and prolamins, (ii) hexameric and aggregated globulins and (iii) β -glucan polysaccharides. After steaming oat groats for 102 °C for 50 mins followed by drying at 110-120 °C, the soluble protein content after extraction decreased from 74.6 wt.% to 35.7 wt%. This correlated to a decrease in the ratio of monomeric protein to globulins from 1.82 to 1.48. Interestingly, the authors suggested that the soluble albumin and prolamins fraction was more susceptible to changes in solubility due to heat treatment compared to the globulin fraction. Further studies using fractionation of oat protein extracts are necessary to fundamentally understand the role of each fraction on protein functionality in solution.

3.1.2 Surface hydrophobicity (H_0)

An important parameter intrinsically linked to solubility and emulsification performance is surface hydrophobicity of plant proteins (Tang, 2017; Voutsinas, Cheung, & Nakai, 1983). Calculating the overall theoretical hydrophobicity via the sum of individual amino acid hydrophobicity values is common when the protein sequence is known. However, functionality is typically defined by hydrophobicity at the surface (H_0), which often does not correlate to predicted values from amino acid composition (Grossmann *et al.*, 2023; Heldt, Zahid, Vijayaragavan, & Mi, 2017). A review by Jamadagni *et al.* highlights that current understanding of hydrophobicity remains a multidimensional challenge, despite it being a key driver of protein self-assembly and other biological processes (Jamadagni, Godawat, & Garde, 2011). Therefore,

understanding hydrophobicity at multiple length scales often requires bringing together experimental, computational and theoretical approaches.

The most common method of experimentally determining surface hydrophobicity involves spectrofluorometric measurements, *i.e.*, using a fluorescent probe to bind to accessible hydrophobic regions of the protein (Cardamone & Puri, 1992). This is a quick and easy method whereby the fluorescence intensity is plotted as a function of protein concentration and the slope is taken as the H_0 value (Nakai, 2003). By far the most prevalent probe for plant-based globulin experiments is 1-anilinonaphthalene-8-sulfonic acid (ANS), since it has good aqueous solubility and a low quantum yield of fluorescence in aqueous environments (0.004) compared to hydrophobic environments (0.63 in *n*-octanol).

Next, we explore and compare ANS binding values reported for oat, pea, rice and soy protein samples. Reasons for discrepancies will be accounted for by internal and external factors affecting surface hydrophobicity, including the nature of Osborne fractions present, pH, temperature, ionic strength of solution and the extraction method chosen.

3.1.2.1 Osborne fractions

Internal factors, such as variations in the composition of major Osborne fractions, are known to influence plant protein surface hydrophobicity. A range of H_0 values determined by ANS binding of oat, rice, pea and soy protein samples are shown in Table 3. Oat globulin H_0 is reported to lie between 50-450, with similar values for soy and pea. However, much higher values from 490 to 2,200 are observed for rice. The large discrepancy is likely due to the macropolymeric nature of the major Osborne fraction in rice protein, glutelin (Figure 3). Oat, pea and soy, however, all contain hexameric globulins as major components of Osborne fractions, resulting in a similar range of H_0 .

The influence of different oat bran protein components, *i.e.* separate Osborne fractions, on protein isolate H_0 has also been investigated. Jing *et al.* fractionated oat bran protein and reported an H_0 trend of prolamin > glutelin > globulin > albumin (Jing, Yang, & Zhang, 2016). Values reported for globulin and albumin were significantly lower (30-60) than prolamin and glutelin (110-120). These values agree with a previous study conducted on soluble and insoluble fractions of oat protein (Ma & Harwalkar, 1988b). Importantly, the H_0 data showed a negative correlation with the aqueous solubility of each fraction and with the number of α helices in the protein structure. The

latter was attributed to a looser, more flexible protein structure upon increasing α helix content. Exposure of buried hydrophobic groups increased the strength of protein-protein interactions, leading to an overall decrease in H_0 due to effective burying of hydrophobic residues within the aggregates. Importantly, the H_0 value for oat bran protein isolate was found to be closest to that of globulin since it is the major fraction in oat.

A similar trend was reported for pea protein, whereby water soluble albumin showed the lowest H_0 of 329, whilst globulin and glutelin fractions exhibited an H_0 of 427 and 523 respectively (Okagu & Udenigwe, 2021). Note that information on prolamins was not supplied due to poor yield. Authors emphasised the higher H_0 for glutelin compared to the other fractions resulted from its larger relative content of hydrophobic residues, including Trp, Leu, Val and Ala. When comparing globulin and glutelin, the effect of glycosylation of 7S globulin was also proposed to strengthen the protein-solvent interactions due to an increase in hydrophilicity. This was highlighted by a reversed-phase high-performance liquid chromatography method (RP-HPLC) study which separated out soy 11S and 7S, with the former showing higher H_0 (Riblett, Herald, Schmidt, & Tilley, 2001). Therefore, it is clear that inherent protein structure can heavily impact surface hydrophobicity and that the major fraction will dominate the H_0 value in protein isolate and concentrate measurements.

Although ANS binding is the preferred method for surface hydrophobicity measurement, a key limitation of using ANS, and anionic probes in general, are that they likely induce electrostatic changes in protein structure in addition to the desired hydrophobic interactions (Alizadeh-Pasdar & Li-Chan, 2000; Nakai, 2003). For example, it is known that ANS can undergo ion pairing with cationic groups in proteins (Matulis & Lovrien, 1998). To illustrate the importance of probe selection, the H_0 of whey protein isolate, β -lactoglobulin and bovine serum albumin were compared using aliphatic, aromatic, anionic and neutral fluorescent probes at pH 2-9 (Alizadeh-Pasdar et al., 2000). Aliphatic and aromatic probes showed opposite trends in H_0 and, at pH 3, H_0 values for anionic probes were higher than that of the neutral probe which suggests that electrostatic interactions were indeed affecting the measurements. Therefore, values should be taken as estimates and probes should be selected based on the desired experimental conditions. Although not covered in this work, alternative methods suitable for measuring protein H_0 include hydrophobic interaction

chromatography and the use of computational modelling (Andrews & Asenjo, 2010; Heldt et al., 2017; Nakai, 2003).

3.1.2.2 pH and temperature

Similar to solubility, H_0 is influenced by external conditions including pH and temperature. Ma *et al.* showed the effect of oat globulin upon alkaline incubation (pH 9.7) on H_0 using *cis*-parinaric acid as a fluorescence probe (Ma, Harwalkar, & Paquet, 1990). At 25 °C, H_0 gradually increased with incubation time. Authors attributed this to alkali-induced conformational changes to oat globulin, causing protein unfolding and exposure of hydrophobic groups over time. However, this relationship was also determined to be temperature dependent. At 37 °C, a sharp increase in H_0 was followed by a steady decrease whilst little change in H_0 was observed when the experiment was repeated at 55 °C. At higher temperature, alkali exposed groups favourably interacted to form aggregates and were essentially buried, *i.e.*, H_0 effectively does not change. These results suggest pH and temperature can be tuned to modify H_0 of oat protein.

3.1.2.3 Ionic strength

Ionic strength is also likely to affect H_0 as a result of its effects on solubility. To our knowledge, this has not been directly explored with oat proteins and requires further investigation. For soy protein isolate, however, H_0 was found to show a negative correlation with solubility at different ionic strengths (Jiang et al., 2015a). This was suggested to be a result of changes in secondary structure using circular dichroism, with higher random coil and lower α -helix content leading to exposure of different amino acid groups at the surface when ionic strength increased.

3.1.2.4 Extraction procedure

Finally, we have emphasised the importance of considering the extraction procedure when comparing functionality throughout this review and, unsurprisingly, this also has an effect on H_0 values. Karaca *et al.* have compared the physicochemical properties, including H_0 , of different legume protein isolates and shown differences based on the chosen extraction method *i.e.*, salt extraction vs. isoelectric precipitation (Karaca et al., 2011). H_0 values were lower in pea and soy when extracted using salt, with values of 77.83 and 50.62 respectively compared to 84.76 and 55.32 when isoelectrically precipitated. However, faba bean and lentil protein isolate showed the opposite effect, with higher surface hydrophobicity upon salt extraction. Notably, in

both cases pea exhibited a higher H_0 than soy, which corresponds to its lower aqueous solubility (Figure 4a). Another study, however, reported that SPI has a higher surface hydrophobicity than PPI despite its higher solubility (Chang, Tu, Ghosh, & Nickerson, 2015).

Generally, same phase properties appear to be defined by protein structure at the experimental conditions chosen. In particular, the nature of exposed residues and the protein's molecular flexibility will influence functionality within a given solvent. Whilst oat protein solubility and H_0 more closely resembles globulin rich pea and soy proteins, little similarity is observed with rice. Two highlighted properties which appear to reduce solubility in oat proteins compared to pea and soy are its high molecular rigidity (high T_d of oat 12S globulin in Table 3) and the exposed Gln-rich octapeptide (Figure 1).

3.2 Interfacial behaviour

Proteins are surface active due to their inherent amino acid structure which provides the amphiphilicity required for interfacial stabilisation. Mechanistically, the first step involves protein diffusion to the interface, followed by exposure of hydrophobic groups (Zhang et al., 2023a). These conformational changes allow for the adsorption of hydrophobic groups at the non-polar interface *e.g.*, oil, whilst hydrophilic residues remain exposed to the bulk polar phase *e.g.*, water. Protein-protein interactions at the interface results in the formation of strong cohesive layers (films) after adsorption that provide the kinetic stability to the droplets against coalescence for certain time periods.

Currently, animal-based proteins are more widely studied due to their high aqueous solubility and their ability to adsorb readily to interfaces. Plant-based alternatives, in particular globulins, have lower surface activity due to their slower adsorption behaviour and lower shear and dilatational moduli values, indicating less rigid film formation (Sagis & Yang, 2022). Recent reviews investigated plant protein interfacial behaviour in detail due to its importance in the development of sustainable food products (Drusch, Klost, & Kieserling, 2021; Sagis et al., 2022; Yang & Sagis, 2021; Zhang et al., 2023a). Briefly, plant proteins showed poorer interfacial stabilisation than animal derived counterparts due to their complex, aggregated particulate structure which slows down the diffusion of the plant proteins to the interface. This increases the time needed for structural organisation and for the formation of a viscoelastic film at the liquid-liquid interface by the relatively insoluble

plant proteins. Impurities upon extraction (polysaccharides, lipids, polyphenols, etc.) also notably influence adsorption and interfacial properties, leading to greater variability in plant protein behaviour at liquid-liquid interfaces (Sagis et al., 2022). In particular, albumin and globulin fractions were reported to have the greatest potential for surface activity due to their partial solubility in water as compared to prolamin fractions (Yang et al., 2021). However, due to the reasons outlined above, these are often modified to improve their interfacial functionality.

In the following section, current knowledge on oat globulin behaviour at the air-water and oil-water interface is explored and complemented where necessary with studies on rice, soy and pea. As seen with their behaviour in aqueous solution, interfacial properties are also defined by a combination of factors, including electrostatic charge, particle size, surface hydrophobicity, *etc.* (de Jongh et al., 2004; Ercili-Cura et al., 2015). We refer to a recent article highlighting to the general effects of experimental conditions (protein concentration, pH, ions, temperature) on plant proteins (Zhang et al., 2023a). Herein, we focus on fundamental behaviour of oat protein extracts at the air-water and oil-water interfaces, relating the findings to structural properties. Papers reporting on emulsification activity and foaming behaviour are considered out of scope. Further information on Pickering emulsions stabilised by the formation of protein particles can be found in another review (Sarkar & Dickinson, 2020). Protein modification, protein-dairy and protein-polysaccharide mixtures are also not covered here (Kumar et al., 2021; Yang et al., 2021).

3.2.1 Air-water interface

Equilibrium surface tension values (γ_0) are commonly used to compare the interfacial stabilisation properties of proteins at the air-water interface. Whilst low molecular weight surfactants typically show a γ_0 range of 22-42 mN m⁻¹, globular proteins have a range of 47-57 mN m⁻¹ (Bos & van Vliet, 2001). A lower surface activity is observed since proteins diffuse more slowly to the interface compared to low M_w surfactants and more time is needed for structural reorganisation (Zhang et al., 2023a).

Surface tension values for oat, rice, pea and soy storage proteins at the air-water interface are reported in Table 3. OPI lies within the globular protein range 45-60 mN m⁻¹, although some values are higher than the other proteins listed (*e.g.*, γ_0 = 35-45 mN m⁻¹ for PPI). Therefore, OPI appears to have slightly lower surface activity

compared to legume and rice isolates. However, we note that a relatively small number of studies have been conducted on OPI, so further testing is needed to confirm this property. Next, we explore factors affecting surface activity of oat proteins at air-water interface.

3.2.1.1 Protein concentration

An important experimental factor which affects surface tension measurements is the protein concentration selected. The effect of changing OPI concentration on dynamic surface tension with a drop shape analyser was investigated by Mohamed *et al.* in 0.5 M NaCl solution (Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009). As the protein concentration increased, the protein showed a sharp reduction in surface tension which equilibrated once a critical concentration (~0.2 mg/mL) was reached. This indicated that enough protein was present at this concentration to effectively form a stable interfacial film.

Mileti *et al.* showed a similar decrease in surface tension when testing soy and brown rice protein. For brown rice, the critical concentration was reported to be 0.1% w/w, whilst larger concentrations were needed for hemp and soy (1% w/w). Another study has similarly reported that RPC exhibited higher surface activity than legumes at the air-water interface (Kontogiorgos & Prakash, 2023). This was attributed to small concentrations of low M_w (10-16 kDa) protein fractions in brown rice which unfolded more easily and exposed the hydrophobic groups at the interface. Other studies have also shown that soy and pea protein fractions present within the sample heavily influence the air-water interfacial behaviour (Martin, Bos, & van Vliet, 2002; Niño, Sánchez, Ruíz-Henestrosa, & Patino, 2005; Rodriguez Patino, Molina Ortiz, Carrera Sanchez, Rodriguez Nino, & Anon, 2003; Santiago *et al.*, 2008; Shen *et al.*, 2023). Deriving this concentration dependency from other plant proteins, the fractions present and their concentrations should be considered when understanding the interfacial behaviour of oat protein.

Another feature of interfacial stabilisation is the lag time, also known as a period in which the protein is at low concentration and little interfacial activity is observed. Differences in lag time have previously been attributed to structural properties which change the speed of adsorption and nature of rearrangement at the interface, such as hydrophobicity, M_w and the presence of disulphide bonds (Mileti *et al.*, 2022; Ruíz-Henestrosa *et al.*, 2007). The latter, for example, may restrict the number of

conformations possible at the interface, therefore reducing the lag time. Whilst lag time has not been explored with oat, interestingly, soy protein adsorption kinetics were found to be faster than brown rice. However, the opposite trend was observed with the kinetics of rearrangement at the interface. Mileti *et al.* suggested that hydrophobic sites are less well exposed in soy, therefore unfolding at the interface requires more time compared to rice (Mileti et al., 2022). Conversely, diffusion was faster for soy due to the lower M_w of globulins compared to macropolymeric rice glutelins, thus increasing the adsorption kinetics.

Recently, understanding was taken a step further by comparing rice and soy using interfacial shear and dilatational rheology (Kontogiorgos et al., 2023; Mileti et al., 2022). The techniques give insight into the properties of protein films at the interface and its response to compression and shear. When comparing the nature of the films formed, soy protein formed a more elastic, solid-like layer (phase angle = 10-20°) than brown rice (phase angle > 45°). Similar results were seen in a separate study, implying weaker in-plane interactions and a less resilient structure in rice (Kontogiorgos et al., 2023). However, interfacial strength was higher in brown rice due to its stronger viscous component. Notably, the viscoelastic response of soy protein films resulted in long-term stability compared to rice protein. However, understanding of oat protein interfacial rheology remains unexplored to date.

3.2.1.2 pH and ionic strength

As discussed previously, ionic strength and pH heavily influence structural properties, thus altering surface activity. Ercili-Cura *et al.* investigated the effect of pH on the adsorption of OPI at the air-water interface using surface tension and AFM (Ercili-Cura et al., 2015). At pH 7.2, OPI particle size was measured to be in an aggregated state (~70 nm). At higher dilution (<0.1 mg/mL), dissociation/ re-association of monomers resulted in polydisperse size distributions with larger sizes measured. When OPI was dissolved in alkaline solution (pH 9.0), less aggregation was seen, and particle size distributions remained the same upon further dilution at a value of ~30 nm. Note that whilst the authors referred to the peak at ~30 nm as the monomeric, native structure, we deem this value high compared to previous light scattering experiments reporting dimensions of 8.5 x 12 nm (Figure 1). At both pH values, high negative surface charges were measured (stock solution ζ -potential values of -36.0 at pH 7.0 and -42.5 mV at pH 9.0). Although smaller than the native

protein, less aggregated globulins at pH 9.0 showed a higher final surface tension (50 mN/m) than at pH 7.2 (45 mN/m) at the same concentration. The higher pH sample also displayed slower adsorption dynamics, indicating poorer interfacial properties. Complementary AFM imaging showed the air-water interface is composed of particles of approximately 11 nm in diameter, matching dimensions in Figure 1. Importantly, a homogenous interfacial layer of individual particles was identified at pH 9.0, whilst large aggregates from tens to hundreds of nm were formed at neutral pH. Overall, the work outlined that retention of native protein state allowed for multilayer formation which promoted high surface activity of OPI compared to its monomeric, dissociated state. The disruption of the native state was clear in Figure 4a, with OPI solubility at pH 7.0 being 41.5%, whilst a dramatic increase to 80% is shown beyond pH 8.0. Whilst further experiments between pH 7.2 and 9.0 are needed to pinpoint the exact pH of dissociation, this is likely to be below pH 8.0.

To date, the effect of pH and ionic strength on individual oat globulin fractions has not been tested, however synergies can be derived from differences in the lag time of soy 11S and 7S at various pH values (Rodríguez Patino, Carrera Sánchez, Molina Ortiz, Rodríguez Niño, & Añón, 2004; Ruíz-Henestrosa et al., 2007). For example, at pH 5.0, lag time significantly increased in both fractions compared to pH 7.0 due to decreased solubility at the pI. However, the lag time more than doubled with the 11S fraction compared to 7S due to its higher molecular weight and the presence of a disulphide bond. This reduced structural flexibility and limited possible conformational rearrangements. Addition of a reducing agent to cleave the disulphide bonds has also been shown to decrease the lag time of soy 11S by a factor of 7 due to facilitated protein unfolding (Rodríguez Patino et al., 2004). Similar to pH, differences due to ionic strength were also observed as this can induce dissociation of 11S soy globulin into its 7S form, therefore changing the 11S/7S ratio of the sample. Therefore, these variables should be carefully controlled when conducting surface tension measurements for oat globulins.

3.2.2 Oil-water interface

To our knowledge, the only fundamental study determining oat protein surface tension at the oil-water interface was conducted with oat bran extract (Ralla et al., 2018). However, the sample contained only 13% protein and authors suggested that surface activity was dominated by oat saponins or saponin-protein complexes. Therefore, information will be complemented with studies on rice, pea and soy.

Importantly, external factors which affect air-water interfacial stabilisation also have an effect here *i.e.*, protein concentration, pH, ionic strength. Since this is covered previously in the air-water interfacial section, instead we focus on key differences between air-water vs. oil-water interface behaviour.

Rice, pea, soy and mung bean protein concentrates were found to behave differently at the oil-water vs. air-water interface despite a similar range of interfacial values shown in Table 3 (Kontogiorgos et al., 2023). Whilst for rice proteins, a higher surface activity was measured in air, the opposite was observed at the oil-water interface. Additionally, changes in surface pressure of all samples were observed when changing the nature of the oil subphase. Triglyceride oil measurements showed overall lower surface pressures than terpene and air. Protein rearrangement was also shown to occur twice as fast at the triglyceride interface ($k_{\text{rear}} \sim 14 \times 10^{-3} \text{ s}^{-1}$) and three times faster in terpene ($k_{\text{rear}} \sim 21 \times 10^{-3} \text{ s}^{-1}$) compared to air ($k_{\text{rear}} \sim 7 \times 10^{-3} \text{ s}^{-1}$). This was attributed to the higher polarity of the triglyceride oil causing an increase of protein solubility in the subphase, therefore facilitating exposure of hydrophobic groups and conformational changes. Generally, all plant protein films at the oil interface were also found to be less rigid ($E' < 35 \text{ mN/m}$) and able to withstand greater strains. This was attributed to hydrophobic amino acids showing greater affinity for the oil phase, leading to weaker protein-protein interactions compared to the air-water interface. These findings emphasise the need for oat protein studies using a variety of techniques, as opposed to simple surface tension measurements and test using various types of oil phases.

In general, multiple phase behaviour of oat may be governed by a subtle balance of structural properties which govern solubility and surface hydrophobicity parameters. These impact the adsorption as well as rearrangement kinetics and subsequently define the film properties. Findings suggested that proteins with high conformational flexibility tend to show the greatest interfacial activity (Karaca et al., 2011; Kinsella, 1979). Based on behaviour of oat proteins at the air-water interface, we speculate similarities to other legumes like pea and soy at the oil-water interface. It is likely that this behaviour will differ from that of rice glutelin due to differences in quaternary structure. As shown with rice, pea and soy protein concentrates, we also predict that the nature of the interface *i.e.*, the polarity of the oil subphases, will be important in determining oat protein interfacial behaviour.

Despite these findings, important gaps remain to be explored to better understand fundamental oat protein interfacial behaviour. To date, the wetting properties of OPI and bulk rheological properties of interfacial films formed have not been systematically investigated to complement interfacial tension measurements (Ercili-Cura et al., 2015). Another technique yet to be tested with oat proteins to understand multi-phase behaviour is quartz crystal microbalance with dissipation (QCM-D) to monitor adsorption onto hydrophobically modified surfaces. Previous QCM-D studies conducted with pea, soy and, more recently, rice hydrosylates have led to insightful studies on surface interactions and hydrated film formation (Kew et al., 2021; Yang et al., 2022c; Zembyla et al., 2021). Finally, there is also a lack of knowledge on fundamental oat protein behaviour at the oil-water interface which needs to be addressed.

3.3 Gelation and coacervation behaviour

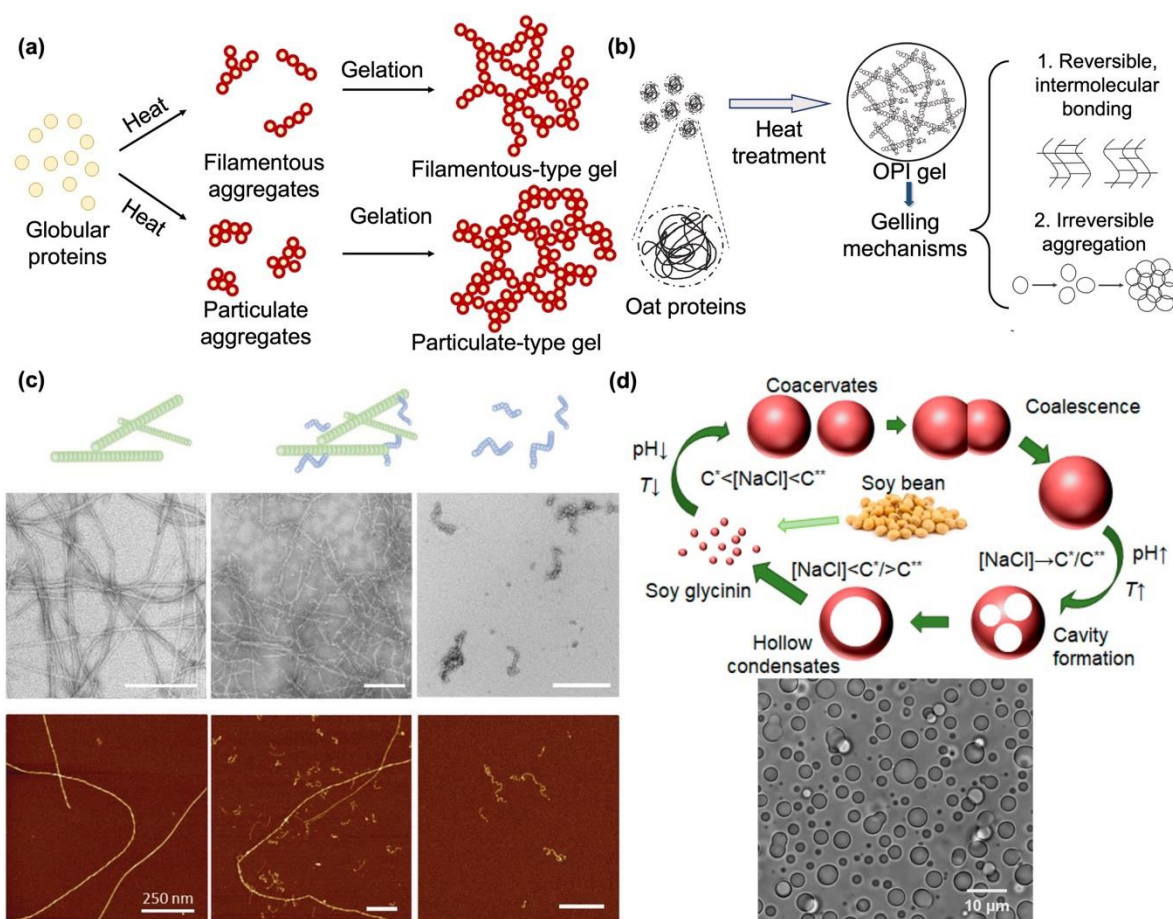


Figure 6. Gelation and coacervation behaviour of plant-derived globular proteins using physical processing. (a) General schematic of globular protein heat-set gelation, forming either filamentous (stranded) or particulate-type gels (Peng, Ren, & Guo, 2016). (b) Proposed mechanism of heat-induced gelation of oat protein isolate (OPI) in aqueous solution via (i) reversible intermolecular interactions or (ii) irreversible aggregation (Kumar et al., 2021). (c) Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) images of mature, worm-like

and elongated worm-like oat globulin amyloid fibrils formed upon incubation at high temperature (90°C) and low pH (pH 2) (Zhou et al., 2022). All scale bars shown are 250 nm. (d) Self-coacervation of soy glycinin into spherical structures and hollow condensates by tuning pH, temperature and salt concentration (Chen, Zhao, Wang, & Dimova, 2020b). Micrograph of coacervate formation included below using soy glycinin solution (5 mg/mL, pH 7.5) with equal volume of 0.2 M NaCl. Images (a) and (b) were reproduced with permission from Elsevier, whilst (c) and (d) are reproduced with permission from Wiley and ACS respectively.

Plant globulins can undergo a sol-gel transition, whereby the protein structure is reorganised due to unfolding, resulting in an elastic, solid-like gel network (Kumar et al., 2021). Similar to gelation of animal-derived globular proteins (e.g. bovine β -lactoglobulin), plant-based globulin heat-set gelation occurs *via* three steps, involving (i) protein denaturation (ii) aggregation and (iii) gel network formation (McSwiney, Singh, & Campanella, 1994; Zheng, Regenstein, Zhou, & Wang, 2022). However, plant protein gels can also be formed below the thermal denaturation temperature, and these are often referred to as cold-set. Figure 6a shows a classification of globular protein gels into either filamentous or particulate-type gels. The type of gel formed is dependent on gelation and heating conditions, including protein concentration, ionic strength and pH (Peng et al., 2016).

3.3.1 Heat-set gelation

Heat-set gels are formed by heating the protein solution, causing groups and side chains to gradually open, thus exposing the hydrophobic protein core and creating a network of aggregates *via* intermolecular interactions between the amino acid chains (Kumar et al., 2021; Zheng et al., 2022). Although studies on oat starch and β -glucan gelation are extensive in the literature due to their importance in the food industry, fundamental understanding of oat protein gelation is less prevalent (Brummer et al., 2014; Du, Meenu, Liu, & Xu, 2019; Lazaridou, Biliaderis, & Izydorczyk, 2003; Punia et al., 2020). A simple schematic proposed by Kumar *et al.* for heat-induced gelation of oat protein isolate (OPI) into a particulate gel is shown in Figure 6b whereby, depending on heating conditions, gels formed can either be reversible or irreversible. We will first attempt to understand the specific interactions and energetics involved in heat-set gel formation, then explore external factors affecting heat-set gelation properties.

3.3.1.1 Energetics of heat-set gelation

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Table 4. Summary of interactions involved in the heat-set gelation of oat, soy and pea globulins.

Protein source	11S/12S Properties (per monomer subunit)			Effect of reagents on gelation properties ^a						Dominant interactions in gel formation ^b	References
	Cysteine residues	SS bonds	Free thiols	Sodium dodecyl sulfate (SDS)	Propylene glycol (PG)/ ethylene glycol (EG)	Chaotropic monovalent salts (e.g. NaCl, NaSCN)	Urea	S-S reducing agents	N-ethylmaleimide (thiol blocker)		
oat	5	2	1	Gel hardness ↓ as [SDS] ↑	Gel hardness ↑ as [EG] ↑	0.2M Na salts: Gel hardness ↑ (greatest for SCN)	Gel hardness ↓ as [urea] ↑	DTT (10mM): great ↓ in gel hardness	10 mM: gel hardness ↓ at alkaline pH	Hydrophobic, electrostatic, H-bonding, disulphide bonds (alkaline pH only)	(Harwalkar, Ma, & Boutin-Muma, 1989; Ma, Khanzada, & Harwalkar, 1988c)
soy	8	2	4	Gel hardness ↓ as [SDS] ↑	Gel hardness ↑; plateau at 10% PG	Gel hardness ↓ as [NaSCN] ↑; Gel hardness ↑ between 0.2 and 0.6M NaCl	Gel hardness ↓ as [urea] ↑	β-mercaptoethanol: Gel hardness ↓	Low T + ionic strength: no change in gel hardness; High T + ionic strength: inhibits aggregation; Slow cooling: gel strength ↓;	Hydrophobic, electrostatic, H-bonding, disulphide bonds	(Adachi et al., 2001; Huang et al., 2022; Mori, Nakamura, & Utsumi, 1986; O'Kane, Happe, Vereijken, Gruppen, & van Boekel, 2004; Utsumi & Kinsella, 1985; Visschers & de Jongh, 2005)
pea	5	2	1	G' ↓ as when immersed in 3% w/v SDS	G' ↑ from 5-20% PG	0.3 M NaCl, NaSCN: ↑ G' due to lower Td (greatest for SCN ⁻); 1 M NaSCN: ↓ in G'; 3 M NaSCN: gel formation inhibited	5 M urea: ↓ in G'	DTT + β-mercaptoethanol: Negligible (<25 mM); higher conc + slower cooling rates: minor SS contribution	Negligible effect on gel formation; Slow cooling: gel strength ↓	Hydrophobic, electrostatic, H-bonding	(Kristensen, Christensen, Hansen, Hammershøj, & Dalsgaard, 2022; O'Kane et al., 2004; Sun & Arntfield, 2010; Zhang, Huang, Feizollahi, Roopesh, & Chen, 2021)

^a Data collected for soy was on individual 11S (glycinin) gels only. Pea and oat data were based on a mixture of globulins fractions.

^b Gel interactions shown here are dependent on the experimental conditions chosen including *e.g.*, pH, ionic strength and cooling rate (O'Kane et al., 2004; Tanger, Müller, Andlinger, & Kulozik, 2022a).

Knowing the dominating interactions involved (hydrogen bonding, hydrophobic interactions, disulphide bonds, *etc.*) is important for mechanistic understanding of the protein gelation process. Typically, this is assessed *via* addition of structure modifying reagents which target specific interactions, then measuring the resulting effect on gel hardness or gel strength (Table 4). Since native rice protein gel interaction studies are comparatively lacking, here we limit oat globulin comparisons to pea and soy alone.

The comparison of sulphur containing amino acids in Table 4 highlights that oat 12S globulin contains the same number of Cys residues as pea 11S globulin (5 in total), whilst soy 11S globulin contains 3 extra free residues which are not involved in disulphide bonding. The location of the cysteine residues in oat 12S globulin are highlighted in Figure 2c. To investigate the effect of disulphide bonding on gelation, addition of a reducing agent (DTT or β -mercaptoethanol) to proteins prior to heating cleaves the disulphide linkage between α and β polypeptides. As shown in Table 4, 10 mM addition of DTT was shown to significantly decrease OPI gel hardness from 5.20 to 1.81 N (Ma et al., 1988c). Aggregation of the dissociated monomers resulted in protein precipitation, thus weakening the gel matrix. Similar results were observed with soy glycinin (Utsumi et al., 1985). Pea globulin gels, however, showed little change in gel strength, with minor effects at high reducing agent concentrations and slow cooling rates. Therefore, disulphide bonds appear to contribute less to gel formation in pea compared to oat and soy globulins.

The importance of sulphur containing residues is further highlighted by the use of a sulfhydryl group (SH) blocking agent, N-ethylmaleimide. Interestingly, the role of SH groups in OPI gelation was reported as more prominent at an alkaline pH due to an increase in reactivity (Ma et al., 1988c). In a pea globulin study, N-ethylmaleimide addition also resulted in slight destabilisation of the gel network (O'Kane et al., 2004). A lower gel strength due to thiol blocking was only observed upon slow cooling ($0.2\text{ }^{\circ}\text{C min}^{-1}$), but not upon fast cooling ($1\text{ }^{\circ}\text{C min}^{-1}$), which indicated that disulphide bonds were not essential but became more involved when the gel cooling rate is slow. Therefore, the gelation mechanism can also be dependent on experimental conditions.

Sodium dodecyl sulphate (SDS), an anionic detergent, acts by binding non-covalently to the protein and increasing its net charge, thus causing denaturation and a decrease in protein-protein interactions (Ma et al., 1988c). Since SDS disrupts

hydrophobic interactions, ionic bonds and hydrogen bonds within the protein whilst maintaining the disulphide bonds intact, it is used as a method of determining the importance of non-covalent interactions in the gelation process (Jung, Savin, Pouzot, Schmitt, & Mezzenga, 2008). This is often complemented by the addition of urea, which primarily disrupts hydrogen bonding in the native protein structure by displacing water molecules due to preferential binding to the protein (Hua, Zhou, Thirumalai, & Berne, 2008). Both SDS and urea were found to decrease the gel forming ability of oat protein which highlights the importance of new non-covalent intermolecular interactions in the formation of the oat protein gel network. Importantly, SDS and urea may disrupt the non-covalent interactions already present within the folded protein structure, whilst also preventing the formation of new interactions. Therefore, results may also suggest that protein native structure must be preserved to a certain extent for optimised gelation.

Addition of chaotropic salts, which disrupt the hydrogen bonding network in water, has a marked effect on protein stability by disrupting electrostatic and hydrophobic interactions. These were found to increase OPI gel hardness at 1.0 M concentration according to the theoretical series developed by Hofmeister which orders ions based on their effects on protein solubility and changes in secondary and tertiary structure (Hofmeister, 1888). As shown in Table 4, legume proteins typically require a lower salt concentration compared to oat protein (<1.0 M) to promote an increase in gel hardness. At 1.0 M concentration and above, gel formation was inhibited in soy and pea proteins. These differences in concentration correlate to the lower solubility of oat proteins and the previously discussed effects of salting in and salting out.

Importantly, such differences in the gel interactions can lead to changes in gel stability. For example, pea and soy protein gels responded differently to re-heating and re-cooling despite their similar protein structures. Soy glycinin gels were classified as thermally reversible, *i.e.* the storage modulus (G') returned to its original value in soy protein, whilst G' in pea legumin became stronger by one log scale after a re-heating/re-cooling cycle (O'Kane et al., 2004). G' modulus values in this article were taken at constant strain (0.015 for legumin and 0.01 for glycinin) and a frequency of 0.1 Hz. The differences between pea and soy are likely a result of the different interactions holding the gel together.

However, it is worth emphasising the difficulty in quantitatively comparing gel properties due to variations in source and extraction method. Whilst PPI gels were sometimes found to be stiffer than SPI ($G'_{\text{PPI}} = 3212.5 \text{ Pa}$; $G'_{\text{SPI}} = 889.5 \text{ Pa}$ at 1.0 Hz), in another study the opposite was true (average shear strain, stress: PPI = 0.78, 14.8 N; SPI = 1.44, 26.9 N) (Shand, Ya, Pietrasik, & Wanasundara, 2007; Sun et al., 2010). Another important property to consider is the effect of different globulin fractions on gelation. Interestingly, the gelation mechanisms of 7S and 11S legumins in pea were shown to be different, with the former being held together by non-covalent bonds alone whilst the latter also showing the formation of covalent disulphide bonds (Messin, Chihi, Sok, & Saurel, 2015). An in-depth mechanistic investigation of gelation fractionated 7S and 11S oat globulins is therefore needed as the 7S/11S ratio changes depending on source and extraction method chosen.

Overall, non-covalent protein interactions (hydrophobic effect, hydrogen bonding, electrostatics) are crucial for both oat and legume proteins. These all appear to be involved in a complex gelation process. However, the contribution of S-containing groups *i.e.*, disulphide-mediated covalent bonding, varies. Despite having the same number of free thiols than oat, comparatively little effect on gel strength is seen with pea protein when DTT was added. Importantly, soy glycinin has a larger number of free thiol groups than OPI, therefore it has the ability to form stronger gels via covalent binding. Once again, further experiments are needed to confirm this under the same environmental conditions. We next analyse conditions affecting gelation properties of OPI gels.

3.3.1.2 Temperature

Heat-set gel formation typically involves protein denaturation and aggregation which is facilitated by an increase in temperature. The temperature at which gelation occurs depends on heating time, as well as other conditions including protein concentration, pH and ionic strength, which will be discussed further. Notably, OPI gel formation at 10 % w/v did not occur below 90 °C after heating for 20 mins (Ma et al., 1988c). Another study by Zhao *et al.* also observed a slow rate of aggregation of an oat globulin dispersion (1 % w/v) at 100 °C, with insoluble aggregates formed after heating for 60 mins (Zhao et al., 2004). Slow gelation under these conditions was attributed to the high stability of oat globulin structure, demonstrated by its high thermal denaturation temperature (Table 3) compared to other plant proteins. In particular, the

study by Ma *et al.* suggested that more energy is required to dissociate subunit monomers, *i.e.*, they are more strongly bound together in hexameric form in oat protein compared to pea and soy. For example, when oat globulin dispersion was heated to 120 °C, the gels formed at pH 8.0 became more elastic and stable, leading to an increase in G' of ~20 kPa at 1.0 Hz compared to 90 °C (Brückner-Gühmann, Kratzsch, Sozer, & Drusch, 2021).

To further understand the energies involved in this first step, a kinetics study of thermal denaturation and aggregation of oat globulin (10%) at pH 7.4 and 1.0 M NaCl was conducted (Ma *et al.*, 1988a). Using Arrhenius plots, the order of oat globulin thermal denaturation and the activation energy (E_a) were found to be 2.5 and 500 kJ mol⁻¹ respectively. These values are similar to β -lactoglobulin (order 2.2, E_a = 525 kJ mol⁻¹) despite oat globulin having a much higher denaturation temperature (Park & Lund, 1984). However, soy globulin fractions have a much lower E_a , with 11S being 215-430 kJ mol⁻¹ and 7S being 175-245 kJ mol⁻¹ (Scilingo & Añón, 1996). Therefore, there is a higher energy barrier for oat protein gelation compared to soy.

Importantly, the rate of heating also affects the nature of aggregates formed by altering protein structure. Zhao *et al.* investigated such changes in OPI using size exclusion chromatography and light scattering techniques (Zhao *et al.*, 2004). Short term heating at 100 °C led to dissociation of oat globulin hexamers and trimers into monomers. The monomers then proceeded to associate to form soluble aggregates. Further heating for >60 min induced the formation of insoluble aggregates whilst increasing the temperature to 110 °C was also shown to increase the rate of hexamer dissociation and insoluble aggregate formation. In a study conducted with pea and soy 11S globulins, decreasing the heating rate from 1 to 0.5 °C/min did not affect the rheology of the gels formed (O'Kane *et al.*, 2004). However, cooling the gel more slowly increased G' . Authors suggested that slower cooling alters the aggregation process by slowing down the reactivity of exposed residues to allow for optimal interactions, *i.e.*, formation of disulphide bonds, resulting in increased gel strength.

We note here that a method to improve dispersibility and give control of particle size is *via* microgelation of plant proteins. This tool allows for rheological modification of the protein gels and has been shown to improve of tribological properties of pea and potato protein (Kew *et al.*, 2023). However, microgelation of oat proteins remains to be investigated.

3.3.1.3 pH and ionic strength

Intuitively, gelation is influenced by pH and ionic strength due to the previously discussed effects on protein solubility (Figure 4). Weak OPI gel networks were formed at neutral and acidic pH due to low solubility of oat globulin (Ma *et al.*, 1988c). Gel hardness increased from 1 to 6 N between pH 8.0 and 10.0 corresponding to improved aqueous solubility in this pH range. Similarly, the strongest gels were formed at low salt concentrations (0.4 M NaCl) due to charge shielding effects. However, unlike oat and legumes, RPC, did not exhibit proper gelation at any pH, likely due to its insoluble nature shown in Figure 4a (Felix *et al.*, 2016).

To understand the underlying mechanism of changes in pH, a sequential Raman spectroscopy study of oat globulin revealed changes in conformation during thermal aggregation and gelation (Ma, Rout, & Phillips, 2003). Upon aggregation at neutral pH, the sample showed reduced α -helical content and higher β -sheet content than unheated oat globulin. Protein denaturation was higher in insoluble aggregates, whilst soluble aggregates typically remained in their native state. Increasing the pH to 9.5 promoted gelation over aggregation; β -sheet content grew as a result of intermolecular β -sheet structure formation. Samples were found to be partially denatured due to the alkalinity of solution, with denaturation becoming more prominent after heating. However, enthalpy measurements by differential scanning calorimetry indicated that the OPI gel still exhibited some native structure, once again suggesting that gelation is caused by conformational rearrangement rather than complete protein unfolding.

3.3.1.4 Protein concentration

The minimum gelation concentration (MGC) is another important parameter which defines the amount of protein needed for a sol-gel transition to take place under defined conditions such as applied temperature, heating time, pH and ionic strength. Ma *et al.* reported the MGC for oat protein isolate as 5 % w/v, with gel hardness increasing linearly as the concentration was increased to 12 % w/v (Ma *et al.*, 1988c). A comparative study of pea legumin and soy glycinin gels showed that the minimum gelling concentrations were higher, at 8.4 and 6.6 % w/v respectively (O'Kane *et al.*, 2004). Another study did not observe gelation of rice proteins despite increasing its concentration to 20%, whilst soybean and pea formed gels at concentrations of 12% and 14% respectively (Zhao *et al.*, 2020). Differences in reported values of MGC are

likely due to variations of source and extraction method between studies, as well as different conditions upon measurements.

3.3.2 Filamentous gelation

To this point, we have solely discussed particulate heat-set gelation of oat protein. At the isoelectric pH range, protein subunits remain intact and the particulate gel structure dominates (Yang, Wang, & Chen, 2017). However, as the pH deviates from the isoelectric point, dissociation of subunits into monomers induces protein unfolding. Unfolded chains can subsequently associate into flexible, linear strands to form filamentous gels. In this case, different to particulate gels, thin filamentous strands are formed upon heating when the pH is largely deviated from the pI. This results in a morphologically different stranded-like network (Figure 6c).

Despite issues with low extraction yields, a recent study proposed a purification and fibrillisation process for oat globulin which allowed for functional material synthesis including aerogels, films, electrodes and membranes (Zhou et al., 2022). The resulting twisted amyloid oat globulin fibrils was several microns in length (Figure 6c) and was formed at pH 2.0 upon long-term heating (90 °C, 18 h) as a result of denaturation and hydrolysis. The mechanism of amyloid formation was suggested to be similar to milk-derived β -lactoglobulin, whereby building blocks (protofilaments) pack laterally to form twisted ribbon multistranded fibrils (Adamcik et al., 2010). Two fibril polymorphs which can coexist were identified via transmission electron microscopy (TEM) and atomic force microscopy (AFM) (Figure 1c): thermally reversible short worm-like fibrils and thermally irreversible mature rigid fibrils. Mature amyloid fibrils showed 3 large height distributions via AFM measurements at 2.2, 4.1 and 6.0 nm whilst the height distribution of worm-like fibrils was between 1 and 3 nm.

Amyloid structures are not unique to oat globulins and have been observed with other plant proteins including soy, pea and rice (Li et al., 2023b; Song, Li, Zhang, & Wang, 2023). Although most experiments were conducted with protein isolates or crude extracts, Zhang *et al.* characterised the kinetics of fibrillation and fibril morphology using individual 7S and 11S globulin fractions of pea and soy (Zhang & Dee, 2023b). Fibril cores were found to be primarily formed by residues in the β -polypeptide chain over the N-terminal region, presumably as a result of their higher hydrophobicity and lower net charge. Interestingly, 7S globulin displayed stronger fibrillation capacity over 11S. In particular, soy 11S globulin showed lower conservation

of amino acid residues in the fibril forming regions due to larger sequence variations, whereas 7S globulins in both pea and soy were highly conserved. Therefore, authors concluded that fibrillation is more prominent among proteins with high amino acid sequence conservation. Experiments also identified differences in morphology between pea and soy-derived fibrils, with the former being predominantly straight and the latter being worm-like.

Whilst work on oat protein fibrillation is still in its early stages, further investigation into the rheological properties of the fibrils and their behaviour at liquid-liquid interfaces remain to be studied. Additionally, further investigation is required to understand whether the presence of minor oat protein fractions (e.g. 7S) influence fibrillation capacity and mechanisms.

3.3.3 Coacervation

In this section, we briefly move away from gelation to discuss coacervation, a unique protein phase transition which is similarly affected by ions, pH, protein concentration and temperature. Simple coacervation is a form of liquid-liquid phase separation which occurs spontaneously at specific environmental conditions to form spherical protein-rich domains (coacervates) in a protein-poor continuous phase (Chen, Nicolai, Chassenieux, & Wang, 2020a). Although heteroprotein coacervation of animal-derived proteins as well as animal-plant protein combinations has been extensively studied, it has recently been discovered that globular plant proteins also have the ability to form coacervates (Adal et al., 2017; Anema & de Kruif, 2013, 2016; de Kruif, Pedersen, Huppertz, & Anema, 2013). A schematic of soy protein coacervate formation and a micrograph of the coacervates formed is shown in Figure 6d.

The stability of coacervates involves a delicate balance of various interactions including electrostatic, hydrophobic, excluded volume and van der Waals (Moulik, Rakshit, Pan, & Naskar, 2022). More information on the theory, factors influencing coacervation and their applications can be found elsewhere (Moulik et al., 2022).

Although until recently unexplored in oat globulins, a recent paper extracted an OPI from non-heat treated kernels and investigated the effect of ionic strength and pH conditions (Wouters et al., 2024). In this fundamental study, OPI experienced extensive centrifugation (50,000 g, 4 hrs) at pH 10.0 and filtration via 200 nm membrane to isolate individual oat globulin hexamers with a hydrodynamic radius of ~ 8 nm. Confocal microscopy images showed microphase separation of OPI into

spherical droplets ($\geq 1 \mu\text{m}$) when 50-300 mM NaCl was present in pH 10.0 solution. Authors suggested a particle nucleation and growth mechanism whereby droplets grow until an equilibrium state is reached. Similar microphase separation was observed when titrating the pH 10.0 solution to pH 7.1-8.2. Therefore, authors reported that oat globulin charge density (α) appears to be dictating the type of colloidal structure formed. As α becomes less negative, unaggregated oat globulin transitioned into microphase separated droplets, followed by the formation of irregular aggregates. As irregular aggregates increased in size, the solubility reduced (pH 5.1). Further investigation into oat protein coacervate formation is a necessary undertaking.

Whilst coacervation behaviour of oat globulins is relatively unknown, this has been mechanistically explored with soy globulins and pea protein isolate/ concentrate. (Chen, Zhao, Nicolai, & Chassenieux, 2017; Chen et al., 2020b; Cochereau, Nicolai, Chassenieux, & Silva, 2019; Kornet et al., 2022). Coacervation occurred under slightly acidic pH (6.2-6.8) and ionic strengths of approximately 0.1 M NaCl. This critical pH was typically slightly above the pI , at the point where the protein charge density is lower, but not low enough to induce aggregation (Chen et al., 2020a; Kornet et al., 2022). The coacervate droplets formed were typically micron-sized (Figure 6d), their advantage being the retention of the protein's native conformation without leading to precipitation. As shown in Figure 6d, further tuning of conditions led to vacuole formation and hollow condensates with potential applications in encapsulation (Chen et al., 2020b).

Other important insights can be drawn from soy and pea which may extend to oat due to the structural similarity of 7S and 11S globulins (Nicolai & Chassenieux, 2019). Kornet *et al.* observed that pea protein coacervates were exclusively formed by the globulin fraction, in particular 11S legumin, over albumins. The results agreed with a soy protein study, which found that 80% of the coacervate phase was made of 11S legumin, whilst only 20% was classified as 7S (Lui, Litster, & White, 2007). Pea protein processing history was also found to influence coacervate formation, with mild fractionation being identified as the most suitable extraction method as opposed to commonly used isoelectric precipitation (Kornet et al., 2022). Authors suggested that these differences are caused by irreversible protein aggregation from changes in protein conformation and surface hydrophobicity induced by isoelectric precipitation.

3.3.4 Cold-set gelation

Cold-set gels are prepared using lower protein concentrations and milder heating conditions than heat-set gels due to the presence of additives including acid, salt and/ or enzymes. Kumar *et al.* recently reviewed studies from the past 10 years on OPI gelation properties, highlighting the positive effects of cold-set gels (Kumar *et al.*, 2021). Herein, we briefly summarise work conducted on oat protein gelation by analysing each additive separately. Current literature has also explored binary gels to improve functionality *i.e.*, via complexation with polysaccharides, however we focused on single component gels only (He *et al.*, 2021b; Lopes-da-Silva & Monteiro, 2019; Nieto-Nieto, Wang, Ozimek, & Chen, 2015; Nunes, Raymundo, & Sousa, 2006; Wang, Yang, Li-Sha, & Chen, 2021; Yang *et al.*, 2022a; Yang *et al.*, 2018).

3.3.4.1 Acidification

Recently, the morphology, rheology and mechanical properties of cold-set OPI gels were studied via glucono- δ -lactone (GDL) acidification (Yang *et al.*, 2017). 5% and 7% w/v OPI solutions were tested over a range of GDL concentrations. Since pH values of the OPI gel were within the isoelectric range for oat globulin (pH 4.4-6.8, Figure 4a), a particulate gel was formed. Authors proposed a microstructure of OPI monomers acting as building blocks and cross-linking to form a percolating, network structure. In all gels tested, the initial storage modulus (G') was always higher than the loss modules (G''), with G' reaching a plateau at approximately pH 6.8. Protein concentration directly influenced gel properties, since 7% OPI gels consistently showed a higher shear strength than 5% OPI. GDL concentration was also shown to be a key parameter since increasing it from 2-15% generally improved the mechanical properties.

At 10% w/w GDL, 7% w/v OPI gel showed comparable compressive strength (30 kPa) to egg white gel (22-32 kPa) (Hammerøj, Larsen, Ipsen, & Qvist, 2001; Yang *et al.*, 2017). This value was also larger than gels made from legume (lupin, pea and faba bean) protein isolates at 20% w/w (5-8 kPa) and GDL-modified 7% whey protein gel when compressed to 80% of its original height (22 kPa) (Cavallieri & da Cunha, 2008; Makri, Papalamprou, & Doxastakis, 2006; Yang *et al.*, 2017).

Although results from OPI gelation via GDL acidification show promisingly high gel strength, information on mechanistic details is lacking. Therefore, understanding of GDL-induced gelation of SPI by Kohyama *et al.* could serve as a model for understanding oat data (Kohyama, Sano, & Doi, 1995). In the first step, soy protein

was proposed to be partially denatured due to heat. GDL was then predicted to aid in aggregate formation by producing H⁺ ions that neutralised the negative charge on the protein surface which decreased electrostatic repulsion and promoted gel network formation. We expect a similar mechanism to take place with OPI samples.

3.3.4.2 Divalent ions

Whilst this cold-set gelation method remains unexplored to date with oat proteins, use of salt (typically containing divalent Ca²⁺) to enhance gel network formation is a common approach employed for soy protein due to its use in traditional tofu manufacturing. Mechanistically, Ca²⁺ ions promote protein aggregation *via* either electrostatic shielding, ion-specific hydrophobic interactions or *via* bridging of two acidic residues or adjacent anionic residues in the protein (Ma, Xiong, & Jiang, 2022b). The major difference between GDL and Ca²⁺ induced gelation in soy protein isolate comes down to kinetics, with an increase of one order of magnitude in the rate of reaction when using salt (Kohyama et al., 1995).

Notably, the type of gel formed (*i.e.*, particulate or filamentous) was found to be dependent on additive concentration in whey and soy proteins (Maltais, Remondetto, & Subirade, 2008; Remondetto & Subirade, 2003; Yang et al., 2017). At high ion concentration, particulate gel network dominated due to weaker protein-protein electrostatic repulsion promoting fast, random aggregate growth. At low Ca²⁺ concentration, filamentous gels dominated whereby repulsive forces encouraged gradual interactions between hydrophobic patches form linear aggregates.

3.3.4.3 Enzymes

Nieto-Nieto *et al.* explored the effects of partial enzymatic hydrolysis (degree of hydrolysis = 5-7%) on the mechanical properties of 15% w/v OPI gels using a selection of different proteases, *i.e.*, flavourzyme, alcalase, pepsin and trypsin (Nieto-Nieto, Wang, Ozimek, & Chen, 2014). This was based on previous work on soy and rice bran protein which report that gelling improvement is highly dependent on the selected enzyme (Hou & Zhao, 2011; Yeom, Lee, Ha, Ha, & Bae, 2010).

Partial hydrolysis changes protein secondary and tertiary structure, leading to exposure of different amino acids on the surface. The enzymes selected for this study have different activities, which are briefly summarised herein. Note that amino acid positions are conventionally categorised as P1, P2, P3 *etc.* when moving left of the hydrolysed bond and P1', P2', P3' *etc.* when moving to the right (Tacias-Pascacio et al., 2020). Enzymes can similarly be categorised as endo or exopeptidases, with the

former recognising amino acids in the middle of the chain whilst the latter cleaving terminal amino acids. Flavourzyme and alcalase are commercial enzymes blends. The former is typically derived from *A. oryzae* fungus and contains both exo-protease and endopeptidase activity due to the presence of aminopeptidase and carboxypeptidase (Waglay & Karboune, 2016). The latter is a blend of non-specific endopeptidases, classified as serine proteases, from a strain of *B. licheniformis*. Pepsin displays broad range protein cleavage at pH ≥ 2.0 , *i.e.*, under the pH conditions of the study (Keil, 1992). Trypsin, conversely, was described by Nieto-Nieto *et al.* as the most specific out of the enzymes in the study, as it preferentially cleaves at Arg and Lys when in position P1 (Nieto-Nieto *et al.*, 2014). When inserting the monomer 12S globulin sequence shown in Figure 2c (UniProt ID: O49258) into an enzyme cleavage site analysis tool, ExPASy PeptideCutter, 128 cleavage sites are predicted in the presence of pepsin at pH ≥ 2.0 , whilst 44 cleavage sites are predicted upon trypsin addition (Gasteiger *et al.*, 2005). Therefore, trypsin treatment is expected to show weaker protein degradation compared to pepsin.

SDS-PAGE results showed that the acidic α polypeptide in 12S globulin is more prone to enzymatic degradation than the basic β polypeptide due to its higher accessibility at the protein surface. The only exception to the norm was pepsin, which also showed degradation of the β subunit. Moreover, as expected, trypsin activity on the α chain was reduced compared to the other enzymes tested due to its more specific activity. Additionally, the authors also suggested that larger quantity of Glu in the α subunit, which is already at high levels in oat 12S globulin (Figure 2a), can favour specific alcalase degradation, as a Glu specific protease has been previously isolated from alcalase blend (Svendsen & Breddam, 1992). Note that alcalase and pepsin treatment resulted in the largest deviation from the protein native structure. The former was likely due to the high Glu contents in the α chain of 12S globulin (Figure 2a, 2c) whilst the latter is presumably due to its broad range specificity, higher temperature (50 °C) and extreme pH treatment needed for pepsin (pH 2.0).

Flavourzyme and trypsin treatment increased gel hardness compared to untreated OPI, especially at higher pH values (8-9) where these showed comparable strength to egg white protein gels (8.70 N) at the same pH. However, previous work conducted with trypsin treatment of oat protein isolate at lower concentrations and temperatures (10% w/v, 100 °C) resulted in weaker gel structures than the native

protein control (Ma & Wood, 1987a, 1987b). Nieto-Nieto *et al.* attributed this to a decrease in the size of polypeptides upon trypsin treatment perhaps reducing their ability to associate into a strong gel network. Therefore, experimental conditions are also essential and should be carefully selected for cold-set gels.

Enzymatically induced cold-set oat globulin gels using microbial transglutaminase have also been synthesised and compared to native heat-induced gels, resulting in improved the protein solubility (Siu, Ma, Mock, & Mine, 2002). Transglutaminase catalyses an acyl transfer reaction, forming an inter- or intra-molecular covalent bond between the carboxamide group of Gln residues and the free amine of Lys residues (Griffin, Casadio, & Bergamini, 2002). G' and G'' for heat-induced gel (10%, pH 9.0, 100 °C for 20 mins) were 34 and 11.8 Pa respectively at 1.0 Hz. Similar values were obtained upon incubation with transglutaminase for 2 hours ($G' = 32.6$ Pa; $G'' = 20.5$ Pa), however increasing the incubation time to 8 hours resulted in maximum values of G' ($G' = 574$ Pa; $G'' = 207$ Pa). Higher storage modulus values for the enzymatically modified gels were attributed to the formation of a chemical gel with covalent $\epsilon(\gamma\text{-glutamyl})\text{-lysine}$ cross links, whilst the heat-set gel resembled a physical gel held together by non-covalent interactions.

Overall, oat protein gelation appears to be qualitatively similar to soy and pea protein gelation, whilst rice protein stands out with a weak gelation potential as a result of its native structure. Direct comparisons should be considered with caution due to the presence of impurities as a result of crop variations or extraction methods (Zheng *et al.*, 2022). Importantly, antinutrients (phytic acid, tannic acid, saponins) were shown to have an effect on heat-set gelation of soy, pea and rice protein isolates as these interact with the proteins, affecting their functional properties *e.g.* solubility (Kaspchak, Silveira, Igarashi-Mafra, & Mafra, 2020). We conclude that limitations in oat protein gel strength at neutral pH can potentially be overcome by cold-set gelation. Importantly, the literature highlights that gelation properties appear to be intricately linked to inherent protein properties, especially solubility and surface hydrophobicity. There is also a clear gap in the literature for a detailed mechanistic understanding of oat protein gelation, in particular cold-set gelation, and the importance of individual 2S, 7S and 12S globulin fractions in determining OPI gel properties. We also highlight the ample opportunity to explore microgelation of oat globulins after recent successful attempts with structurally analogous pea and soy globulins.

4. CONCLUSIONS AND FUTURE OUTLOOK

Whilst oat proteins have the potential to increase their widespread application as a plant-based alternative, it is clear that fundamental understanding of structure-function relationships is a much-needed endeavour which seems to be overlooked in literature to date. After a compilation of work conducted on oat proteins and complementing studies on rice, pea and soy, oat protein structure was found to differ from most other cereals since its major Osborne fraction is globulin. Whilst at the amino acid scale, oat was previously reported to strongly resembled rice glutelin, its folded quaternary state is homologous to that of 11S legumins from pea and soy. Previous studies also highlighted a unique exposed Gln-rich octapeptide in oat 12S globulin. This differentiates its structure from rice glutelin and other plant storage globulins, whilst likely influencing oat protein functionality.

Protein functionality studies suggested that behaviour of plant proteins in aqueous solution (solubility, surface hydrophobicity, surface charge) is crucial and these appear to define subsequent multiple phase and phase transition properties. However, due to lack of complete data in oat, this needs further confirmation. It was also noted that functionality is highly variable on internal (growth conditions, genetic variations) and external factors (extraction method, experimental conditions). These differences can often be linked to resulting differences in the protein structure and consequently performance. Overall, oat protein behaviour was shown to resemble more closely that of legumes than rice, indicating that quaternary structure, *i.e.*, the protein's native folded state, is likely defining its functionality.

The review has also emphasised major areas that remain to be explored in the field of oat proteins. A thorough investigation of three-dimensional oat 12S globulin structure *via* X-ray diffraction is needed to corroborate current protein structure prediction and to fully appreciate the structural aspects of oat protein. Whilst studies have focused on oat protein modification empirically, there is a lack of fundamental understanding of oat protein functionality compared to legumes (*e.g.*, surface tension measurements at the oil-water interface, lack of small angle neutron and X-ray scattering). This can help design a bottom-up approach for defining the protein modification required for a specific application. Finally, there are currently limited rheological and no tribological or adsorption studies using oat proteins. We believe

these are necessary to provide quantitative understanding of mouthfeel attributes to incorporate oat proteins into plant-based food design.

DECLARATION OF COMPETING INTEREST

Authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the Engineering and Physical Sciences Research Council (EPSRC) funded Centre for Doctoral Training in Soft Matter for Formulation and Industrial Innovation (SOFI²), Grant Ref. No. EP/S023631/1 for financial support. This work was co-funded by Science and Technology, Oatly, U.K.

LITERATURE CITED

- Adachi, M., Kanamori, J., Masuda, T., Yagasaki, K., Kitamura, K., Mikami, B., & Utsumi, S. (2003). Crystal structure of soybean 11S globulin: glycinin A3B4 homohexamer. *Proceedings of the National Academy of Sciences of the United States of America*, 100(12), 7395-7400.
- Adachi, M., Takenaka, Y., Gidamis, A. B., Mikami, B., & Utsumi, S. (2001). Crystal structure of soybean proglycinin A1aB1b homotrimer. *Journal of Molecular Biology*, 305(2), 291-305.
- Adal, E., Sadeghpour, A., Connell, S., Rappolt, M., Ibanoglu, E., & Sarkar, A. (2017). Heteroprotein Complex Formation of Bovine Lactoferrin and Pea Protein Isolate: A Multiscale Structural Analysis. *Biomacromolecules*, 18(2), 625-635.
- Adamcik, J., Jung, J.-M., Flakowski, J., De Los Rios, P., Dietler, G., & Mezzenga, R. (2010). Understanding amyloid aggregation by statistical analysis of atomic force microscopy images. *Nature Nanotechnology*, 5(6), 423-428.
- Alizadeh-Pasdar, N., & Li-Chan, E. C. Y. (2000). Comparison of Protein Surface Hydrophobicity Measured at Various pH Values Using Three Different Fluorescent Probes. *Journal of Agricultural and Food Chemistry*, 48(2), 328-334.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389-3402.
- Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017). The composition, extraction, functionality and applications of rice proteins: A review. *Trends in Food Science & Technology*, 64, 1-12.
- Amagliani, L., O'Regan, J., Schmitt, C., Kelly, A. L., & O'Mahony, J. A. (2019). Characterisation of the physicochemical properties of intact and hydrolysed rice protein ingredients. *Journal of Cereal Science*, 88, 16-23.
- Andrews, B. A., & Asenjo, J. A. (2010). Theoretical and Experimental Evaluation of Hydrophobicity of Proteins to Predict their Partitioning Behavior in Aqueous

- Two Phase Systems: A Review. *Separation Science and Technology*, 45(15), 2165-2170.
- Anema, S. G., & de Kruif, C. G. (2013). Coacervates of lysozyme and β -casein. *Journal of Colloid and Interface Science*, 398, 255-261.
- Anema, S. G., & de Kruif, C. G. (2016). Phase separation and composition of coacervates of lactoferrin and caseins. *Food Hydrocolloids*, 52, 670-677.
- Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P., & Stubbs, J. M. (1975). The structure, physical and chemical properties of the soy bean protein glycinin. *Biochimica et Biophysica Acta (BBA) - Protein Structure*, 412(2), 214-228.
- Bos, M. A., & van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a review. *Advances in Colloid and Interface Science*, 91(3), 437-471.
- Boukid, F. (2021). Oat proteins as emerging ingredients for food formulation: where we stand? *European Food Research and Technology*, 247(3), 535-544.
- Brückner-Gühmann, M., Kratzsch, A., Sozer, N., & Drusch, S. (2021). Oat protein as plant-derived gelling agent: Properties and potential of modification. *Future Foods*, 4.
- Brummer, Y., Defelice, C., Wu, Y., Kwong, M., Wood, P. J., & Tosh, S. M. (2014). Textural and Rheological Properties of Oat Beta-Glucan Gels with Varying Molecular Weight Composition. *Journal of Agricultural and Food Chemistry*, 62(14), 3160-3167.
- Buggenhout, J., Brijs, K., & Delcour, J. A. (2013). Impact of processing conditions on the extractability and molecular weight distribution of proteins in parboiled brown rice. *Journal of Cereal Science*, 58(1), 8-14.
- Burgess, S. R., Shewry, P. R., Matlashewski, G. J., Altosaar, I., & Mifflin, B. J. (1983). Characteristics Of Oat (*Avena sativa* L.) Seed Globulins. *Journal of Experimental Botany*, 34(10), 1320-1332.
- Cardamone, M., & Puri, N. K. (1992). Spectrofluorimetric assessment of the surface hydrophobicity of proteins. *Biochemical Journal*, 282 (Pt 2)(Pt 2), 589-593.
- Cavallieri, A. L. F., & da Cunha, R. L. (2008). The effects of acidification rate, pH and ageing time on the acidic cold set gelation of whey proteins. *Food Hydrocolloids*, 22(3), 439-448.
- Chang, C., Tu, S., Ghosh, S., & Nickerson, M. T. (2015). Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, 77, 360-367.
- Chen, N., Nicolai, T., Chassenieux, C., & Wang, Y. (2020a). pH and ionic strength responsive core-shell protein microgels fabricated via simple coacervation of soy globulins. *Food Hydrocolloids*, 105.
- Chen, N., Zhao, M., Nicolai, T., & Chassenieux, C. (2017). Exploiting Salt Induced Microphase Separation To Form Soy Protein Microcapsules or Microgels in Aqueous Solution. *Biomacromolecules*, 18(7), 2064-2072.
- Chen, N., Zhao, Z., Wang, Y., & Dimova, R. (2020b). Resolving the Mechanisms of Soy Glycinin Self-Coacervation and Hollow-Condensate Formation. *ACS Macro Letters*, 9(12), 1844-1852.
- Cochereau, R., Nicolai, T., Chassenieux, C., & Silva, J. V. C. (2019). Mechanism of the spontaneous formation of plant protein microcapsules in aqueous solution. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 562, 213-219.

- Crippa, M., Solazzo, E., Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., & Leip, A. (2021). Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, 2(3), 198-209.
- Cui, L., Bandillo, N., Wang, Y., Ohm, J.-B., Chen, B., & Rao, J. (2020). Functionality and structure of yellow pea protein isolate as affected by cultivars and extraction pH. *Food Hydrocolloids*, 108, 106008.
- Dai, H., Zhan, F., Chen, Y., Shen, Q., Geng, F., Zhang, Z., & Li, B. (2022). Improvement of the solubility and emulsification of rice protein isolate by the pH shift treatment. *International Journal of Food Science & Technology*, 58(1), 355-366.
- de Jongh, H. H., Kusters, H. A., Kudryashova, E., Meinders, M. B., Trofimova, D., & Wierenga, P. A. (2004). Protein adsorption at air-water interfaces: a combination of details. *Biopolymers*, 74(1-2), 131-135.
- de Kruif, C. G., Pedersen, J., Huppertz, T., & Anema, S. G. (2013). Coacervates of Lactotransferrin and β - or κ -Casein: Structure Determined Using SAXS. *Langmuir*, 29(33), 10483-10490.
- Deng, R., Mars, M., Van Der Sman, R. G. M., Smeets, P. A. M., & Janssen, A. E. M. (2020). The importance of swelling for in vitro gastric digestion of whey protein gels. *Food Chemistry*, 330, 127182.
- Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry*, 15(1), 3-24.
- Dhanjal, N. I., Sharma, S., & Prakash, N. T. (2016). Quantification and In Vitro Bioaccessibility of Selenium from Osborne Fractions of Selenium-Rich Cereal Grains. *Cereal Chemistry*, 93(4), 339-343.
- Dickinson, C. D., Hussein, E. H., & Nielsen, N. C. (1989). Role of posttranslational cleavage in glycinin assembly. *The Plant Cell*, 1(4), 459-469.
- Drusch, S., Klost, M., & Kieserling, H. (2021). Current knowledge on the interfacial behaviour limits our understanding of plant protein functionality in emulsions. *Current Opinion in Colloid & Interface Science*, 56.
- Du, B., Meenu, M., Liu, H., & Xu, B. (2019). A Concise Review on the Molecular Structure and Function Relationship of β -Glucan. *International Journal of Molecular Sciences*, 20(16), 4032.
- Dunwell, J. M., Gibbings, J. G., Mahmood, T., & Saqlan Naqvi, S. M. (2008). Germin and Germin-like Proteins: Evolution, Structure, and Function. *Critical Reviews in Plant Sciences*, 27(5), 342-375.
- Ercili-Cura, D., Miyamoto, A., Paananen, A., Yoshii, H., Poutanen, K., & Partanen, R. (2015). Adsorption of oat proteins to air–water interface in relation to their colloidal state. *Food Hydrocolloids*, 44, 183-190.
- Felix, M., Romero, A., & Guerrero, A. (2016). Development and evaluation of rheological and bioactive properties of rice protein-based gels. *Journal of Cereal Science*, 72, 91-100.
- Friedman, M. (2018). Analysis, Nutrition, and Health Benefits of Tryptophan. *International Journal of Tryptophan Research*, 11, 1178646918802282.
- Fukushima, D. (1991). Structures of plant storage proteins and their functions. *Food Reviews International*, 7(3), 353-381.
- Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. e., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). *Protein identification and analysis tools on the ExPASy server*. Springer.
- Griffin, M., Casadio, R., & Bergamini, C. M. (2002). Transglutaminases: nature's biological glues. *Biochemical Journal*, 368(Pt 2), 377-396.

- Grossmann, L. (2023). Structural properties of pea proteins (*Pisum sativum*) for sustainable food matrices. *Crit Rev Food Sci Nutr*, 1-21.
- Grossmann, L., & McClements, D. J. (2023). Current insights into protein solubility: A review of its importance for alternative proteins. *Food Hydrocolloids*, 137.
- Gwiazda, S., Schwenke, K. D., & Rutkowski, A. (1980). Isolation and partial characterization of proteins from pea (*Pisum sativum* L.). *Food*, 24(10), 939-950.
- Hammerøj, M., Larsen, L. B., Ipsen, R. H., & Qvist, K. B. (2001). Effect of hen egg production and protein composition on textural properties of egg albumen gels. *Journal of Texture Studies*, 32(2), 105-129.
- Harwalkar, V. R., Ma, C. Y., & Boutin-Muma, B. (1989). Changes of oat globulin during storage with sodium dodecyl sulfate. *Canadian Institute of Food Science and Technology Journal*, 22(4), 387-389.
- He, T., Wang, J., & Hu, X. (2021a). Effect of heat treatment on the structure and digestion properties of oat globulin. *Cereal Chemistry*, 98(3), 740-748.
- He, Z., Ma, T., Zhang, W., Su, E., Cao, F., Huang, M., & Wang, Y. (2021b). Heat-induced gel formation by whey protein isolate-Lycium barbarum polysaccharides at varying pHs. *Food Hydrocolloids*, 115, 106607.
- Heldt, C. L., Zahid, A., Vijayaragavan, K. S., & Mi, X. (2017). Experimental and computational surface hydrophobicity analysis of a non-enveloped virus and proteins. *Colloids and Surfaces B: Biointerfaces*, 153, 77-84.
- Hofmeister, F. (1888). On the understanding of the effects of salts. *Archiv für experimentelle Pathologie und Pharmakologie*, 24, 247-260.
- Holt, L. A., Milligan, B., & Roxburgh, C. M. (1971). Aspartic acid, asparagine, glutamic acid, and glutamine contents of wool and two derived protein fractions. *Australian Journal of Biological Sciences*, 24(3), 509-514.
- Hou, Y., & Zhao, X. (2011). Limited hydrolysis of two soybean protein products with trypsin or neutrase and the impacts on their solubility, gelation and fat absorption capacity. *Biotechnology*, 10(2), 190-196.
- Hu, H., Wu, J., Li-Chan, E. C. Y., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food Hydrocolloids*, 30(2), 647-655.
- Hua, L., Zhou, R., Thirumalai, D., & Berne, B. J. (2008). Urea denaturation by stronger dispersion interactions with proteins than water implies a 2-stage unfolding. *Proceedings of the National Academy of Sciences*, 105(44), 16928-16933.
- Huang, Z., Sun, J., Zhao, L., He, W., Liu, T., & Liu, B. (2022). Analysis of the gel properties, microstructural characteristics, and intermolecular forces of soybean protein isolate gel induced by transglutaminase. *Food Science & Nutrition*, 10(3), 772-783.
- Hyde, A. M., Zultanski, S. L., Waldman, J. H., Zhong, Y.-L., Shevlin, M., & Peng, F. (2017). General Principles and Strategies for Salting-Out Informed by the Hofmeister Series. *Organic Process Research & Development*, 21(9), 1355-1370.
- Immonen, M., Chandrakusuma, A., Hokkanen, S., Partanen, R., Mäkelä-Salmi, N., & Myllärinen, P. (2022). The effect of deamidation and lipids on the interfacial and foaming properties of ultrafiltered oat protein concentrates. *Lwt*, 169.

- Jamadagni, S. N., Godawat, R., & Garde, S. (2011). Hydrophobicity of Proteins and Interfaces: Insights from Density Fluctuations. *Annual Review of Chemical and Biomolecular Engineering*, 2(1), 147-171.
- Jiang, L., Wang, Z., Li, Y., Meng, X., Sui, X., Qi, B., & Zhou, L. (2015a). Relationship Between Surface Hydrophobicity and Structure of Soy Protein Isolate Subjected to Different Ionic Strength. *International Journal of Food Properties*, 18(5), 1059-1074.
- Jiang, Z., Sontag-Strohm, T., Salovaara, H., Sibakov, J., Kanerva, P., & Lojonen, J. (2015b). Oat protein solubility and emulsion properties improved by enzymatic deamidation. *Journal of Cereal Science*, 64, 126-132.
- Jing, X., Yang, C., & Zhang, L. (2016). Characterization and Analysis of Protein Structures in Oat Bran. *Journal of Food Science*, 81(10), C2337-C2343.
- Ju, Z. Y., Hettiarachchy, N. S., & Rath, N. (2001). Extraction, denaturation and hydrophobic Properties of Rice Flour Proteins. *Journal of Food Science*, 66(2), 229-232.
- Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Zidek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A. W., Kavukcuoglu, K., Kohli, P., & Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873), 583-589.
- Jung, J.-M., Savin, G., Pouzot, M., Schmitt, C., & Mezzenga, R. (2008). Structure of Heat-Induced β -Lactoglobulin Aggregates and their Complexes with Sodium-Dodecyl Sulfate. *Biomacromolecules*, 9(9), 2477-2486.
- Jung, R., Scott, M. P., Nam, Y. W., Beaman, T. W., Bassüner, R., Saalbach, I., Müntz, K., & Nielsen, N. C. (1998). The role of proteolysis in the processing and assembly of 11S seed globulins. *Plant Cell*, 10(3), 343-357.
- Kamal, N., Tsardakas Renhuldt, N., Bentzer, J., Gundlach, H., Haberer, G., Juhasz, A., Lux, T., Bose, U., Tye-Din, J. A., Lang, D., van Gessel, N., Reski, R., Fu, Y. B., Spegel, P., Ceplitis, A., Himmelbach, A., Waters, A. J., Bekele, W. A., Colgrave, M. L., Hansson, M., Stein, N., Mayer, K. F. X., Jellen, E. N., Maughan, P. J., Tinker, N. A., Mascher, M., Olsson, O., Spannagl, M., & Sirijovski, N. (2022). The mosaic oat genome gives insights into a uniquely healthy cereal crop. *Nature*, 606(7912), 113-119.
- Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9), 2742-2750.
- Kaspchak, E., Silveira, J. L. M., Igarashi-Mafra, L., & Mafra, M. R. (2020). Effect of antinutrients on heat-set gelation of soy, pea, and rice protein isolates. *Journal of Food Science and Technology*, 57(11), 4201-4210.
- Katsube-Tanaka, T., Duldulao, J. B., Kimura, Y., Iida, S., Yamaguchi, T., Nakano, J., & Utsumi, S. (2004). The two subfamilies of rice glutelin differ in both primary and higher-order structures. *Biochim Biophys Acta*, 1699(1-2), 95-102.
- Keil, B. (1992). *Specificity of Proteolysis*: Springer Berlin Heidelberg.
- Kew, B., Holmes, M., Lamas, E., Ettelaie, R., Connell, S. D., Dini, D., & Sarkar, A. (2023). Transforming sustainable plant proteins into high performance lubricating microgels. *Nature Communications*, 14(1), 4743.

- Kew, B., Holmes, M., Stieger, M., & Sarkar, A. (2021). Oral tribology, adsorption and rheology of alternative food proteins. *Food Hydrocolloids*, 116.
- Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemists' Society*, 56(3), 242-258.
- Kitamura, K., & Shibasaki, K. (2014). Isolation and Some Physico-chemical Properties of the Acidic Subunits of Soybean 11S Globulin. *Agricultural and Biological Chemistry*, 39(5), 945-951.
- Klose, C., & Arendt, E. K. (2012). Proteins in oats; their synthesis and changes during germination: a review. *Critical Reviews in Food Science and Nutrition*, 52(7), 629-639.
- Kohyama, K., Sano, Y., & Doi, E. (1995). Rheological characteristics and gelation mechanism of tofu (soybean curd). *Journal of Agricultural and Food Chemistry*, 43(7), 1808-1812.
- Kontogiorgos, V., & Prakash, S. (2023). Adsorption kinetics and dilatational rheology of plant protein concentrates at the air- and oil-water interfaces. *Food Hydrocolloids*, 138.
- Kornet, R., Roozalipour, S. L., Venema, P., van der Goot, A. J., Meinders, M. B. J., & van der Linden, E. (2022). Coacervation in pea protein solutions: The effect of pH, salt, and fractionation processing steps. *Food Hydrocolloids*, 125.
- Kramer, R. M., Shende, V. R., Motl, N., Pace, C. N., & Scholtz, J. M. (2012). Toward a molecular understanding of protein solubility: increased negative surface charge correlates with increased solubility. *Biophysical Journal*, 102(8), 1907-1915.
- Kristensen, H. T., Christensen, M., Hansen, M. S., Hammershøj, M., & Dalsgaard, T. K. (2022). Mechanisms behind protein-protein interactions in a β -lg-legumin co-precipitate. *Food Chemistry*, 373, 131509.
- Kumamaru, T., Uemura, Y., Inoue, Y., Takemoto, Y., Siddiqui, S. U., Ogawa, M., Hara-Nishimura, I., & Satoh, H. (2010). Vacuolar processing enzyme plays an essential role in the crystalline structure of glutelin in rice seed. *Plant & Cell Physiology*, 51(1), 38-46.
- Kumar, L., Sehrawat, R., & Kong, Y. (2021). Oat proteins: A perspective on functional properties. *Lwt*, 152.
- Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β -glucans in solution and gels. *Food Hydrocolloids*, 17(5), 693-712.
- Li, R., & Xiong, Y. L. (2021a). Sensitivity of oat protein solubility to changing ionic strength and pH. *Journal of Food Science*, 86(1), 78-85.
- Li, R., & Xiong, Y. L. (2021b). Ultrasound-induced structural modification and thermal properties of oat protein. *Lwt*, 149.
- Li, R., & Xiong, Y. L. (2023a). Disulfide cleavage to improve interfacial behavior and emulsification properties of oat protein. *Food Chem*, 404(Pt A), 134511.
- Li, S., Yang, X., Zhang, Y., Ma, H., Liang, Q., Qu, W., He, R., Zhou, C., & Mahunu, G. K. (2016). Effects of ultrasound and ultrasound assisted alkaline pretreatments on the enzymolysis and structural characteristics of rice protein. *Ultrasonics Sonochemistry*, 31, 20-28.
- Li, T., Zhou, J., Wu, Q., Zhang, X., Chen, Z., & Wang, L. (2023b). Modifying functional properties of food amyloid-based nanostructures from rice glutelin. *Food Chemistry*, 398, 133798.

- Liama, E., Connell, S. D., & Sarkar, A. (2023). Frictional behaviour of plant proteins in soft contacts: unveiling nanoscale mechanisms. *Nanoscale Advances*, 5(4), 1102-1114.
- Liu, F., & Tang, C.-H. (2016). Soy glycinin as food-grade Pickering stabilizers: Part. I. Structural characteristics, emulsifying properties and adsorption/arrangement at interface. *Food Hydrocolloids*, 60, 606-619.
- Liu, G., Li, J., Shi, K., Wang, S., Chen, J., Liu, Y., & Huang, Q. (2009). Composition, secondary structure, and self-assembly of oat protein isolate. *Journal of Agricultural and Food Chemistry*, 57(11), 4552-4558.
- Liu, Y., Li, X., Zhou, X., Yu, J., Wang, F., & Wang, J. (2011). Effects of glutaminase deamidation on the structure and solubility of rice glutelin. *Lwt*, 44(10), 2205-2210.
- Lopes-da-Silva, J. A., & Monteiro, S. R. (2019). Gelling and emulsifying properties of soy protein hydrolysates in the presence of a neutral polysaccharide. *Food Chemistry*, 294, 216-223.
- Lui, D. Y. M., Litster, J. D., & White, E. T. (2007). Precipitation of soy proteins: Particle formation and protein separation. *American Institute of Chemical Engineers Journal*, 53(2), 514-522.
- Ma, C., & Harwalkar, V. R. (1988a). Studies of Thermal Denaturation of Oat Globulin by Differential Scanning Calorimetry. *Journal of Food Science*, 53(2), 531-534.
- Ma, C., Rout, M. K., & Phillips, D. L. (2003). Study of Thermal Aggregation and Gelation of Oat Globulin by Raman Spectroscopy. *Spectroscopy*, 17, 752027.
- Ma, C., & Wood, D. F. (1987a). Functional properties of oat proteins modified by acylation, trypsin hydrolysis or linoleate treatment. *Journal of the American Oil Chemists' Society*, 64(12), 1726-1731.
- Ma, C., & Wood, D. F. (1987b). Functional properties of oat proteins modified by acylation, trypsin hydrolysis or linoleate treatment. *Journal of the American Oil Chemists' Society*, 64(12), 1726-1731.
- Ma, C. Y. (1983). Preparation, Composition and Functional Properties of Oat Protein Isolates. *Canadian Institute of Food Science and Technology Journal*, 16(3), 201-205.
- Ma, C. Y., & Harwalkar, V. R. (1988b). Study of thermal denaturation of oat globulin by ultraviolet and fluorescence spectrophotometry. *Journal of Agricultural and Food Chemistry*, 36(1), 155-160.
- Ma, C. Y., Harwalkar, V. R., & Paquet, A. (1990). Physicochemical properties of alkali-treated oat globulin. *Journal of Agricultural and Food Chemistry*, 38(8), 1707-1711.
- Ma, C. Y., Khanzada, G., & Harwalkar, V. R. (1988c). Thermal gelation of oat globulin. *Journal of Agricultural and Food Chemistry*, 36(2), 275-280.
- Ma, K. K., Greis, M., Lu, J., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022a). Functional Performance of Plant Proteins. *Foods*, 11(4).
- Ma, T., Xiong, Y. L., & Jiang, J. (2022b). Calcium-aided fabrication of pea protein hydrogels with filler emulsion particles coated by pH12-shifting and ultrasound treated protein. *Food Hydrocolloids*, 125.
- Mäkinen, O. E., Sozer, N., Ercili-Cura, D., & Poutanen, K. (2017). Chapter 6 - Protein From Oat: Structure, Processes, Functionality, and Nutrition. In S. R. Nadathur, J. P. D. Wanasundara & L. Scanlin (Eds.), *Sustainable Protein Sources* (pp. 105-119). San Diego: Academic Press.

- Makri, E. A., Papalamprou, E. M., & Doxastakis, G. I. (2006). Textural properties of legume protein isolate and polysaccharide gels. *Journal of the Science of Food and Agriculture*, 86(12), 1855-1862.
- Maltais, A., Remondetto, G. E., & Subirade, M. (2008). Mechanisms involved in the formation and structure of soya protein cold-set gels: A molecular and supramolecular investigation. *Food Hydrocolloids*, 22(4), 550-559.
- Martin, A. H., Bos, M. A., & van Vliet, T. (2002). Interfacial rheological properties and conformational aspects of soy glycinin at the air/water interface. *Food Hydrocolloids*, 16(1), 63-71.
- Matulis, D., & Lovrien, R. (1998). 1-Anilino-8-naphthalene sulfonate anion-protein binding depends primarily on ion pair formation. *Biophysical Journal*, 74(1), 422-429.
- McSwiney, M., Singh, H., & Campanella, O. H. (1994). Thermal aggregation and gelation of bovine β -lactoglobulin. *Food Hydrocolloids*, 8(5), 441-453.
- Mel, R., & Malalgoda, M. (2021). Oat protein as a novel protein ingredient: Structure, functionality, and factors impacting utilization. *Cereal Chemistry*, 99(1), 21-36.
- Mel, R., Rampitsch, C., Zvomuya, F., Nilsen, K. T., Beattie, A. D., & Malalgoda, M. (2024). Determining the Impact of Genotype \times Environment on Oat Protein Isolate Composition Using HPLC and LC-MS Techniques. *Journal of Agricultural and Food Chemistry*.
- Mession, J.-L., Chihi, M. L., Sok, N., & Saurel, R. (2015). Effect of globular pea proteins fractionation on their heat-induced aggregation and acid cold-set gelation. *Food Hydrocolloids*, 46, 233-243.
- Mileti, O., Baldino, N., Carmona, J. A., Lupi, F. R., Muñoz, J., & Gabriele, D. (2022). Shear and dilatational rheological properties of vegetable proteins at the air/water interface. *Food Hydrocolloids*, 126.
- Moelbert, S., Emberly, E., & Tang, C. (2004). Correlation between sequence hydrophobicity and surface-exposure pattern of database proteins. *Protein Science*, 13(3), 752-762.
- Mohamed, A., Biresaw, G., Xu, J., Hojilla-Evangelista, M. P., & Rayas-Duarte, P. (2009). Oats protein isolate: Thermal, rheological, surface and functional properties. *Food Research International*, 42(1), 107-114.
- Moreira, M. A., Hermodson, M. A., Larkins, B. A., & Nielsen, N. C. (1979). Partial characterization of the acidic and basic polypeptides of glycinin. *Journal of Biological Chemistry*, 254(19), 9921-9926.
- Mori, T., Nakamura, T., & Utsumi, S. (1986). Behavior of intermolecular bond formation in the late stage of heat-induced gelation of glycinin. *Journal of Agricultural and Food Chemistry*, 34(1), 33-36.
- Moulik, S. P., Rakshit, A. K., Pan, A., & Naskar, B. (2022). An Overview of Coacervates: The Special Disperse State of Amphiphilic and Polymeric Materials in Solution. *Colloids and Interfaces*, 6(3).
- Nakai, S. (2003). Measurement of Protein Hydrophobicity. *Current Protocols in Food Analytical Chemistry*, 9(1), B5.2.1-B5.2.13.
- Nicolai, T., & Chassenieux, C. (2019). Heat-induced gelation of plant globulins. *Current Opinion in Food Science*, 27, 18-22.
- Nieto-Nieto, T. V., Wang, Y., Ozimek, L., & Chen, L. (2016). Improved thermal gelation of oat protein with the formation of controlled phase-separated networks using dextran and carrageenan polysaccharides. *Food Research International*, 82, 95-103.

1851 Nieto-Nieto, T. V., Wang, Y. X., Ozimek, L., & Chen, L. (2014). Effects of partial
1852 hydrolysis on structure and gelling properties of oat globular proteins. *Food*
1853 *Research International*, 55, 418-425.

1854 Nieto-Nieto, T. V., Wang, Y. X., Ozimek, L., & Chen, L. (2015). Inulin at low
1855 concentrations significantly improves the gelling properties of oat protein – A
1856 molecular mechanism study. *Food Hydrocolloids*, 50, 116-127.

1857 Niño, M. R. R., Sánchez, C., Ruíz-Henestrosa, V. P., & Patino, J. M. R. (2005). Milk
1858 and soy protein films at the air-water interface. *Food Hydrocolloids*, 19(3),
1859 417-428.

1860 Nivala, O., Makinen, O. E., Kruus, K., Nordlund, E., & Ercili-Cura, D. (2017).
1861 Structuring colloidal oat and faba bean protein particles via enzymatic
1862 modification. *Food Chemistry*, 231, 87-95.

1863 Nunes, M. C., Raymundo, A., & Sousa, I. (2006). Rheological behaviour and
1864 microstructure of pea protein/k-carrageenan/starch gels with different setting
1865 conditions. *Food Hydrocolloids*, 20(1), 106-113.

1866 O'Kane, F. E., Happe, R. P., Vereijken, J. M., Gruppen, H., & van Boekel, M. A. J. S.
1867 (2004). Heat-Induced Gelation of Pea Legumin: Comparison with Soybean
1868 Glycinin. *Journal of Agricultural and Food Chemistry*, 52(16), 5071-5078.

1869 Okagu, O. D., & Udenigwe, C. C. (2021). Molecular Interactions of Pea Globulin,
1870 Albumin and Glutelin With Curcumin: Formation and Gastric Release
1871 Mechanisms of Curcumin-loaded Bio-nanocomplexes. *Food Biophysics*,
1872 17(1), 10-25.

1873 Omura, M. H., de Oliveira, A. P. H., Soares, L. d. S., Coimbra, J. S. d. R., de Barros,
1874 F. A. R., Vidigal, M. C. T. R., Baracat-Pereira, M. C., & de Oliveira, E. B.
1875 (2021). Effects of protein concentration during ultrasonic processing on
1876 physicochemical properties and techno-functionality of plant food proteins.
1877 *Food Hydrocolloids*, 113.

1878 Osborne, T. B. (1907). *The Proteins of the Wheat Kernel*: Carnegie Institution of
1879 Washington.

1880 Park, K. H., & Lund, D. B. (1984). Calorimetric Study of Thermal Denaturation of β -
1881 Lactoglobulin. *Journal of Dairy Science*, 67(8), 1699-1706.

1882 Peng, I., Quass, D., Dayton, W., & Allen, C. (1984). The physicochemical and
1883 functional properties of soybean 11S globulin-A review. *Cereal Chem*, 61(6),
1884 480-490.

1885 Peng, X., Ren, C., & Guo, S. (2016). Particle formation and gelation of soymilk:
1886 Effect of heat. *Trends in Food Science & Technology*, 54, 138-147.

1887 Peterson, D. M. (1978). Subunit structure and composition of oat seed globulin. *Plant*
1888 *physiology*, 62(4), 506-509.

1889 Plietz, P., Damaschun, G., Müller, J. J., & Schwenke, K. D. (1983). The Structure of
1890 11-S Globulins from Sunflower and Rape Seed. *European Journal of*
1891 *Biochemistry*, 130(2), 315-320.

1892 Plietz, P., Drescher, B., & Damaschun, G. (1988). Structure and Evolution of the 11S
1893 Globulins: Conclusions from Comparative Evaluation of Amino Acid
1894 Sequences and X-ray scattering Data). *Biochemie und Physiologie der*
1895 *Pflanzen*, 183(2), 199-203.

1896 Plietz, P., Zirwer, D., Schlesier, B., Gast, K., & Damaschun, G. (1984). Shape,
1897 symmetry, hydration and secondary structure of the legumin from Vicia faba in
1898 solution. *Biochimica et Biophysica Acta*, 784(2-3), 140-146.

1899 Punia, S., Sandhu, K. S., Dhull, S. B., Siroha, A. K., Purewal, S. S., Kaur, M., &
1900 Kidwai, M. K. (2020). Oat starch: Physico-chemical, morphological,

1901 rheological characteristics and its applications - A review. *International Journal*
1902 *of Biological Macromolecules*, 154, 493-498.

1903 Ralla, T., Salminen, H., Edelmann, M., Dawid, C., Hofmann, T., & Weiss, J. (2018).
1904 Oat bran extract (*Avena sativa* L.) from food by-product streams as new
1905 natural emulsifier. *Food Hydrocolloids*, 81, 253-262.

1906 Reichelt, R., Schwenke, K., König, T., Pähitz, W., & Wangermann, G. (1980). Electron
1907 microscopic studies for estimation of the quaternary structure of the 11S
1908 globulin (Helianthinin) from sunflower seed (*Helianthus annuus* L.). *Biochemie*
1909 *und Physiologie der Pflanzen*, 175(7), 653-663.

1910 Remondetto, G. E., & Subirade, M. (2003). Molecular mechanisms of Fe²⁺-induced
1911 beta-lactoglobulin cold gelation. *Biopolymers*, 69(4), 461-469.

1912 Riblett, A. L., Herald, T. J., Schmidt, K. A., & Tilley, K. A. (2001). Characterization of
1913 β -Conglycinin and Glycinin Soy Protein Fractions from Four Selected
1914 Soybean Genotypes. *Journal of Agricultural and Food Chemistry*, 49(10),
1915 4983-4989.

1916 Robert, L. S., Nozzolillo, C., & Altosaar, I. (1985a). Characterization of oat (*Avena*
1917 *sativa* L.) residual proteins. *Cereal Chemistry*, 62(4), 276-279.

1918 Robert, L. S., Nozzolillo, C., & Altosaar, I. (1985b). Homology between rice glutelin
1919 and oat 12 S globulin. *Biochimica et Biophysica Acta*, 829(1), 19-26.

1920 Rodríguez Patino, J. M., Carrera Sánchez, C., Molina Ortiz, S. E., Rodríguez Niño,
1921 M. R., & Añón, M. C. (2004). Adsorption of Soy Globulin Films at the
1922 Air–Water Interface. *Industrial & Engineering Chemistry Research*, 43(7),
1923 1681-1689.

1924 Rodríguez Patino, J. M., Molina Ortiz, S. E., Carrera Sanchez, C., Rodriguez Nino,
1925 M. R., & Anon, M. C. (2003). Dynamic properties of soy globulin adsorbed
1926 films at the air-water interface. *Journal of Colloid and Interface Science*,
1927 268(1), 50-57.

1928 Romero, A., Beaumal, V., David-Briand, E., Cordobes, F., Guerrero, A., & Anton, M.
1929 (2012). Interfacial and emulsifying behaviour of rice protein concentrate. *Food*
1930 *Hydrocolloids*, 29(1), 1-8.

1931 Ruiz-Henestrosa, V. M. P., Martinez, M. J., Patino, J. M. R., & Pilosof, A. M. R.
1932 (2012). A Dynamic Light Scattering Study on the Complex Assembly of
1933 Glycinin Soy Globulin in Aqueous Solutions. *Journal of the American Oil*
1934 *Chemists' Society*, 89, 1183-1191.

1935 Ruíz-Henestrosa, V. P., Sánchez, C. C., Escobar, M. d. M. Y., Jiménez, J. J. P.,
1936 Rodríguez, F. M., & Patino, J. M. R. (2007). Interfacial and foaming
1937 characteristics of soy globulins as a function of pH and ionic strength. *Colloids*
1938 *and Surfaces A: Physicochemical and Engineering Aspects*, 309(1-3), 202-
1939 215.

1940 Runyon, J. R., Nilsson, L., Alftren, J., & Bergenstahl, B. (2013). Characterization of
1941 oat proteins and aggregates using asymmetric flow field-flow fractionation.
1942 *Anal Bioanal Chem*, 405(21), 6649-6655.

1943 Runyon, J. R., Sunilkumar, B. A., Nilsson, L., Rascon, A., & Bergenståhl, B. (2015).
1944 The effect of heat treatment on the soluble protein content of oats. *Journal of*
1945 *Cereal Science*, 65, 119-124.

1946 Sagis, L. M. C., & Yang, J. (2022). Protein-stabilized interfaces in multiphase food:
1947 comparing structure-function relations of plant-based and animal-based
1948 proteins. *Current Opinion in Food Science*, 43, 53-60.

1949 Santiago, L. G., Maldonado-Valderrama, J., Martín-Molina, A., Haro-Pérez, C.,
1950 García-Martínez, J., Martín-Rodríguez, A., Cabrerizo-Vílchez, M. A., &

1951 Gálvez-Ruiz, M. J. (2008). Adsorption of soy protein isolate at air–water and
 1952 oil–water interfaces. *Colloids and Surfaces A: Physicochemical and*
 1953 *Engineering Aspects*, 323(1-3), 155-162.

1954 Sarkar, A., & Dickinson, E. (2020). Sustainable food-grade Pickering emulsions
 1955 stabilized by plant-based particles. *Current Opinion in Colloid & Interface*
 1956 *Science*, 49, 69-81.

1957 Schubert, R., Bäumlein, H., Czihal, A., & Wobus, U. (1990). Genomic sequence of a
 1958 12S seed storage protein gene from oat (*Avena sativa* L. cv. 'Solidor'). *Nucleic*
 1959 *Acids Research*, 18(2), 377.

1960 Scilingo, A. A., & Añón, M. C. (1996). Calorimetric Study of Soybean Protein
 1961 Isolates: Effect of Calcium and Thermal Treatments. *Journal of Agricultural*
 1962 *and Food Chemistry*, 44(12), 3751-3756.

1963 Scott, M. P., Jung, R., Muntz, K., & Nielsen, N. C. (1992). A protease responsible for
 1964 post-translational cleavage of a conserved Asn-Gly linkage in glycinin, the
 1965 major seed storage protein of soybean. *Proceedings of the National Academy*
 1966 *of Sciences of the United States of America*, 89(2), 658-662.

1967 Sha, L., & Xiong, Y. L. (2022). Comparative structural and emulsifying properties of
 1968 ultrasound-treated pea (*Pisum sativum* L.) protein isolate and the legumin and
 1969 vicilin fractions. *Food Res Int*, 156, 111179.

1970 Shand, P. J., Ya, H., Pietrasik, Z., & Wanasundara, P. K. J. P. D. (2007).
 1971 Physicochemical and textural properties of heat-induced pea protein isolate
 1972 gels. *Food Chemistry*, 102(4), 1119-1130.

1973 Shen, Q., Luo, Y., Zheng, W., Xiong, T., Han, F., Zuo, J., Dai, J., Li, B., & Chen, Y.
 1974 (2023). Nonlinear rheological behavior and quantitative proteomic analysis of
 1975 pea protein isolates at the air-water interface. *Food Hydrocolloids*, 135.

1976 Shen, Q., Xiong, T., Dai, J., Chen, X., Wang, L., Li, Y., Li, J., Li, B., & Chen, Y.
 1977 (2022). Effects of pH-shifting treatments on oil-water interfacial properties of
 1978 pea protein isolates: Identification and quantification of proteins at interfacial
 1979 protein layer. *Food Hydrocolloids*, 133.

1980 Shigeru, U., Chan-Shick, K., Toshiro, S., & Makoto, K. (1988). Signal sequence of
 1981 preproglycinin affects production of the expressed protein in *Escherichia coli*.
 1982 *Gene*, 71(2), 349-358.

1983 Shotwell, M. A. (1999). Oat Globulins. In P. R. Shewry & R. Casey (Eds.), *Seed*
 1984 *Proteins* (pp. 389-400). Dordrecht: Springer Netherlands.

1985 Shotwell, M. A., Afonso, C., Davies, E., Chesnut, R. S., & Larkins, B. A. (1988a).
 1986 Molecular characterization of oat seed globulins. *Plant physiology*, 87(3), 698-
 1987 704.

1988 Shotwell, M. A., Afonso, C., Davies, E., Chesnut, R. S., & Larkins, B. A. (1988b).
 1989 Molecular characterization of oat seed globulins. *Plant Physiol*, 87(3), 698-
 1990 704.

1991 Simm, S., Einloft, J., Mirus, O., & Schleiff, E. (2016). 50 years of amino acid
 1992 hydrophobicity scales: revisiting the capacity for peptide classification.
 1993 *Biological Research*, 49(1), 31.

1994 Siu, N.-C., Ma, C.-Y., Mock, W.-Y., & Mine, Y. (2002). Functional Properties of Oat
 1995 Globulin Modified by a Calcium-Independent Microbial Transglutaminase.
 1996 *Journal of Agricultural and Food Chemistry*, 50(9), 2666-2672.

1997 Song, Y., Li, T., Zhang, X., & Wang, L. (2023). Investigating the effects of ion strength
 1998 on amyloid fibril formation of rice proteins. *Food Bioscience*, 51.

1999 Spaen, J., & Silva, J. V. C. (2021). Oat proteins: Review of extraction methods and
 2000 techno-functionality for liquid and semi-solid applications. *Lwt*, 147.

- Subirade, M., Gueguen, J., & Schwenke, K. D. (1992). Effect of dissociation and conformational changes on the surface behavior of pea legumin. *Journal of Colloid and Interface Science*, 152(2), 442-454.
- Sugimoto, T., Tanaka, K., & Kasai, Z. (1986). Molecular Species in the Protein Body II (PB-II) of Developing Rice Endosperm. *Agricultural and Biological Chemistry*, 50(12), 3031-3035.
- Sun, X. D., & Arntfield, S. D. (2010). Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Research International*, 43(2), 509-515.
- Svendsen, I., & Breddam, K. (1992). Isolation and amino acid sequence of a glutamic acid specific endopeptidase from *Bacillus licheniformis*. *European Journal of Biochemistry*, 204(1), 165-171.
- Tacias-Pascacio, V. G., Morellon-Sterling, R., Siar, E.-H., Tavano, O., Berenguer-Murcia, Á., & Fernandez-Lafuente, R. (2020). Use of Alcalase in the production of bioactive peptides: A review. *International Journal of Biological Macromolecules*, 165, 2143-2196.
- Tandang-Silvas, M. R., Fukuda, T., Fukuda, C., Prak, K., Cabanos, C., Kimura, A., Itoh, T., Mikami, B., Utsumi, S., & Maruyama, N. (2010). Conservation and divergence on plant seed 11S globulins based on crystal structures. *Biochimica et Biophysica Acta*, 1804(7), 1432-1442.
- Tang, C. H. (2017). Emulsifying properties of soy proteins: A critical review with emphasis on the role of conformational flexibility. *Critical Reviews in Food Science and Nutrition*, 57(12), 2636-2679.
- Tanger, C., Müller, M., Andlinger, D., & Kulozik, U. (2022a). Influence of pH and ionic strength on the thermal gelation behaviour of pea protein. *Food Hydrocolloids*, 123.
- Tanger, C., Utz, F., Spaccasassi, A., Kreissl, J., Dombrowski, J., Dawid, C., & Kulozik, U. (2022b). Influence of Pea and Potato Protein Microparticles on Texture and Sensory Properties in a Fat-Reduced Model Milk Dessert. *ACS Food Science & Technology*, 2(1), 169-179.
- Utsumi, S., & Kinsella, J. E. (1985). Forces Involved in Soy Protein Gelation: Effects of Various Reagents on the Formation, Hardness and Solubility of Heat-Induced Gels Made from 7S, 11S, and Soy Isolate. *Journal of Food Science*, 50(5), 1278-1282.
- Van Der Borght, A., Vandeputte, G. E., Derycke, V., Brijs, K., Daenen, G., & Delcour, J. A. (2006). Extractability and chromatographic separation of rice endosperm proteins. *Journal of Cereal Science*, 44(1), 68-74.
- Villareal, R. M., & Juliano, B. O. (1978). Properties of glutelin from mature and developing rice grain. *Phytochemistry*, 17(2), 177-182.
- Vischers, R. W., & de Jongh, H. H. (2005). Disulphide bond formation in food protein aggregation and gelation. *Biotechnological Advances*, 23(1), 75-80.
- Vitale, A., & Hinz, G. (2005). Sorting of proteins to storage vacuoles: how many mechanisms? *Trends in Plant Science*, 10(7), 316-323.
- Vlădescu, S.-C., Agurto, M. G., Myant, C., Boehm, M. W., Baier, S. K., Yakubov, G. E., Carpenter, G., & Reddyhoff, T. (2023). Protein-induced delubrication: How plant-based and dairy proteins affect mouthfeel. *Food Hydrocolloids*, 134, 107975.
- Voutsinas, L. P., Cheung, E., & Nakai, S. (1983). Relationships of Hydrophobicity to Emulsifying Properties of Heat Denatured Proteins. *Journal of Food Science*, 48(1), 26-32.

- Waglay, A., & Karboune, S. (2016). Enzymatic generation of peptides from potato proteins by selected proteases and characterization of their structural properties. *Biotechnology Progress*, 32(2), 420-429.
- Wakasa, Y., Yang, L., Hirose, S., & Takaiwa, F. (2009). Expression of unprocessed glutelin precursor alters polymerization without affecting trafficking and accumulation. *Journal of Experimental Botany*, 60(12), 3503-3511.
- Wang, Y. R., Yang, Q., Li-Sha, Y. J., & Chen, H. Q. (2021). Structural, gelation properties and microstructure of rice glutelin/sugar beet pectin composite gels: Effects of ionic strengths. *Food Chemistry*, 346, 128956.
- Wen, T. N., & Luthe, D. S. (1985). Biochemical characterization of rice glutelin. *Plant physiology*, 78(1), 172-177.
- Wouters, A. G. B., & Nicolai, T. (2024). Self-assembly of oat proteins into various colloidal states as function of the NaCl concentration and pH. *Food Hydrocolloids*, 149, 109603.
- Xu, X., Liu, W., Liu, C., Luo, L., Chen, J., Luo, S., McClements, D. J., & Wu, L. (2016). Effect of limited enzymatic hydrolysis on structure and emulsifying properties of rice glutelin. *Food Hydrocolloids*, 61, 251-260.
- Xu, X., Sharma, P., Shu, S., Lin, T.-S., Ciais, P., Tubiello, F. N., Smith, P., Campbell, N., & Jain, A. K. (2021). Global greenhouse gas emissions from animal-based foods are twice those of plant-based foods. *Nature Food*, 2(9), 724-732.
- Yang, C., Wang, X., Hu, H., Feng, Y., Tang, H., Zhang, W., & Wang, J. (2022a). Cold-set oat protein isolate--gellan gum binary gels with various microstructures: Fabrication, characterization, formation mechanism, and controlled release properties. *Food Hydrocolloids*, 131.
- Yang, C., Wang, Y., & Chen, L. (2017). Fabrication, characterization and controlled release properties of oat protein gels with percolating structure induced by cold gelation. *Food Hydrocolloids*, 62, 21-34.
- Yang, C., Wang, Y., Lu, L., Unsworth, L., Guan, L. L., & Chen, L. (2018). Oat protein-shellac beads: Superior protection and delivery carriers for sensitive bioactive compounds. *Food Hydrocolloids*, 77, 754-763.
- Yang, J., Mocking-Bode, H. C. M., van den Hoek, I. A. F., Theunissen, M., Voudouris, P., Meinders, M. B. J., & Sagis, L. M. C. (2022b). The impact of heating and freeze or spray drying on the interface and foam stabilising properties of pea protein extracts: Explained by aggregation and protein composition. *Food Hydrocolloids*, 133.
- Yang, J., & Sagis, L. M. C. (2021). Interfacial behavior of plant proteins — novel sources and extraction methods. *Current Opinion in Colloid & Interface Science*, 56.
- Yang, Z., Dai, L., Sun, Q., McClements, D. J., & Xu, X. (2022c). Effect of molecular weight on the interfacial and emulsifying characteristics of rice glutelin hydrolysates. *Food Hydrocolloids*, 128.
- Yeom, H.-J., Lee, E.-H., Ha, M.-S., Ha, S.-D., & Bae, D.-H. (2010). Production and physicochemical properties of rice bran protein isolates prepared with autoclaving and enzymatic hydrolysis. *Journal of the Korean Society for Applied Biological Chemistry*, 53(1), 62-70.
- Yousefi, N., & Abbasi, S. (2022). Food proteins: Solubility & thermal stability improvement techniques. *Food Chemistry Advances*, 1.
- Yusuf, D., Davis, A. M., Kleywegt, G. J., & Schmitt, S. (2008). An Alternative Method for the Evaluation of Docking Performance: RSR vs RMSD. *Journal of Chemical Information and Modeling*, 48(7), 1411-1422.

- Zembyla, M., Liamas, E., Andablo-Reyes, E., Gu, K., Krop, E. M., Kew, B., & Sarkar, A. (2021). Surface adsorption and lubrication properties of plant and dairy proteins: A comparative study. *Food Hydrocolloids*, 111, 106364.
- Zhang, S., Huang, W., Feizollahi, E., Roopesh, M. S., & Chen, L. (2021). Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, 67, 102567.
- Zhang, X., Ren, X., Zhao, X., Wang, M., Liu, H., Zhang, L., & Zhu, Y. (2022). Comparative effects of extraction methods on functional and structural features of subunits from 11S soy protein fraction. *Journal of Food Measurement and Characterization*, 16(5), 3855-3867.
- Zhang, X., Wang, Q., Liu, Z., Zhi, L., Jiao, B., Hu, H., Ma, X., Agyei, D., & Shi, A. (2023a). Plant protein-based emulsifiers: Mechanisms, techniques for emulsification enhancement and applications. *Food Hydrocolloids*, 144, 109008.
- Zhang, Y., & Dee, D. R. (2023b). Morphology, Formation Kinetics and Core Composition of Pea and Soy 7S and 11S Globulin Amyloid Fibrils. *Journal of Agricultural and Food Chemistry*, 71(11), 4755-4765.
- Zhao, C.-B., Zhang, H., Xu, X.-Y., Cao, Y., Zheng, M.-Z., Liu, J.-S., & Wu, F. (2017). Effect of acetylation and succinylation on physicochemical properties and structural characteristics of oat protein isolate. *Process Biochemistry*, 57, 117-123.
- Zhao, H., Shen, C., Wu, Z., Zhang, Z., & Xu, C. (2020). Comparison of wheat, soybean, rice, and pea protein properties for effective applications in food products. *Journal of Food Biochemistry*, 44(4), e13157.
- Zhao, Q., Xiong, H., Selomulya, C., Chen, X. D., Huang, S., Ruan, X., Zhou, Q., & Sun, W. (2012). Effects of Spray Drying and Freeze Drying on the Properties of Protein Isolate from Rice Dreg Protein. *Food and Bioprocess Technology*, 6(7), 1759-1769.
- Zhao, Y., Mine, Y., & Ma, C. (2004). Study of Thermal Aggregation of Oat Globulin by Laser Light Scattering. *Journal of Agricultural and Food Chemistry*, 52(10), 3089-3096.
- Zheng, L., Regenstein, J. M., Zhou, L., & Wang, Z. (2022). Soy protein isolates: A review of their composition, aggregation, and gelation. *Comprehensive Reviews in Food Science and Food Safety*, 21(2), 1940-1957.
- Zhou, J., Li, T., Peydayesh, M., Usuelli, M., Lutz-Bueno, V., Teng, J., Wang, L., & Mezzenga, R. (2022). Oat Plant Amyloids for Sustainable Functional Materials. *Advanced Science*, 9(4), e2104445.