final report

Project Code: A.COP.0057

Prepared by: Ingrid Appelqvist, Mickey Huson, Mike Gidley, Sandani Udabage, Katia Strounina, Michael Kelly, Jeff Church, Leif Lundin, Amirtha Puvanenthiran, Danyang Ying, Elliot Gilbert and Jitendra Mata

Date published: September 2010

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Protein research collaboration project

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government and contributions from the Australian Meat Processor Corporation to support the research and development detailed in this publication.

Executive Summary and Major Findings

The goal of the project was to categorise the behaviour of a range of food proteins in the solid state, and predict their response to formulation variables likely to be found in food manufacturing processes and products.

Major Findings

The research conducted in the Protein Syndicate have shown that

- The glassy state behaviour of proteins is dependent on the primary structure and the proportion of ordered and non-ordered amorphous structure.

- The least structurally-ordered proteins such as gluten and MPC exhibit the sharpest and most easily measured glass transition (though still broader than for synthetic polymers), while highly folded and ordered proteins such as native BSA did not show a detectable glass transition.

- An ageing endotherm was shown for all proteins in the glassy state and relates to the thermal history of the sample.

- Proteins exhibit a broad glass transition (spanning up to 50ºC) in comparison with homologous polymers, and is a consequence of the heterogeneous nature of the molecular structure and the sequence of amino acids.

- Reduction in molecular weight and increase in the proportion of amorphous to ordered regions (salting in) in proteins lowers the glass transition temperature for a given moisture

- Differences in high order supra-molecular and network structures has a limited effect on protein glassy state behaviour but has a profound affect on their functional properties

- Moisture sorption and solubility behaviour is driven by changes in supra-molecular and macro structures such as networks of proteins and not by the glass transition.

- Protein-specific behaviour for protein-water interactions could only be detected at about 20% moisture i.e. above the glass to rubbery transition.

1. Project Summary

1.1. Whole of Project

1.1.1. Background

The intention of the Protein Syndicate research was to provide fundamental understanding of biophysical properties of food products containing high levels of protein and aimed to develop design rules for their manipulation. The work represented the first systematic application of a wide range of analytical and characterisation techniques to define and probe the structure and dynamic behaviour of commercially and biologically important proteins and has enabled the development of a coherent description of properties based on various structurally 'ordered' and 'disordered' segments in proteins that respond to moisture environment differently.

The goal of the project was to categorise the behaviour of a range of food proteins in the solid state, and predict their response to formulation variables likely to be found in food manufacturing processes and products.

Multiple analysis techniques were used to show how characteristic features of the ordered/rigid regions of the protein (as probed by small angle x-ray scattering (SAXS), nuclear magnetic resonance (NMR) and infrared spectroscopy) and mobile segments (as probed by NMR, dynamic mechanical analysis (DMA) and differential scanning calorimetry DSC) behave as a function of limited water content, and how the information can be integrated within a mobility-transformation framework. This information and understanding should provide food manufacturers with new scope for designing consumer-preferred products with, for example, an improved nutritional profile or optimised functionality after drying and rehydration.

The key objectives of the research program were to

- Develop underlying basic principles and design rules for protein molecular behaviour
- Understand mechanisms of protein conformational change and behaviour in limited water
- Build structure-function relationships of pure and mixed proteins in different environments
- Demonstrate proof of principle for ageing phenomena, solubility behaviour and structural mobility.

1.1.2. Advancement of our understanding of protein behaviour in limited water

1.1.2.1 *Generic learnings on protein behaviour in limited water*

The data generated in this program has enabled the categorisation of proteins investigated in terms of properties such as the balance between 'amorphous' and 'ordered' segments (presence and location of glass transition), and the degree of unfolding. This combination of structural and dynamic properties is a subtle function of protein sequence, secondary structure and tertiary folding and is responsible for the diversity of both physical and biological properties of proteins.

The techniques applied have demonstrated their ability to clearly distinguish between classes of proteins (network, micellar, globular, semi-crystalline) and for the proteins investigated (milk protein concentrate (MPC), gluten and bovine serum albumin (BSA)) can identify those features that are common across the protein range and those that are protein specific.

Common features among the proteins studied were:- limited interaction with water molecules at low moisture, a broad glass transition which decreased in accordance with the Fox/Gordon- Taylor equation as a function of moisture, and the development of an ageing endotherm.

Protein-water interactions at low moisture

It was shown from proton T_2 NMR, which is sensitive to the mobility of water protons and their rate of exchange with protein protons, that below 8% moisture there is surprisingly limited interaction between water and protein. The relatively long water relaxation times at low moisture suggest that water proton exchange with protein protons is slower than it is at higher moisture contents where the water molecules are expected to be further away from the protein surface. We propose that the water surrounding the protein at low moisture interacts preferentially with itself rather than with the protein surface, independent of protein molecular structure. This suggests that the water associates closely within clusters rather than directly via hydrogen bonding to hydrophilic groups on the protein surface; this may be a feature of the level of drying that the protein has been exposed to, inferring that there is an optimum level of moisture for storage conditions where there is minimum damage (due to over drying) of the protein and maximum storage stability where the water is not molecularly associated with the protein. This appears to be between 5 to 8% water content. SAXS shows that, irrespective of the degree of long-range order, the protein structure expands with increasing moisture content. This is most obvious in native glycinin where clear features of crystalline order are observed but appears to be generic across the proteins studied.

Glass transition and plasticization

Another feature common to all the proteins studied was that they exhibit a glass transition (Tg), although depending on the level of crystallinity the transition may be difficult to detect by DSC e.g. in the case of native BSA, no calorimetric Tg could be measured. The heterogeneous nature of proteins in general results in a broad spectrum of relaxation times, and hence a very broad glass to rubber transition often spanning almost 40ºC. The breadth of the transition is proposed to be due to the superposition of separate transitions arising from different local segments within the proteins. These arise because individual protein chains contain both ‘amorphous’ and ‘ordered’ segments with a spectrum of conformational states and, because of the multi-sequence nature of proteins; they are essentially copolymers with many different monomer (amino acid) units. It has become customary to report Tg as a single value (often the midpoint); however it is important to remember that in proteins, Tg-sensitive properties such as hardness, diffusion coefficients, and physical ageing can start to change at temperatures well removed from these stated glass transitions. One recommendation from this research is that the glass to rubber transition for proteins is identified through three temperature values indicating the start, mid-point and end of the transition. This may be very important since modification of proteins by denaturing or hydrolysis for example can have a

significant effect on part or all of the broad glass transition temperature. The nature of these changes is system-specific, and illustrates the diversity and heterogeneity in proteins. Knowing this information will allow the food ingredient industry to identify more accurately storage-temperature conditions that will protect and give the longest shelf-life stability.

In all cases, the addition of water resulted in plasticisation/softening of the proteins and decreases in the glass transition temperature and the mechanical response observed in DMA. This behaviour is analogous to the plasticization of synthetic polymers by low molecular mass organic molecules and, for all systems, could also be modelled using either the Fox or Gordon-Taylor equations. A further common feature for proteins is that reduction of molecular weight by hydrolysis and/or degradation results in a reduction in Tg for a given moisture and as in common with synthetic polymers, proteins can also be plasticised by low molecular weight species.

DMA measures the Tg by mechanical means and is typically more sensitive to changes at Tg than DSC. Comparison of Tg behaviour by the two techniques was very similar for all the proteins investigated in that both transitions were plasticised to lower temperatures and the slope of the Tg versus moisture curves were similar and suggests that the mechanical and thermal transitions have a common origin. In general the mechanical softening transition in DMA occurred at about 10-20ºC above that of the thermal transition for the same moisture content, which is consistent with the polymer literature and demonstrates the frequency dependency of the glass transition. More importantly it implies that any bulk mechanical changes, such as softening, particle stickiness, mechanical flow properties and collapse, need to be preceded by a thermal transition. In other words, the proteins need to go through their glass to rubber transition and be in a fully rubbery state in order to gain sufficient flexibility and mobility in the molecular backbone to change their mechanical properties. DMA has been a valuable method in that it can be more reliable and easier to measure the mechanical glass transition than the calorimetric glass transition, and that this parameter is more closely related to the bulk properties and behaviour of proteins in processing.

The research also showed that the least structurally-ordered proteins such as gluten and MPC exhibited the sharpest and most easily measured glass transition (though still broader than for synthetic polymers), while highly folded and ordered proteins such as BSA in the native state were impossible to measure by DSC. Only after the protein was denatured, with some loss of ordered structure, could a glass transition for BSA be measured.

Physical ageing and de-ageing

When the proteins were stored below their glass transition, physical ageing occurred often within minutes of storage and led to a gradual hardening of the protein and the appearance of an ageing endotherm in the DSC trace. A similar feature has been observed in polysaccharides and it is indicative of kinetically-trapped states within the system. Physical ageing was fully reversed by heating the proteins to a temperature above their glass transition temperature. This process is often referred to as a “stress relaxation”, or “de-ageing”, and has been observed in many biopolymers. However, of great interest was the observation that at selected

annealing temperatures, both ageing and de-ageing could occur simultaneously because the temperature selected, although above the mid-point Tg, was actually still within the broad glass transition range. The consequence of this is that some parts of protein molecules relax rapidly leading to accelerated ageing and other parts, that were above their local Tg, become de-aged. Further work is needed to determine what this will mean for protein bulk properties but the expectation is that the properties will be intermediate between those of the fully relaxed and fully aged sample enabling the potential for subtle control of behaviour and functionality.

Another generic behaviour for proteins is that the ageing endotherm can be moved up to higher temperatures with increased storage temperature and that, as the annealing temperature moves closer to the glass transition temperature, the endotherm can become coincident with the glass transition often exhibited as an overshoot in the DSC trace. When ageing at a fixed temperature was followed as a function of time the endotherm peak increased in height and moved to higher temperature as noted for polysaccharides and simpler synthetic polymers. Thus ageing is a function of the thermal history of the sample and is accelerated the higher the temperature of exposure to the protein. In other words, in newly de-aged proteins, the rate of increase of the ageing endotherm is faster with increased storage temperature.

1.1.2.2. Comparisons between protein behaviours

Protein-water interactions

Increasing the moisture content led to the proteins becoming more mobile since the water acts as a plasticiser causing the proteins to move through their glass to rubber transition at low temperature, e.g. below room temperature for moisture contents above about 15%. This resulted in an increase in proton exchange between the water and protein for all the proteins examined, and indicates that water above about 10% moisture undergoes more rapid exchange with the protein surface than at lower moisture. Differentiation of behaviour between the proteins could only be detected, if at all, above about 20% moisture i.e. above the glass transition. Folded globular proteins (such as BSA, lysozyme and native glycinin), which have a high level of ordered structure, and are subject to a limited amount of flexibility, showed an increase in water T_2 relaxation values above 20% due to increasing average distances of water molecules from protein surfaces; this is indicative of surface accessibility for proton exchange with water becoming restricted at this water content. In contrast, high surface area proteins (for example, unfolded proteins such as denatured glycinin, gluten, evaporated MPC and sodium caseinate) have sufficient available surface for close average water/protein contact up to at least 30% water. The implication is that high surface area proteins require more water to hydrate and that this could impact on their dispersibility and solubility.

Glass transition and plasticisation behaviour

It has already been mentioned that the least structurally ordered proteins such as gluten and MPC show the sharpest and most easily measured glass transition, while for highly folded and ordered proteins, such as BSA in the native state, a Tg could not be measured by DSC except when it was thermally denatured resulting in some loss of ordered structure.

Comparison of the behaviours of water plasticisation of the proteins showed that MPC (evaporated and non-evaporated), and BSA (denatured) had the highest Tg followed by gluten and finally hydrolysed gluten which had the lowest glass transition for the same moisture content. Reduction in the molecular weight and additional plasticising from low molecular weight protein fractions could contribute to the lower glass transition observed for hydrolysed gluten. The implication of this for the food industry is that storage conditions for different proteins to optimise the shelf-life of powders may need to be different. For example, if all the proteins studied were at 12% moisture content and the storage room conditions were subject to temperatures of 35ºC then it is likely that the hydrolysed gluten will be stored close to or slightly above its glass transition temperature which means it will be subject to greater deterioration in comparison to MPC which will be well below its glass transition i.e. in the glassy state). Similarly hydrolysed gluten powder particles, with moisture contents >11%, were shown to have started to mechanically flow together during storage at room temperature whereas BSA showed no such tendencies even up to a moisture content of 30%. This suggests that even at very high moisture, BSA powder particles are more rigid in nature and are not as sensitive to environmental changes as hydrolysed gluten powder particles. This observation is consistent with evidence for greater molecular mobility for hydrolysed gluten than in the highly folded globular BSA protein

The susceptibility to plasticisation also illustrates differences in the mobility and flexibility of the proteins, indicating that MPC and BSA are the least flexible and that hydrolysed gluten is the most flexible protein structure of those studied. NMR spectroscopy evidence also indicated that gluten has more internal molecular flexibility than any other protein studied by the syndicate. Within the relatively rigid fraction of the gluten (probed using cross polarisation methods), water-plasticised increases in kHz molecular mobility are detected below the DSC Tg temperature. Within the relatively mobile fraction (probed using direct polarisation, DP) methods, sharper lines are seen for side chain signals (even at <5% water) than for any other protein studied, suggesting an unusually high level of side-chain motion for both native and hydrolysed gluten. Quasielastic neutron scattering (QENS) also indicated a change in protein mobility in the picosecond timescale which we believe relates to methyl side groups spinning and is unrelated to the glass transition at room temperature.

Native versus denatured proteins

Unfolding and denaturation of proteins (in the dry state) is induced by heating above the temperature of a denaturation endotherm that is observed in DSC, which is moisture-dependent and moves to lower temperatures as the moisture is increased. Denaturation (in solution) for some proteins such as BSA and glycinin can also be induced by solvent effects such as low pH and high ionic strength.

Some generalisations can be made about the thermally-induced sol-to-gel transformations of globular proteins. It is confirmed from the FT-IR data for both glycinin and BSA that generation of β-sheet structure occurs during aggregation, as observed for several other globular proteins previously. During the unfolding and protein-protein aggregation processes, the particular identities of the various native proteins become less obvious, and changes in the secondary structure take place involving a decrease in helix content and an increase in β-sheet and disordered

structure. The extent of these changes is dependent on the ratio of these secondary structures present in the native state and the degree to which β-sheet formation plays a significant part in the aggregation process. While β-sheet is an important conformational component of the aggregated state, for BSA the secondary structures did not vary greatly for two different (linear and globular) network architectures that contain widely different bonding patterns and levels of network homogeneity. This indicates that bonding of BSA aggregates has little effect on the overall β-sheet content and implies that these are largely internal to the protein molecules that form the aggregates i.e. trapped by hydrophobic contacts between the aggregating molecules.

In practice, this means that, for globular proteins, a continuous spectrum of behaviour exists between systems where the extent of β-sheet structure is so great that the aggregates can in any sense be described as crystalline e.g. insulin to proteins such as BSA where the networks in these gels can be described as composed of folded globular particles linked together. In the case of native glycinin, crystallinity is due to the arrangement of individual proteins on a lattice with respect to each other and not to the nature of the internal structure within an individual protein (e.g. β-sheet content). Thermal denaturation of glycinin results in a loss of long-range crystalline order although there is no significant change to the glass transition temperature for given moisture, indicating that supramolecular changes do not impact on glassy behaviour but will impact on functional properties such as moisture sorption.

1.1.2.3. Protein-specific learnings of behaviour in limited water

MPC

All MPC powders lost some solubility on storage although they were stored well below their glass transition temperature (Tg). In common with all other proteins studied, an ageing endotherm developed at around 60ºC which indicates the powders were stored at least at or below room temperature. The size and the temperature of the ageing endotherm changed with storage time, moisture content (MC) and storage temperature. An increase in MC and storage temperature which reduced the difference in temperature between storage conditions and Tg resulted in increased molecular mobility and accelerated ageing which allowed for some supramolecular reorganisation within the protein leading to an increased loss of cold water solubility of the MPC powders. No change in lactose crystallization was observed as a function of storage temperature and water content. The solubility loss was not due to the formation of insoluble material rather a decrease in the rate of release of casein micelles from the dispersed powder particles. MPC powders have ~ 2.5 fold more protein than skim milk powders and therefore for a given volume contain more protein and less lactose. This means there is a decrease in the distance between two casein micelles with a possible collapse of the casein hairy layer which will promote interactions leading to skin formation near the surface of the protein particles and loss of solubility. Technological fixes such as lowering micellar calcium through ion exchange or chelation can counteract protein aggregation and

hence delay the loss of solubility but might not necessarily eliminate the issue. SAXS data also indicated that changes in morphology of the powder surface can influence the solubility of MPC.

Evaporated versus non-evaporated – water/protein interaction

Evaporated and non-evaporated MPC exhibited interesting differences in behaviour at ca 30% water. The non-evaporated sample shows behaviour similar to BSA in which a restricted available surface exists for water / protein interactions; at this moisture content, most of the water was sufficiently far away from the protein to result in long T_2 relaxation times. In contrast, the evaporated sample shows behaviour similar to unfolded proteins such as denatured glycinin and sodium caseinate where at 30% moisture the water was still interacting closely with the protein surfaces, as indicated by short T_2 relaxation times. This suggests that the evaporation process has resulted in the exposure of more protein surfaces to water and, with the exposure of new surfaces, further protein-protein interactions can take place that lead to reduced dispersability and solubility of the protein.

Spray-dried and freeze dried powder- solubility

Removal of a critical amount of water (to a MC of <1%) during the drying process had a negative effect on the solubility of the freeze dried powders. No bulk properties were shown to change between spray-dried (SD) and freeze-dried (FD) powders, as measured by NMR or DSC, indicating that bulk properties of polymer mobility and water interactions with protein surfaces are not responsible for solubility differences. However, significant differences in structure on the nanoscale were observed with SAXS. In addition, we have demonstrated that SAXS enables the nano-structure and interfacial properties of the spray-dried and freeze-dried powders to be successfully correlated with their differences in solubility.

We observe a marked difference in the interfacial scattering between FD and SD powder samples by SAXS. This difference may result from bulk surface area and/or interfacial surface of casein micelles. We have demonstrated that, within the samples investigated, a large positive deviation from q^{-4} (all FD samples) is found for materials with low solubility; this is associated with interfacial effects. Scattering techniques may therefore be valuable to check the interfacial structure during the production of MPC powders and to study modification of the casein micellar (and surface) structure. We have shown the internal structure of casein micelles is preserved in both FD and SD powders.

Effect of Conditioning

SAXS demonstrates that the casein micelles have internal structure comprising of mono-dispersed colloidal calcium phosphate nanoparticles separated by a characteristic length. The separation of calcium phosphate nanoclusters within the casein micelle expands with increasing moisture content, indicating the penetration of water and relaxation of casein micelles with increase of moisture content.

Gluten

Highly flexible polymer

Water was shown by proton NMR to interact directly with gluten at low moisture, which was somewhat different from other proteins, where the water preferred to associate with itself. The difference in behaviour, we believe, is because gluten was found to be the most flexible of the proteins studied; even at low moisture (less that 5%), therefore, whilst the protein held at room temperature is still well below its glass transition temperature, side chain mobility could be detected. It is possible that this is related to the unique network properties of gluten.

Hydrolysed versus vital gluten

The reduction of molecular weight by the hydrolysis of gluten resulted in lowering of the glass transition temperature compared to the parent vital gluten for the same water content. The results indicate that control of molecular size provides the potential to modify the glass transition temperature and therefore the bulk properties that are associated with the glass to rubber transition such as mechanical properties, flow, density etc. In terms of NMR-detected molecular mobility, hydrolysed and vital gluten show a high level of similarity, suggesting that differences in DSC/DMA transition temperatures (hydrolysed gluten ca 10-20 ^{O}C lower Tg values) are due to molecular size effects. Thus it may be possible to tailor the response to moisture of gluten ingredients through control of molecular weight profiles of ingredient components.

Effects of pH and salt

The pH values (2-11) or NaCl contents (up to 5%) of solutions from which either vital or hydrolysed gluten were dried did not markedly affect transition temperatures. In contrast, drying gluten samples from solutions containing 0.1M sodium bromide and, particularly, 0.1M sodium thiocyanate (NaSCN) resulted in a reduction in transition temperatures compared to sodium chloride. This ‘salting in’ effect suggests that the glassy state is stabilised by hydrophobic interactions, as ‘salting in’ anions (Br^- and, particularly, SCN^-) counteract the hydrophobic interactions between proteins that can result in aggregation. This implies that ‘salting in’ affects the balance between amorphous and ordered regions in the protein, increasing the non-ordered structure of the protein through disruption of ordered segments.

SAXS highlights a difference in interfacial scattering for gluten samples dried from pH 4.2 compared with pH 5.2/5.8 at both low and high moisture content on a length scale of at least 60 nm, FT-IR analysis showed a change between pre-drying pH values of 5.2/5.8 and 4.2 consistent with changes in carboxyl group ionisation, resulting in small changes in protein secondary structure. This suggests that the pH before drying affects not only local molecular structure (FT-IR), but also longer-range packing effects (SAXS).

SAXS profiles show clear evidence for structural rearrangement in gluten dried from NaCl solutions. Increasing salt concentrations caused a small but systematic contraction of local structure at the nm length scale but enhanced surface scattering consistent with salt-induced aggregation. It is possible that salt-induced aggregation arises from solubilisation of hydrophilic gluten components and consequent association of undissolved hydrophobic proteins. This difference in the interfacial scattering of glutens dried from >1.0% NaCl compared to those dried from lower (or

no) NaCl is apparently unrelated to changes in protein secondary structure (FT-IR) or molecular mobility (DSC, DMA, NMR).

Effects of lipid co-components

Gluten contains about 8% lipid as a co-component, and although this is a relatively low level, flour lipids have both a physical and structural role to play in gluten network performance, influencing the visco-elastic nature of the network and therefore its processability. Defatting of flour prior to gluten extraction resulted in different SAXS behaviour compared with gluten extracted from non-defatted flour. SAXS data and electron microscopy images are consistent with each other, and indicate a role for lipid in holding protein components together and potentially forming a surface active coating.

BSA

Comparison of BSA aggregated gel powders

There is potential to modify the performance and the properties of BSA protein by controlling the mechanism of gelation and aggregation to produce a range of gel structures from globular opaque gels to fine filament translucent gels, by varying the conditions of concentration, pH, ionic strength and heat load applied. The gels varied in texture from clear, rubbery, water-retaining, visco-elastic solids to opaque, water-releasing globular coagulates. These systems were dried to produce BSA protein powders that demonstrated differences in their behaviour such as moisture sorption and solubility. Comparison of the glass transition of the original (thermally unfoldeded) BSA powder and the powder produced from the clear gel showed that, as a function of moisture content, the slope of the Tg curve was different. DSC traces indicated that the glass transition differences in BSA between the original and the pH-adjusted samples, particularly at low moisture content, was due to higher levels of degradation induced in the latter samples (during DSC measurement), possibly leading to a decrease in molecular weight of BSA and therefore a decrease in Tg. The globular particle gel powder adsorbed less moisture than the clear threadlike particle gel powder, potentially because the transparent gel formed a more open structure which could swell more and take up more moisture on rehydration. This indicates that changes to functional properties of protein can be induced by altering the pre-treatment or thermal process of BSA solutions and powders. The results also showed that differences in moisture sorption uptake for example, between the BSA samples while still in the glassy state (i.e. below Tg) was small compared to above the glass transition where differences were much greater indicating that it is changes in the supramolecular structure that control functional properties such as moisture sorption rather than governed by Tg.

1.1.3. Use of different techniques

In this study, structural features have been probed using SAXS over a size range from 1 to several 100 nm and with FT-IR to study vibrational modes on the sub-nm range; these probes provide information on the second to kilosecond timescale. Dynamic properties have been investigated at the sub-molecular level using ^{13}C NMR spectroscopy over the microsecond to millisecond timescale while the thermal and mechanical behaviour of the bulk material has been studied by DSC and DMA on the second to kilosecond timescale. The motional changes at room temperature are observed at different moisture contents due to the different characteristic

frequencies of DSC (11-13% water; close to static), cross-polarisation NMR (17-21% water; kHz) and direct polarisation NMR (21-26%; MHz). Thus, at the same temperature, higher moisture (plasticiser) contents are needed to observe a mobility transformation at higher frequencies. When faster probes are used (e.g. quasielastic neutron scattering on the GHz frequency scale), the mobility changes are observed to occur above ca. 25% moisture content but at significantly lower temperatures (ca. -170ºC); this so-called dynamical transition is absent below this moisture content.

Together, this suite of techniques provides a high level of complementary information that can now be integrated into a model for the behaviour of a range of food proteins under low moisture conditions. By determining the structure and behaviour of such food-relevant proteins, it becomes possible to identify and develop design criteria for the properties of proteins in relation to dried foods and ingredients.

1.1.4. Future direction for protein research

- Relate and identify the relative importance of different amino acids in modulating the glassy state and ageing behaviour.
- Investigate routes to modify the relative amounts of non-ordered and ordered structures of protein to modify the glassy behaviour.
- Investigate the possibility to use low molecular weight peptides to plasticise proteins and change their glassy behaviour. (Peptide choice potentially based on identifying which amino acids affect the glassy behaviour).
- Investigate structural changes that occur during the drying and rehydration process as a function of different drying and rehydration kinetics. This includes the effect of the co-solute on this process, e.g. decreased pH and increased salt concentrations during drying.
- Investigate the hydration and structural function of proteins which have been dried from different structural states.
- Relate the solution state and supramolecular structure of proteins to digestibility and satiety
- Intermediate moisture conditions where food manufacture occurs e.g. dough formation for bread and biscuits, processing of milk to make cheese.

1.1.5. Syndicate papers in pipeline

- Structure of Casein Micelles in Milk Protein Concentrate Powders via Small Angle X-ray Scattering' is targeted for the peer reviewed journal Soft Matter. (submitted to Syndicate partners for approval)

- Understanding solubility of MPC – SD vs FD MCP powders, SAXS measurements

- Ageing phenomena in proteins - broad endotherms, annealing temperatures and acceleration of ageing kinetics

- DMA technique development – sample holder development, powder compression,

- ^{1}H-T_2 NMR as a function of moisture content and protein type

- BSA gel networks leading to new functionality –thermal and pH effects, adsorption hysteresis, Tg
- Effect of salt and pH on gluten functionality