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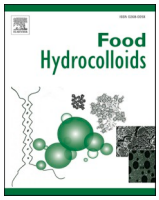

Boosting structural food science using X-ray and neutron techniques

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A B S T R A C T

Knowledge about food structures at different length scales is key for the continued development of sustainable, tasty and healthy foods. It is critical to control, model and predict the supramolecular architecture of foods along the whole value chain: from raw materials, to their changes during processing, all the way to how products form structures during consumption and digestion. Today, advanced physical methods enable us to obtain structural information from the nanoscale-to the microscale with unprecedented resolution. The structural details can then relate to the mesoscale and microscale functionalities, important for the appeal and consumption of food products. X-ray and neutron techniques expand and strengthen the food structure characterisation toolbox. They enable *in situ* and *in operando* investigations with greater detail as well as new types of measurements that are not possible with other techniques. The knowledge gained will complement compositional and functional data obtained by other techniques, providing robustness to the interpretation of complex structural information. There are several intrinsic scientific challenges to overcome: from the lack of relevant sample environments to advanced data processing and modelling tools that consider the complexity of the food. The new frontier in food structural science can be gained through interdisciplinary collaborations not only in academia but also from the wider innovation ecosystem. This review showcases how the use of X-ray and neutron techniques is already leading to transformational knowledge in structural food science with a perspective that points to the future of this new multidisciplinary discipline.

1. Introduction

There is a great need for healthier food and more sustainable food production and consumption solutions to achieve UN's sustainable development goals. To progress, we must employ food sources that are grown, produced and manufactured in a less resource intensive way. In the future, we will not use only traditional raw materials, but less known ingredients, such as those from plant- or blue-based feedstocks, or obtained from fermentations and biotechnologies. However, to deliver on this much needed dietary transition, an in depth understanding of how their composition and structure affect their technological and nutritional functionality is needed. Research on food structure-function relationships is then a prerequisite to achieve the necessary knowledge enabling their inclusion in future dietary solutions, which are not only appealing to consumers but also healthy and sustainably produced.

Structure-function studies are at the basis of structural food science, a discipline that focuses on the investigation of food with a more holistic approach, beyond just composition. The objectives of this review are to

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highlight some recent advancements in food structure science, with particular attention to X-ray and neutron tools. As identified in recent reviews by Gilbert, such techniques have provided, and will continue to provide, transformational knowledge that advance food science and its applications (E. P. Gilbert, 2019) (E. P. Gilbert, 2023b). This work aims to inspire and bring together scientists from different fields with focus on food structure, experimental structure characterisation techniques, modelling and understanding of structure-functionality relationships of foods. By working together and sharing competences, it will be possible to accelerate the much-needed food systems innovation in society. This work should also be regarded as response to the need to increase the overall awareness and competence in using X-ray and neutron-based techniques by a wider research community in universities, institutes, industry and society to analyse food structure and provide the food for the future.

Food structure is intimately related to many functional properties, such as texture, processability, storage stability, appearance, aroma and flavour release, sensory properties, digestive breakdown and bioavailability of nutrients (Boire et al., 2019; Capuano & Pellegrini, 2019; Somaratne et al., 2020). Hence, to design more attractive, new and innovative food products, food structure needs to be investigated at more fundamental levels, to improve our ability to derive principles rather than being limited to product specific properties. This demands the use of multiple structural techniques adequate for different time and length scales. The most established means to analyse food structures today are techniques such as light microscopy, confocal microscopy, or electron microscopy, rheological analysis or light scattering (Dahl et al., 2025; de Kort et al., 2018; Erich Schuster, et al., 2014). Furthermore, spectral imaging techniques have also been recently developed for food structural characterization (Dahl et al., 2023; Sala et al., 2024). Advanced structural characterisation techniques based on X-ray and neutron sources have already shown their potential to complement and strengthen this toolbox (Martinez-Sanz et al., 2019; Olakanmi et al., 2023), and to provide the opportunity to understand structure with details that open up possibilities for a mechanistic understanding. Obtaining this level of information gives us a further advantage to develop food products that are not only safe, healthy and sustainable, but also taste good and are well accepted by consumers.

The importance of the study of food structure extends beyond food appearance and texture. For example, a full understanding of the structural dynamics occurring during digestion will help derive mechanistic knowledge of nutritional differences between foods, even with the same composition, and will facilitate the design of impactful dietary interventions. The *in situ, in operando* effects are critical in food science, as this complex matrix is affected by extrinsic factors, such as temperature, shear, processing or simply storage time. Obtaining a more detailed and fundamental understanding on the process-structure-property relationships will then provide access to the principles underpinning how processing dynamics influence food properties. Such principles enable understanding of the impact on health and nutrition and will accelerate innovation. Indeed, the lack of knowledge of structural changes during processing leads to considering processing a "black box", leaving only the option of a full characterization of specific critical points in the process, such as the raw material or the final product, and extrapolation of processing dynamics.

Food materials include a complex hierarchy of various components assembled into interconnected structures, spanning multiple length-scales, as illustrated in Fig. 1. The interplay between these structures gives the food its quality and functionality. In Fig. 1, we also highlight the multitude of techniques that can be and in some cases are necessary to use to characterize the structures at the different time and length scales. Detailed knowledge requires a combination of techniques. To comprehend the relationships between the components and their role in creation of different hierarchy of structures, a soft matter approach to the study of food science is needed in order to understand food from first principles (Boire et al., 2019; Mezzenga et al., 2005; Ubbink et al., 2008). Here multiple length and time scale dynamics must be considered as well as the multicomponent character of food systems. Thus, the functional properties of a specific component, or an ingredient are strongly coupled to the interactions with the other components in the system. For example, in an emulsion, not only the emulsifiers affect the properties but also other component like, proteins, polysaccharides and salt and their interactions have to be considered. Such interactions can be modified by the processing conditions like whipping or freezing. This

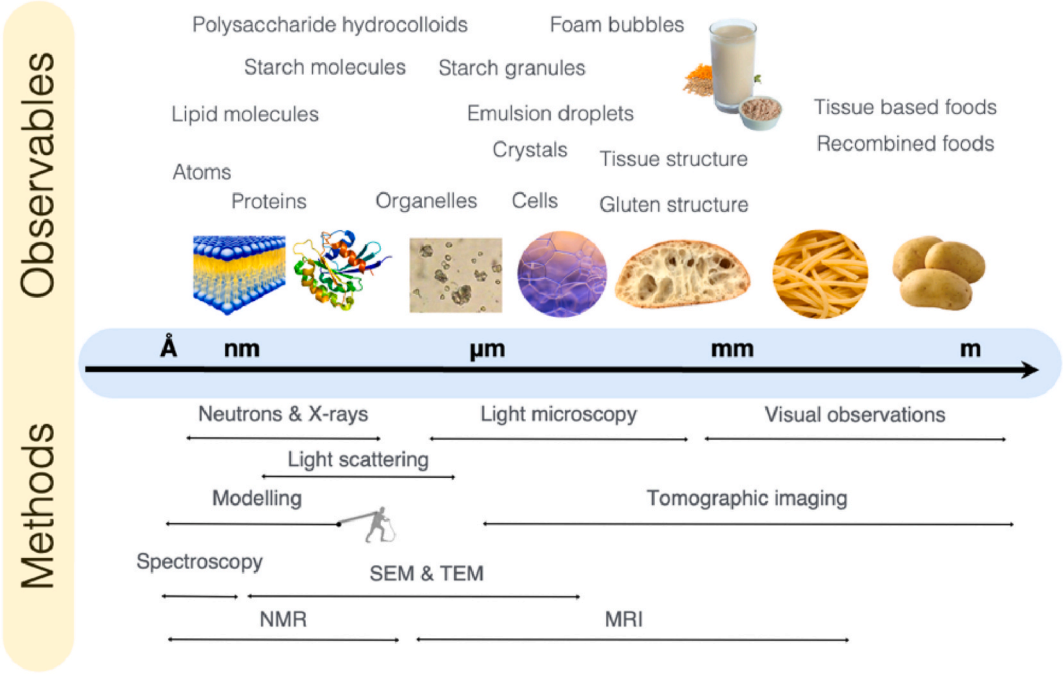

Fig. 1. Illustration of the hierarchical structure of food and food raw materials, and their relationship with techniques that can be used to study these structures at different characteristic length scales (courtesy: Mikael Lund, Lund University. All used pictures with creative commons license).

raises complex research questions that require a wide range of complementary and advanced physico-chemical techniques. When making a food product, all steps, from raw materials to the final food product, including consumption, digestion and absorption, lead to the disruption, disassembly, and reassembly of structures in foods. Hence, studying food structure often requires carefully crafted experimental designs, and non-invasive *in situ* analysis.

The experimental data acquired using advanced physical methods need to go hand in hand with the creation of models and simulations to derive important mechanistic principles (Vitrac & Hayert, 2020). In the past decade, much progress has been made to use modelling requiring the needed knowledge of the forces that govern the formation of structures in biological systems (Khalid & Rouse, 2020). Such principles can also be applied to food systems as discussed in section 2. It is therefore timely to bring together scientists in different fields with a common focus on the study of food structure, the interrelationship between structure and function, and how a combination of techniques can lead to increased understanding, based on first principles, of the changes occurring in structure and function of food in complex matrices.

2. Combining powerful sets of tools

A range of methods are available to characterize the physical properties and structure of food (Sarkar et al., 2022). Many of them are challenging to access and each of them have their limitation in terms of requirements, measurement environment, how the measurement themselves affect the studied samples and last but not least modelling and interpretation of the recorded data. Imaging, together with advancements in omics techniques has open up new avenues to link composition to structure. Multi-component analyses of large numbers of food samples and appropriate chemometric approaches provide the required foundations for advancing the field of structural food science. Microscopy techniques have been widely employed to identify the different structures as well as the spatial distribution of the components (Hagsten et al., 2016; Öhgren et al., 2019). This has made it possible to observe the extent of the hierarchical distributions present in the food matrix. This approach including Confocal Laser Scanning Microscopy (CLSM) involves staining that can affect the system and the resolution is limited to the micrometer range. Recent advances of super-resolution microscopy techniques have shown that orders of higher resolution (down to tens of nanometer) than traditional microscopy can be achieved (Gallegos-Cerda et al., 2023). Even here staining must be used. Cryogenic transmission electron microscopy (Cryo-TEM) gives the possibility to achieve nanometer resolution imaging without labels but requires samples that can be prepared as thin layers under cryogenic conditions (Cui et al., 2007). An alternative method to perform high resolution imaging is to use atomic force microscopy (AFM), which not only give information about the morphological properties, but also on the mechanical properties (Gunning & Morris, 2018; Shi et al., 2019).

We are now at the crossroads, as research with X-ray and neutron techniques has demonstrated the potential for further understanding of structures down to the atomic level in biological systems including food. As discussed below x-ray and neutrons are complementary. Combining x-ray and neutron imaging that covers the micrometer scale with scattering that goes down to nanometer scale allows us to cover the whole length scale relevant for understanding food structure. When used together, X-rays and neutrons provide complementary information on food structures, as neutrons interact with the nucleus of the atom and X-rays with the electron cloud. Both neutron and X-rays can be used in a range of techniques and combined with various sample environments. However, it should be borne in mind that synchrotron x-ray radiation is very intense and although this gives high spatial and temporal resolution, the risk for beam damage of the sample should be considered. In addition, isotopic substitution and use of D_2O alone or in mixtures with water can affect the structure to be studied by neutron scattering techniques.

The X-ray radiation, even using a laboratory source is orders of magnitude more intense than the neutron radiation, which is only available at large scale facilities. Therefore, X-rays give higher temporal and spatial resolution as well as better energy selection for certain techniques. It is also possible to focus the X-ray beam to a much smaller spot compared to neutrons. Small angle X-ray scattering (SAXS), wide angle X-ray scattering (WAXS) and X-ray scanning tomography techniques are becoming increasingly common in soft matter, biomedicine and food science. Noteworthy is that x-ray interact stronger with heavy elements than neutrons. Regarding heavy elements, the most relevant for food and food contamination are lead (Pb), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), arsenic (As), nickel (Ni), zinc (Zn) and mercury (Hg) as discussed by (Scutaraşu & Trincă, 2023). Both the health risks and the global situations as well as strategies to reduce the risks requires accurate methods. A range of x-ray techniques are available to detect these elements like, X-ray fluorescence (XRF), X-ray photoelectron spectroscopy (XPS), X-ray absorption spectroscopy (XAS) and X-ray fluorescence microscopy (μ-XRF). Here synchrotron-based techniques offer the advantage of higher sensitivity and resolution (Kopittke et al., 2017). In particular, X-ray fluorescence microscopy provides the possibility to locate a particular element within the food raw material and product.

Neutrons interact weakly with matter, but can detect light elements like in particular hydrogen, therefore acting as a non-destructive probe to study food-related materials and living tissues where hydrogen containing components are important (E. P. Gilbert, 2023b) Therefore, key components in food such as water, lipids, protein and carbohydrates can be studied separately within more complex mixtures and their contribution to structure and function more readily determined. Neutrons are isotopically sensitive, especially to deuterium and hydrogen, which can be utilized in imaging as well as scattering experiments.

The capability to vary the neutron scattering contrast through replacement of hydrogen by deuterium is a key driver for the use of neutron scattering and imaging methods. This can be achieved most simply for solution studies through the mixing of H_2O and D_2O to selectively match the scattering properties of the solvents to different components of the sample. In the case of food systems, proteins, starches, lipids, and sugars all have different natural scattering length densities and so this simple external contrast variation can already be very powerful. The further application of chemical- and bio-deuteration can be used to change the scattering length density of components in model systems, further enhancing information about co-location and structure of individual components (Duff et al., 2022; Kelman, 2016; Russell et al., 2015). It should be noted, however, that contrast variation methods assume that hydrogen and deuterium are chemically identical and so there is no effect on the structure or dynamics of the system under study. This is not always valid, especially where hydrogen bonding is a driver of system behaviour, or close to phase transitions, and care must be taken to check that the deuterated sample has the same thermodynamic, kinetic, and mechanical properties as the non-deuterated sample (Bryant et al., 2019; Fisher & Helliwell, 2008; White et al., 2018).

Neutrons also easily penetrated matter, making it possible to see through metals such as aluminium, a feature of particular interest when studying food processing in real time, under realistic conditions. Another feature is that neutrons have a magnetic moment which allow polarized neutron techniques to be used and these are increasingly used in the study of soft matter (Dikaia et al., 2024; J. K. Zhao et al., 2013).

Both these families of techniques are particularly powerful when combined with a thorough understanding of the composition of the samples and other complementary structural information. Hence, the application of foodomics' approaches (based on nuclear magnetic resonance (NMR) spectroscopy, chromatographic and mass spectrometric approaches) are combined with these techniques, to ensure that specific assumptions can be made while modelling the experimental data.

As an example of how X-rays have transformed a scientific discipline,

one could consider the advances in drug discovery and structural biology resulted from X-ray crystallography and electron cryo-tomography. Furthermore, when combined with neutron crystallography, which can define the location of water and hydrogen bonding, complex biological systems can be described in great detail (Hjorth-Jensen & Budayova-Spano, 2024). This is relevant also in the case of food matter, as the control of water in these structures are of the utmost importance, where developments combining X-ray and neutron tomography show indeed great promise for the analysis of water location (Tengattini et al., 2020).

A range of techniques can be used with X-rays and neutrons, and applications and sample environments continue to evolve due to the development of ever sophisticated beam lines (Le Brun & Gilbert, 2024). Small-angle scattering measurements can provide information of the structure at various length scales, from subnano-to hundreds of nanometers, as well as of the inner structure of supramolecular complexes. The technique can be combined with imaging to cover length scales from subnanometer to micrometer. Imaging techniques make it possible to obtain *in situ* knowledge from two-dimensional all the way to a time-resolved 3D structures. However, it should be noted that evolution of the structure during the scanning can be a limitation that influences the quality of the obtained 3D structure and care must be taken to adjust the scanning parameters accordingly. Spectroscopy is also a powerful technique when using electromagnetic radiation or neutrons, as specific responses can be collected, for example, focusing on the ions and their environments in a complex matrix, (DeBeer, 2018; Sala et al., 2024). These techniques are used to identify trace substances, derive information on chemical composition, local electronic structure, and geometry and can be used to study metal uptake in tissues, and investigate molecular interactions on catalytically active surfaces (Neal et al., 2013; Niimi et al., 2023; Oli et al., 2016; Zhang et al., 2018). Neutron spectroscopy allows for the study of the motion of molecules on picosecond to microsecond timescales, and in particular the motion of water and its binding state within complex materials such as food (E. P. Gilbert, 2023b; Nagao et al., 2017).

Imaging techniques such as electron microscopy, confocal imaging, and atomic force microscopy are a familiar toolkit of many food scientists. These powerful tools provide important information, which often requires additional confirmation using complementary techniques, due to potential artefacts due for example, to sample preparation (specific staining, cryo-freezing, deposit on silicon wafers). Neutron and X-ray scattering techniques are obvious complementary tools, which provide robust ensemble average information, but in the reciprocal space. Furthermore, X-ray and neutron imaging techniques provide critical insights into the external and internal structures of materials across multiple dimensions, offering spatial resolutions ranging from the macroscopic to the nanoscopic scale. These methods leverage the contrast generated by differences in density, absorption, or scattering properties, allowing for the visualization of structural, chemical, and functional information. Scanning small angle X-ray scattering combines imaging with scattering, providing spatially resolved structural information, offering a detailed map of features, such as particle size, shape and orientation. This technique is particularly effective in studying biological tissues, hydrogels and polymers. Phase contrast imaging exploits instead variations in the refractive index rather than absorption, and it is particularly sensitive to structural interfaces. Phase contrast, in synchrotron applications can achieve nanometer resolution, providing new information. Phase contrast imaging has been used in foods (Z. Wang et al., 2018; Indore et al., 2022) to study for instance meat (Miklos et al., 2015), beans (Hidaka et al., 2022) and seeds (Ashe et al., 2025). These imaging methods, including fluorescence imaging will offer a comprehensive toolkit for visualizing complex food matrices (Buffiere & Baruchel, 2015; Donoghue et al., 2006). When paired with tomographic approaches, which will extend into three dimensions, volumetric reconstruction of intricate food structures will be possible.

The D22 SANS instrument at Institute Laue-Langevin (ILL) now offers the possibility of simultaneous SANS and SAXS measurements that generates time-resolved complementary structural information at the nanoscale (Metwalli et al., 2021). Using software like SASFit (Bressler et al., 2015) the data obtained from SAXS and SANS can be co-refined. An example of models based on combining data from scattering and another technique is the combination of NMR and small angle X-ray scattering data to reveal structure and dynamics of biomolecules (Mertens & Svergun, 2017). It should be noted that these approaches have so far been limited to simple systems and further work is needed to apply these approaches to more complex systems like food. Simultaneously recorded data with other complementary techniques are useful in defining more elaborate models. Neutron and contrast matching offers a way to address this and facilitates application of "classical" scattering models as discussed above. As mentioned in the introduction the biophysics field the formation of structures in biological systems have rather successfully been mapped by a combination of modelling tools (Khalid and Rouse, 2020). Simulations can also be employed to calculate x-ray and neutron scattering profiles (see e.g. (Majumdar et al., 2024)) and thereby link them together. Clearly AI has been helpful in modelling experimental scattering data as high-performance neutron and synchrotron generate large data sets (Akepati et al., 2024; Heil et al., 2023). In the protein field the use of AI has had a large impact with the development of tools, e.g. AlphaFold, for computational protein design and protein structure prediction that was awarded the 2024 Nobel Prize in Chemistry (Jumper et al., 2021). Similar approaches for advancing our understanding of food structure are certainly within reach. Even though, additional data revealed by AI can aid in building better models, the sample preparation before and during the experiment as well as transforming the analysed data into realistic and applicable models remain challenging.

3. Structure of food raw materials

The multiscale structure of food raw materials is complex, not only in terms of composition (i.e. macronutrients such as polysaccharides, proteins, lipids and micronutrients such as phenolics, vitamins inorganic ions and other metabolites), but also the supramolecular organisation of the components and their heterogeneities. This structural complexity arises from their evolutionary path to fulfil specific storage and structural functions in the original organisms (i.e., seeds, muscular tissue, milk).

3.1. Structural studies on plant tissues

Plant tissues from cereals, pulses, fruits and vegetables are fundamental food raw materials rich in macro- and micronutrients, with complex multiscale organisation from the individual molecules (nm) to the whole tissue level (mm/cm) (Meldrum and Yakubov, 2024). An example of the complexity of plant raw materials can be found in the multiscale organisation of cereal grains, as described in Fig. 2. In these matrices, the starchy energy-rich endosperm is surrounded by multiple cell wall layers that act as protective and structural support (Fig. 2). Native and processed starch assemblies have a complex organisation, as the original amylose/amylopectin polymers arrange in semicrystalline lamellar layers that further assemble in microscopic granules (R. G. Gilbert et al., 2013). X-ray and neutron scattering techniques have been widely used for the determination of the crystalline arrangements of starch glucose chains and the lamellar architecture of semicrystalline growth rings of intact starch granules in plant tissues (Blazek & Gilbert, 2011). In parallel, the molecular structure of the plant cell wall components (cellulose, lignin, and non-cellulose polysaccharides such as hemicellulose and pectins) and their complex supramolecular architecture at the nanoscale (Fig. 2) have been widely studied using NMR, supported by glycomic approaches (Mansfield et al., 2012; Sivan et al., 2024; T. Wang & Hong, 2016; W. Zhao et al., 2020). The use of WAXS, SAXS and small-angle neutron scattering (SANS) techniques can

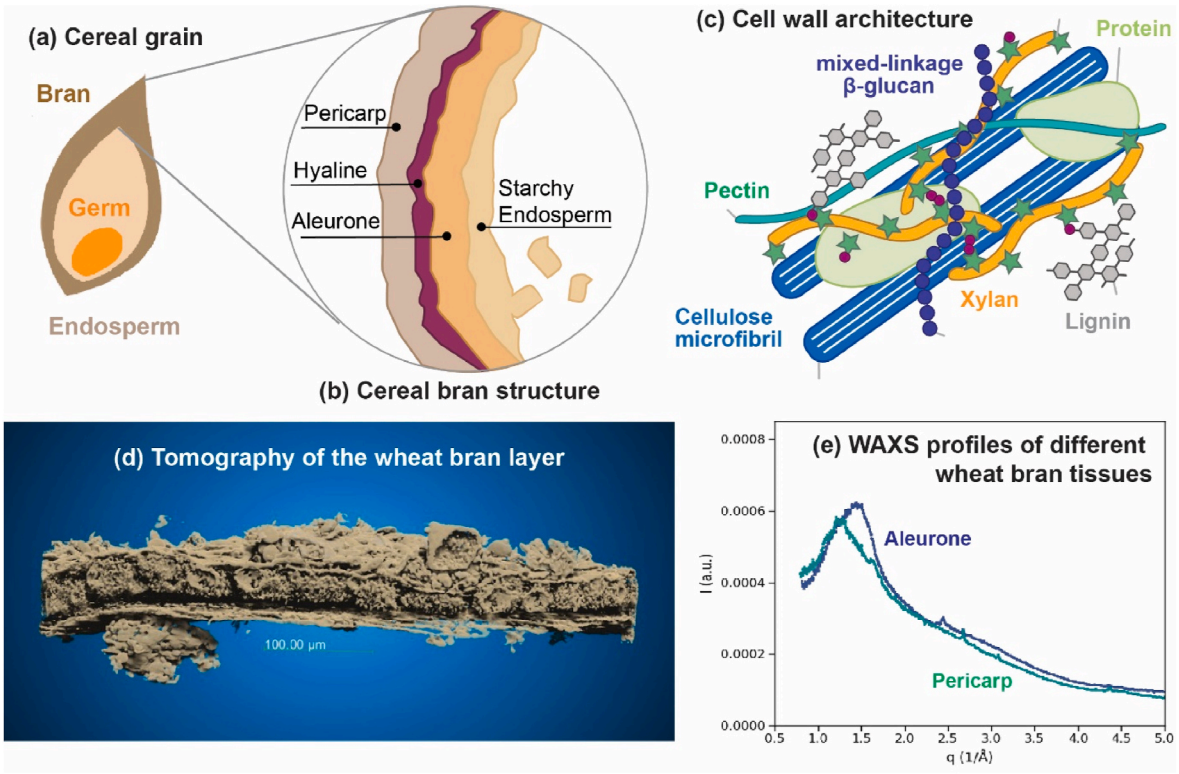

Fig. 2. (a) Structure of the cereal grain, with the endosperm, germ and bran components. (b) Cereal bran multilayer structure (aleurone, hyaline and pericarp layers). Partially redrawn and reprinted from (Rudjito et al., 2019) through a Creative Commons CC BY public use license. (c) Nanoscale architecture of the cereal cell wall (including the cellulose microfibrils embedded in a matrix of crosslinked hemicelluloses, lignin, pectin and proteins). Partially redrawn and reprinted from (Rudjito et al., 2020) through a Creative Commons CC BY public use license. (d) Example of tomography imaging of a wheat bran sample, where the residual endosperm can be observed, with the typical aleurone, hyaline and pericarp cells. (e) WAXS profiles of aleurone-rich and pericarp-rich wheat bran regions. The WAXS profile corresponding to a pericarp-rich region has well-defined peaks compared to the aleurone-rich layer, which can be related to the higher abundance of semicrystalline cellulose and hemicellulose components in the pericarp region, compared to the aleurone layer richer in amorphous proteins (gluten).

complement such approaches, to reveal the crystalline arrangement of the cellulose microfibrils, their interactions with matrix components (hemicellulose and lignin) and their further network organisation in terms of interfibrillar distance and network packing (Fernandes et al., 2011). Similar approaches have been used to understand the poro-viscoelastic properties and the role of cellulose-pectic interactions in the hydrated primary plant cell walls found in vegetables and fruits (Lopez-Sanchez et al., 2020). Neutron spectroscopy has been used to study the binding of water in cellulose (O'Neill et al., 2017).

The next challenge for scattering techniques is to provide, with spatial and temporal resolution, information on the changes occurring in the multiscale structure of plant raw materials, in different plant tissues, and also at their different developmental stages. Here, the combination of X-ray tomography imaging, SAXS, SANS and WAXS offers a powerful strategy to visualise the microscopic structural features and to simultaneously localise the nanostructural organisation of the macromolecular components (Duncan et al., 2022). Spatially resolved, synchrotron-based X-ray fluorescence (XRF) techniques have been used to map the mineral content of seeds (Neal et al., 2013; Oli et al., 2016; Zhang et al., 2018), which may direct crop development as well as understanding food mineral availability. X-ray fluorescence microscopy and scanning electron microscopy (SEM) can also be employed, as they have shown great promise to investigate the spatial distribution of sodium, nitrogen and magnesium between starch and gluten in bread with high spatial resolution and sensitivity (Sala et al., 2024).

We have recently employed synchrotron tomography and scanning wide-angle X-ray scattering (SWAXS) to reveal the organization of the cereal cell wall components at the different structural levels (unpublished data), from the network scale (nanometer level, provided by the SAXS/WAXS data) to the histological scale (micrometer level, provided by the tomography imaging), as illustrated in Fig. 2. Tomography imaging of wheat bran clearly reveals the different histological layers, from the remaining endosperm starch granules, the larger aleurone cells, and the elongated and thin pericarp layers, see Fig. 2. In addition, WAXS can provide information about the distinct organisation of the lignocellulosic components in ordered semicrystalline domains in the pericarp and aleurone layers, see Fig. 2. This example (Fig. 2) highlights the potential of integrating biochemical multiomics approaches, providing molecular information of the individual food components, with synchrotron imaging and X-rays/neutron scattering techniques, to fully elucidate, to great detail, the structure of complex plant-based food raw materials.

3.2. *Milk structural studies*

Another example of a food widely studied for its complex structure is milk, nature's response to the need for a unique food suited for energy, growth and development of the newborn. Milk has a complex composition and contains several functional supramolecular structures, such as emulsified fat, protein aggregates as well as solid nanoparticles namely, colloidal calcium phosphate nanoclusters (CCP). Casein proteins assembling into large structures of a few hundreds of nanometers, termed "micelles", have been of particular interest due to their importance for nutrition, as well as their function as building blocks to provide structure and texture in e.g. cheese. The structure of these assemblies is quite complex, showing various characteristic length scales, from a few nanometers all the way to their average diameter of about 200 nm (Pedersen et al., 2022). These structures became evident only after SANS and SAXS observations (de Kruif et al., 2012; Holt, 2004). The

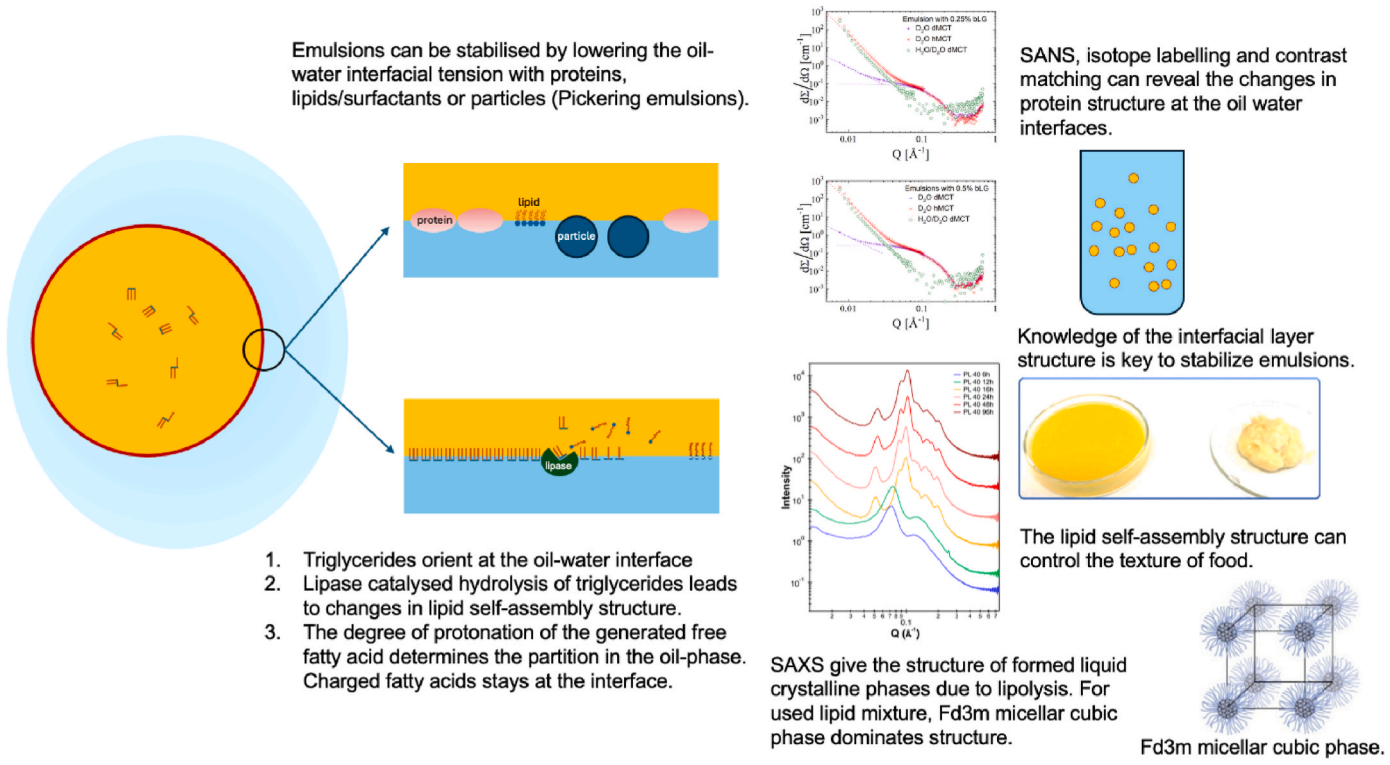

Fig. 3. Schematic illustration of how the interfacial processes can affect the stability of the emulsion and the lipid self-assembly structure. The SANS data is for β-lactoglobulin stabilized emulsions of MCT-oil at pH 7. The data for is for two protein concentrations 0.25 % (top) and 0.5 % (bottom). To separate out the contributions for the different components different isotopic contrasts are used, D_2O and d-MCT-oil highlights the protein, D2O and h-MCT-oil highlights the oil, H2O/D2O (60/40 ratio) and d-MCT-oil matches out the protein, while H2O and h-MCT-oil matches out bulk oil but give weak protein contrast. Data from Heiden-Hecht et al. (Heiden-Hecht et al., 2024). The SAXS data from Gladkauskas et al. show the effect of adding lipase to oat oil samples that contains 40 wt% polar lipids (Gladkauskas et al., 2025). The photo to the right shows the texture of the sample after different incubation time, where the photo to the far right one for the micellar cubic phase (photo kindly provided by Dr. Cecilia Tullberg, Lund University).

experimental results collected from scattering experiments on casein micelles have also been, employed to build a structural model of these complex protein particles, from empirical description to advanced theoretical models (Hansen et al., 1996; Ingham et al., 2016; Pedersen et al., 2022). These protein particles contain CCP and neutron-based experiments, using contrast matching approaches, have been particularly useful to reveal the formation, presence and role of CCPs, and their unique synergistic interactions with the casein proteins. This led to colloidally stable protein aggregates with calcium and phosphate concentrations beyond the solubility of inorganic calciumphosphate (Lenton et al., 2016; Lenton et al., 2020). These complexes have also been noted in other biofluids, like blood and saliva, making it possible for soft and hard tissues to co-exist in the same organism (Lenton et al., 2020). In addition to protein assemblies, milk also contains lipid droplets stabilized by a unique membrane rich in complex phospholipids and proteins. These droplets have also been extensively studied by SANS and SAXS (Peyronel et al., 2020). In addition, the structural evolution of protein coagulation and degradation at nano- (CCP and micelles) and micro-metre (gel network) has been investigated under gastric environment conditions using SANS, ultra small-angle neutron scattering (USANS), small-amplitude oscillatory rheometry, and CLSM (M. Yang et al., 2025). It was found that heat treatment retarded coagulation but accelerated curd proteolysis and that general proteolysis followed coagulation at high pepsin concentrations.

Milk products have also been widely investigated with X-ray and neutron techniques, due to the presence of different components and their various characteristic length scales. For example, *in situ* studies to better understand the formation of yogurt and cheese curds have been reported using USANS (Z. Yang et al., 2023) and SAXS (Li et al., 2022). The use of spin echo small angle neutron scattering (SESANS) also makes it is possible to cover a wider size range from nanometer up to 3 μm, allowing to observe different milk components and their aggregates (Tromp & Bouwman, 2007). Furthermore, the presence of calcium and phosphate in milk is not only important for the formation casein micelles, but also affect interfacial behavior of caseins, and nanocluster complexes and their interaction at surfaces, and have been analysed using neutron reflectometry (Follows et al., 2011; Follows et al., 2004). In these studies, it was possible to show that the surface layer at low temperature is limited to 10–30 nm. This is of relevance during for e.g. fouling during heat treatment. During high temperature processing of milk, thick deposits rich in CaP form on heated surfaces causing the formation of a hierarchical fouling layer composed of an interpenetrating network of protein and crystalline apatite (Hagsten et al., 2016). The response of casein micelles to high pressure treatment has also been studied in-situ using neutrons (Jackson & McGillivray, 2011). The hydration of milk powders has been examined using neutron spectroscopy techniques to try to develop an understanding of product performance parameters such as shelf life, stability, and reconstitutability (Loupiac, 2016).

The last decades have seen a boom of plant-based replacement of milk, starting with oat-based drinks in the 1990s (Ahlden et al., 1997). Since then, a range of products from various plant sources have entered the market. The use of X-rays and neutron techniques can be beneficial for the development of such plant-based food products. One challenge is the colloidal stability of plant-based replacement of milk. Here scattering techniques can be a useful tool to study the influence of processing on the colloidal stability of such system as shown for example for dispersions using faba bean protein isolate (FBI) (Hu et al., 2024).

4. Food colloids and structured interfaces

From a structural point of view, food contains different colloidal entities, i.e., emulsions, dispersions, foams and gels, and frequently a given food contains more than one of these elements. The high complexity and hierarchical structures of these systems render them challenging to fully understand. Therefore, a multitude of techniques are needed to provide a robust understanding of the compositional complexity and spatial distribution affecting structure and function.

The following section is dedicated to examples of how X-ray and neutrons have been employed to probe structures at the nanoscale, and evaluate structural dynamics in emulsions, gels, foams and liquid crystalline systems. We will also discuss how these learnings are now applied to more complex and far less studied soft matter systems and how the knowledge gained will lead to an in depth understanding of structure and dynamics in real foods.

4.1. Colloidal dispersions of lipid liquid crystalline phases and oil droplets

Lipids play an essential role in foods, forming a variety of structures when interacting with aqueous environments. Lipid-based structures are widely used in food and pharma to deliver functional molecules, enzymes, and nutrients. X-ray and neutron scattering techniques have proven particularly useful for evaluating lipid crystalline phases, vesicles and emulsions. These methods have provided a robust theoretical foundation for controlling the formation and stability of such structures. The scientific basis was established nearly 40 years ago by Larsson and co-workers, who used X-ray diffraction to demonstrate that partially stabilized fragmented non-lamellar, cubic phase lipid particles could be prepared from monoacylglycerols, with bile salts or caseins serving as dispersing agents (Larsson, 1989).

It is interesting to note this concept was first applied to food grade ingredients and discovered thanks to fundamental studies of the self-assembly structures using X-ray diffraction, which provided crucial information. Since then, the number of formulations and applications have increased, using a range of lipid liquid crystalline structures can be formed in response to composition, water content and temperature. Such systems have been explored for delivery of functional food components, like nutraceutical and enzymes (Barauskas and Nylander, 2008; Sagalowicz et al., 2006), but in particular for delivery of bioactive components and drugs (Barriga et al., 2019). Here SAXS and SANS have contributed to understanding of the internal structure as well as the overall core-shell structure of these particles (Valldeperas, Dabkowska, et al., 2019; Wadsäter et al., 2015), which are crucial for the encapsulation capability as well as the delivery and release of the active substance. For instance, mixtures of mono- and diacylglycerides can form highly swollen sponge phases (L3), well defined nanoparticles in excess water, able to encapsulate a range of food related enzymes and other proteins such as aspartic protease (34 kDa), β-galactosidase (460 kDa) and heme proteins for iron dietary supplements (Barauskas and Nylander, 2008; J. Gilbert et al., 2023; J. Gilbert et al., 2019; Valldeperas, Talaikis, et al., 2019). Formulations can also be made to create liquid crystalline phases and dispersions which can be used as structure builders in foods (Freire & Salentinig, 2024). Furthermore, X-ray diffraction experiments have been combined with a series of advanced physical techniques to study the formation of crystalline structures *in situ,* under laminar shear flows, improving our understanding of the effect of processing factors (Mishra et al., 2023).

The structure and distribution of components in different types of lipid bilayers, such as in liquid crystalline phases or in cell membranes, can be studied using neutron reflectometry and contrast matching (Caselli et al., 2022; Caselli et al., 2024). The ongoing development of *in situ* grazing incidence small angle X-ray scattering (GISAXS) and small angle neutron scattering (GISANS) has allowed detailed characterisation of the structure of the lipid/water interfaces. For example, recently the lipase catalysed digestion of lipid liquid crystalline coatings from GMO/triolein assemblies was followed *in situ* by GISAXS (Freire, Tran, et al., 2024). This approach clearly shows great promise to give structural evolution details, following both the liquid crystalline structure and the orientation of the domains.

Emulsions are lipid phases separated from the aqueous phase by a stabilizing layer of emulsifiers and stabilizers in foods, like milk and milk analogues, and mayonnaise. Their structural characteristics are closely linked to their functional properties, which have been studied extensively using different types of advanced techniques. Light scattering is an important technique for investigation of emulsions. However, concentrated systems must be diluted which can influence the structure and the droplet sizes. Light scattering has provided average droplet size distribution, while X-ray and neutron techniques have revealed important structural features, such as lipid organization within the droplets and emulsifiers arrangement at the interfaces. Already in 1968, Bursh et al. (Bursh et al., 1968) suggested that glycerides at a fat-water interface align similarly to multilayers. Since then, a combination of spectroscopic ellipsometry, coarse grain modelling and SAXS have been used to demonstrate that e.g. triolein can take up pockets or clusters of water (Stamm et al., 2018). More recently, Frigerio et al. (Frigerio et al., 2024) combined ellipsometry at the liquid/liquid interface, tensiometry, scanning small angle X-ray scattering and modelling to show that pH could trigger changes of the structure at the triolein/water interface. These structural insights into the triolein/aqueous interface and its dependence on the aqueous solution have important implications for emulsifier interactions and, hence, for emulsion stability.

As the stability of the emulsions is related to the structure of the interface of the oil droplets, interactions between oil droplets with the emulsifiers are often studied using neutron reflectometry, SAXS and SANS, see Fig. 3. Complex interfaces, composed of electrostatic protein-polysaccharide multilayers have been studied by neutron reflectometry demonstrating their transient binding depending on environmental pH (Bertsch et al., 2019). In a recent study, Heiden-Hecht et al. (Heiden-Hecht et al., 2024) used a combination of SAXS and SANS with contrast variation to provide new insights on the structure of β-lactoglobulin (β-lg) stabilized emulsions of hydrogenated medium-chain triglyceride-oil, MCT, see Fig. 3. Coarse grain modelling (Koutsioubas et al., 2016) showed that at low concentration, β-lg was unfolded while β-lg dimers dominated the interface at high concentration. The combination of proteins and polar lipids, like phospholipids or mono- and diglycerides (MDG) and the structures that they form at the interface have been studied using SAXS and WAXS (MacWilliams et al., 2023) showing the potential of these techniques to follow polar lipid crystallization at the oil–water interface, an quantify the extent of crystallization at the interface (Yesiltas et al., 2019).

In addition to the fundamental work on specific structural features in food systems, it is now possible to carry out more applied work, for example, *in situ* studies to evaluate crystallization of lipids in complex food matrices using synchrotron small-angle and wide-angle X-ray diffraction (Smith et al., 2022) or to highlight differences between natural and common fat mimetics used in foods (Marangoni et al., 2020; Salentinig et al., 2015). In particular, oleogels, semi-solid gel structures, have been suggested as versatile systems to reduce dietary lipid intake without compromising the structure of food. Small angle and ultra-small angle scattering X-ray and neutron techniques can reveal important details on the structure of these highly organized systems, when studied *in situ,* from the primary building blocks to the larger network structures (E. P. Gilbert, 2023a; Tsung et al., 2020). Oleogels are particularly suited for neutron studies due to the benefits of contrast variation and selective deuteration.

4.2. Protein and polysaccharide hydrogels and protein aggregation

In situ techniques are particularly key in determining precursors to structure formation, transient structures, aggregates and their

precursors. In these non-equilibrium systems, a minimal perturbation will affect their structural development. Few techniques are hence available to study their development, limiting our mechanistic understanding of how larger structures form from colloidal particles, water-in-water phase separated systems or gels.

Advanced physical techniques have been employed to study the self-assembly of proteins in aqueous solutions. For example, SAXS is notably used to follow the modification of casein supramolecular assemblies (Wijaya et al., 2021) or the propensity of food proteins to form amyloid fibrils which could be used as functional materials (Cao & Mezzenga, 2019). By understanding and being able to follow the changes occurring at characteristic length scales in complex environments, under industrial relevant conditions, it will be indeed possible to develop strategies to broaden our toolbox and improve food protein functionalities.

The models employed to study the formation of aggregates and gels in foods are also not as well-developed and defined as for classical colloidal systems. The heat-induced gelation kinetics of milk proteins or hen egg white proteins has been revealed by combining X-ray photon correlation spectroscopy along with ultra-small-angle X-ray scattering to cover the dynamics and the evolution of the gel-network (Begam et al., 2021; Li et al., 2022). The evolution of the network features is a reaction-limited aggregation process, where the heterogeneity increases with decreasing length scale.

It is becoming increasingly clear that these techniques will allow to investigate the structural evolution of food proteins in complex systems. Recently, the gelation of different protein isolates from yellow pea, faba bean, and soy were investigated using USANS, SANS, USANS with SEM and rheology (Tiong et al., 2025). The gelation was found to be related to the method used to extract the protein, i.e. the fraction of insoluble protein or particles in the sample. For samples with a high fraction of insoluble protein, fractal networks formed, while more homogenous networks were formed when soluble proteins dominated.

Rheology, combined *in situ* with scattering techniques have also been utilized to better understand formation of alignment, not only in lipid crystals but also for protein and complex carbohydrate gels (Bianco et al., 2024). Using rheo-SAXS tools it is indeed possible to study *in situ* orientation of structures at multiple length scales. The formation of protein fibres under shear is of particular importance. As an example, fibrous calcium caseinate can form through shearing of dense calcium caseinate dispersions. Using SANS it is possible to determine the dimensions of these fibres (Tian et al., 2020). Enzymatic treatment (transglutaminase) gives longer fibrils, which can be aligned by increasing shear. Microfocus SAXS has been used to monitor the whey protein fibril orientation in a microchannel and compare the assembly processes of PNFs (protein nanofibrils) of distinct morphologies (Kamada et al., 2017), and potato-PNF has been evaluated with wide angle X-ray fibre diffraction, showing characteristic cross-β reflections (Josefsson et al., 2020). This provides new leads for the build-up of hierarchical structure in new complex foods.

Polysaccharides, extracted from an array of available terrestrial and marine feedstock, or produced via fermentation, are often added to food to improve texture, gel structures, stabilisation, or as dietary fibre. One fourth of the global sales of polysaccharides are used within the food and beverage sector (Global Polysaccharides Products Market Size, Share 2032; accessed December 2024). The largest sale is that of starch, followed by cellulose, pectin, agar, alginate and xanthan gum. Due to their large molecular weight and extended conformation, often they form colloidal dispersions when added to aqueous solutions. Scattering (light, X-ray, neutrons) techniques have been particularly useful to evaluate the characteristic length-scales from the polysaccharide single chains (Å to nm) to the polysaccharide (tens to hundreds of nm) clusters (Alba et al., 2018; Maire du Poset et al., 2020), impacting solution properties (Doutch & Gilbert, 2013).

Advances in the understanding of starch structures and granules hydration have been made using a combination of techniques, including SAXS, WAXS and SANS. In particular, it has been possible to follow the various stages of gelatinization, and using neutrons, water hydration (Doutch and Gilbert, 2013; Jenkins & Donald, 1998), using industrially relevant conditions. SAXS can distinguish the semi-crystalline lamellar structure of the granules, and at extended q ranges (0.002–004 Å), it may be possible to distinguish differences in the dry starch granules depending on botanical source. The effects of starch retrogradation in rice from cooking have also been studied using neutron spectroscopy techniques with the cooking done in D_2O to allow the changes to the starch dynamics to be separated from water dynamics (Hirata et al., 2023).

Pectin with molecular dimensions (radii of gyration) ranging from 6 to 40 nm have been studied using SAXS (Alba et al., 2018), and their structural flexibility as a function of pH could be evaluated. In addition, the clusters formed by pectins in solution, via hydrogen bonding, ranging between 100 and 200 nm, can be measured (Alba et al., 2017). This polysaccharide forms gels at acidic conditions and in the presence of multivalent ions. Acid pectin gels are characterised as a clustered network composed of flexible cylindrical elements, as well noted from TEM images showing flexible rod-like (Mansel et al., 2015). Differences in scattering intensity of the acid pectin network in D_2O compared to H_2O further highlights the importance of hydrogen bond formation in these networks (Mansel et al., 2015). Similarly, alginate polymers and their gels have been characterized. (Hermansson et al., 2016; Maki et al., 2011; E. Schuster et al., 2017; Stokke et al., 2000; Yamamoto et al., 2019).

In addition to charged polysaccharides, structural changes in solution of neutral polysaccharides such as xanthan, agar and carrageenan, have been evaluated, and specifically, the kinetics underpinnig the formation of double stranded conformations using SAXS (Kitamura et al., 1991; Liu et al., 1987; Tomofuji et al., 2022). Agar is another polysaccharide forming double helices and bundles upon gelation. Combination of time resolved SAXS and SANS have been used to demonstrate that the gelation mechanism of the agar had little influence on the nanostructure of the gels, even though it impacted mechanical properties (Martínez-Sanz, Ström, et al., 2020). Carrageenan is another polysaccharide commonly used in foods since its properties can be easily tailored by biopolymer concentration, counter ions and cooling rates during processing. Its gelation mechanism has been investigated using transmission electron microscopy (Lorén et al., 2009) and XRD. Gelation of κ-carrageenan is initiated upon cooling by a conformational change from random coils to double helices. The random coil to double helix transition is then followed by aggregation of the double helices into gel strands and network formation. X-ray scattering and molecular dynamics simulations have been used to reveal the secondary structure of κ-carrageenan in solution (Westberry et al., 2022). Finally, the combination of WAXS, SAXS and SANS has been fundamental to understand the biochemical and biophysical mechanisms that govern the formation of arabinoxylan hydrogels upon enzymatic and physical crosslinking, mediated by a combination of covalent, backbone and side chain interactions (Yilmaz-Turan et al., 2022; Yu et al., 2019; Yu et al., 2018).

4.3. Foams

In foams, as in emulsions, the interfacial structure is crucial for stabilizing air bubbles. However, studying foams is quite challenging due to the dynamic nature of foam formation and destabilization. Neutron reflectometry can be used to investigate multi-component interfacial structures (Bertsch et al., 2019), while classical experiments, such as the over-flowing cylinder, combined with neutron reflectometry can quantify surface concentrations, layer density profiles and thickness under dynamic conditions (van Well & Brinkhof, 2000). Since these earlier studies, both neutron scattering methods and data modelling have evolved, enabling structural probing of liquid foams *in situ* (Lamolinairie et al., 2022; Li et al., 2025). In addition, scattering has been used to probe macroscopic foam down to nanometer resolution (Chiappisi, 2024). This development has allowed investigation of single films within

macroscopic foams to provide insights into foaming and destabilization processes, though data modelling and interpretation is still not trivial. The recent results clearly demonstrate the new possibilities linked to neutron scattering techniques to obtain more detailed insights in the composition and interactions in foams. Additionally synchrotron X-ray microcomputed tomography (μCT) can capture foam microstructure in 3D, which is particularly valuable for food related foams in non-equilibrium states (Li et al., 2025; Schott, Dollet, et al., 2023; Schott, Isaksson, et al., 2023). Recent work has also reported the characterization of triglyceride-based oleofoams using μCT (Mader et al., 2012; Metilli et al., 2022).

5. Revealing structure of food during processing

During food processing and consumption, the structure is in continuous evolution. Hence, in structural food science, there is an increased challenge in evaluating structures *in operando*. Only with an in-depth understanding of these changes, observed in relevant environments and conditions, it will be possible to develop the necessary principles to optimize product quality (Ubbink et al., 2008).

The structure changes during the processing span length scales from nm to cm, and time scales from nanoseconds to minutes. In addition, interfaces are continuously created and/or removed due to rupture, coalescence and aggregation of phases, heavily influenced by surface-active components and viscosity. In the past, much of the understanding of processing effects was obtained either by analysing the structure before and after, or with stop-flow techniques. However, in *situ/in operando* investigations are now becoming increasingly possible. Indeed, even if stop-flow approaches may help in analysing a specific stage of the process, it is a compromise, since many reactions are at a non-equilibrium state during the processing. For *in situ/in operando* investigations, high temporal and spatial resolution as well as high sensitivity/high contrast are needed. This is where synchrotron X-ray and neutron techniques can respond to the challenges. Neutrons can penetrate metals like alumina and titanium, which is an advantage, when wanting to investigate food processing reactions occurring in sealed vessels. In addition, these time-resolved and high-resolution measurements come with additional challenges, related to the creation of extensive data sets, requiring for new approaches for effective data analysis pipelines non existing today.

5.1. In situ studies

Processing of multiphase food materials (baking, cooking, drying etc.) is often approached as a black box. One example of such processes is extrusion, a common approach used to texturize plant-based ingredients into fibrillar structures to be used in meat replacers. Recently, this process was studied by neutron scattering *in situ,* after designing an extruder cooling die environment to be subjected to experiments in a large-scale facility. The fibre evolution evaluated during these experiments covered length scales from 1.3 to 436 nm (Guan et al., 2024). When using soy protein concentrate (SPC) as an ingredient for the process, the scattering pattern showed that the building blocks of the fibre structure were densely packed 40 nm protein aggregates formed from 9 nm sized globular proteins. These globular proteins were suggested to form chain-like arrangements. The complementarity of SANS and SAXS proved to be a powerful tools to reveal the structure of these fibres created by the proteins (Garina et al., 2024; Velichko et al., 2019). Another successful example of *in situ* studies using relevant environment is the work on starch gelation, whereby by using a rapid viscoelastic analyzer in line with a SANS/SAXS, it is possible to evaluate time resolved hydration (Blazek & Gilbert, 2011). In addition to scattering, tomography techniques have been used to follow the proofing of bread (Primo-Martín et al., 2010), foaming of extruded starch fortified by spirulina (Martínez-Sanz, Larsson, et al., 2020), and cooking of meat fibers (Scussat et al., 2017). Neutron and X-ray imaging allow non-destructive observation of large objects at micrometer resolution. Recent work showed the ability of a synchrotron X-ray micro-tomography to observe *in situ*, real time, the evolution of a bread foam during proofing and baking in a combined microwave-conventional oven (Schott, Isaksson, et al., 2023), see Fig. 4. This seminal work demonstrated the ability of these techniques to follow the porosity as well as mean pore volume and coordination number, when imaging is combined with advanced data treatment. The combined use of NMR and neutron tomography allowed studies of the compositional and structural changes during cooking of meat at different temperatures. The tomography revealed fiber contraction and increase in defects with temperature, which were located on the surface of the meat. These changes were found to be accompanied with decrease in water diffusion coefficient as measured be NMR (Scussat et al., 2017).

Another food system studied during crystallization using SAXS and WAXS is chocolate. During its production, the structure goes from liquid state to a semi-solid state with a three-dimensional fat crystal network with interspersed liquid oil. Different fat polymorphs are formed during the cooling and heating treatments. Bayes-Garcia et al. studied cooling and heating of POP at different rates using a combination of DSC and synchrotron SWAXS and could map the polymorphic transformation pathways (Bayés-García et al., 2013). Combinations of SAXS, WAXS, USAXS, polarized light microscopy, DSC and rheology are excellent tools to investigate the fat crystal formation (Simone et al., 2024) and how different ingredients affect chocolate tempering (Chen et al., 2021). Cracking in chocolate has been investigated using X-ray tomography (Reinke et al., 2016). X-ray diffraction and polarized light microscopy have been used to investigate effects of adding various minor non-triglyceride lipidic components on chocolate tempering and formation of the desired polymorph V (Chen et al., 2021). X-ray techniques have also played an important role in investigating the relationship between fat bloom and microstructure (Delbaere et al., 2016). The phase separation of cocoa butter substitute from cocoa butter in compound chocolate drives the formation of fat bloom, but the crystallization kinetics can minimize this problem as shown in a combined DSC and SR-SWAXS study (Koizumi et al., 2022). The rate of transformation from β' to β form for CBS is related to the strain of the crystals (Koizumi et al., 2023).

Freeze drying of probiotics is carried by first forming frozen pellets by dripping a dispersion of probiotic bacteria in a solution of lyoprotectants (e.g., maltodextrin, sucrose, amino acids and antioxidants) into liquid nitrogen. The frozen pellets are thereafter transferred to a freeze-dryer and dried. Annealing of the frozen pellets before freeze-drying strongly influences the structure of the final dried pellets as studied by μCT (Palmkron, et al., 2023, 2024). The advantage of μCT is that the structure can be quantified to determine the pore size and the wall thickness, where a thick wall is beneficial for bacterial viability after drying, storage and rehydration. Recently, the structural evolution during freezing, annealing and drying was followed *in situ* using synchrotron μCT, and it was possible to distinguish ice, freeze concentrate and voids by using phase contrast imaging (Bai Palmkron et al., unpublished data). Synchrotron μCT has also been used to investigate freeze-drying in vials to establish structure as well as supporting heat- and mass transfer modelling in the freeze-drying process (Foerst et al., 2019; Thomik et al., 2022). Recently, a pilot-scale freeze-dyer for *in situ* neutron imaging was designed and implemented (Hilmer et al., 2024).

The advancement of tomographic X-ray imaging techniques at synchrotrons have open up new possibilities to study membranes and membrane processes in 3D with a spatial resolution down to 40 nm, which was not possible before (Rudolph-Schöpping et al., 2024). Furthermore, the relationship between the rheological behaviour of deposits and the corresponding microstructure during membrane filtration has been characterized by SAXS (Pignon et al., 2004).

Further advances in sample environments for neutron scattering allows studies under more realistic conditions, but also when simultaneously combined with e.g. IR and UV spectroscopy and light

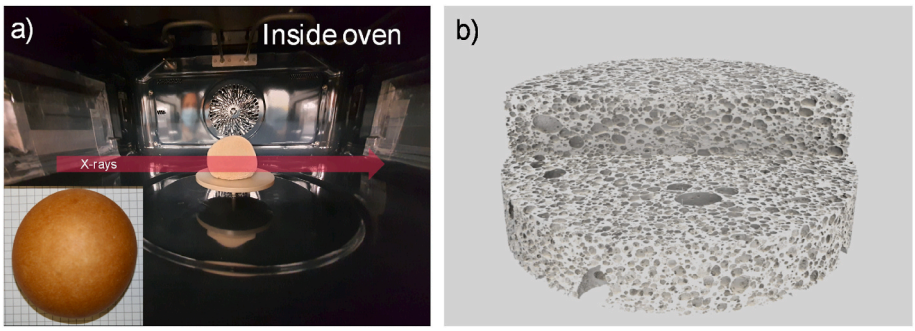

Fig. 4. X-ray tomography imaging of *in situ* baking inside a combined conventional and microwave oven. a) Image showing the custom-built rotation table inside the oven and a picture of a baked bun. b) 3D structure of the bread close to the beginning of the baking (Schott, Isaksson, et al., 2023).

microscopy as well as x-ray scattering on the same sample, can provide fundamental insight on the mechanisms involve during processing (Le Brun & Gilbert, 2024).

6. In-vitro digestion and nutrition

The digestion of food in the gastrointestinal tract is an example where structural changes are directly linked to physiological events, as pointed out already in the 1970s by Patton & Carey, 1979; Patton & Carey, 1979). Synchrotron scattering techniques have provided insights on the changes in lipid assembly structures that occur due to lipolysis in pure systems (Barauskas and Nylander, 2008; Salentinig et al., 2011; M. Wadsäter et al., 2018) as well as changes within single triolein particles (Manca et al., 2023) and changes in lipid assemblies during in-vitro digestion in more complex substrates like milk. Time-resolved SAXS has also been used to follow simulated gastrointestinal digestions in vitro of milk and mayonnaise (Clulow et al., 2018; Salentinig et al., 2017; Salentinig et al., 2015; Salentinig et al., 2013), during which the

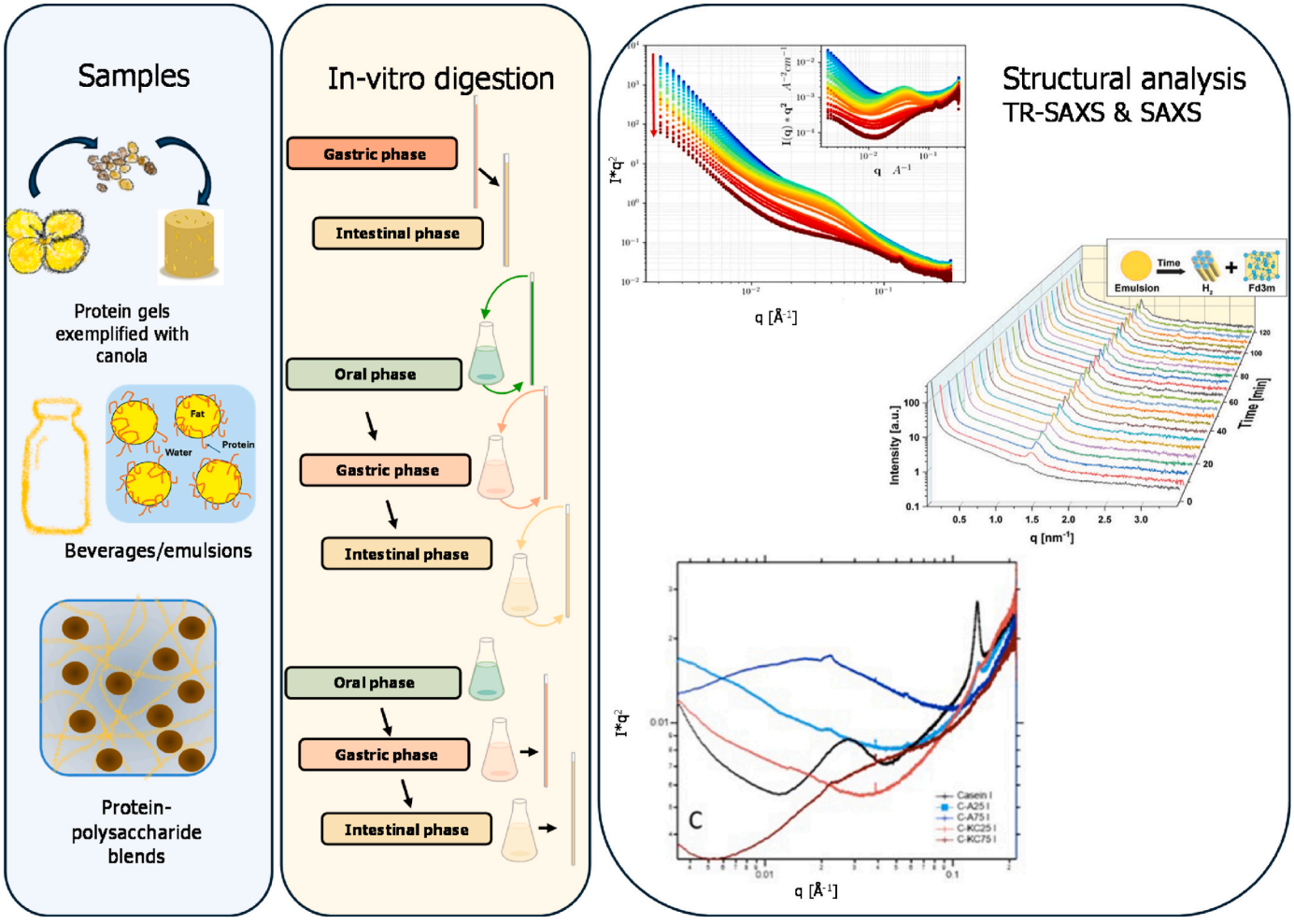

Fig. 5. Structure formation and break-up of protein gels, lipids in beverages, proteins within polysaccharide gels or in presence of polysaccharides (left) during the different phases of digestion (middle) have been monitored using SAXS and time resolved (TR) - SAXS (right). Top image right depicts the scattering profile of canola proteins during digestion (Napieraj et al., 2024), middle right depicts structure formation of buriti oil emulsion during digestion (Freire et al., 2023), and lower right casein within polysaccharide gels (Fontes-Candia et al., 2023).

formations of highly ordered nanostructures were detected and connected to the intestinal uptake of lipids and lipophilic nutrients.

Similarly, time resolved SAXS has been used to follow simulated gastrointestinal digestion of canola protein gels, in which changes to gel and protein structure was captured (Napieraj et al., 2024), see Fig. 5. The changes in nanostructure of casein and casein – polysaccharide (agar and κ-carrageenan) complexes occurring after gastric and intestinal digestion have also been reported (Fontes-Candia et al., 2023), see Fig. 5. In this case, the INFOGEST protocol was followed and aliquots of digesta was analysed. The results presented by Fontes-Candia and co-workers suggested interaction between bile salts and peptides release from the casein hydrolysis (Díaz-Piñero et al., 2024). The suggestion was confirmed, and increasing amount of bile salts influenced nanostructures formed. In addition, bile salts interacted also with agarose, which reduced proteolysis of casein. Xu and co-workers investigated the fate of conformational diversity of β-lg monomer and amyloid fibril during gastric and intestinal in-vitro digestion using SAXS, showing distinct differences in scattering prior digestion, but where β-lg monomer and β-lg amyloid fibril showed similar scattering after intestinal digestion. The authors conclude that there is no detectable conformational diversity between the β-lg monomer and amyloid fibril solution after simulated gastro-intestinal digestion (Xu et al., 2023).

Laboratory-based SAXS, in combination with 13C NMR, has also been used to study the behaviour of soluble dietary fibres, beta-glucan and arabinoxylan, and their interaction with bile and bile salts to mimic the environment in the duodenum and the small intestine (Gunness et al., 2016).

Digestion of starch, which has been studied with SAS showed an increase in surface roughness of the starch granules after digestion (Lopez-Rubio et al., 2007). Time resolved neutron scattering, in combination with other techniques showed the lamellar peak intensity decreases during digestion, especially for type-A starches (Blazek & Gilbert, 2010).

In even more complex food matrices, understanding the availability of micronutrients derive from the knowledge of the structure of the raw food components. Salentinig and co-workers showed the formation of highly ordered geometric nanostructures during in-vitro intestinal digestion of commercial dairy milk (Clulow et al., 2018; Salentinig et al., 2013), as well as commercial mayonnaise (Salentinig et al., 2017) and human breast milk (Salentinig et al., 2017). These formations of highly ordered nanostructures might be connected to the intestinal uptake of lipids and lipidophilic nutrients (Risbo et al., 2023).

7. Conclusions and perspectives

To be able to tackle big societal challenges, and to achieve a healthier and more sustainable food production and consumption, it is crucial to collaborate and approach the research problems holistically. Structural studies of food need to integrate and advance the new X-ray and neutron tools with other physical and chemical techniques, for a multi-pronged approach, deriving information at various length scales. Food hierarchical structures are complex, however studying their complexity needs to be elevated from trial-and-error approaches to model systems, allowing to derive important mechanistic principles. In addition, model systems, such as structures with a limited number of ingredients, must be researched in relevant environments, to be able to derive information on the dynamics of structure formation of complex foods and processes, and ultimately aid in designing new and innovative food products which are more sustainable and resilient.

To get the full mechanistic understanding of food, it is key to have a broad perspective. Food science brings together multiple disciplines from materials science to food processing and advanced computation to medicine. Here, the large-scale research facilities (i.e., synchrotrons, neutron reactors and spallation sources) and the technologies that they offer are central to obtaining the necessary information for a fast and effective development of innovative, healthy and sustainable foods. They are crucial to understanding the complexity of food and food processing and offer a new additional toolbox with unsurpassed spatial, chemical and temporal resolution.

Future advancements in X-ray-based methods will transform food science by providing new insights into the structural, compositional, and functional properties of food. These techniques enable the exploration of food at multiple scales, from the molecular arrangement of nutrients to the macroscopic organization of complex systems, such as emulsions, gels, and porous matrices. As time-resolved and *in situ* studies become more accessible, dynamic processes such as cooking, fermentation, extrusion, storage can be studied with such details that it unlocks pathways to enhance food quality, shelf life, and sustainability and supporting the development of innovative food products, more efficient processing methods, and solutions for addressing global challenges like malnutrition and food security.

Many of the X-ray techniques can in principle be performed using laboratory instruments, but with significantly lower resolution in space or time and less sensitivity. However, often these experiments are necessary in foods, to ensure that the right experimental conditions and environments are tested and optimized before embarking in X-ray synchrotron experiments. Neutron techniques, on the other hand, are only available at large scale research facilities. The food science community is faced with significant challenges in accessing such facilities, which include the absence of relevant environments or models that are adapted for the study of food soft matter. The food science community has so far exploited these opportunities to a much lower extent compared to other life science disciplines and biophysics. To fully exploit the potential of X-ray and neutrons to understand the hierarchical food structure and the dynamics in the future, we need to:

- Develop sample environments that are adapted for complex materials and the research questions within food science. Examples include homogenizers and extruders.
- Analyse the structure formation and the structure breakdown *in situ* during conditions which resemble those occurring in processing equipment or during digestion.
- Determine the suitability of laboratory sources or synchrotron/neutron facilities to answer the appropriate research questions.
- Combine X-rays and neutrons with other complementary advanced techniques (food-omics approaches, electron microscopy, light microscopy etc.) and being able to describe in detail the composition of the material at hand.
- Develop mathematical models for hierarchical structures, assisted by recent development of AI assisted modelling, that allow translation into mechanistic understanding of processes and structure formation and breakdown.
- Enable handling of the big data that is generated by large scale research infrastructures (LSRI). Here data evaluation with very large data sets can be helped by AI but still require input data on the properties and structure of the system. Here an interdisciplinary approach is needed where experts on food science, food structure, physics, chemistry, biology, computer science and AI/mathematics/statistics work together.

Indeed, to address the complexity of food with respect to composition, structure on a wide range of length scales and the evolution of structures in processing requires a combined effort including physics, chemistry and mathematics. By regarding food as soft matter, and widening the community studying food matter, better insights and mechanistic understanding of food structure can be obtained, thus providing the scientific basis for future food development.

The limited examples given in this review highlight the potential of X-ray and neutron techniques within food science. Recent years have seen many advances particularly in the understanding and development of plant-based foods. The combination of advanced materials characterisation and data evaluation will be a paradigm shift of how we

produce the sustainable food of the future, and the results will be of value beyond academia, to small and large companies engaged in food innovation. In this context, it will be helpful to investigate food structure using a materials science approach, and strengthen the view of food as soft matter, albeit with complex composition and structure. Education and training programs as well as communication efforts provide means to reach out to all stakeholders, from students to early career researchers, to industry and society. These are also important ways to disseminate and share the benefit of the advanced analytical approaches for future food development discussed here.

CRediT authorship contribution statement

Milena Corredig: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Anna Millqvist Fureby:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Henrik Hansson:** Writing – review & editing, Writing – original draft. **Andrew Jackson:** Writing – review & editing. **Maud Langton:** Writing – original draft, Funding acquisition, Conceptualization. **Selma Maric:** Writing – review & editing, Funding acquisition, Conceptualization. **Emma Nordell:** Writing – review & editing, Project administration. **Tommy Nylander:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Marjorie Ladd Parada:** Writing – review & editing. **Jens Risbo:** Writing – review & editing. **Elisabet Rytter:** Writing – review & editing. **Anna Ström:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Francisco Vilaplana:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Niklas Lorén:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

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Data availability

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

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