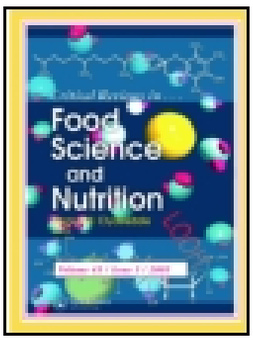

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Application of molecular dynamic simulation to study food proteins: A review

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ABSTRACT

This review presents an overview of the application of molecular dynamic simulation to study food proteins. Processing of food using thermal, chemical, radiation, electromagnetic, and mechanical techniques is subject to its macromolecular bio-components such as carbohydrates and proteins to extreme heat, ionic strength, pH, and mechanical deformation. These processing factors affect protein's functional properties such as emulsification, dough formation, gelation, etc., which are associated with changes in their structure. It is difficult to study the structural changes of protein during processing using standard methods like Circular dichroism, Nuclear Magnetic Resonance (NMR), and X-ray diffraction. Hence, in this manuscript application of molecular dynamic simulation to visualize and analyze the protein dynamics during processing has been evaluated. Effect of external stresses such as hydration, temperature, and electric field on protein structure have been analyzed and related mechanisms are explained. The response of food proteins to these stresses demonstrated that it is necessary to gain insight into protein dynamics to be able to develop novel and/or modify existing food processing techniques to improve the overall nutritional and organoleptic qualities of processed food products.

Abbreviations: AMBER: Assisted Model Building with Energy Refinement; CFF: Consistent Force Field; CHARMM: Chemistry at HARvard Macromolecular Mechanics; FT-IR: Fourier Transform Infrared; GROMACS: GROningen MAchine for Chemical Simulations; GROMOS: GROningen MOlecular Simulation; HVP: High Voltage Processing; LINCS: Linear Constraint Solver; MD: Molecular Dynamics; MMFF: Merck Molecular Force Field; NAMD: Not just Another Molecular Dynamics; NMR: Nuclear Magnetic Resonance; PBC: Periodic Boundary Conditions; PDB: Protein Data Bank; PEDC: Path Exploration with Distance Constraints; PEF: Pulsed Electric Field; RMSD: Root Mean Square Deviation; SHP: Soybean Hydrophobic Protein; SPC: Simple Point Charge; SPC/E: Simple Point Charge Extended; STI: Soybean Trypsin Inhibitor; TIP3P: Transferable Intermolecular Potential with 3-Points; TIP4P: Transferable Intermolecular Potential with 4-Points; TIP5P: Transferable Intermolecular Potential with 5-Points

KEYWORDS

Molecular dynamics; molecular simulations; protein dynamics; hydration; high electric field; thermal processing

1. Introduction

Proteins are an essential component of diet. They play a central role in determining the overall nutritional and organoleptic quality of the food. In addition to their nutritional and sensorial role, proteins also impart the structural basis of various functional properties of food. Their functional role in food is not contributed only by virtue of its physico-chemical properties; rather it is attributable through its complex interactions of several intrinsic and extrinsic properties. These properties of proteins are based on their structure (Singh et al., 2013a), which is classified as primary structure referring to the linear sequence of amino acid polypeptide chain; secondary, which are the highly regular local substructures such as α-helix, β-sheet conceived due to formation of hydrogen bonds between main-chain peptide groups; tertiary structure referring to the three-dimensional form of a single protein; and quaternary referring to the complex three-dimensional structure of multi-subunit protein (Fig. 1).

The functional properties of proteins such as solubility, viscosity, water binding, gelation, cohesion/adhesion, elasticity, emulsification, and foaming are related to their physico-chemical and structural properties such as shape, size, amino acid sequence, hydrophobicity, hydrophilicity, charge, and ability of the protein structure to change in a given environment (Kinsella, 1979). The primary structure of the protein, that is, its amino acid sequence governs the final three-dimensional structure of the protein, its thermodynamic stability and the distribution of hydrophilic and hydrophobic patches on the surface of the protein.

The folding of the protein is dictated by the need of reaching a thermodynamically stable state; in order to do so the hydrophobic groups are buried inside and the hydrophilic and charged residues are on the surface, but in several food proteins the presence of hydrophobic groups on the surface, due to structural constraints, influences the functional properties such as solubility of the protein (Amadei et al., 1993; Budi et al., 2004). Many proteins such as casein and gelatin have high proline (amino acid) content, which results in the high flexibility of these proteins which exhibit important functional properties such as gelation, emulsification, and foaming properties. (Hayakawa and Nakai, 1985; Damodaran, 1994). The flexibility

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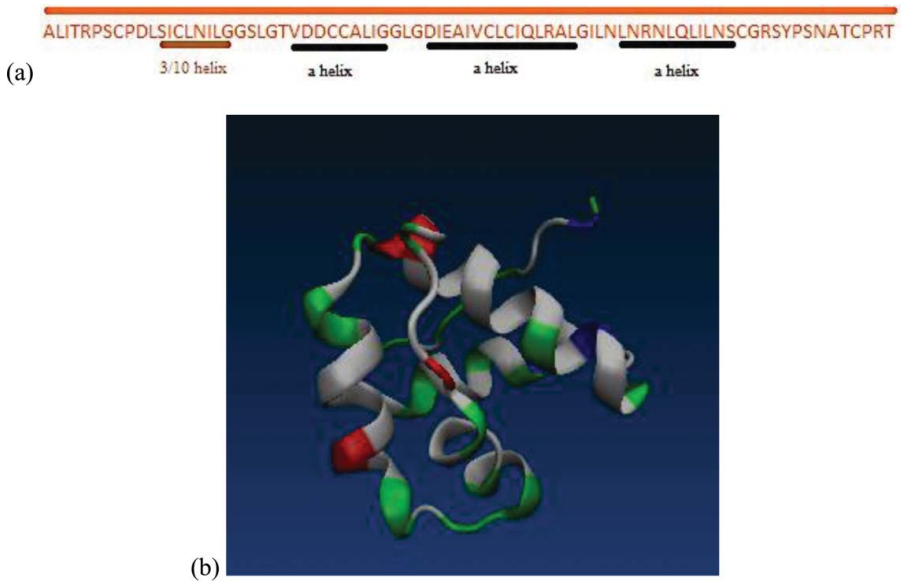

Figure 1. (a) FASTA sequence of soybean hydrophobic protein (SHP) (PDB Accession Code: 1HYP) (Primary structure) (b) Tertiary structure of soybean hydrophobic protein (SHP) representing 3 α-helical and a 3/10 helical substructures (Secondary structure) (Amadei et al., 1993; Singh et al., 2013b).

described here is the overall flexibility of the protein rather than just an amino acid. Because of the presence of proline amino acid (which is not very flexible because of the aromatic structure), it does not let the protein to fold and form secondary structures making the protein stable and rigid (Damodaran, 1994). Due to lack of stable structures, these proteins are flexible and lead to better gelation and foaming properties. In casein, the proline content is about 9.5% which is the second most abundant amino acid next to glutamine (14.5%) and in case of gelatin the proline ranges from 14% to 17% depending on the source (McMeekin et al., 1949; Eastoe, 1955).

Processing of foods and food products using conventional and novel techniques to improve their quality and shelf life, subjects food proteins to varied physical and chemical environment and these bring changes in their functional properties. Physical treatments such as addition of heat or milling; chemical treatments such as addition of acids, alkalis, enzymes, and fermentation can unfold or even break the polypeptide chain into smaller fragments impacting the protein's functional properties. Sometimes these changes are desirable, such as improvement in the digestibility of food proteins but in most cases they are negative and undesirable (Table 1).

Major protein modification during processing occurs due to proteolysis, protein cross-linking, reaction induced due to high temperature and pressure, and protein interaction with polyphenols (Amadei et al., 1993; Aguilera et al., 2003). Proteolysis is the process of breaking down polypeptide into smaller peptide fragments. It is achieved by adding specific enzymes during processing of the food. Proteolysis releases bioactive compounds and peptides, which may have beneficial nutritional effect or sometimes even detrimental, as in the case of the `Maillard reaction, where a chemical reaction between amino acids and reducing sugars leads to the formation of bioactive and carcinogenic compounds like acrylamide (Ames, 1998).

Cross-linking between different proteins under the influence of high temperature, extreme pH, and presence of oxidizing agents results in the reduction in their digestibility due to structural modifications and proteins cannot bind to the enzymes active site for hydrolysis. Cross-linking is important for some food products such as milk where it brings out the gelling of milk proteins (Gerrard, 2002). Oxidative reactions lead to modification of amino acids including methionine, cysteine, tryptophan, and lysine resulting in cross-linking of proteins, affecting their functional properties. Application of heat and

Table 1. Effect of food processing techniques on amino acids and proteins (Adapted from Damodaran (1997).

Processing Conditions	Phenomenon	Nutritional Effects
Heat treatment	Protein denaturation	Improvement in intrinsic digestibility
	Heat-sensitive amino acids	Different residues exposed
	Intramolecular reaction	Destruction
	Reaction with sugars	Crosslink formation
	Racemization	Maillard reaction
		Destruction of lysine
		Bioavailability
High pressure	Protein denaturation	Improvement in intrinsic digestibility
		Different residues exposed
pH change	Solubilization	Improvement of solubility
	Acid/alkaline hydrolysis	Unspecific peptide bond breakage
	pH-sensitive amino acids	Destruction
		Crosslink formation
		Racemization
Enzymatic reaction	Proteases	Oxidation of amino acids through lipid or polyphenol oxidation
	Oxygenases	
Modifications to improve food properties	Structural modifications	Reductive alkylation, acylation
	Enzymatic modifications	Proteolysis, cross-linkages, loss of amino acids, less allergenic, amino acid fortification
	Maillard reaction	
	Proteolytic enzymes	
	Covalent fixation of amino acids	

Table 2. Health-functional properties of proteins (Adapted from Damodaran (1997)).

Function	Mechanism
Antibiotic	Specialized protein with ability to attach to foreign particles such as bacteria. Their attachment depends on the surface topography of the protein. This is governed by the distribution of hydrophilic and hydrophobic residues on the surface of the protein.
Hormone	Small peptides, which regulate many biochemical functions within the body. Their ability to interact with other cellular components depending on their surficial properties.
Structural	Collagen, keratin, elastin are involved in formation of structures such as skin and exoskeleton. Ability to make networks helps in providing the structural stability.
Energy storage	Egg ovalbumin, milk casein and almost all other protein can be easily digested and used as energy source.

high pressure denatures the protein molecules improving their digestibility but also makes them susceptible to chemical reactions such as the Maillard reaction.

From a nutritional and clinical point of view, proteins are vital for human growth. They are considered as one of the building blocks of the body and act as a source of energy, producing 4 kcal/g just like carbohydrates and lipids. Table 2 presents some of the health-functional properties of proteins. The structure of the protein plays a pivotal role in defining their role in maintenance of human health. Proteins are also responsible for certain allergenic diseases. Most of the food allergens are proteins and some of the most commonly reported are related to peanuts, cow's milk, hen eggs, fish, and wheat. Many of the known food allergens can be grouped into cupin and prolamin families, which are storage proteins in seeds and cereals (Vanga et al., 2015c; Vanga and Raghavan, 2016). It is important to note that many factors play a role in determining whether a protein can trigger allergic reactions. Some of these factors are primary, secondary, and tertiary structure of proteins, food processing methods, their quantity in food, and their ability to resist gastric digestion (Breiteneder and Radauer, 2004).

It is therefore important for food engineers to understand how the proteins behave during processing and how their structural modification leads to changes in their functional properties. Techniques such as nuclear magnetic resonance (NMR) (Fadel et al., 2005) and X-ray diffraction (Garman, 2014) have been widely used to understand protein structure and the relation to its functionality (Canduri et al., 2008; Garman, 2014). But in order to gain insight into the mechanism of the interactions between processing conditions and proteins, it is necessary to investigate and understand the influence at molecular or even atomistic level (Singh et al., 2013b; Singh et al., 2015a; Vanga et al., 2016).

The field of molecular dynamic (MD) simulation studies the atomic and molecular interactions that take place within a physical system and how it governs its microscopic and macroscopic behavior (Singh et al., 2013b). The technique has been widely used to develop novel drug systems in the field of pharmaceutical sciences (F de Azevedo, 2011). However, its application in food process engineering has been negligible. Only a handful of articles in the literature are available on the application of MD simulations of food proteins. Few of the food proteins on which MD simulations have been performed include lysozyme (Gilquin et al., 2000; Soares et al., 2004; Buck et al., 2006; English and Mooney, 2007), soybean hydrophobic protein (Singh et al., 2013b), soybean trypsin inhibitor (STI) (Vagadia et al., 2016), peanut peptides (Apostolovic et al., 2016), and Ara h 6 (peanut protein allergen) (Vanga et al., 2015a). This review presents a concise but in-depth introduction to MD simulations and how it can be applied to define the structural and functional dynamics of food proteins under thermal and electrical processing conditions.

2. Molecular dynamic simulations of proteins

For decades, scientists have been interested in deciphering the connection between structure and function of proteins. In early 1951, Linus Pauling and Robert Corey proposed the 2 main structural features of the protein: α-helix and β-sheet, which now are known to be the building blocks for tens of thousands of proteins (Pauling and Corey, 1951a, b, c, d, e, f; Pauling et al., 1951); these structures were deduced from the amino acid interactions leading to structural integrity, whose data were obtained from X-ray diffraction and Pauling's resonance theory of chemical bonding, which predicted the planar peptide groups. Later in 1953, Linus Pauling and Robert Corey introduced the scale models representing the molecules of amino acids and related compounds (Corey and Pauling, 1953; Pauling and Corey, 1953), which was improved by Walter L. Koltun in 1965 and since then it has been widely used to study and make accurate structural measurements of proteins (Koltun, 1965). This traditional method provides only the ensemble-averaged information, that is, it can only define the mean of a physical quantity as a function of the micro-state of a system. With the advent of supercomputer-based simulation techniques, it has been possible to overcome the limitation of traditional method by visualizing the atomistic details of the system.

The computer simulation process can be organized into 5 broad categories including *ab-initio*, which calculates properties of the system like proteins by numerically solving Schrödinger's equations. In this method the interaction between atoms is determined by their electronic configuration and position in the system. The second method is the *semi-empirical method*, which simplifies the calculations of the *ab-initio* method and can be used to study large systems. The *Molecular mechanics* method is widely used to study larger systems using *Born–Oppenheimer approximation* in which the state of the system can be determined using the positions and velocities of the atoms (Rohs et al., 1999). This method is typically used to study systems with thousands of molecules like proteins and carbohydrates and is widely used to study the thermodynamic and interaction of molecules. In this method, the interactions between each atom are estimated using interatomic potentials derived from the *ab-initio* method and the two methods implemented in these systems are Monte-Carlo and MD (Rohs et al., 1999).

2.1. Molecular dynamics

The basis of MD lies in the heart of the molecular mechanics method, which uses *Born–Oppenheimer approximation*. By virtue of the approximation theory, only the positions and

velocities of the atoms are required to describe the microscopic state of the system, in our case a protein. Equation 1 is used to express the Hamiltonian of the system as a function of nuclear variables (Leimkuhler, 1994).

$$H(q,p) = K(p) + V(q) \tag{1}$$

where, H is the Hamiltonian, $K(p)$ is the kinetic energy as a function of momenta, that is, it contains the momenta of each atom, and $V(q)$ is the potential energy as the function of generalized coordinates, that is, it contains the details of the interatomic interactions. For better understanding, it helps in understanding what exactly molecular mechanics does. Molecular mechanics considers the atomic composition of a molecule such as a protein to be a collection of masses that are interacting with each other through harmonic forces. Further simplification suggests that in molecular mechanics the atoms are considered as balls and the bonds between them are considered as springs. Now to visualize and study the dynamics of this system we need to apply a force. It is possible to generate an equation of motion in Newtonian form using the Hamiltonian (Equation 2) (Leimkuhler, 1994).

$$\frac{d^2 r_i}{dt^2} = \frac{F_i}{m_i} \tag{2}$$

where, F_i is defined as the total force experienced by an atom i in the direction r, m_i is the mass of the atom, and r_i is the position of the atom i. So if we consider that we have the position and velocity of the atom i at a given time t, then the position and velocity at time $t + \delta t$, where δt is the time interval between 2 simulation snapshots, can be estimated by solving Equation 2 using an integration scheme. The most commonly used scheme is the Verlet algorithm (Alder and Wainwright, 1959; Van Der Spoel et al., 2005; Adcock and McCammon, 2006). For the Verlet algorithm, we require the values for the current position, $r(t)$; acceleration, $a(t)$; and position of the atom from previous step, $r(t - \delta t)$. The position and velocities of the atom i for the next step can then be found using Equations 3 and 4, respectively (Karplus and Petsko, 1990; Van Der Spoel et al., 2005).

$$r(t+\delta t) = 2r(t) - r(t-\delta t) + \delta t^2 a(t) \tag{3}$$

$$v(t) = \frac{r(t+\delta t) - r(t-\delta t)}{2\delta t} \tag{4}$$

Equations 3 and 4 are the basic form of Verlet algorithm and are prone to truncation error of the order of δt^4 for position and δt^2 for the velocities. Estimation of velocities is important as it is used to compute the kinetic energy; computation of kinetic energy is required to test the conservation of total energy. This test is one of the most important steps during MD simulation as it verifies the stability and correctness of the MD simulation. To combat the problem of truncation errors that are associated with the basic form of the Verlet algorithm, several variants of the Verlet algorithm have been developed. One of the most notable and commonly used variants is the Velocity Verlet algorithm. In this the position and velocities of the atom are estimated using a modified form of the Verlet algorithm (Equations 5,6) (Van Der Spoel et al., 2005).

$$r(t + \delta t) = r(t) + v(t)\delta t \frac{1}{2}\delta t^2 a(t) \tag{5}$$

$$v(t + \delta t) = v\left(t + \frac{\delta t}{2}\right) + \frac{1}{2}a(t + \delta t)\delta t \tag{6}$$

In molecular mechanics, the forces acting on the atom i can arise from both internal and external sources. The internal sources are basically the interaction forces acting between bonded and non-bonded atoms. The external sources can be environmental stresses including electric field, heat, and pressure, which are imposed on the system externally. To determine the forces, it is important to obtain the potential energy of the system that can be expressed as a sum of bonded, non-bonded, and cross-term interactions. The derivatives of the potential energy function are known as force fields (Equation 7) (Van Der Spoel et al., 2005).

$$E_{\text{total}} = E_{\text{bonded}} + E_{\text{non-bonded}} + E_{\text{cross-term}} \tag{7}$$

Bonded interactions are also termed as valence interactions and incorporate diagonal terms such as bond-stretching, angle-bending, dihedral-angle torsion, inversion, or out of plane interactions. Since in molecular mechanics it is assumed that the interactions are through harmonic forces, the potential energy associated with bond-stretching can be represented as

$$E_{\text{stretching}} = \frac{1}{2}k_b(b - b_0)^2 \tag{8}$$

where, k_b is force constant for the bond length, b_0 is the equilibrium bond length, and b is the actual bond length. Similarly for angle-bending, the simple harmonic expression would be

$$E_{\text{angle-bending}} = \frac{1}{2}k_\theta(\theta - \theta_0)^2 \tag{9}$$

where, k_θ is the force constant for bond angles, θ_0 is the equilibrium bond angle, and θ is the actual bond angle. The dihedral-angle torsion potential energy is represented as a cosine expression (Equation 10).

$$E_{\text{torsion}} = \frac{1}{2}k_\phi(1 + cos(n\phi - \phi_0)) \tag{10}$$

where, k_ϕ is the force constant for the dihedral angle, ϕ_0 is the reference torsion angle, and ϕ is the actual torsion angle. All the aforementioned potential energy functions belong to *Class I* force-fields, which only incorporate diagonal terms and non-bonded interactions (Brooks et al., 1983; Karplus and Petsko, 1990; Karplus and Sali, 1995). This class of force-field is widely used to simulate complicated systems such as proteins, as one has to consider different degree of freedoms associated with it. Some of the widely used force-fields of *Class I* are CHARMM, AMBER, and GROMOS (Brooks et al., 1983; Case et al., 2005; Christen et al., 2005). The non-bonded interactions defined in this class of force-fields include electrostatic and van der Waals

interactions. The electrostatic interactions can be described as an interaction between two charged particles and is expressed with a Coulombic potential function (Equation 11).

$$E_{\text{electrostatic}} = \sum_{ij} \frac{q_i q_j}{4\pi D r_{ij}} \tag{11}$$

where, q is the charge of the particle, D is the dielectric constant of the material, and r_{ij} is the distance between the centers of the particles i and j.

The van der Waals interaction between non-bonded atoms can be accounted for using the Lennard–Jones potential (Karplus and Sali, 1995; Lindahl et al., 2001; Van Der Spoel et al., 2005). In molecular mechanics, the simplest potential energy function is the hard sphere potential. This assumes that the particles would travel in a straight line until hitting another particle, which will lead to elastic scattering. This potential does not include the attractive and repulsive components, which make it inappropriate for the study of huge systems such as proteins. The Lennard–Jones potential included both attractive and repulsive components and can be expressed using Equation 12.

$$E = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \tag{12}$$

where, ε is the depth of the potential well, r is the distance between the particles, and σ is the distance at which the inter-particle potential is 0. This potential function is also called as 12–6 potential, which represents the exponent terms for repulsive and attractive components, respectively (Abbasgolipour et al., 2010). Using Equation 12, the van der Waals interaction between non-bonded particles can be presented as

$$E_{\text{vdw}} = \sum_{ij} \varepsilon_{ij}\left[\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{\min,ij}}{r_{ij}}\right)^{6}\right] \tag{13}$$

where, r_{ij} is the distance between the particles, ε_{ij} is the minimum value for van der Waals term, and $R_{\min}$ is the radius where the van der Waals term is minimum (Abbasgolipour et al., 2010).

Some modern force-fields including CFF, MM3, and MMFF94 also include another class of force-fields, *Class II*, which incorporates the cross-term interaction between the particles. These cross-terms are required to reproduce the experimental vibrational frequencies of molecules and include the bond-bond, angle-angle, bond-angle, bond-dihedrals, angles-angles-dihedrals, and bond-bond-dihedrals (Van Der Spoel et al., 2005). All these terms account for effects such as distortion of bond angles due to stretching of the bonds or changes in bond length due to changes in the other in an opposite direction. The purpose of these force-fields is to describe the complete potential energy surface of the molecule as accurately as possible. If we want to study the effect of external stresses on a molecule, we can look at the changes in the potential energy functions and determine how they are affected by stress (Alder and Wainwright, 1959).

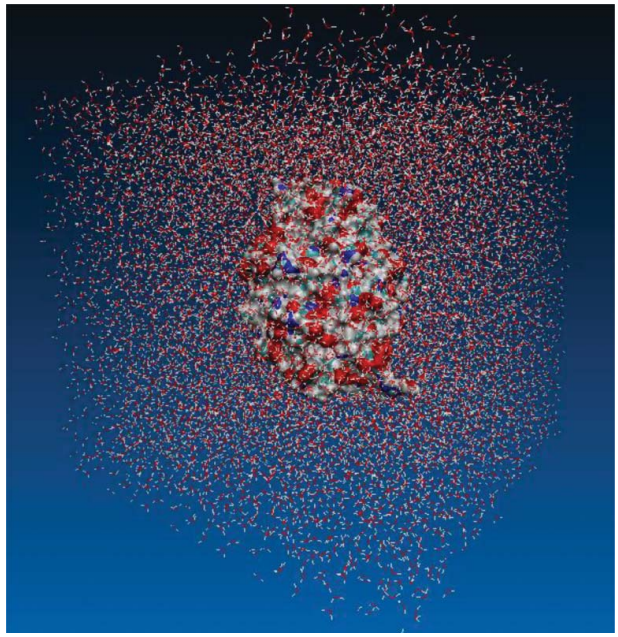

Figure 2. A typical cubic simulation box with protein in the center, surrounded by explicit solvent atoms (water).

All of the aforementioned force-fields are used in molecular simulations of proteins to provide the parameters of mathematical functions used to describe the potential energy of all the atoms of the protein system (Alder and Wainwright, 1959; Karplus and Sali, 1995). In MD, proteins are studied in an aqueous environment and to simulate these systems it is necessary to understand the interaction between the particles at the surface and the particles within. Fig. 2 presents a protein in an aqueous environment system (Bekker et al., 1995). The forces experienced by the particles on the surface are transcended across the system and this influences the forces experienced by the particles within the system. If we consider a system, which has small number of water molecules surrounding the protein, the surface-to-volume ratio of this system will be large and so will be the surface effect (Bekker et al., 1995). The surface effect includes the imbalance of forces between the solvent and the vacuum surrounding the solution droplet. Water molecules in a simulation system behave just like a real solution and would try to escape into the surrounding vacuum, that is, evaporate and this will change the dynamics of the system and make it difficult to accurately study the system. Hence, when simulating a bulk system like protein in an aqueous environment it is important to minimize or eliminate the surface effect. Application of periodic boundary conditions (PBCs) helps in completely eliminating the surface effect (Berendsen et al., 1995; Budi et al., 2004). In PBC, the simulation box, that is, protein with the water molecules surrounding it (Fig. 2) is replicated in 3 dimensions to form an infinite lattice; that is, exact images of the box (system = protein + water molecules) are stacked over each other in all directions, such that there are no surface to the solution. This helps eliminating any surface tension effect and if a water molecule leaves the simulation box the net effect is such that a copy of the same molecule enters the box, and this way the water molecules never escape (Van Der Spoel et al., 2005). Fig. 3 presents a three-dimensional PBC.

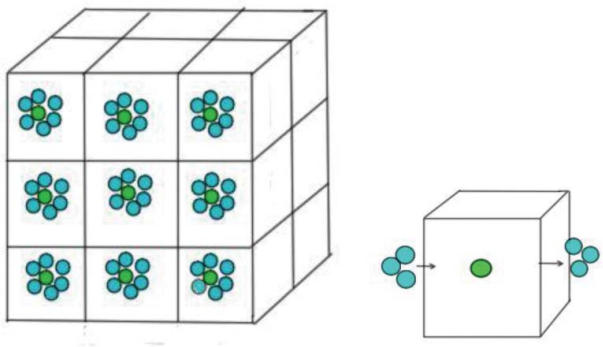

Figure 3. Three-dimensional illustration of periodic boundary conditions. The protein is represented in green in the center. The box in the center is the main simulation box.

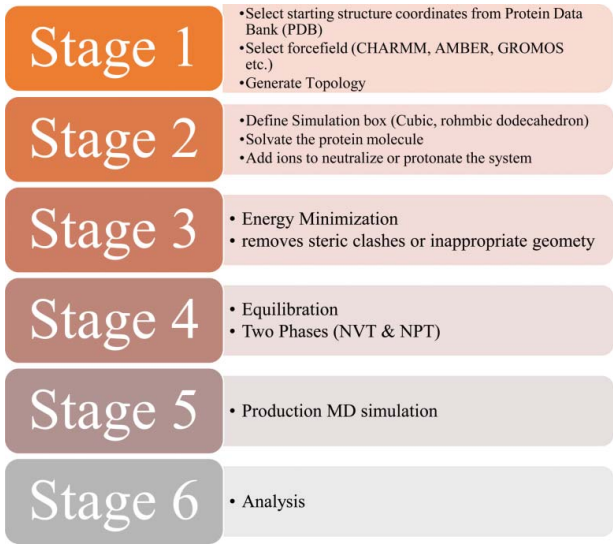

Figure 4. Outline of the simulation stages commonly followed while using GROMACS MD simulation package.

2.2. Simulation methodologies and stages

MD is routinely applied for the investigation of dynamic properties and processes in the field of structural biochemistry, molecular biology, pharmaceutical science, and biotechnology. This tool helps researchers to generate a trajectory of macromolecules such as protein, that is, it generates a progress of simulated structure with respect to time. The most common methodologies incorporated in the study of food proteins include classical molecular dynamics, which is implemented into various software packages such as GROningen MAchine for Chemical Simulations (GROMACS) (Van Der Spoel et al., 2005), Not just Another Molecular Dynamics program (NAMD) (Kalé et al., 1999), Assisted Model Building with Energy Refinement (AMBER) (Case et al., 2005), and Chemistry at Harvard Macromolecular Mechanics (CHARMM) (Brooks et al., 1983). Various experimental conditions can be simulated using MD. In earlier protein studies conducted using MD, it was common to consider the protein molecule as isolated entities present in a vacuum. Slowly, with further advancement in MD techniques/simulations, simulations can be performed in explicit water and neighboring protein molecules as in a crystal environment (Van Der Spoel et al., 2005; Anderson et al., 2008). Therefore, we use the PBCs as discussed in the previous section. Typically, a PBC is implemented on a cubic system; however, it is not necessary and several other geometries including rhombic, dodecahedron, and truncated octahedron can be used (Bekker et al., 1995). These geometries significantly reduce the number of solvent atoms required in the system, which in turn reduces the computational time.

The length of the simulation is determined by various factors, including the number of interactions that needs to be evaluated at each time step and number of time steps and degrees of freedom that need to be produced. Hence, to improve the simulation and computational efficiency, several algorithms are used including the Verlet integration algorithms discussed in the previous section. Other algorithms such as SHAKE, RATTLE, and LINCS are commonly implemented to improve the MD efficiency by increasing the time-step without compromising the accuracy of the simulation (Budi et al., 2004).

Typically, in an MD simulation, it is important to accurately replicate the experimental conditions. To obtain the starting structure coordinates of the protein of interest, researchers search the Protein Data Bank (PDB) (Astrakas et al., 2011; Singh et al., 2013a). The structures available in the PDB were obtained by using X-ray diffraction and NMR techniques. In GROMACS (Fig 4), it is necessary to generate a topology file, which contains all the information including bonded and non-bonded parameters required to define the molecule within the simulation (Van Der Spoel et al., 2005).

Once the required force-fields are selected, the protein structure is put into a simulation box and immersed in water. It is important to note that in nature most proteins are at least partially within an aqueous environment; hence it is justifiable to immerse a protein structure into water containing an ion or without. Ions are added to the system (protein + water) to neutralize or protonate the system. Solvent effects on the simulation results are important as they contribute to specific interactions necessary to mediate protein structure or function. Some of the explicit water models commonly used in the simulation of food proteins are TIP3P, TIP4P, TIP5P, SPC, and SPC/E (Lindahl et al., 2001). For the simulation, the parameters of the water models are so adjusted that they reproduce the enthalpy of vaporization and density of water during the simulation. The dipole moment of the aforementioned water models is about 2.8 D instead of 1.85, which is the experimental gas-phase value (Van Der Spoel et al., 2005). One of the demerits of these models is with respect to the temperature dependence of density of water; except for TIP5P none of the models define this dependence accurately. Once the system has been neutralized, the solvated electro-neutral system is relaxed to ensure that it has no steric clashes or inappropriate geometry. After minimization, the system undergoes NVT (fixed number of atoms, N, fixed volume, V, and fixed temperature, T) and NPT (fixed number of atoms, N, fixed pressure, P, and fixed temperature, T) equilibration. At this point, the system is called as an ensemble, which is defined as a collection of all possible systems that have varied microscopic state, but have a single thermodynamic state. Physical experiments are mostly carried out at constant temperature and pressure or constant temperature and volume; hence it is desirable to conduct simulation at the two states.

These states are also called as canonical ensemble (NVT) and isobaric-isothermal ensemble (NPT). Once the system has been equilibrated, MD simulations are carried out with or without the presence of an external stress (Van Der Spoel et al., 2005; Singh et al., 2013b).

The second approach used is the use of membrane proteins for MD simulations. The first simulations of membrane proteins were performed in mid-90s and from that point researchers have successfully investigated various membrane protein systems over the years which include trans-membrane peptides, fusion proteins, channel and pore proteins, etc. (Woolf and Roux, 1994; Edholm et al., 1995; Ash et al., 2004; Gumbart et al., 2005). These simulations can also include lipid models, but in these cases, United-atom force-fields are found to have higher computations efficiency compared to all-atom force-fields with a slight reduction in accuracy of the result (Kukol, 2009).

3. Present and future application of molecular dynamics in studying food proteins

The forces involved in the stability of the protein structure are quite complex and can be grouped into two categories: (a) intramolecular interactions involving forces intrinsic to the protein molecule (steric strains and van der Waals interaction), and (b) intermolecular interactions involving the surrounding environment including the solvent and other food components (hydrogen bonds, electrostatic interactions, hydrophobic interactions, and disulfide bonds) (Dunker et al., 2002). All the aforementioned intramolecular interactions and intermolecular interactions can be studied using MD. In food, which is a complex blend of major and minor components, the native structure of the protein is the net result of the various attractive and repulsive forces emanating from the intermolecular and intramolecular interactions. Changes in the environment such as pH, surrounding solvent ionic strength, solvent composition, temperature, and so forth can alter proteins (Arntfield and Murray, 1981; Korhonen et al., 1998). When the environment in which the protein is situated is changed, it undergoes a conformational adaptability stage, where it acquires a molecular conformation that does not alter its molecular architecture, but when external stresses such as thermal, chemical, and electrical are involved in changing the surrounding, then major changes in the secondary, tertiary, and quaternary structures take place without cleavage of any backbone peptide bonds, and this is termed as denaturation (Korhonen et al., 1998). Physical agents such as temperature induce denaturation of food proteins to varying degrees during processing. The mechanism involved is destabilization of hydrogen bonds and electrostatic interactions, which are exothermic in nature. Increase in temperature stabilizes the endothermic processes including hydrophobic interactions. The amino acid composition of protein also influences its thermal stability. The presence of hydrophobic amino acid residues greatly increases proteins' thermal stability. The basic and acidic residues within the core of the proteins form salt bridges which greatly improves the stability of the secondary and tertiary structures of the protein molecule. Further, the stability can increase due to the presence of cysteine amino acids that leads to formation of disulfide bonds within the protein structures. Thus, the stability of proteins is mainly due to the formation of salt bridges and disulfide bonds between non-polar residues buried deep inside the protein structure and hydrogen bonds, both on the surface and within the core of the protein (Ma, 1990). Hydration simply classified as presence of water greatly facilitates protein denaturation. Dry protein powders are extremely stable to thermal denaturation, and as the water content increases thermal stability decreases. This is because in a low-moisture state the proteins have static state and as the water content increases so is the hydration and penetration of water into surface cavities of proteins; hence their backbone mobility and flexibility increases and when this structure is subjected to high temperature, and it ends up in a denaturation state (Halle et al., 2004). Other external stress which too can affect protein structural stability includes hydrostatic pressure, presence of chemical agents that alter the pH of the surrounding solvent, presence of detergents, and chaotropic salts (Balasubramaniam and Farkas, 2008). For this review, we will evaluate the applicability of MD studies in understanding the dynamics of food protein hydration and the effect of thermal and electrical stresses.

3.1. Hydration of proteins

Several rheological and textural properties of food depend on the interaction of the food macromolecules and water. Functional properties such as swelling, solubility, viscosity, gelation, emulsification, etc. are determined by the modification of physico-chemical properties of food proteins caused by water–protein and protein–protein interactions. For example, for food products such as bread and other bakery products, balance between these interactions is key for their acceptability by consumers. The water binding capacity, commonly known as the hydration capacity of protein is largely controlled by its amino acid composition (Hess and Van Der Vegt, 2006). When a protein molecule is hydrated, the primary interaction is between the water and the surface of the protein. Since proteins are a defined three-dimensional arrangement of several secondary structures, the interactions with water or any other solvent is based on the surface property of that particular secondary structure of the protein, which is facing the water or other solvent molecules (Halle et al., 2004).

In general, water plays a pivotal role in maintaining the surface properties of the protein due to the availability of hydrophobic and hydrophilic binding sites (Levy and Onuchic, 2004). It is important to note that the amount of hydration over the surface of the protein is an important factor in stabilizing the protein and also any variation can lead to changes in their functional properties (Ansari et al., 1992; Levy and Onuchic, 2004). Several researchers have studied experimentally the effect of hydration on the structure of the protein and in turn the changes in its biological functions (Halle et al., 2004; Levy and Onuchic, 2004).

In a study on soy protein, Were et al. (1997) evaluated various modification treatments and found out that the alkali treatment increases the hydration capacity of the protein by 20 times. They proposed that this rise in hydration capacity of the protein molecule is due to changes in its quaternary structure. It is possible to apply MD simulation to visualize the results reported in the aforementioned study in real time and also obtain an insight into the dynamics involved. Apart from

acting as a visual aid, MD simulation will also provide statistical information such as hydration energy, efficiency, and even the involved kinetics. For example, Mobley et al. (2009) conducted an MD simulation study to estimate the hydration free energy of 504 organic compounds in explicit TIP3P water. They reported a very good correlation between their simulated data and experimental data. When they compared the calculated and experimental hydration free energies, the rms error was found to be 1.26 ± 0.01 kcal/mol with a mean error of 0.676 ± 0.002. The correlation coefficient was found to be 0.0889 ± 0.006. Application of similar methodology to study the hydration energies of food proteins is possible, where the hydration energy can be defined as the quantification of the effect of hydration on solvent-free energies and the protein– water intermolecular interactions evaluated using both the attractive forces and the repulsive forces (Alfatni et al., 2008).

Another probable application of hydration studies using MD simulation technique is for estimation of efficiency of various solvent extraction techniques. It is well known that the solvent extraction efficiency depends largely on the interaction between the desired compound and the solvent in question; and if the desired compound is a protein it would be wise to understand the relation between a chosen solvent and the protein molecule before using the solvent for extraction. Hence, understanding the dynamics of a protein's interaction with various solvents can provide beneficial information and hints on the efficiency of an extraction process. For example, Micaêlo and Soares (2007, 2008) performed an MD simulation study on the hydration mechanism of the enzyme serine protease cutinase in non-aqueous media. In their study, they differentiated the solvents into 3 different classes: polar organic solvents (Hexane, di-isopropyl ether, and 3-pentanone), non-polar organic solvents (Ethanol and Acetonitrile), and water. They evaluated the dynamics and structure of the enzyme used in the simulation at different hydration levels and concluded that the water stripping capacity of a solvent is directly dependent on the nature of the solvent and that the protein undergoes structural modifications due to variations in its hydration capacities and intermolecular interaction between solvent molecules and protein side chains. Hence, an understanding of protein interaction with water or any given solvent at molecular level can help researchers to predict the behavior of the process flow including efficiency of extraction or how protein will behave structurally or functionally.

3.2. Thermal treatment of protein

Several food processing techniques such as pasteurization, sterilization, boiling, etc. require subjecting protein to thermal stresses. Thermal stress is associated with an increase in heat or thermal energy, which forces proteins to adopt conformational phases or stages that are irreversible. Changes in conformation of protein affect their functional properties; this phenomenon makes an interesting subject of investigation at both macro and molecular level. At the macro level, when a protein is subjected to an increase in temperature beyond a threshold value represented as the transition temperature, it undergoes a transition from its native state to its denaturation state. The primary mechanism involved in this is the effect of temperature on the noncovalent interactions, i.e., an increase in hydrophobic interactions and destabilization of hydrogen bonding and electrostatic interactions are observed. The net stability of a protein at a given temperature is defined as the total sum of all of these interactions (Davis and Williams, 1998). But not all proteins are affected at high temperature, some food proteins such as lysozyme denature at lower temperature (75 °C) compared to Ovalbumin (84 °C), Ovamucoid (79 °C), Globulin (92.5 °C), and Avidin (85 °C) (Li-Chan et al., 1995; Stadelman et al., 1995). Hence, a question arises on how does lysozyme protein behave under the influence of higher thermal stress. This is an important question because in today's era, the commercially sold eggs are normally treated to high temperature to extend their shelf-life by disinfecting any surface pathogens present on the egg shell (Froning et al., 2002). Various methods of thermal treatment of eggs require exposing the egg to a certain temperature for a predetermined period of time to destroy the pathogens over the shell. But, due to the exposure to high temperatures and other forms of stress, there can be changes that take place to the various properties of the egg that are later consumed (Denmat et al., 1999; Min et al., 2005). Furthermore, novel pasteurization techniques also involve exposing eggs to various electromagnetic radiations including microwave (Dev et al., 2008) and radio waves (Ball Jr et al., 1997). Apart from pasteurization, eggs are processed in various ways, which can all affect the functional properties of the egg protein; these changes can be attributed to the structural change in the egg proteins.

Several researchers have tried studying the effect of temperature on the structural and functional properties of the egg protein. For example, Gilquin et al. (2000) conducted an MD simulation study on the role the inter-domain interactions in the secondary structure play in the unfolding path followed by the hen egg lysozyme at 300 K. They conducted the simulations for 1.2 ns and used Path Exploration with Distance Constraints (PEDC) to find out the least energy structures as a function of the RMSD in the simulation. They concluded that the results obtained from the simulations were completely in accordance with experimental results obtained from fluorescence spectroscopy. They stated that the loss of stability was caused by changes in the hydrophobicity of the protein core due to conformational changes of the protein structure.

In another work, Mark and Van Gunsteren (1992) studied the thermal denaturation of the hen egg lysozyme in water under wide range of temperatures using the MD simulation technique. They analyzed the unfolding (conformational change) of the hen egg lysozyme as a function of the radius of gyration, RMSD, and hydrogen-bonding pattern during the period of simulation. They came to a similar conclusion as those of Gilquin et al. (2000). Similarly, as it is possible to evaluate the effect of thermal stress on lysozyme protein, it is also possible to evaluate the dynamics of folding and unfolding of other food proteins using MD technique, but all within the limitations of MD.

3.3. Electrical treatment of protein

As mentioned before, traditional thermal processing methods can lead to deterioration of food components, such as

proteins, starch, and vitamins, resulting in possible changes in its nutritional and organoleptic properties. In recent years, several non-thermal processing methods such as pulsed electric field processing (PEF), high voltage processing (HVP), ultrasonication, etc. have garnered huge attention by their advantages over conventional thermal processing methods. PEF has been widely investigated for its ability to kill microorganisms while maintaining the original nutritional and organoleptic properties. In PEF processing, application of high electric field pulses inactivates microorganisms and several enzymes responsible for deterioration of food, but its effect on the structural modification of proteins, enzyme, and other food components has not been widely studied. For example, Xiang et al. (2011a, b) studied the effect of pulsed electric field on structural conformation of whey and soy protein isolates using fluorescence spectroscopy. They reported the structural modification as a function of changes in the surface hydrophobicity of the protein. For whey protein isolate, they stated that application of electric field intensities of 12, 16, and 20 kV/cm and number of pulses (10, 20, and 30) resulted in an increase in the intrinsic tryptophan fluorescence intensity indicating shift in the polarity of the tryptophan residues microenvironment from less polar to a more polar environment. Since the hydration and several functional properties of the protein depend on the surface properties of the protein which in turn are governed by their structural conformation, it is possible to conclude that application of electric field will certainly have an effect on the structural conformation of the protein to bring about changes (folding or unfolding) in its microenvironment. Since these changes are intrinsic and can be visualized, it is difficult to study these changes at a molecular level without using expensive experimental setups such as X-ray diffraction or NMR. It is here, where MD simulation can be used to evaluate the effect of external stresses such as static or oscillating electric field. Singh et al. (2013b) studied the effect of static external electric field on the conformation of soybean hydrophobic protein. They subjected the soybean hydrophobic protein to three electric field intensities 0.002 V/nm, 0.004 V/nm, and 3 V/nm. In their study when nominal electric field intensities (0.002–0.004 V/nm) were applied, no major effect on the structural and surficial properties of the protein was observed, but when the field strength was increased to 3 V/nm, the protein underwent unfolding and significant changes in the surface properties were observed. Their study was one of the few, which targeted application of MD simulation for the study of food proteins. In another, Singh et al. (2013a) also assessed the effect of an external electric field stress on conformation of gliadin protein, which is an important component of wheat gluten protein and they reported that application of an electric field actually varied the hydrogen-bonding pattern of the protein and that the intensity of the field plays an important role. They validated their observation on varying hydrogen-bonding patterns of gliadin protein in their study on electrohydrodynamic drying of wheat and its effect on wheat protein conformation (Singh et al., 2015b). They utilized Fourier transform infrared (FT-IR) spectroscopy to study the wheat protein and reported that the analysis of the Amide I (1720–1580 cm^{-1}) of wheat protein, the FT-IR spectra showed distinct valleys at 1682–1686 cm^{-1} (β-sheets), 1674 cm^{-1} (β-sheets), 1664–1667 cm^{-1} (turns), 1654–1657 cm^{-1} (α-helices), 1651 cm^{-1} (α-helices), 1645–1647 cm^{-1} (Random coils), and 1633–1634 cm^{-1} (β-sheets). Further analysis of the spectral data by peak fitting using Gaussian band shapes suggested that exposure to electric field influenced the hydrogen-bonding pattern of wheat protein resulting in shifts between low and high frequency bands (Singh et al., 2016; Vanga et al., 2016).

A recent study conducted by Vanga et al. (2015b) using MD simulation to evaluate the effect of static and oscillating electric field in the microwave region (2450 MHz) and with an intensity of 0.05 V/nm at different temperatures including 300 K, 380 K, and 425 K on the conformation of Ara h 6 peanut protein allergen, they reported that an increase in temperature and application of external electric field both static and oscillating had significant effect on the conformation of Ara h 6 protein. Their study demonstrated that an exposure to external stress including thermal and electrical will induce conformation changes in the protein structure. Their study provided an insight into the behavior of Ara h 6 allergen under the influence of external stresses and how various food processing techniques might affect the allergenic properties of peanut proteins. In another recent study, Vagadia et al. in 2016 used MD simulation technique to investigate the unusual stability of STI protein under thermal and electrical processing conditions. Through MD simulation, they subjected STI to 4 temperatures 300 K, 343 K, 373 K, and 394 K under the influence of external oscillating electric field strength of 0.5 V/nm with frequency of 2.4 GHz. They reported that significant structural rearrangements took place within the protein especially in secondary structures "turns" and "coils." Their study also demonstrated that aromatic residues of the protein structure played an important role in stabilizing STI protein (Vagadia et al., 2016, 2017). This study is one of the evidences of how MD simulation technique can provide an insight into food proteins behaviors under the influence of processing conditions through knowledge gained at molecular level.

4. Conclusion

In the past few decades, the understanding of structure and function of proteins in model food systems has seen great advances from early application of chemical or enzymatic modifications, to present-day application of molecular biology tools. This trend is likely to further advance into more futuristic technologies as the "-omics era" takes shape. MD simulation for study of food proteins is still in its novice state and prone to limitations such as the availability of defined crystalline structure of food proteins, but still it can be a beneficial tool to study the molecular configuration of food protein interactions with other food components and how they contribute to the organoleptic and nutritional properties of a food. The understanding gained through MD simulation into behavior of food components, especially proteins

can also be used to optimize the processing parameters and design of processing technologies.

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