Journal of Agriculture and Food Research 16 (2024) 101177

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Recent advances in cold plasma technology for modifications of proteins: A comprehensive review

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ARTICLE INFO

Keywords:
Cold plasma
Proteins
Amino acids
Modification
Structure
Function

ABSTRACT

The increasing quest for the minimally processed foods and demand for nearly fresh foods from the consumer segment has paved the way for the emergence of Non thermal processing technologies. A significant development in the food and bioprocessing sector is the evolution of cold plasma processing which is renowned for its preservation efficacy and shelf life enhancement of foods. The growing application of cold plasma has expanded its role in the modification of food biomolecules in order to meet the diversified requirements of food industries for varied applications. The previous investigations have shown its proven benefits in the microbial destruction, enzyme inactivation, modification in the polymer matrices for food packaging etc. Cold Plasma processing is a promising candidate with the benefit of minimizing the negative effects of thermal processing to the food matrix particularly to the protein molecules which are prone for denaturation at relatively higher temperatures. Proteins which are the polymers of amino acids are highly valued with commercial importance because of the techno-functional properties they possess along with its nutritional potential. The cold plasma induced modification of proteins are known to enhance the functionality of native proteins by altering the structure-functional relationship. The interaction of cold plasma and its reactive species on the amino acid structure will lead to a functional change in the protein molecule and can propel its application in diverse fields like 3-D printing, novel gel structures formation, thermostable foams and emulsions, improved solubility and enhanced organoleptic property. Considering the growing importance of the process technology, review aims to shed light on the impact of cold plasma processing on the protein modification and its associated molecular understanding with its future path of research. Future scope of considerations along with the challenges are also discussed which need to be taken on priority to get the potential benefit and commercial realization of the technology.

1. Introduction

Safety and Security of food product plays a crucial role in protecting the consumer's health and public welfare. As there is an increasing trend in the production of food grains, the preservation of harvested produce and its safer delivery to the intended consumer with minimum loss in nutrition calls for a technological process. Food quality gets deteriorated in the supply chain owing to various factors like microbial

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https://doi.org/10.1016/j.jafr.2024.101177
Received 23 February 2024; Received in revised form 11 April 2024; Accepted 20 April 2024
Available online 25 April 2024

contamination, enzymatic damage, non-enzymatic spoilage etc leading to loss and damage in the nutritional, physiological and sensorial properties of the food thus making food unacceptable in the market. Consumers have strong affinity for minimally processed foods with reduced additives and chemicals and this has seen the emergence of novel food segments (low sugar foods, low fat foods, protein rich foods) [1]. The commonly followed thermal process for preservation and processing of food includes pasteurization, sterilization, ultra-heat temperature (UHT), baking, frying, drying, evaporation etc. [2]. But these process comes with their inherent disadvantages with the significant loss in nutrition, loss of original flavor and color, textural damage and organoleptic changes which has driven consumers to look for more of a fresh like qualities in the food they consume [3,4].

Non thermal processing technologies offer a promising alternative for minimizing the heat induced damage to the food properties and a way for the retention of thermally labile nutrients. Non thermal processing technologies includes high pressure processing [5–8], pulsed electric field [9–13], ultrasound [14–16], gamma irradiation technology [17,18] and cold plasma. These novel technologies are increasingly being explored by the food industries and researchers to minimize the changes induced due to thermal damage and to maintain the nutritional profile of the food products as natural as possible. Cold plasma is a proven non thermal technology to improve food safety and quality with the advantage of not leaving chemical residues making it an environmental friendly approach. The relatively lower time requirements of cold plasma processing coupled with lower energy requirements has highlighted its importance in the field of sterilization and quality control [87]. Proteins are the nutritionally important macromolecules along with the unique properties it can provide on the physicochemical, rheological, textural, organoleptic attributes of the food system. But the native protein often faces challenges to meet the requirements of the food industries. Therefore, it becomes essential to modify the proteins and cold plasma is being viewed as sustainable solution to achieve the same. The effect of plasma processing on proteins precisely works by the interaction of reactive species on the protein structure leading to reactions not limited to oxidation, dimerization, deamidation, nitration, sulfoxidation, hydroxylation of amino acids bringing necessary change in the techno-functional properties of the protein molecule. Previous reviews have discussed the cold plasma induced modification of dairy proteins [36] but a comprehensive review on the effect of cold plasma on all the protein sources was lacking. This review was undertaken to provide complete information on the plasma generation principles and its effects on improving various techno-functional benefits of protein (seed based proteins, dairy based proteins, animal based proteins). It has also highlighted the modifications and its underlying mechanism which can provide scientific understanding of the process and deliver its application to the targeted stakeholders.

2. Cold plasma principle and applications

Cold plasma is an emerging environmentally friendly, solventless approach which finds vivid uses and applications. Irving Langmuir coined the term plasma in 1928 as the fourth state of matter which gets generated due to partial or complete ionization of gas. He described it as a region containing balanced charges of ions and the electrons. Plasma can be seen as a quasi-neutral molecule comprising of ions, photons and free electrons along with atoms in their fundamental state of excited state with neutral charges [19]. It mainly consists of excited species of atoms and molecules with the reactive species of electrons, negative and positive ions, reactive oxygen species (OH, O_2^-, O_3, $^1O_2$, O, H_2O_2), reactive nitrogen species (NO, N_2O, NO_2), free radicals, ultraviolet light, atoms and molecules in ground state, charged particles etc [20,21]. Plasma generation occurs in two stages [22] - Primary plasma which leads to the formation of ionization, dissociation and excitation between the electrons and the secondary plasma which refers to the formation of reactive oxygen species, reactive nitrogen species and UV radiations in smaller amounts due to collisions between the heavy particles. Plasma can be divided based on the temperature of electron generation and density as thermal plasma and low temperature plasma [23]. Thermal plasma is formed at higher pressures of ($>10^5$ Pa) and 50 MW power for its propagation and will be in a state of thermal equilibrium with the heavy species and electrons [24], while low temperature plasma gets further subdivided into thermal plasma which is at quasi equilibrium state and non-thermal plasma existing at non equilibrium state and are formed at lower pressure and do not require any power [25–27]. Thermal plasma is commonly seen in the field of aeronautics and metal welding industry and not recommended for food application due to higher temperature associated defects in the food and the need for higher energy requirements and a larger quenching effect by chemicals species [28–30].

Cold atmospheric plasma falls under the category of non-thermal plasma which can be used for heat sensitive materials [31,32]. The gas persists in lower temperatures due to the cooling of ions and charged particles [33] though the electrons are at a higher temperature. The mass difference between the heavy species and electrons leading to no momentum transfer can also be a reason for temperature difference. The energy of the discharged heavy particles and internal degrees of freedom will be lower than the electrons. Cold plasma gets generated when electric current is applied to gas which are at a state of potential electrical difference leading to collisions and release of ions, radicals and radiation of varying wavelengths [34]. Thermal, nuclear and electric are the commonly used methods to ionize the gases but electric energy due to its ease of application is commonly used as compared to rest. The amount of electrical energy needed to ionize the gas molecules is referred to as breakdown voltage, which inversely depends on the product of distance between the electrode and pressure. If the breakdown voltage is high, it results in fewer collisions and vice versa [35]. The gases can be individual or in combination of argon, air, helium, nitrogen and oxygen [36]. As the electrons are released they gets accelerated in the electric field and leads to collisions between the atoms, molecules and gas electrons. As the electrons moves at a very high speed it will hit the other atom and removes an electron from that atom and this will continue leading to a cascading effect. Due to mass difference between the electrons and heavy species, there is no momentum transfer involved and hence the heavy particles generated will be at room temperature. When the gas molecules shift from insulated condition to conductance stage, the plasma gets created [37]. Ionization of gas formed at relatively lower electrical difference in the range of 1–10 ev and electronic density of up to 10^{10} cm^3 generates cold plasma which is in a state of thermal non-equilibrium with the electron energy and heavy species generated [38]. The mechanism of plasma generation is said to follow the Townstend theory with electron avalanche mechanism and Paschens law.

According to Townstends theory, ionization of gas gets initiated when the applied voltage to the electrodes increases thus increasing the flow of electrons from cathode (ground electrode) to anode (high voltage electrode). Free electrons on flowing collides with the neutral gas molecules and leads to ionization creating ion avalanches. Thus it results in the creation of electron avalanche and ion avalanche with gas as the medium. The generated electron avalanches lead to secondary emission of reactive species. The rush of ion avalanche to the cathode leads to the release of electrons from ground electrodes thus forming a self-perpetuating reaction mechanism as the applied voltage comes to a breakdown stage [39]. The elastic collisions between the electrons at the beginning moves to a state of inelastic collision capable of ionizing the gas molecules. The increase in collision leads to an increase in the gas ionization. The generated electrons get accelerated with the applied electric field leading to electrons, positive ions and electron avalanche. After reaching the ultimate stage of ionization it leads to the disruption of gas and finally becoming conductive [40–43]. Paschens law of plasma generation states that the voltage required for induction of plasma from gas molecules depends on the pressure and distance between the

electrode [35]. Plasma can get generated at atmospheric pressure or reduced pressure [21]. Atmospheric pressure generation of plasma is widely used in the food industry since it is easy to generate plasma and no need to create vacuum condition [44]. Method of cold plasma generation has been depicted in Fig. 1. Cold plasma is increasingly finding its application in the life science, material science, packaging, electronics sector, dental application, textiles fields, water and air purification etc. [45–47]. The plasma application in the food and packaging sector relies on microbial decontamination [48–54], degradation of endogenous enzymes [55–57], germination of seeds [58–62], modification in the packaging material [63–66], surface hydrophobicity modification, mass transfer reactions modification [67].

3. Methods of plasma generation

The generation of plasma can be formed at atmospheric pressure and reduced pressure. Dielectric barrier discharge (DBD), radiofrequency discharge, corona discharge, gliding arc discharge generates the plasma at atmospheric pressure and requires the presence of electrodes but the microwave based plasma generators do not require the presence of electrodes. These methods differ primarily on the method of excitation to generate the plasma. Photo-ionization, photo-emission, photo absorption, ion-ion neutralization, ion-molecule reactions, penning ionization, quenching, three-body neutral recombination, and neutral chemistry can be seen as possible mechanisms for the plasma generation.

3.1. Dielectric barrier discharge

Most commonly used method of plasma generation owing to its simple design and construction (selection of electrode and dielectric material) and ease of use [47,68]. The discharge can be in micro discharge or stream mode of discharge [69]. When a voltage of sufficiently high energy is inducted between two electrodes of which one or both the electrodes are covered with a dielectric substance (eg. quartz, glass, mica, ceramic, polymer) [70], it leads to the generation of plasma. Here the dielectric material acts to stabilize the discharge property. The relative ease of use along with adoption of varied electrode geometry, combination of gases and reduced gas flow rate and streamlined uniform discharge of ionized gas are the merits of using DBD. However, the requirement of higher voltages of >10 kV for ignition adds to the cost factor. There are two types of DBD based on the configuration of the electrode-planar and cylindrical. The following requirements need to be satisfactory for effective plasma generation [71], gas pressure between

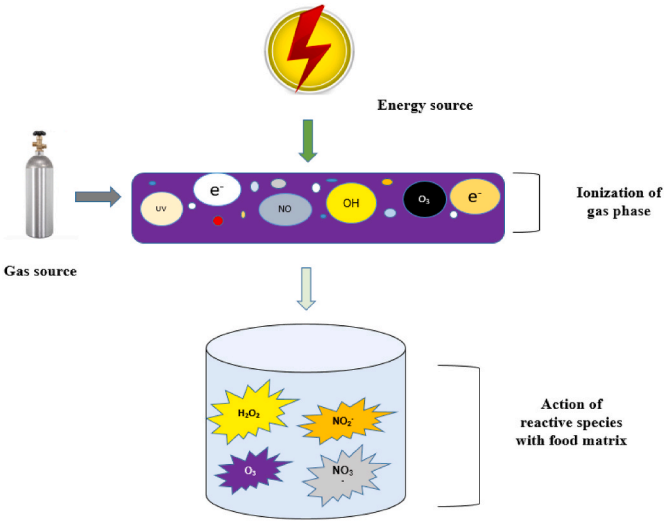

Fig. 1. Schematic representation of plasma generation.

1×10^4 and 1×10^6 Pa with 10–50 MHz of frequency range and alternating current (AC) or pulsed direct current (DC) with voltage amplitude oscillating between 1 and 100 KV_{rms}.

3.2. Plasma jet method

Commonly referred to as atmospheric pressure plasma jets. The plasma ejects out more steadily, homogenously and constantly at atmospheric pressure. It has the advantage of generating the plasma discharge at a very low electric field and utilizes the coaxial electrodes. When the electric energy of suitable power is fed to a gas phase, the gas particles moves in between the live electrode and ground electrode and discharge the plasma. The distance between the plasma discharge electrode and receiving substrate is kept at few centimeters range which is mediated by the working gas. The plasma is being discharged as a stream of jet into the open environment [42,73]. It can be used with relatively smaller sample size application.

3.3. Radiofrequency generated plasma

The system consists of radio frequency discharge, ceramic nozzle, and grounded electrode, gas supply system and generates the ions at high frequency and high voltage. The nozzle is attached with two electrodes needle electrode and active ring electrode. When an oscillating electric voltage of high capacity is applied between two electrodes a radiofrequency power source of 1 KW and frequency of 13.56 MHz is created [74]. This is being applied to the electrodes which generates the plasma. The oscillating electromagnetic field thus creates collision between the gas molecules and leads to plasma generation. The intensity of the plasma depends on the gas composition, gas flow rate and applied voltage potential [75]. RF type of plasma generation can be divided into 3 types based on the mode of RF generation - inductively coupled plasma, capacitively coupled plasma and helicon wave source.

3.4. Microwave based plasma generation

In Microwave based plasma generation method the microwaves generated by magnetron is used to create electromagnetic field which causes ionization of gases as a result of elastic collisions thus releasing the energy as photons of UV and visible [76]. The discharge from the microwave sourced plasma is called as plasma torches which can work with air or noble gases. The working frequency for the transfer of energy lies below the thermal radiation and operates in the frequency range of 300 MHz–10 GHz [77]. The excited discharge gets absorbed by the gas which leads to inelastic collisions along with the ionization. Motrescu et al. [78] used the microwave based plasma for arginine vasotocin peptide modification with argon as the working gas with frequency and power of 2.45 GHz and 600 W was used. The major advantage in using the microwave based plasma generation is its ease of use and no requirements of electrodes coupled with high electron density in the ionized gas. But the major lacuna in large scale use is its surface reach effect as a result of its lower penetrating efficiency.

3.5. Corona discharge

The electrodes are non-homogenous which gets bombarded by high voltage power leading to ionization of gas [79]. Plasma occurs at pointed ends at which the strength of the electric field is high. When a high electric field is applied to a sharp pointed electrode, gas molecules gets ionized at a higher intensity either at atmospheric pressure or reduced pressure. The energy discharges at the flumes at the tip of the electrode in a non-uniform manner which is solely dependent on the shape of electrode. The electrode on which the corona emits is called as active electrode [80]. Glow discharge and spark discharge are the classifications of corona discharge.

3.6. Gliding arc discharge

It consists of two principal electrodes, two auxiliary electrodes and a generator set up. The plasma ejection is at higher temperatures. The gas gets feeded at the wider top inlet which moves down and simultaneously by the applied electric field of higher voltage plasma gets released at the narrowest arc electrode and the airflow at the end supports in transporting the plasma to the substrate [76,81].

4. Factors affecting the cold plasma generation

The effectiveness of plasma treatment depends on the source variables (electrode, gas flow, gas composition, gas temperature, gas combinations, source of electromagnetic field, type of dielectric material, power input, frequency, pulse), processing variables (design and mode of electrodes, mode of exposure, time of exposure, temperature, density of electrons created, characterization of ions and free radicals generated, method of plasma to the substrate) and the nature and type of commodity (nature of commodity, initial microbial load, moisture, surface geometry of the produce, phase of product, volume of the sample, effect of different additives and ingredients) to which the treatment is intended for. Selection of gas plays an important role as it directly affects the type of plasma reactive species generated. Air at present is the easy and cheaply available gas source used for plasma generation. Mixed gas, Co_2 rich gas and rare gases like Helium (He), Argon (Ar) are also available and it dictates the type of reactive species emission as the gas rich in O_2 and N_2 can lead to more amount of ROS and RNS are created respectively. Cold plasma treatment has been successful in liquid foods but in solid matrix it mainly acts on the surface with lesser penetration and the extent of penetration depends on the moisture content and porosity of the solid material. The half-life of reactive species is important along with the flow rate of plasma and its interaction with the food matrix determines the end benefits associated with it. Typically, the half-life of reactive species ranges from 1 ns (OH^-) over 1 m s ($^1O_2$, O_2^-) to 1 m s (H_2O_2) [82].

5. Food proteins and functional properties of proteins

Food proteins are the important biomacromolecule along with carbohydrates and fats which has its role in structure building of muscles, regulating the metabolic functions, growth, repair and maintenance of body tissues etc. Rightly called as the body building component of the diet. It is also known for possessing several structural and functional roles in the human body [83,84]. Proteins are valued not just for their nutritional content but also for its techno-functional properties. They have a significant effect on the rheological, textural, physicochemical and organoleptic properties of the foods, therefore many processing techniques has been researched for increasing or modifying the functional properties which can fit its application in the food industries. Milk, meat, eggs, fish are the recognized as rich source of proteins but in recent years as the trend of sustainability is emerging people are moving towards the plant based proteins (pulses, almonds, soy, hemp, and cashew). The schematic representation of functional application of proteins is presented in Fig. 2.

Proteins are the polymers of amino acids which gets linked by peptide bonds formed by α carbonyl group of an amino acid with α amino group of the another amino acid. Further the amino acid structure can be seen as α amino ($-NH_2$) group linked with α carboxyl group (-COOH), hydrogen atom and a variable R group [85]. At present 20 amino acids are classified as essential and needs to be met through the diet. The nature of arrangement of amino acids will guide the structure and function of protein molecule. Depending on the spatial arrangement of the protein chains, four types of structural classification is observed - primary, secondary, tertiary and quaternary. The primary structure gives the amino acid sequence in the polypeptide chain which are formed by peptide bond, secondary structure refers to the folded structures formed between the polypeptide chains from the background atoms and are grouped as α helix, β pleated sheets characterized by the type of molecular interactions and stability between the side chains. Occasionally other types of secondary structures get formed (β turns, random coils) which are stabilized by hydrogen bonds. The tertiary structures refer to the spatial arrangement and three dimensional matrix of the polypeptide chain stabilized by various interactions like covalent bonds, disulfide bond, hydrogen bonds, ionic interactions, van Der Waals force, hydrophobic bond etc. and forming the native structure of the protein molecule. Quaternary structure refers to the combination and complex interaction between several protein chains to form single unit complexes [86,87]. The amino acids linked polypeptide chain has been stabilized by various bonds and interactions mainly hydrogen bonds, salt bridges, vanderwaals force, hydrophobic linkage, salt bridges, ionic interactions, disulfide bonding etc. Proteins can exist as monomer, dimer, trimer in the aqueous solutions based on the pH, ionic

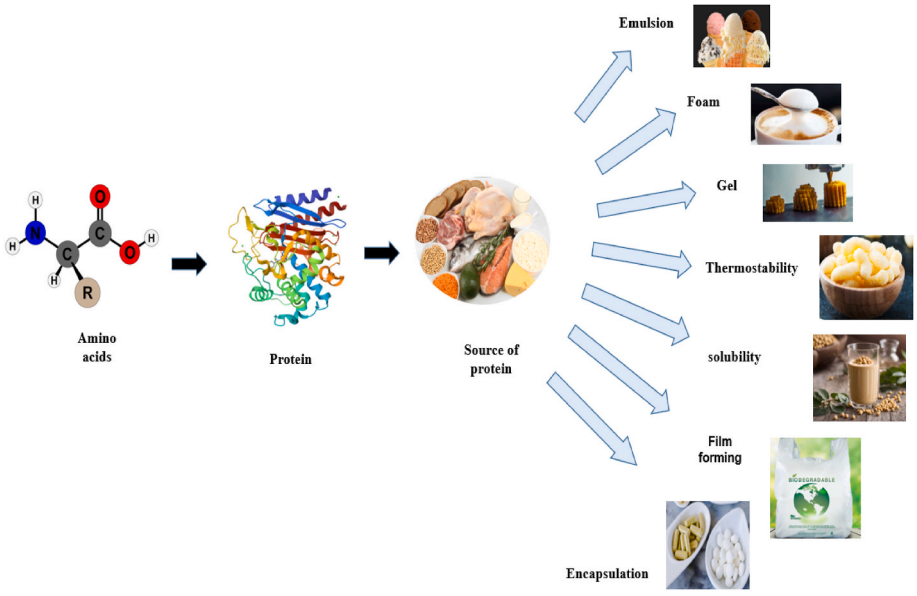

Fig. 2. Schematic representation of functional application of proteins.

interactions and ionic strength. They also exert a charge upon itself which is dependent on the surrounding media pH and presence of charges at amino and carboxyl residues. Proteins can be polar or non-polar based on the side chains in the amino acids present and this is also a responsible factor for the solubility behavior of the protein molecules. On the basis of solubility food derived proteins are classified as albumins (soluble in water), globulins (soluble in salt solutions), glutelins (soluble in acids, bases, detergents), prolamins (soluble in alcohol) [88]. A brief classification of amino acids based on the chemical classification is depicted in Table 1.

6. Functional properties of proteins

The interaction between the structure, composition, conformation, physicochemical properties of amino acids in proteins defines its functional properties [89] and is being depicted in Table 2. Interaction results in the development of networks (gels and films), emulsions and sol [90,91]. This functional characteristics of proteins are valued for its application in various fields of food, beverage, pharma, biomedical sectors. The structure of proteins has a direct relationship with the functional properties and digestion behavior of the proteins. Proteins undergo numerous changes in response to the external conditions be a change in temperature, pH, processing conditions etc. owing to its structure, net charges on the surface, interactions with the other ingredients and gets reflected in its change in the functional properties. The functional properties of protein exhibit a multi factor relationship with the protein structure, spatial conformation of the protein and its associated surrounding conditions (temperature, pH, ionic concentration, salts).

The functional properties of proteins have been grouped under the following categories [92,93].

6.1. Hydration properties

Hydration properties - solubility, water holding property, water absorption capacity, fat absorption capacity, wettability, thickening.

6.2. Interfacial properties

Interfacial properties-hydrophobicity, hydrophilicity, surface net charge distribution which can affect the emulsion and foaming.

Table 1
Classification of amino acids based on chemical property.

Classification	Amino acids
Non polar amino acids	Glycine
	Alanine
	Valine
	Leucine
	Isoleucine
	Methionine
Polar amino acids	Serine
	Threonine
	Cysteine
	Proline
	Aspargine
	Glutamine
Aromatic group	Phenylalanine
	Tyrosine
	Tryptophan
Positively charged amino acids	Lysine
	Arginine
	Histidine
Negatively charged amino acids	Aspartic acid
	Glutamic acid

6.3. Rheological properties

Rheological properties - modification in the protein structure which can affect properties like elasticity, adhesion, gelling, viscosity and aggregation.

These properties of the proteins are driving its application in various fields and application like soups, beverages, bakery products, whipped creams etc. [94]. However, there are certain functional properties which are tailor-made for specific group of proteins for example renneting for dairy proteins which is known for the curdling of milk at the specified pH range. During the course of processing, protein biomolecules are drastically affected thus exhibiting a change in its structure which gets reflected in the functions and applications in the end product. Processing of food mainly follows the thermal treatment thus exposing the food to higher temperatures for reduction of microbes (spoilage, pathogenic), browning reactions, it also leads to significant reduction in the nutritional property and associated negative impacts to the food system. Proteins, a chain of amino acids are vulnerable to any form of extreme treatment procedures as it may directly lead to a denaturation effects and eventual release of amino acids and may lead to the development of off flavors and glycation end products on account of maillard reaction with sugar moieties. Hence there exists a need for the non-thermal based methods which can assist in the modification of proteins with minimum impact to the biomolecule and keeping in view the circular economy for the way to solvent less approach in a greener perspective. Proteins are being modified by physical (heat, freezing, extrusion) [95–97], chemical (deamidation, crosslinking) and enzymatic methods (transglutaminase) to improve its functional properties. The impact of cold processing treatment on functional properties in various groups of proteins are being reviewed in this particular search.

7. Effect of cold plasma on functional properties of proteins

Cold plasma treatment has known to benefit the processors and consumers in reducing the heat and temperature induced negative properties in the food matrix. It is known to alter and modify the chemical and structural composition of proteins (precisely on the amino acids) and bring a desirable change in the functionality of the protein molecule. Further the associated benefits also depend on the interaction of reactive species with the protein molecule, nature and state of the protein chain, temperature, pH condition and processing variables of the plasma source. Emitted radical species is known to increase the carbonyl content and reduce the sulfhydryl groups in the protein chain due to oxidation effect [98]. Direct exposure of cold plasma species leads to the release of degradative end products of –COOH and $–NH_2$ of alanine and valine respectively [99,100]. The reactive species like hydroperoxides and hydroxyl radicals leads to cleavage of peptide bond via amidation reaction [101]. The effect of cold plasma affects the functionality of the proteins. The interaction between the ionized molecules and the food proteins will lead to a modification in the structural property which is seen at the end in the change of techno-functional property of the proteins.

7.1. Solubility

Solubility of the protein refers to the thermodynamic state of equilibrium established between the protein-protein and protein-solvent molecule which is dependent on the particle size of the protein molecule interacting with the solvent. The molecular weight, surface charge, hydrophilic-hydrophobic ratio, denaturation percentage are the prime factors responsible for the solubility function [102]. The hydroxyl radicals induced cleavage of peptide bonds and oxidation of aromatic amino acids led to a change in the 3-dimensional matrix of the protein which shown an increase of 113 % solubility of the pea protein [97]. There will be a relative increase in the proportion of active sites for the absorption of water due to plasma exposure [122]. Reducing the hydrophobicity

and increasing the repulsion behavior (increase in the electrostatic repulsion forces) are known to increase the solubility of proteins. Exposure of proteins to cold plasma reactive species can lead to the increase in surface charge of protein and eventually creating an etching effect thus getting opportunity for the creation of more hydrophilic groups. Hence these newly created hydrophilic structures helps in holding the water molecules at the surface and increase in polarity. Increase in polarity of proteins can also be attributed to acidic conditions formed by the electrolysis reactions and an associated reduction in pH of the surrounding media. Polarity can also be due to etching induced activation of sites by the reactive species for the hydrophilic attachment [63]. The positive impact of solubility raise can also lead to a favorable output in the viscosity, rheology and emulsions of the food matrix [103]. The negative impact of long term exposure to plasma gas can be seen in subsequent reduction in solubility and increase in aggregation of molecules due to crosslinking and suprahydrodynamic cavitation [104]. These aggregates are known to reduce the molecular weights of protein as observed in the studies of Abarghoei et al. [140]. Reduction in the quantity of effective reactive sites is often noticed as a result of over exposure due to competitive effect between the molecules. Intense oxidation of proteins by the reactive species interaction with the protein can reduce the solubility due to precipitation. Sharifian et al. [105] observed a reduction in the emulsifying property of myofibrillar proteins when exposed above 20 min because of the aggregates formed due to oxidation of amino acids. Oxidation is believed to occur from the reaction of reactive species like O2 and O_2^- and its protonated form HO_2^- [138]. The aggregates thus formed will eventually reduce the foaming capacity of the proteins. The initial exposure of plasma to the protein molecule leads to the unfolding and denaturation effect which actually lets the amino acid chains to move freely to the interface and to establish the thermodynamic equilibrium between the hydrophilic and hydrophobic moieties which gets reversed on the over exposure.

7.2. Gelation

Proteins are known to provide structure and body to the food textures and it is purely dependent on the ability of the protein to gel. Gelation behavior of proteins is mainly attributed for its application in the jellies, puddings etc. It is induced by heat, enzymes or chemicals and is measured by least gelation concentration index. Gels are the intermediated between solids and liquids (they act as solids with liquids inside them), hence they are unique textures which has received profound research interest. It has a direct relationship with the prevention of synersis by increasing the water retention. Exposure to cold plasma leads to oxidation of amino acids along with the cross linking can have a positive role in increasing the gelation strength [84]. The duration of exposure and oxidation effects in protein, branching pattern, denaturation percent, surface hydrophobicity are the determining factors for the perfect gelation behavior. The disulfide bonds upon oxidation of sulfur containing amino acids are shown to play a critical role in the formation of protein gel. Sulfur containing amino acids (methionine, cysteine) are the major targets for reactive species leading to oxidation effects by the addition of hydrogen and oxygen which leads to RSH and RSO formation. Increased oxidation by the reactive species leads to an increase in the surface hydrophobicity [106]. Increase in the dough strength was noticed when the strong and weak wheat flours were exposed to plasma treatment of 60–70 kV for 5–10 min due to modification in the secondary structure of gluten along with the increase in the disulfide bonds thus contributing stability to the formed dough [107]. Decrease in the temperature of gelation was observed for the plasma treated pea protein in the range of 70–90 °C due to the unfolding of protein molecules and the formation of fibrillary aggregates thus aiming for the premature gelation before denaturation [108].

7.3. Nutritional property enhancement

The nutritional property of proteins in the human body has been directly influenced by the nature of amino acids (essential, non-essential, semi essential), type of amino acids (acidic, basic, neutral), structure of amino acids, its behavior at the intended pH, temperatures, digestibility status, and absorption rate etc. Cold plasma has known to affect the amino acid content by forming various reactions and cross linkages and is dependent on the processing variables (voltage, time, frequency). There was an increase in the proportion of aromatic amino acid and corresponding decrease in the proportion of acidic and basic amino acid residues occurred when the short and long grain rice flours are exposed to cold plasma treatment along with the significant reduction in citrulline in short grain type due to the interaction of ozone species with the amino acids causing oxidation reactions which can lead to the loss of certain category of amino acids though the exact reason is unknown [109].

7.4. Structural changes

The structural changes caused by the plasma treatment is due to interactions of reactive species with amino acids. The conformational and steric property of the protein chains plays a very important role in emulsification, foaming, solubility, film forming etc. Plasma treatment has been investigated in detail on the effects it has on the protein chains and found that mostly the secondary and tertiary structure gets modified [110,111] during the course of treatment and very limited information is available on its effect on primary structure of amino acids. The increase in concentration of carbonyl residues generated after the plasma treatment can be used as an indicator for the oxidation of amino acids formed due to the breakdown of peptide chains and leading to fragmentation and aggregation [110,113]. On the other end higher release of carbonyl side chains will have a negative effect in reducing the solubility by forming cross links via schiff's base [114]. Hydroxyl radicals (OH •) converts sulfhydryl group to sulfhydryl radicals which eventually due to its instability proceeds to the formation of thiol peroxylradicals (SOO •) which gets converted to stable disulfide linkage by the interaction with the ozone species during plasma treatment [115,116]. The amino acids get derivatised via different reactions like hydroxylation and nitration of aromatic amino acid (tryptophan, tyrosine), sulfoxidation of methionine, ring opening and amidation of histidine and proline etc. Intrinsic fluorescence spectral measurements from tryptophan, tyrosine, phenylalanine provides a reliable indication on the conformational changes in the secondary and tertiary structure of proteins. Fluorescence of the tryptophan reduced drastically following plasma treatment [112]. Due to its aromatic nature tryptophan is located in the hydrophobic core of interior proteins at the interface of two protein domain and subdomains, hence they are sensitive to the surrounding environment and this changes in tryptophan can relate to the conformational change in the protein molecule. In the studies of Yu et al. [124] there was reduction in the absorption intensity of tryptophan and tyrosine which was due to covalent bonding between tryptophan, tyrosine with the lactose.

FT-IR (Fourier transform infrared spectroscopy) studies additionally provides information on the change in functional groups upon plasma exposure. The amide bond I (1600-1690 cm^{-1}), amide bond II (1480-1575 cm^{-1}) and amide bond III (1300-1200 cm^{-1}) is most sensitive vibrational band of the protein backbone and directly provides an insight on the secondary structure and hydrogen bonds of the proteins. The reduction in α helix is known to reduce or destroy the orderly structure of the protein as seen in the studies of Segat et al. [122] the reduction in α helix was found to have an increment in the proportion of random coils which could contribute to enhanced emulsification. There was a reduction in the α helix and change in the β turns and β sheets and random coil structure noticed in the treatment of soybean protein. It is observed that the random coil patters prior to the plasma treatment gets

oriented and change in the secondary structures is noticed. While β turns is responsible for hydration property, β sheets will connote the interaction effects between the peptide chains [117]. Contrarily there was an increase in the contents of α helix in the studies of Yu et al. [124] due to the introduction of hydroxyl groups promoting covalent linkage between the conjugates. The increase in α helix can be attributed to the increased denaturation of proteins.

7.5. Emulsions

Proteins are well recognized additives for reducing the interfacial tension by itself getting adsorbed on the oil -water interface and stabilizing the structure owing to its thermodynamic behavior [118]. Therefore, proteins are used in bakeries and confectionary segments (whipped creams and toppings), mousses, margarine, ice-creams, beverages to create a stable emulsion. By acting as an emulsifier, protein holds the water to oil or oil to water by forming a bubble film around the oil/water [119] which is mainly due to its amphiphilic nature. The amphiphilic nature of the protein leads to adsorption at the interface of oil and water, coating of oil droplets and film formation. By optimizing the size of protein, conformational structure, flexibility of the protein chain, nature and viscosity of the immiscible liquids, temperature, pH and the ratio of hydrophobic to hydrophilic side an ideal emulsion structure gets created. Formation of a cohesive continuous viscoelastic films at the interface is primarily important for the ideal emulsion. Cold plasma is known to positively impact on the emulsion formation and stability due to the reduction of mean particle size reduction of surface charges and bringing a balance in the hydrophobic to hydrophilic content. This again is governed by the nature and type of reactive species generated, gas composition used for plasma creation, current flow, voltage pattern, exposure treatment time [36].

Cold plasma is known to reduce the protein aggregates and increase the unfolding of the molecule thus exposing more of the active sites for the emulsion formation. Generally, plasma treated proteins have a tendency to get rearranged at air water interface as compared to oil water interface [120]. There is a sudden increase in the charge of the proteins on exposure to plasma particles which leads to immediate movement to the air-water interface. Higher frequency of exposure to plasma can have a negative effect on the emulsion property as observed in the findings of Zhang et al. [110]. The increase in molecular weight of the protein coupled with excess oxidation of the amino acids will have a negative effect on the emulsion formation and stabilization as it may reduce the flexibility of the strands by aggregates formation. Oxidation primarily depends on the availability of O_2, O_2^- and HO_2^- radicals [121]. Emulsion formation was more effective at the basic pH in which proteins unfolded more and reduced the interfacial tension [122]. Additionally, the emulsion formation and stability is also dependent on the interaction of protein with other molecules (including sugars, fats, bioactive compounds, cell wall and cell components) in the matrix. Increase in the stability of emulsion is seen in the flaxseed protein emulsion due to its interaction with lignans, mucilage and phenolics which further tends to enhance the interfacial activity by coacervation complex formation [124].

7.6. Thermostability

The thermostability of protein plays an important role in food processing industry as the food components are exposed to higher temperatures for inactivation of microbes and destruction of endogenous enzymes and this property gives an idea about the denaturation temperatures, enthalpy of heat required etc which can be obtained by Differential scanning calorimetric (DSC) studies. It gives an idea of the orderly structure and configuration of proteins. Cold plasma has been shown to alter the thermal profile of the proteins mainly by the interaction of reactive species with the side chains of proteins causing a structural and conformational change, cleavage of peptide bonds and reducing the surface hydrophobicity which tends to reduce the enthalpy [123]. Studies on the impact of cold plasma to foods has shown to reduce the thermostability of protein chains mainly due to etching along with the breaking of peptide bonds and intermolecular forces on exposure to reactive species [63]. However certain pre-modification of protein chains can actually prevent the negative impact. J. Yu et al. [124] chemically modified the pea protein with lactose sugar as protectant molecule which aids in marinating the structural integrity of protein upon plasma exposure. Thus the collective contribution from protein chain strength and complexity, structural conformation of the molecule, nature and number of bonds (precisely the strong hydrogen bonds) will determine the effect of cold plasma on the protein change configuration and impacting the stability at higher temperatures.

7.7. Encapsulation

Cold plasma treatment can increase the effectiveness of encapsulation by increasing the stability of coacervates complex. Decrease in the size of the particles and reduction in the aggregates by reaction with reactive species tends to increase the active sites for encapsulation. Resveratrol loaded nanoparticles encapsulation efficiency increased from 51.8 % to 82.7 % with the cold plasma treatment of 40 V indicating the assembly and interaction in the zein-chitosan complex coacervates leading to better dispersion stability [125].

7.8. Film forming property

Polymer films derived from the petroleum sources tends to have a negative impact on the environment, hence the focus has been shifted towards utilizing the biodegradable polymers derived from the natural sources. Among which protein based films is extensively studied for improving its properties and making it a better alternative to commercially available polymers. Naturally derived biopolymer lacks rigidity, and possess a lower barrier property, hence cold plasma treatments can be used for chemically modifying the biopolymer chain through increasing the polar groups and increasing the surface energy which finds its applicability in better adhesion and printability. G. Chen et al. [125] used a partial chemical modification by means of composting of zein protein with chitosan followed by exposure to cold plasma od 100 W for 60 s which resulted in the dual benefit of increasing the solubility and wettability and a corresponding reduction in hydrophobicity. The effect of cold plasma on protein functionality changes has been seen in Fig. 3.

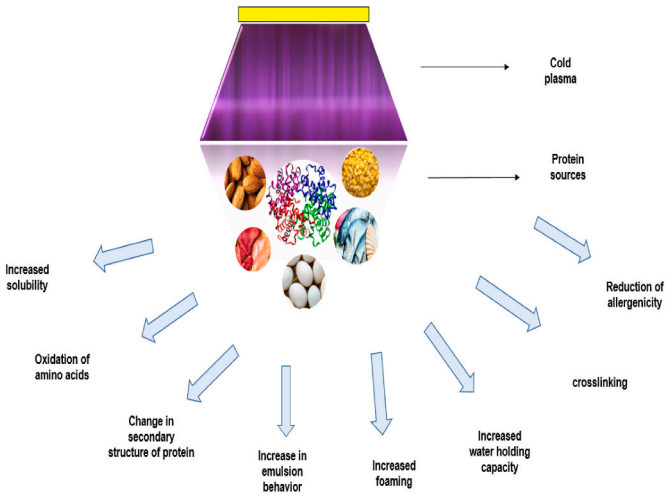

Fig. 3. Effects of cold plasma on protein functionality.

8. Effect of cold plasma treatment on seed proteins

Cereals and pulses are the largest contributors of starch and protein in the human diet and are considered as staple food providing a balanced caloric distribution. Besides starch and proteins, these group has a significant amounts of bioactive compounds, essential fatty acids, dietary fibre etc. Cereals falling under the Graminacea family consists of rice, wheat, maize, barley, oats, rye, sorghum, pearl millet, small millets etc. It generally contains 70–72 % carbohydrates, 7–15 % proteins and 1–12 % fats [126]. Seed storage proteins are known to store and supply essential nutrients to the developing embryonic tissues [123]. It mainly consists of globulins, glutelins and prolamins [127]. While cereals (except oats and rice) are known to contain prolamins as the major fractions, pulses contain globulins as the major fraction. Cereal proteins like gluten in wheat is a boon to the baking industry as the protein leads to the development of rigid structures to the breads, biscuits, cookies upon baking. Gelatinized starch on proofing and baking process gets stabilized and entrapped by the protein network. Pulses comprising pea, chickpeas, lentils, beans contain 17–30 % proteins and 4–23 % carbohydrates and are known to complement cereals in the diet providing a sustainable protein source [128]. Apart from providing proteins, pulses are known to contain large amounts of vitamins, minerals, and dietary fiber. Pulse proteins are generally available as flours (<65 % protein on dry weight basis), concentrates (>65 % protein on dry weight basis), isolates (>90 % on dry weight basis). Functional properties of the pulse proteins generally include solubility, emulsion, foaming, thickening, water binding, fat binding, gelation, flavor binding etc [123]. They are related to chain length, branching pattern, molecular weight, hydrophobicity [129]. Cold plasma exposure cereals and pulses derived proteins can significantly increase the techno-functional properties and paves way for the large scale utilization of the proteins.

The treatment of atmospheric cold plasma on soy protein isolate at 80 Hz for 1–10 min was found to increase the solubility due to unfolding and exposure of hydrophobic groups but the solubility tends to get reduced when exposed to 100 Hz for 5 min due to overcrowding effect and consequent reduction in the available active sites for water binding [110]. Increase in the treatment combinations (voltage and time) leads to increase in the solubility by creating more active sites for the water molecules to diffuse [113,130]. Reduced solubility due to aggregates was seen in the treatment to flax seed proteins which was formed by electrostatic repulsion at the isoelectric pH thus further contributing to reduced solubility [124]. The solubility of the protein molecule depends on the hydration capacity of the proteins, swelling power, pH of the surrounding medium and the interaction between the protein and solvent with a thermodynamic equilibrium between them. Increase in the amount of soluble proteins and polar amino acids at the initial interaction from the reactive species along with an increase in the ionization of polar amino acids lead to increased solubility [131]. Establishing a balance between hydrophilic and hydrophobic groups is important when the food matrix gets exposed to plasma species as excess of hydrophilic groups can aggregate and leads to reduce the solvent interaction with the protein molecule [132]. As the proteins possess isoelectric point (pI) (net charge at specific pH) any change in pH will have a reduction in its solubility of the protein due to the modification brought about by change in the functional groups. Reduction in the pH caused by hydroxyl acids are known to initiate and strengthen the cross linkage between the depolymerized starch particles and stabilize the matrix [109]. Increase in hydrophilicity was seen in the zein based protein films when exposed to plasma by the addition of oxygen to carbon atoms at the surface [19].

The bombardment of reactive species will lead to modification in the secondary structure of proteins and cause the structural changes. The α helix which is known for the rigidity of the protein gets reduced and eventually the β sheets and random coils gets increased thus providing flexibility to the protein. The similar effect is seen in the studies on pea protein isolate [133]. Manoharan et al. [134] studied the effect of cold plasma bubbling on the sesame milk with at 200 V for 10, 20 and 30 min which was successful in reducing the I_gE binding epitopes by 23 % due to structural change caused by oxidation of proteins as confirmed by circular dichroism and FTIR spectra, whereas the allergenicity of the sesame milk increased at 30 min treatment due to the creation of new neotypes of the allergen. The increase in the exposure time can have a deleterious effect in creation of neotypes which should be a needs to be a concern in its optimization. To the present state, no studies has been done on the interaction benefits of reactive species and its impact on the nutritional status. Mehr & Koocheki [113] investigated on the effect of dielectric cold plasma treatment at 9.4 and 18.6 kVpp at two time combinations 30 s and 60 s with 20 kHz as the oscillation frequency on the grass pea protein isolate and studied on its interfacial properties and emulsification activity. The reactive species composition from the dielectric plasma was characterized by optical emission spectroscopy. Plasma treatment of 18.6 kVpp for 60 s had a positive impact on the creaming stability owing to the reduced size due to etching which increased the thermodynamic stability and surface hydrophobicity with increased electrostatic repulsion. This is further evidenced by the lower interfacial tension values due to the ordered arrangement of secondary and tertiary structure coupled with the formation of protein in the form of nanoparticles getting observed on the oil water interface. Treatment with cold plasma was able to bring about more ordered stability in secondary and tertiary structure of proteins and resulted in the enhanced stability with respect to interfacial and emulsion forming property [135]. Increase in hydrophobic groups is known to increase the surface hydrophobicity [136,137].

Apart from proteins lipids are known to get modified on exposure to reactive species. Bahrami et al. [72] treated the matured wheat flour to dielectric barrier discharge of cold plasma at the power of 15 V and 20 V for 60 s and 120 s with a constant frequency of 9 kHz has shown a drastic increase in the lipid oxidation markers PV, FFA (primary) and hexanal (secondary marker) indicating the oxidation behavior of lipid fractions in the wheat flour at higher treatment times. Proteins got oxidized leading to an increase in molecular weight fractions and solubility thus modifying the flour which suits to the new product developments as a result of strong dough developments which is required for bakery products. but there was no change in the microbial profile of the wheat flour due to its reduced moisture level indicating that the condition of sample plays an important role along with the treatment effect. This was even confirmed by Misra et al., [107]. Proteins with aromatic side chains (tyrosine, tryptophan, phenylalanine) are more prone to get oxidized from the reactive species [112,138,139]. Cold plasma assists in the release of polyphenols from the protein polyphenol complex due to etching and structural change in the amino acid thus releasing the bound phenols [111]. Plasma induced release of bioactive compounds is an interesting field which will help in valorizing the waste for extracting the bound and hidden bioactive materials in a greener approach.

Plasma treatment was investigated for the wheat flour functionality improvement in which the discharge of plasma had the ozone molecules which was quantified for 100 ± 20 ppm and 200 ± 40 ppm for 60 kV at 5 and 10 min and 160 ± 40 and 240 ± 20 ppm for 70 kV for 5 and 10 min respectively. Ozone has the highest half-life period and chemically stable with higher oxidation potential [107]. There was changes observed in the secondary structure of proteins (increased for weak flour and reduced in strong flours). Improvement in dough strength and mixing property was noticed due to disulfides formation in the glutenins subunits which makes the dough more elastic by increasing the cross linkages. A predominant increase in the oxidation of proteins chains can be evidently seen in the results [107]. The oxidation effect has a more usage in improving the wheat flour functionality for the baking conditions. The added benefit here is since the glutenin molecules are tyrosine rich they are more prone to get oxidized owing to its amino acid structure and yield the desired result. Presently chemicals like azodicarbanamide, ascorbic acid is used for this effect for which plasma mediated increase in oxidation can be a suitable option and further

studies are to be done for the optimization and long term effects. Pea protein isolate and pea starch fractions were exposed to cold plasma for 10 min. Increase in the values of water binding capacity and fat binding capacity was noticed upon plasma exposure to 10 min Reduction in pH and increase in solubility up to 327 % was observed [97]. The buffering capacity of the surrounding matrix can lead to a stable pH condition. Dielectric barrier plasma exposure to peanut proteins had an impact on reducing the secondary structure and increasing the flexibility by decreased β turns and α helix and subsequent increase in the β sheets and random coils [122]. The effect of cold plasma on the secondary structure of proteins has a significant importance and is reflected in its change in the end application of proteins.

9. Effect of cold plasma treatment on dairy proteins

Dairy based milk refers to the class of milk obtained from the bovine species which is produced consumed and exported as fresh and processed formats to a greater extent. Recently milk from the non-bovine sources are also getting in limelight as plant based milks, milk derived from the goats, camels etc. The bovine milk consists of water as the major component and the remaining part is contributed by the protein, fats, minerals and salts etc. It is viewed to be a dispersion of fats and other molecules in the aqueous system. Proteins approximately constitute 32 g/L to 38 g/L in the whole milk. Caseins and whey are the two major fractions in the total protein component present to the tune of about 80 % and 20 % respectively [144]. The casein fraction of milk protein is again subdivided as α_{s1}, α_{s2}, (β+γ.) and κ casein present in the ratio of 0.45:0.11:0.33:0.11 [145]. Casein is known to form stable aggregates by means of colloidal complex formation with calcium and phosphorous., thereby stabilizing the calcium and phosphorous content. Due to the presence of larger propyl residues casein lacks well organized secondary structures. Casein protein generally exists as micelle complex with α and β in the core of the complex and stabilized by κ fractions at the surface thus exhibiting a strong electrostatic repulsion which prevents collapse and flocculation of the milk matrix [146,147]. They are phosphorylated and possess a limited number of α helix and β pleated sheets. Whey proteins are comprised of α -lactalbumin, β-lactoglobulin serum albumin and immunoglobulins [90]. Whey protein are generally non phosphorylated and globular type of proteins with variable number of disulfide linkages [148] having significantly more number of secondary structures including α helix, β pleated sheets, β turns and aperiodic structures. Since whey proteins have more stable and organized secondary structures they are prone to denaturation effects due to heat and harsh environmental conditions [145].

Current process of handling the milk includes exposing to heat treatments like 62.8 °C or more than 65.6 °C for at least 30 min or not less than 71.7 °C for at least 15 s which no doubt has proven to reduce the microbial load and enhance the safety of liquid milk but it comes with the problems of denaturing the proteins and associated change in flavor, non-enzymatic browning and loss of essential vitamins [149]. These proteins on exposure to heat during the processing in excess of the recommended temperatures are known to have a negative impact on the solubility, heat induced protein aggregates formation, increased viscosity and denaturation of the proteins leading to whey and serum separation, κ casein dissociation, maillard reactions, dephosphorylation of the caseins etc. which altogether affects the keeping quality and organoleptic properties of the milk [150]. Therefore, the non-thermal methods of inactivating the microflora and endogenous enzymes in the milk has been researched for optimizing the output along with energy efficiency as compared to thermal treatments (pasteurization, sterilization).

Cold plasma is known to affect the different properties of proteins thus exhibiting a change in the specific functional behavior. The associated changes include the changes in the color, viscosity, taste factor, flavor, particle size and the effect on allied nutrient content (proteins, minerals, carbohydrates). Generally, an increase in the viscosity of the milk is observed on increasing the treatment time due to mild shear thinning behavior of the other molecules present (lactose, fats and proteins). In the treatment of milk to cold plasma the viscosity increased from 1.8 to 2.2 mPa s when the voltage increased from 40 V to 80 V [151]. An optimization in the processing variables (voltage, power and time of exposure) need to be taken for a desired viscosity and consistency in the milk and milk based beverages. The proteins get exposed to plasma reactive species and thus gets altered and modified depending upon the time of exposure as it is observed from the study of Manoharan et al. [152] that no effect on the content of protein was seen 35.3 g/L ± 0.06 as compared to control sample 34.7 g/L ± 0.17. Ng et al. [142] investigated on reducing the antigenic proteins in bovine milk and found that there was reduction in the levels of α lactalbumin and casein for the exposure duration of 30 min. Due to aggregation, cross linking and fragmentation induced changes in primary and secondary structure of proteins solubility was drastically reduced [19]. Radicals generated are known to cause cross linkage and strengthen the polymer network [153]. This property finds its application in the development of barrier packaging films. Segat et al. [120] studied on the effect of cold plasma on whey protein isolate for different timing starting with 1 min–60 min and found a reduction in the pH which affected the solubility and had an increased carbonyl content with increase in the emulsion and foaming behavior. Though foaming capacity decreased on long exposure (after 15 min) but there was an increase in foam stability The increase in hydrophobicity is responsible for the emulsion and foaming property.

10. Effect of cold plasma treatment on animal derived proteins

Proteins derived from the animal source tends to have a similar amino acid pattern as required for the humans. Biological value of the egg protein is high indicating its higher digestibility and a good source of easily available protein. Eggs, chicken meat, fish are the prominent source of proteins from the animal origin.

10.1. Meat derived proteins

The meat derived proteins are grouped as sarcoplasmic proteins (myoglobin, hemoglobin, cytochrome proteins, endogenous enzymes), myofibrillar proteins (myosin, actin, tropomyosin, m-protein, alpha-actinin, beta-actinin, c-protein, troponin T, I, and C) and stromal proteins (collagen and elastin). The properties like hydration, heat induced aggregations, gelation, water holding property, emulsifying properties are the functional properties majorly investigated in the meat proteins. The myofibrillar proteins accounts for 55–60 % of the total meat proteins and are the major determinants of texture, yield after processing and taste of the meat. They are excellent gel formers and binders in the comminuted and emulsified meat products but often the sulfur side chains make them susceptible to oxidation [154]. The same is evident in the competitive plasma treatment experiments on amino acids conducted by Takai et al. [155] found that the methionine is highly oxidized by the plasma exposure due to its higher chemical reactivity. Around 14 amino acids are prone to get oxidized by plasma species, among them the sulfur containing groups (cysteine, methionine), aromatic amino acids (tyrosine, tryptophan, phenylalanine) are prone to selectively get oxidized [21]. Factors affecting the functionality of meat proteins includes the intrinsic parameters (body pH, method of stunning, rigor (pre and post), structure, content and nature of amino acids) and processing factors (pH, metal salts, processing conditions, temperature, effect of additives and other ingredients). Denaturation, coagulation, cross linking, aggregation of proteins reducing the solubility, water holding capacity are the commonly encountered problems in thermal processing of meat with reference to proteins. Accompanying with these are the flavor and color changes due to maillard reactions. Modifying and improving the myofibrillar protein matrix is generally followed by traditional processing methods, additives, oxidation mechanisms and non-thermal technologies like cold plasma is being adapted for modifying the

functional attributes of myofibrillar group of proteins due to its environmental friendly approach and with a benefit of reducing the amount of additives [156].

Pérez-Andrés et al. [102] investigated the atmospheric cold plasma treatment to haemoglobin, pork gelatin and bovine lung protein at 80 kV for 15 min and shown that it had a negative effect in reducing the emulsifying property due to the over exposure and unfolding leading to the more hydrophilic chain over hydrophobic which drastically lowered the emulsion property, increased water holding capacity in pork gelatin and lung protein isolate due to partial denaturation was noticed which can find its applicability in the fabricated foods for the superior gelling property. Maximum proteolytic activity was seen in the acidic pH and reduction in the activity of crude protease extract was seen and the destruction of enzyme was related to the voltage, time of exposure and structure of enzyme along with the surrounding buffer matrix. Acidification of the food matrix is due to nitrous acid (HNO_2), nitric acid (HNO_3), H_3O + ion [158–160]. At a higher treatment exposure, the emulsion behavior decreased due to aggregates formation [161]. The textural properties of the gels (hardness, adhesiveness, cohesiveness) was increased due to crosslinking reactions mediated by reactive species [98]. Luo et al. [143] investigated on the aroma binding property of myofibrillar proteins in dry cured bacon and shown that flavor compound (aldehydes) binding capacity increased by the myofibrillar proteins due to hydrophobic amino acids and increased chain length due to unfolding of secondary structure. Interaction between the myofibrillar proteins and flavor compounds was mediated by the intermolecular forces (hydrogen bond, van der Waals's forces, disulfide bond, ionic interactions [162]. Proteins gets involved in binding reaction with the different flavor and aromatic compounds in the meat and the reaction can be reversible or irreversible [163] which is dependent on the amino acid structure, hydrogen bond, hydrophobic interaction, intermolecular interactions.

10.2. Fish and marine derived proteins

Based on solubility fish proteins are classified as myofibrillar proteins, sarcoplasmic proteins, and stromal proteins [157]. The nature and sequence of amino acids, surface charge, size of amino acids, conformation and intermolecular bonding are the major factors determining the functional properties. The solubility of the fish proteins is mainly pH dependent as it tends to increase due to change in the protein-protein interaction to protein-solvent interaction above and below the isoelectric point. Freshness of fish, rigor mortis condition, nature of storage, type of processing plays a role in determining the functional properties of proteins. Solubility, Gelation, Viscosity, Emulsion, Foaming, Water holding capacity are the major techno-functional properties of the fish derived proteins.

De Souza Silva et al. [164] studied on the physicochemical properties effect of cold plasma on myofibrillar proteins which was extracted from white shrimp (*Litopenaeus vannamei*) and found a reduction in the pH from 6.77 ± 0.02 to 6.07 ± 0.02 and a lowered value for protein solubility due to coagulation, aggregation and precipitation with an increase in the mean particle size due to modification in the secondary and tertiary structure of proteins. Ekezie et al. [116] investigated on the effect of argon based plasma on the structural and physicochemical properties of actomysosin extracted from tiger prawn and shown that the pH reduction and increase in the emulsion and foaming capacity is due to increase in the hydrophobicity and oxidation of sulfhydryl groups which is due to the unfolding of actomysoin complex. The effect of cold plasma on the seed protein, dairy proteins and meat derived proteins are provided in Table 2.

11. Modification of protein based films by cold plasma

Cold plasma is used for the preparation and fabrication of protein based films and edible coatings. The modification in the protein is

Table 2
Summary of effect of cold plasma on different sources of protein.

Food matrix	Treatment details	Key changes observed	Reference
Wheat flour	Frequency - 9 kHz; Voltage - 15 V and 20 V; Gas - Air Method - DBD type	Increase in the higher molecular weight glutenin subunits; oxidation of lipids and proteins.	[72]
Aqueous solution of naturally occurring amino acids	Frequency - 13.9 kHz; Voltage - (AC) −3.5 kV to +5.0 kV; Gas -Helium Method - Low frequency plasma jet	Hydroxylation of aromatic amino acids; sulfonation of cysteine side chain amino acids; sulfoxidation of methionine; ring opening of histidine and proline; reduced pH of the sample	[155]
Flax seed proteins	Frequency - 5 kV; Voltage - 40 kHz Gas - compressed air under pressure Method - Plasma jet	Reduction in the solubility and pH; increase in the levels of bound flavonoids and phenolic contributing to increased antioxidant and change in spatial conformation; increase in surface hydrophobicity due to unfolding of tyrosine side chains	[111]
Soft and hard flours	Voltage - 60 kV for 5 & 10 min and 70 kV for 5 & 10 min	Change in the secondary structure of proteins (increased for weak flour and reduced in strong flours); reduction in β-sheets and increase in α helical and β turns; Increase in viscoelasticity; lower tan δ and increased modulus.	[107]
Pea protein fractions (pea protein isolate, pea starch and pea protein isolate)	Frequency - 3 kHz; Voltage - 8.8 kV	Ablation of proteins; Reduction in pH; Increase in solubility (191 %); Increase in water holding capacity (113 %) and fat holding capacity by (116 %).	[97]
Peanut protein	Voltage - 35 V; DBD type; treatment duration −1,2,3,4 min	Reduction in pH, Increase in the solubility for up to 3 min and then decreased; increase in water holding capacity and mean particle size to 1208 ± 33.2 nm for 4 min exposure; reduction in β turns and α helix and increase of β sheets and random coils (2.23 %)	[122]
Glycosylated conjugates (high temperature peanut protein isolate and lactose)	Frequency - 90 W; treatment duration- 1,2,3,4,5 min	Increase in solubility (1.34 mg/ml) and reduction in hydrophobicity; Increase in degree of glycosylation; Reduction in the browning intensity due to stable cross linking.	[124]
Wheat germ protein isolate	Voltage- 25 kV; Time - 5, 10, 20, 40 min; DBD type	Reduction in the content of amino acids as compared to control; Increased values for emulsion absorption	[140]

(*continued on next page*)

Table 2 (*continued*)

Food matrix	Treatment details	Key changes observed	Reference
		index (EAI), emulsion stability index (ESI), foam absorption index (FAI), foam stability index (FSI) more pronounced at 5 min exposure at basic pH condition (pH −10).	
Pea protein isolate	Voltage - 170, 200, 230 V; Time - 5, 10, 15 min	Increase in solubility by 66.94 %; emulsion activity and emulsion stability increased by 25.25 % and 10.8 %; foaming capacity and foam stability by 57.28 % and 6.08 %; Reduction in the values for oil holding capacity; Increase in β sheet and random coils by 25 % and 32 % respectively.	[133]
Pea protein	Frequency - 3500 Hz; Voltage output 0–30 kV; current output – 0–1 A; pulse width - 10 μs exposure for 2 min; DBD type	Enhanced gelling property; Increased hydrophobicity with an increase in water holding capacity; Increase in G′ and modulus of the gels; decrease in fluorescence intensity; Increase in β-sheet and anti-parallel β sheets conformation	[108]
Short and long rice grain flour	Voltage - 60 and 70 kV; Time - 5 and 10 min	Increase in pH; Increase in the content of glutamic acid, asparagine, serine histidine, threonine, .-aminobutyric acid, tryptophan, isoleucine, phenylalanine and proline	[109]
Sesame milk	Voltage - 200 V; Time - 10, 20 and 30 min; DBD type	Reduction of 23 % in IgE binding epitopes of the protein; oxidation of proteins; Increase in α helix proportion and reduction in β turns and β coils content.	[134]
Whey protein isolate	Power - 40 and 50 W; Time - 10,20,30,40, 50 s	Reduction in α helix, increase in β sheets; Increased emulsifying capacity and stability of the WPI based emulsion	[141]
Bovine milk proteins	Voltage - 20 kV; Frequency - 20–70 kHz; Time-10,20,30 min	Reduction in the antigenic fractions of α lactalbumin and casein; Reduction in the content of overall amino acids was seen; Increase in α helix and β turns content.	[142]
Crude protease extract from squid mantle	Voltage - 60 kV; Time - 15, 60, 120, 180, 240 and 300 s	Increase in water holding capacity; reduction in the activity of crude protease extract; textural property of the gels increased	[98]
Chinese dry cured bacon	Voltage - 50, 60, 70 kV	Increased the flavor binding capacity of myofibrillar proteins	[143]
Myofibrillar proteins from beef (*Longissimus dorsi*)	Voltage - 12 kV; Frequency - 7 kHz; Time - 5,10,15,20 min	Increase in water holding capacity, emulsion and foaming capacity	[105]
Extracted actomyosin protein from king prawn	Voltage - 7 kV; Frequency - 50 kHz	Increase in emulsion and foaming capacity	[116]

brought about by changes in the structural, mechanical and barrier properties. Often the protein can be used as a medium for encapsulating and releasing agent for bioactive and antimicrobial components. Cold plasma reacts with the biopolymer and is known to bring changes in the surface of the films by etching, cleaning etc. and are known to increase the diffusibility of functional compounds. A brief overview of cold plasma on the protein based films is presented. A list of proteins application for film development is presented in Table 3.

12. Future prospects

Cold plasma application on the protein functionality modification and improvement has been increasingly researched in recent years yet the molecular understanding and scientific unravelling about the effect and changes observed post plasma exposure is still lacking. Poor reproducibility has been seen in many of the experiments as some may had increasing level of functional property at the same voltage while some group had the lower values for functional property. Standard operating protocols needs to be developed for uniformity in operation and control of equipment. Studies related to amino acid sequence recognition by the reactive species and biochemical changes associated with the protein molecule need to be focused to have a better understanding of the effect. The effect of amino acid modification needs to be assessed for allergenicity and toxicity as in some cases there are evidence of generation of neotypes at a higher voltage and higher exposure time which is a prime concern. The cytotoxic effect of plasma activated water and has been reported for eukaryotic cell lines [170]. The reactive species need to be characterized and its effect on the protein molecule should be investigated. Till now, chemistry of plasma generation has been studied, the kinetics involved in the release of reactive species and its interactions are poorly understood. Although plasma is able to bring

Table 3
Effect of cold plasma treatment on the biopolymer based films derived from proteins.

Protein source	Source and working conditions	Treatment effects	Reference
Zein	Atmospheric cold plasma (Voltage - 65 V; Time - 5,15,30,45,60 s)	Increase in tensile strength and surface hydrophilicity	[165]
Gluten and whey protein	Low pressure glow discharge 50 kW for 5,10 and 15 min	Reduction in oxygen transmission rate; Increase in tensile strength for both protein based films	[166]
Myoproteins from Whitemouth croaker fish	Glow discharge plasma 4. kV for 2 and 5 min	Increase in tensile strength and elongation a break values for 5 min exposure	[167]
Gelatin from bovine hide	Dielectric barrier discharge 230 V and 50 kHZ	Increased the surface polarity and hydrophilicity of the films	[169]
Sodium caseinate	Dielectric barrier discharge with voltage - 60 and 70 kV	Increase in surface roughness; increase in hydrophilicity; reduction in Tg (glass transition temperature)	[168]

out improvements in functionality of protein, the modified proteins need to be used in the product development in order to assess the functionability of the modified proteins at the processing conditions. Since the plasma generation and application is a surface phenomenon it finds its lacuna in reaching the deeper tissues, though the concept of harmonic plasma has come in recent days its effectiveness needs to be studied. Cold plasma is highly researched topic but the output of the experiments is still at a lab stage. It is still in its developmental stage as it is being adapted close to around 2 % [171]. The feasibility studies for its scale up and commercialization need to be geared up. The feasibility should not only focus on initial investment but also on the energy and economic requirements for its sustained benefit. Although a comparative data has shown that the cost of energy incurred for the use of cold plasma assuming >50 kV voltage and a current of 1–2 mA is equivalent to the energy consumption by a light bulb [27]. Handling and storing of reactive species requires additional safety precautions and economic requirements. The invitro and invivo studies need to be studied to investigate the long term effect of reactive species on the human health and wellness and to develop the framework for the standards and limits for the plasma usage. The consumers and the end stakeholders need to be satisfied and confident about the use of plasma for proteins modification for which knowledge gap should be reasonably reduced and the potential benefits should be highlighted.

13. Conclusions

Cold plasma is increasingly finding its importance in diverse areas due to its potential benefits. New and novel methods of usage of plasma to the food molecules has been the focus. Most successful and industry adapted technology is the plasma enhanced chemical vapor deposition (PECVD) to coat the PET (Polyethylene terephthalate) polymer with thin layer of barrier materials. Plasma treatment is being used as inpackage plasma and encapsulated plasma in which the packaging material itself acts as dielectric medium and an increasing innovation in the scale up of plasma generators is being developed like microplasma arrays, surface and coplanar dielectric barrier discharge. The use of plasma to modify the proteins is rapidly undergoing innovations to improve the digestibility and functionality in various application. The review highlights the effect of cold plasma on protein functional property which has been investigated by structure-function relationship and the impact of reactive species on the functional properties. Ablation, Etching and cleavage of peptide bonds can be seen as starter effect of cold plasma on proteins followed by the unfolding of protein molecule, changes in secondary structure coupled with increase in surface hydrophobicity, reduction in surface charge and oxidation reaction of amino acids (particularly the sulfur containing amino acids) leading to a increase in solubility, water holding capacity, foaming, emulsion and increased cross linkage between the protein chains. The combined effect of proteins (amino acid structure, chain length, functional groups, molecular weight), process parameters (source of power, gas composition, gas combination, voltage, time of exposure, distance between the source and substrate) are the critical parameters for the effective optimization and usage of cold plasma. Further the cold plasma can be branded as clean label since no additives has been added to the food matrix. Hence cold plasma can be a potential and novel technology which can drive the future of food and nutrition security.

Funding

Project No. TKP2021-NKTA-32 has been implemented with support from the National Research, Development, and Innovation Fund of Hungary, financed under the TKP2021-NKTA funding scheme, and supported by the University of Debrecen Program for Scientific Publication.

CRediT authorship contribution statement

N. Sharath Kumar: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Aamir Hussain Dar:** Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Kshirod Kumar Dash:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Bhupinder Kaur:** Methodology, Resources, Supervision, Validation, Visualization. **Vinay Kumar Pandey:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation. **Anurag Singh:** Formal analysis, Resources, Software, Validation. **Ufaq Fayaz:** Data curation, Software, Validation, Visualization. **Rafeeya Shams:** Writing – review & editing, Visualization, Validation, Data curation, Formal analysis, Software. **Shaikh Ayaz Mukarram:** Writing – review & editing, Data curation, Formal analysis, Funding acquisition, Visualization. **Béla Kovács:** Visualization, Validation, Supervision, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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