

Improvements in Emulsifying Properties of Protein by High Hydrostatic Pressure Treatment

Nasir Mehmood Khan^{1*}, Farman Ali Khan², Ziaullah Khan³

¹Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, Dir Upper 18000, Pakistan.

²Department of Chemistry, Shaheed Benazir Bhutto University, Sheringal, Dir Upper 18000, Pakistan.

³College of Bio-system Engineering and Food Sciences, Zhejiang University Hangzhou, 310058, P. R. China

Abstract

High hydrostatic pressure is a method different to heat processing and more convenient for food product modifications. The modification by high hydrostatic pressure did not cause loses of any components of food which is considered to be essential in foods. Emulsifying characteristic of protein give a valuable position to protein in food emulsions and the improved properties of protein have been achieved by high hydrostatic pressure technology. This review covers the research conducted by high hydrostatic pressure in field of improving emulsifying properties of protein and how high hydrostatic pressure effect structure of proteins.

1. Introduction

Emulsion is the mixture of two immiscible liquids in which one of the liquid is dispersed in other liquid making unstable mixture [1]. These two liquids make a boundary in mixture which is called interface and this boundary between these two phases is count for stability of emulsion. The aim of the making emulsion is to reduce the interfacial tension between two immiscible liquids either by mechanical agitation or action of surface active agents [2]. There are two types of emulsion [i] Micro emulsion and [ii] nano emulsion. micro emulsion is thermodynamically stable and transparent and with low viscosity in which oil and water stabilized by an interfacial film of the surfactant.

The particle size of the micro emulsion is range from 50-1000 nm. While the nano emulsion is oil in water emulsion with mean droplet diameters ranging from 5 to 100 nm. Smaller the particle size greater the stability of emulsion. Many of the natural or formulated foods contain emulsion wholly or making some of its parts during their production [1]. Food emulsion of oil in water (O/W) created by homogenization of oil and liquid phase together in the presence of one or more than one emulsifier [3-5]. The study of food emulsion is complex if many interactions are present in emulsion due to different components. An emulsifier is a surface active substance that adsorb at the surface of droplets formed during homogenization, where it reduce the interfacial tension and facilitates further droplets disruption [6,7]. Emulsifier can be divided into two types (i) small molecules such as mono and diglycerides, sucrose ester sorbitan ester (span), polysorbates (tween), steroyllactylates, lecithin and derivatives (ii) macromolecule: protein such as bovine serum albumin, β -lacto globulin, lysozymes and ovalbumin. Protein is a surface active molecule that is commonly used to stabilize emulsion in food products. During emulsification protein molecules are immediately adsorbed on the newly formed droplet surfaces, and reduce the interfacial tension between water and oil and provide protective coating. Protein facilitates the formation of oil droplets and then play role in emulsion stability and produces desirable physiochemical properties in oil in water emulsions [8]. Improvement of the functional property of protein in

emulsion is depend on structure of protein and for course of improvement of emulsifying properties of protein may be achieved by modifying the protein structure by chemical, enzymatic and physical treatment [9-11].

1.1: Heat Method: Heat treatment changes the protein conformation by disrupting the Vander Waals interactions, hydrogen bonds and electrostatic forces [12, 13] exposing some hydrophobic protein residues previously hidden inside its globular structure. The importance of exposing a certain number of the hydrophobic groups of globular proteins in the improvement of protein functionality has been discussed [14, 15]. It has been reported for soy protein isolates the heat treatment unfolds the protein and improves surface properties like hydrophobicity, emulsification capability, and stability [16]. In the solution of soy protein in proper heat treatment can make protein moderate modifications, improve the function of proteins; however the existing state of aqueous solution heat under high protein concentration defects, easily to form a gel, gathered precipitation or water phase heat treatment is difficult to control etc.

1.2: Chemical Method: For the better utilization of the protein it is necessary that protein cover wide variety of functional and nutritional characteristic. This is usually difficult because many native proteins having limited functionality, therefore there is technical need for the development of methodology to improve functional properties of protein. Chemical derivatization through acylation of amino acid residue with acetic and succinic anhydride has been used to improve functional properties of many plant proteins [17]. O.S. Lawal (2007) reported for bambarra groundnut that acylation of bambarra bean protein concentrate produced pronounced changes in its functional properties; their study also revealed that solubility of protein concentrate improved following acetylating and succinylation and this improved both emulsifying and foaming properties of the native protein concentrate [18]. These modifications have been applied to many plant proteins including mucuna bean, jack bean, soybean, peanut, sunflower, pea, winged bean, rapeseed, and cotton [19-26].

1.3 : Enzymatic Method : Many proteins, particularly those in plants, require structural modifications to improve their functional properties for expanded use. Several chemical and enzymatic methods are described for food protein deamidation to improve solubility, emulsification, foaming, and other functional properties of the proteins. The use of enzymes in protein modification is more desirable than chemical

treatments because of their speed, mild reaction conditions, and their high specificity [27]. Lin Chen (2011) reported for soy protein isolates that combined extrusion pre-treatment and controlled enzymatic hydrolysis using pancreatin led to significant changes in physico-chemical properties and interfacial properties of SPI, and produced prominent benefits in improving emulsifying capability of SPI and the stability of their emulsions [28]. María Del Mar Yust (2010) reported for chickpea proteins that the partial hydrolysis of chickpea protein isolate with immobilised Alcalase is a helpful strategy to improve some functional properties of intact proteins, such as solubility, oil absorption capacity, and foaming capacity and stability [29].

2. High hydrostatic pressure and protein

High hydrostatic pressure is an emerging technology for the modification of protein. High pressure mainly effects the noncovalent attraction in protein molecule such as hydrogen bonds, hydrophobicity and electrostatic interaction which are concerned with stabilization of tertiary structure of protein[30]. High pressure can affect the protein conformation and can lead to protein denaturation, aggregation or gelation which depending on the protein structural configuration, pH, ionic strength and duration of the applied parameter of pressure and temperature. If the protein is subjected to high hydrostatic pressure in dilute solution then on release of high pressure the formation of new bonds can lead to modified structure of protein which have alert functional properties as compared to native protein [31]. Recent development in high pressure technology is now reliable where some of the food stuff can be commercially processed isobarically at pressure in hundreds of MPa [32]. Typical pressure used in commercial processes are in the range of 100-600 MPa up to 30 minutes which is sufficient for bringing the changes up to desired level of processing of extended shelf life and molecular changes [33,34]. It is already known that high pressure induced changes in structure of protein from 200 MPa to 500 MPa causing unfolding of protein which lead to aggregation and formation of gel structure [35]. The real unfolding mechanism of protein molecule by high pressure is yet not fully understood [36]. However it has been suggested that pressure cause change in protein molecule by rupturing of non-covalent interaction and weak hydrogen bonds. This consequently ensures the retention of essential nutrients and thereby improving the quality of product obtained.

3. Emulsifying properties of protein and high hydrostatic pressure

Protein is well known as an emulsifier to reduce the interfacial tension between oil and water emulsion. Protein is often used in emulsion to increase their stability. Protein is much larger and more complex emulsifier. Formation of protein stabilized emulsion require the protein reach the water /oil interface and then unfold so that its hydrophobic group can contact with lipid phase. In the native protein structure the hydrophobic group lying in the interior of the molecule. As high pressure is well known for rupture of non-covalent attraction and unfolding of protein so the hydrophobic interaction is affected by high pressure to expose the hydrophobic group of protein molecule in solution to contact with the lipid molecule in emulsion and to reduce the interfacial tension and give stability to emulsion. Surface and emulsifying properties of protein are very strongly concerned to the protein structure [37]. Emulsifying activity of protein is determined by protein surface hydrophobicity and solubility while molecular flexibility of protein play role in emulsion stability [38, 39]. High pressure can play a role to change the structural properties of protein and give them new shape for improved emulsifying properties. Many of the plant protein show improved emulsifying properties by high hydrostatic pressure however these all improvements are achieved at different concentration of protein, pH, duration and level of high hydrostatic pressure treatment. Among plant protein up to date soy protein get a lot of attention from researchers because they have high nutritional value. Other plant protein such as chick pea, cowpea, lupin, wheat, also show improved emulsifying properties by high hydrostatic pressure [40-43].

4. Effect of high pressure on molecular basis of protein

It is important that the forces and energies involved in the achievement and keep maintain the protein native structure is described after the treatment of high hydrostatic pressure.

4.1: Hydrophobic Interaction: The tendency of hydrocarbons (or of lipophilic hydrocarbon-like groups in solutes) to form intermolecular aggregates in an aqueous medium, and analogous intramolecular interactions. The name arises from the attribution of the phenomenon to the apparent repulsion between water and hydrocarbons. For protein as a emulsifier it is must that protein become unfold at interface and expose hydrophobic group to contact with the lipid phase. In

native protein most of the nonpolar amino acid chains are located in the inner part of the molecule. Protein have charged group at the surface of the molecule and in contact with water molecules. If the protein molecule reaches to the interface there is less opportunity for the charged group to interact with solvent. High hydrostatic pressure play role in exposing the hydrophobic group of the protein in aqueous phase due to unfolding of protein. Once a protein unfolds the hydrophobic groups are inserted into the lipid phase. In theory a measure of the relative hydrophobicity of a protein should be related to its ability to function as an emulsifying agent [44, 45]. High hydrostatic pressure is one of the methods to change the arrangement of hydrophobic group of protein and give them unfold shape for performing good emulsifier in oil/water emulsion. High hydrostatic pressure effect on hydrophobicity is reported by Xian-Sheng et.al for soy protein isolates that gradual and significant ($P < 0.005$) increase of hydrophobicity at concentration 1 and 3 % when the pressure increased from 0.1 to 600 MPa while a significant increase in hydrophobicity was observed at 5% of protein concentration at 400 MPa. These results indicate that high hydrostatic pressure treatment resulted in the exposure of the hydrophobic regions to exterior of the protein molecule [46]. Nicolas Chapleau et.al reported for myofibrillar protein that increasing in pressure up to 400 MPa lead to the exposure of the hydrophobic groups [47]. Increase in hydrophobicity is also reported by Cecilia Puppo et.al for soy protein isolates at different pH medium (pH8, pH3). These results indicating that pressure treatment produced a molecular unfolding of the protein which results in exposure of the hydrophobic groups to the medium [48]. Molina et.al also reported the increase in hydrophobicity for fractions 11S and 7S of soy protein isolates [49].

4.2: Electrostatic Interaction: Electrostatic interaction is count for determination of protein molecular structure [50]. Protein contains a number of amino acids that can ionize to form either positively charged ions or negatively charged ions [51]. If the protein contains many similar charged groups then it will have more extended configuration because increase in average distance between the charges and therefore minimizes the unfavorable electrostatic repulsion. And if the protein contains many oppositely charged groups, it is more likely to fold up into a compact structure that maximizes the favorable electrostatic attraction [52]. Electrostatic interaction also play role in determining the aggregation of proteins in solution. The high pressure induced the non-covalent interactions with in protein molecules which may imply formation of new complexes by means of intra and intermolecular bonds.

Masson (1992) reported that if high pressure is higher than 200 MPa it modified the electrostatic interactions which lead to structure modifications as far as protein aggregation [53]. N.Chapleau, (2003) reported for lupin protein emulsion that flocculation's index decreased with increase in pressure, this is because of modification in electrostatic interaction of the proteins by high pressure. The electrostatic repulsion between droplets was higher when protein has been pressurized which lead to better emulsifying properties of lupin protein [54].

4.3: Hydrogen Bonding: Hydrogen bonds are a relatively strong type of molecular interaction and therefore a system attempts to maximize the number and strength of the hydrogen bonds formed. Study of model system showed that hydrogen bonds are stabilized by high pressure [55]. This resulted from the smaller inter-atomic distances in the hydrogen bonded atoms. The stabilizing effect of pressure on hydrogen bonding is indicated by the infrared spectra of the α -helix in myoglobin [56]. The lengths of existing hydrogen bonds within proteins have been observed to shorten under hydrostatic pressure. Shortening of hydrogen bonds at high pressure was first detected ^1H Nuclear Magnetic Resonance (NMR) for liquid water and ethanol [57, 58]. The shortening of hydrogen bonds, in addition to the collapse of internal cavities, can contribute to the compression of proteins under pressure [59, 60].

4.4 : SS Bonding: The three dimensional structure of proteins can be stabilized by covalent and non-covalent interactions. Covalent interactions consist of disulfide bonds. Several approaches have been used to modify disulfide bonds and to test whether the resulting protein has enhanced emulsifying properties [61, 62]. Protein molecule that contain crosslinks such as disulfide bonds are more rigid than it is not easy to unfold and then not effective in emulsion formation. The presence of crosslinks is related to stability of the native protein structure and makes the molecules resistant to unfolding or denaturation. The changes in SS bonding are often assayed when exploring the properties of protein in foods [63]. High hydrostatic pressure modifies the structure of protein by disrupting the interactions between protein molecules so that is making reason for changing in the SS bonding.

4.5: Secondary structure: As protein upon reaching the surface and exposing hydrophobic groups for performing the function of emulsifier then it is important to highlight the relevance of the secondary structure connect64, 65. Due to the changing in non covalent bonding in protein structure after high

pressure we should be notice the secondary structure as it have different characteristic compared to native structure. Chuan-He Tang (2009) notice for soy protein isolates that change in secondary and tertiary structure induced by high pressure treatment [66]. L.-A Tedford (1999) notice the change in secondary and tertiary of ovalbumin, lysozymes, and beta-lacto globulin. These results show that all these three proteins are pressure-induced process had greatest effect at 600 MPa [67]. Nicolas Chapleau (2003) result for isolated bovine myofibrillar protein show high pressure processing induced the protein to adopt a new structure reminiscent of the molten globule state. Functional properties such as gelling, emulsifying and foaming are highly linked with the structure of proteins; these properties will be modified by high pressure processing. The aggregation of myofibrillar protein by high pressure was accompanied by enhanced binding of aniline-1-naphthalene-8-sulphonic acid which indicates an increase in hydrophobic bonding of myofibrillar protein [68].

5. Conclusions

Physical (heat), chemical (acylation, phosphorylation) and enzymatic methods have all been used to modify the functional properties of proteins of the different sources to get the desirable improvement in emulsifying, foaming and other surface properties of protein, however high hydrostatic pressure is a technique by which the functional characteristic of protein can be modified for improved emulsifying properties of protein in consumer friendly and play role in the product safety and green way which lead to product of added value. Despite of modification and improving functional properties such as emulsifying properties by treatments such as heat, chemical and enzymatic method modification processes raise a problem of safety regarding to food. These treatments cause serious physical and chemical change that affect the protein functionality. Although these technologies have helped to ensure a high level of food safety, the heating and cooling of foods may contribute to the degradation of various food quality attributes. The colour, flavour and texture of foods processed by heating may be irreversibly altered. To ameliorate the undesirable thermal effects on foods, considerable efforts has been made in commercial and academic circles to develop non-thermal technologies other than heating or cooling operation.

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