Enzymatic modification of proteins

Modification of the molecular structure of food proteins with enzymes is an attractive way of improving the functional and nutritional properties of these proteins. Enzymes provide several advantages including fast reaction rates, mild conditions and high specificity. Proteases and transglutaminase are the most frequently used enzymes for modifying the polypeptide backbone of food proteins. Functional properties of proteins (solubility, gelation, and emulsification and foaming) are closely related to their size, structural conformation, and level and distribution of ionic charges. Limited proteolysis can enhance their functional properties over a wide pH range and processing conditions. These changes in the functional properties are attributed to changes in charge, hydrophobicity and molecular mass in going from protein to peptide mixtures.

1. Limited enzymatic hydrolysis

Endopeptidases cleave the peptide linkage between two adjacent amino acid residues in the primary sequence of a protein yielding two peptides. The specificity of an enzyme is a big factor influencing both the number and location of the peptide linkages that are hydrolysed. Proteolysis can proceed either sequentially or through the formation of intermediates that are further hydrolysed to smaller peptides often termed Zipper mechanism. One of the key parameters in protein hydrolysis is the degree of hydrolysis (DH). This is defined as the percentage of peptide bonds cleaved. The enzymatic hydrolysis of proteins depends on pH, temperature and the concentration of substrate and enzymes.

Hydrolysis of peptide bonds causes three distinct changes in proteins:

i. The NH3+ and COO- content of protein increases which increases its solubility.
ii. The molecular weight of the protein/polypeptide decreases.
iii. The globular structure of the protein is destroyed and the buried hydrophobic groups become exposed.

Enzymatically hydrolysed proteins are classified based on the molecular weight distribution of the resultant hydrolysate. Larger peptides (2-5 kDa) are mainly used as functional ingredients or in personal care products. Medium sized peptides (1-2 kDa) are used in clinical nutrition. Smaller peptides (<1 kDa) are used in infant food products required reduced allergenicity.

2. Liberation of biologically functional peptides

Until, recently, food proteins were regarded mainly as a source of amino acids essential for the proper functioning of the human body. Recent years have witnessed a growing interest in the use of biologically active peptides (BAP) in the production of functional foods. In addition to its basic function, every protein can play the role of a precursor of biologically active peptides, which are activated only after they have been released from the protein chain by proteolytic enzymes**.** Biologically active peptides found in food proteins usually contain 2 to 9 amino acid residues. Some, however, may comprise even 20 or more amino acid residues. For example, caseinomacropeptide contains 64 amino acid residues and shows many types of activity. Biologically active peptides may be released from their protein

precursors in three ways: during (a) enzymatic hydrolysis by digestive enzymes; (b) fermentation processes involving proteolytic starter cultures; and (c) proteolysis involving enzymes of animal and plant origin or microbial enzymes.

In most cases, these protein hydrolysates and peptides have demonstrated better bioactivity compared to their parent proteins, and this shows that hydrolysis of peptide bonds is important in liberating the potent peptides. Several factors affect the bioactive properties of the peptides including the enzymes used for hydrolysis, processing conditions, and the size of the resulting peptides, which greatly affects their absorption across the enterocytes and bioavailability in target tissues. Most reported BAPs are produced by *in vitro* enzymatic hydrolysis or fermentation. After selecting an appropriate food protein, enzymatic hydrolysis is performed using single or multiple specific or nonspecific proteases to release peptides of interest.

Upon oral administration, BAP may affect the major body systems, namely, the cardiovascular, digestive, immune and nervous systems, depending on their amino acid sequence. These beneficial health effects may be attributed to numerous known peptide sequences exhibiting antimicrobial, antioxidative, antithrombotic, antihypertensive and immunomodulatory activities. Once these peptides released from parent proteins, they may act in the body as regulatory compounds with a hormone-like activity.

3. Enzymatic cross- linkage reaction

Transglutaminase (TG EC 2.3.2.13, glutaminyl-peptide, amine-γ-glutamyl transferase,) catalyzes an acyl transfer reaction between the γ-carboxyamine group of a protein-bound glutamyl residue and a primary amino group of various substrates including the ε – amino group of lysine or lysyl residues in proteins, resulting in polymerization or amine incorporation (Fig. 5, 6) (Buchert, et al., 2010). The crosslink formed is called a ε – (γ-glutamyl)-lysine isopeptide bond (Kumagai, 2012). In the absence of amines, water serves as acyl acceptor leading to the conversion of glutamines to glutamic acid (deamidation) but is more frequently used for attachment of amino acids and especially cross-linking (reactions with primary amines). Cross-linking results in the formation of bonds between glutaminyl and lysyl residues from the same protein molecule (intramolecular) or from two separate molecules (intermolecular). By this intermolecular cross-linking covalent interactions between proteins are brought about resulting in, for instance, enhanced gel strength or network formation in milk, meat and bakery products.

The applications of enzymatic cross – linking of proteins have been used in cereal products, milk products and meat products.

i. Cereal products:

Cross linking of cereal proteins has major potential in modification of technological and sensory properties of cereal products. TG mediated protein crosslink results in tailoring of rheological properties of gluten by decreasing dough extensibility, increased water absorption and hinder the growth of air bubbles in the dough, thereby decreasing volume of bread. In the preparation of pasta and noodles, cross linking of gluten causes an increase in resistance for thermal processing.

ii. Milk products:

Creation of inter-/ or intra molecular covalent bonds by TG cross-linking of milk proteins results in modification of textural and water binding properties of milk products especially, in low fat or protein products with acceptable texture.. In the production of fermented milk product such as yogurt, the introduction of covalent bond by enzymatic cross linking into gel network results in increase in gel firmness and sensory properties.

iii. Meat products:

Enzymatic cross linking of the myofibrillar protein myosin improves the texture and water holding properties of meat or fish products and add value to meat and fish of poorer quality. In addition to restructuring, TG is successfully exploited in improving textural properties of heated meat products such as hams and sausages and enhancing surimi (fish paste) gelation and textural properties.

Changes in functional properties:

a) Solubility

Proteolytic modification has special importance for the improvement of solubility of proteins. This effect becomes significant even after very limited proteolysis. Zein, the maize protein that is highly insoluble at pH 2-5, exhibited good solubility (30-50%) at this pH range when only 1.9% of the peptide bonds were split by treatment with trypsin. Hydrolysis of casein to DH 2 and 6.7% with *staphylococcus aureus* V8 proteases increased the isoelectric solubility to 25 to 50%, respectively.

b) Emulsifying property

Emulsifying properties of proteins are sensitive to proteolytic modification. Limited hydrolysis (DH 2 and 6.7%) of casein decreased the emulsifying activity (EA) at all pH whereas the emulsion stability (ES) at DH=2% was higher than native casein. The EA of casein was reported to decrease with increasing net charge and with the decreasing hydrophobicity due to proteolysis. The beneficial effect of limited proteolysis of globular proteins on EA and ES may be due to exposure of buried hydrophobic groups, which may improve the hydrophobic – hydrophilic balance for better emulsification. The loss of hydrophobic peptides from the surface of the proteins may directly result in an increase of the surface hydrophobicity, thus favouring surface adsorption. The detrimental effect of excessive digestion is related to the loss of globular structure and optimum size of split peptides, resulting in formation of a thinner protein layer around the oil droplets and a loss of stable emulsion.

c) Foaming property

The excellent foaming properties of soybean protein hydrolysate made using acid aspartic protease from *mucor miehei* might be due to narrow molecular weight distribution of peptides produced by this enzyme. This enzyme is very specific and can hydrolyze very few of the peptide bonds in soy protein. At 0.5% DH, the soy protein hydrolysate made with this enzyme behaves like an egg white substitute with excellent foaming properties. Protamex hydrolysates of sodium caseinate (DH 0.5 and 1.0%) displayed increased from expansion at pH 2, 8 and 10 as compared to native casein.

d) Gelation

Hydrolysis was presumed to be detrimental to the gelling properties of protein because of the reduced hydrophobicity of hydrolysates. The increased net charge on the protein results in increased charge repulsion between peptides, decreasing their gelling ability. The loss of gelation ability of soy protein isolate is used to advantage in manufacture of soy protein hydrolysate that can be heat processed without changing their flow properties. Limited proteolysis of whey protein isolate exhibited gelation characteristic that were different from those of untreated whey protein isolate.