Crosslinking Food Proteins for Improved Functionality

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Abstract

Different possibilities for protein crosslinking are examined in this review, with special emphasis on enzymatic crosslinking and its impact on food structure. Among potential enzymes for protein crosslinking are transglutaminase (TG) and various oxidative enzymes. Crosslinking enzymes can be applied in cereal, dairy, meat, and fish processing to improve the texture of the product. Most of the current commercial applications are based on TG. The reaction mechanisms of the crosslinking enzymes differ, which in turn results in different technological properties.

INTRODUCTION

Food structure is a very important sensory property that affects consumer choices and food stability. Food structure can be tailored in many ways, including by addition of hydrocolloids and thickeners or by creating covalent crosslinks between food biopolymers, i.e., proteins or carbohydrates. Different possibilities for protein crosslinking are reviewed in this chapter with special emphasis on enzymatic crosslinking and its impact on food structure. Crosslinks can be introduced to food matrices by chemical, enzymatic, and physical means as reviewed by Singh (1991) and Gerrard (2002).

Enzymatic crosslinking of food biopolymers is an attractive option because of the specificity of enzymes and mild reaction conditions needed. The crosslinking can be a result of direct enzymatic catalysis of crosslink formation or occur indirectly by enzymatic production of a crosslinking agent, such as H_2O_2, which in turn is able to oxidize reactive structures with subsequent crosslink formation. Proteins have several reactive groups for crosslinking enzymes, such as glutamine, lysine, tyrosine, and cysteine residues. The reactions obtained are dependent on the type of an enzyme used, the accessibility of the target reactive groups in the biopolymer, and on the process conditions used.

NONENZYMATIC CROSSLINKING OF PROTEINS

Disulfide crosslinking has a significant impact on protein functionality and affects gelling (Tadayasu et al. 1980, Lauber et al. 2000), heat stability (Tatu et al. 1990, Singh 1991), emulsifying capability, foaming capability (Làsztity et al. 1998), thermo-rheological properties (Lee et al. 1997a), and dough properties (Gerrard et al. 1998). This crosslinking reaction can be induced via enzymatic catalysis, mechanical mixing, or heat treatment and proceeds via the oxidation of the sulfhydryl groups of two cysteine residues in a protein. Intentional creation of inter- and intramolecular disulfide bonds can lead to the formation of a protein network, which adds rigidity to the system and endows the protein with an enhanced stability and resistance to proteolysis. Therefore, on properties such as stability, firmness, viscosity, and elasticity, introduction of disulfide crosslinks often has a strengthening role.

The Maillard reaction is a complicated naturally occurring reaction; however, much is still unknown about its reaction mechanism. In an early stage of Maillard reaction, reactions occur between amine groups of proteins and carbonyl groups often within a reducing sugar, finally resulting in the formation of Amadori products. During the intermediate stage and advanced stage, reactive intermediates such as sugar-derived dicarbonyl compounds (e.g., methylgloxal, 3-deoxyosones, or glyoxal) are produced, eventually resulting in the formation of a large variety of polymerized compounds (Biemel et al. 2001, Grandhee & Monnier 1991, Meade et al. 2003). Heat treatment significantly accelerates this reaction without any extraneous chemicals or enzymes. This makes the heat-induced Maillard reaction a very promising approach for the modification of the functionality of food proteins (Gerrard et al. 2002). Additionally, the Maillard reaction introduces covalent bonds between proteins and carbohydrates, which are both food components. Extensive research has been carried out to utilize this reaction to improve the functionality of proteins.

Alkaline and/or heat treatment of proteins leads to the formation of inter- or intramolecular crosslinks based on the formation of a dehydroprotein, which tends to react with ε-amino groups or sulfhydryl groups. An unfavorable side effect here is that this reaction is associated with the production of several types of crosslinked amino acids such as dehydroalanine, lysinoalanine, and histidinoalanine, which may affect the nutritional value of the treated protein. Friedman (1999) described the chemical mechanism of the formation of these crosslinked amino acids during alkali treatment and how they affect the food protein product. Another nonenzymatic crosslink induced by severe heat treatment is isopeptide crosslinking, in which pure proteins can be polymerized as a result of the formation of ε-N-(γ-glutamyl)lysine or ε-N-(γ-aspartyl)lysine crosslinks via condensation of the lysine residues with the amide groups of glutamine or asparagine residues (Singh 1991).

Dityrosine and trityrosine crosslinks are widely observed in nature in long-lived structural proteins such as collagen, keratin, elastin, resilin, and fibroin. Under photo-oxidative conditions, tryptophan and tyrosine side chains are coupled, giving fluorescent biaryl products (Stachel et al. 1996, Amadó et al. 1984). Biaryl crosslinks share the same topology as disulfide crosslinking, although the reactions are more demanding on conditions (a harsh reaction condition is needed) than the formation of disulfide bonds (Giulivi & Davies 1993). Formation of dityrosine can also be promoted by metal-peptide complexes or hydrogen peroxide (Brown et al. 1995, Singh 1991). This type of crosslinking was found to play a role in the formation of crosslinked gluten protein in wheat and is studied because of the importance for the structure of the gluten network (Peña et al. 2006, Tilley et al. 2001).

Proteins can also be crosslinked via chemical crosslinkers that are bifunctional chemical compounds that possess specific reactive moieties for sulfhydryl, amine, or carbohydrate functional groups, and inter- or intramolecular crosslinks are formed via covalent bonds. Furthermore, other more rare cases of crosslinking such as lipid peroxidation, uncharacterized Maillard reaction, and hydrocarbon crosslinking are reported (Singh 1991, Friedman 1999).

ENZYMOLOGY AND MODE OF ACTION OF CROSSLINKING ENZYMES

Transglutaminase

Transglutaminases (TGs; EC 2.3.2.13, γ-glutamyl-peptide, amine-γ-glutamyl transferase) belong to the group of acyltransferases, which catalyze acyl-transfer reactions between a γ-carboxyamine group of a peptide- or 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 (**Figure 1*a,b***). The crosslink formed is called an ε-(γ-glutamyl)lysine isopeptide bond (Folk & Finlayson 1977, Griffin et al. 2002). During the reaction, one molecule of ammonia is generated per crosslink. If the amine substrates are not available as acyl acceptors, TG can catalyze deamination of glutamyl residues using water as an acyl acceptor (**Figure 1*c***). In contrast to their limited glutamine (an acyl donor) substrate specificity, TGs possess a wide specificity for the acyl acceptor substrates.

The first TG, cytoplasmic TG 2, was identified in guinea pig liver almost 50 years ago (Sarkar et al. 1957), and since then TGs have been identified in various mammals, fish (Ha & Iuchi 2003, An et al. 1996), and plants (Serafini-Fracassini et al. 1995). Biotechnical exploitation of TG in various food and nonfood applications was launched more than ten years ago after these enzymes had been discovered in bacteria such as *Streptomyces mobaraense* (Ando et al. 1989), *S. ladakanum* (Tsai et al. 1996), *S. cinnamoneum* (Duran et al. 1998), and *Bacillus subtilis* (Kobayashi et al. 1998).

Properties of TGs vary considerably depending on the source. Mammalian TGs are generally dependent on the presence of Ca^{2+} (Ichinose et al. 1990). In contrast to the mammalian TGs, the *Streptomyces* TGs are independent of a Ca^{2+} cofactor. *S. mobaraensis* TG is a monomer with a molecular mass of 38 kDa (Ando et al. 1989), and that of *S. ladakanum* has a molecular mass of 30.5 kDa (Tsai et al. 1996). *S. mobaraense* TG is a secreted protein, which is activated outside the cytoplasm membrane by posttranslatorial processing (Pasternack et al. 1998). The enzyme participates in mycelial growth and has a role in morphological differentiation. The enzyme

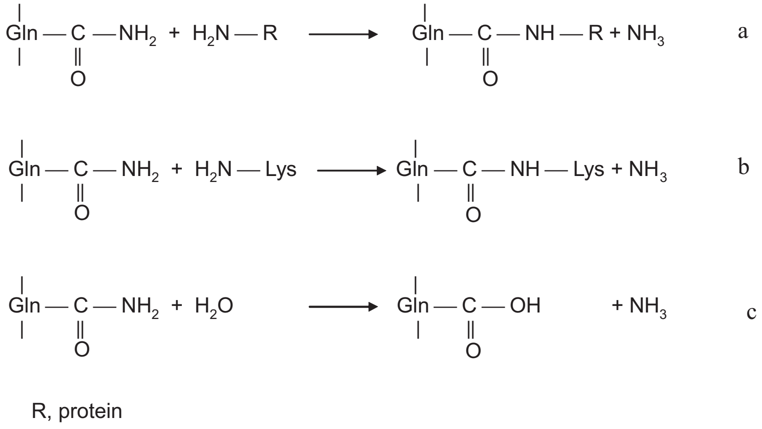


Figure 1

TG-catalyzed reactions: (*a*) crosslinking via a lysyl residue, (*b*) incorporation of an ε-amino group of lysine and (*c*) deamidation.

shows an optimum in the pH range of 5–8, retains full activity at 40°C for 10 minutes but is rapidly inactivated at 70°C (Ando et al. 1989). Microbial TGs are hitherto the only commercially available food-grade protein crosslinking enzymes.

TGs have been reported to crosslink myofibrillar proteins (Kahn & Cohen 1981), gelatin (Broderick et al. 2004), milk proteins (reviewed by Jaros et al. 2006a), soy proteins (Nio et al. 1985, Babiker 2000), egg yolk and white (Sakamoto et al. 1994, Lim et al. 1998), fish proteins (Joseph et al. 1994, Tsai et al. 1996), and cereal proteins (Larré et al. 2000, Basman et al. 2002a,b). Modification of proteins by TG may be exploited in a number of applications in the food industry such as by improving rheological properties, improved gel formation and gel properties, modification of protein solubility, foaming properties, and water-holding properties (Matheis & Whitaker 1987, Nielsen 1995, Seguro et al. 1995, Kuraishi et al. 1997, Motoki & Seguro 1998, Zhu et al. 1999, Jaros et al. 2006b).

Tyrosinase

Tyrosinase (monophenol, *o*-diphenol:oxygen oxidoreductase, EC 1.14.18.1) belongs to the type-3 copper proteins. The active site contains two copper atoms; each copper atom is coordinated by three highly conserved histidines (Lerch et al. 1986). Tyrosinases are ubiquitous in nature; they are found in mammals, plants, fungi, and microorganisms. The biological function of tyrosinase is related to the biosynthesis of melanin pigments (Ito et al. 1984). In plants, tyrosinases play a defense role by being upregulated in wounded tissues (van Gelder et al. 1997). Plant tyrosinases are also responsible for the browning of fruits and vegetables. In fungi, tyrosinases are mainly located in the cytoplasm and are involved in the formation and stability of spores and resistance to stress such as dehydration and UV and gamma radiation (Halaouli et al. 2006, Mayer 2006).

Tyrosinase is a bifunctional enzyme as it catalyzes ortho-hydroxylation of monophenols (monophenolase or cresolase activity) and subsequent oxidation of diphenols (diphenolase activity) to quinones (**Figure 2**). Molecular oxygen is used as an electron acceptor, being reduced to water without hydrogen peroxide formation (Robb 1984). One mole of O_2 is required to oxidize 1 mole of monophenol, whereas one half of a mole of O_2 is needed to oxidize 1 mole of diphenol. In both activities, the final product released is a quinone, which is the active precursor of

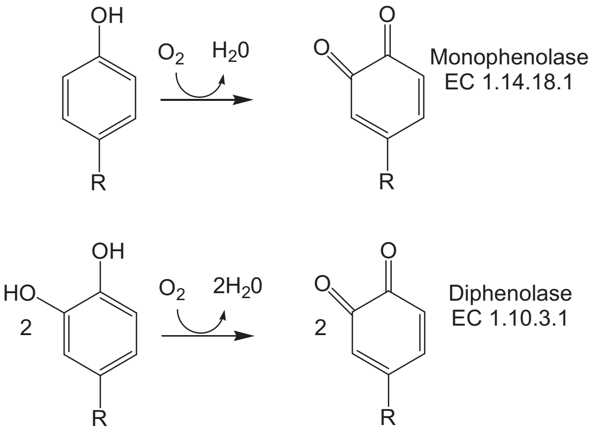


Figure 2

Reactions of phenolic substrates catalyzed by tyrosinases.

pigments constituting melanins. The reaction mechanism of tyrosinase is comprehensively reviewed by Sánchez-Ferrer et al. (1995) and Rescigno et al. (2002). Tyrosine and L-dopa are the natural substrates of tyrosinase in the melanogenesis pathway. In addition, tyrosinase reacts on various monophenolic and diphenolic small compounds, such as phenol and catechol or phloretic acid and hydrocaffeic acid. Tyrosinase can also oxidize tyrosine in the side chain of proteins to the quinone, which can further crosslink with lysyl, tyrosyl, and cysteinyl residues present in a different protein (Selinheimo et al. 2008).

Most eukaryotic tyrosinases are multimeric, whereas the bacterial enzymes appear to be either monomeric (*Streptomyces* sp.) or dimeric (*Bacillus thuringiensis*) (Liu et al. 2004, Claus & Decker 2006). The monomeric forms of tyrosinase from *Agaricus bisporus* have molecular masses of 43 kDa and 13.4 kDa (Kim & Uyama 2005). The molecular mass of the tyrosinase monomer isolated from the fungus *Trichoderma reesei* is 43 kDa (Selinheimo et al. 2006a). The largest tyrosinases have been characterized in mammals, the human tyrosinase monomer having a molecular weight of 62–67 kDa (Kwon et al. 1987, Kim & Uyama 2005). Many microbial and plant tyrosinases have their pH optima in a slightly acidic pH range and often lose their activity within few minutes at temperatures higher than 70°C (Zawistowski et al. 1991).

During the past decade the knowledge of fungal tyrosinases has increased. The best characterized fungal tyrosinases are from cultures of *Agaricus bisporus* (Wichers et al. 2003), *Neurospora crassa* (Lerch 1983), *Pycnoporus sanguineus* (Halaouli et al. 2005), and *Trichoderma reseei* (Selinheimo et al. 2006a). *A. bisporus* tyrosinase is today the only tyrosinase preparation commercially available for research purposes. In contrast to the other characterized tyrosinases, *T. reesei* tyrosinase is an extracellular enzyme showing good crosslinking capabilities (Selinheimo et al. 2006a, 2007a).

Tyrosinase from the filamentous fungus *T. reesei* can polymerize random coil proteins such as α- and β-caseins (Selinheimo et al. 2006b, 2007b, Monogioudi et al. 2009, Mattinen et al. 2008) as well as short tyrosine-containing peptides (Mattinen et al. 2008). Tyrosinase catalyzed oxidation of the phenolic ring of tyrosine side chain is assumed to proceed via L-dopa (3,4-dihydroxy-L-phenylalanine) intermediate to diquinone as in the case of single tyrosine amino acid (Kim & Uyama 2005). These diquinones are extremely reactive and can further react with various amino acid side chains as sulfhydryls, amines, amides, indoles, and other tyrosines commonly present in proteins, resulting in the formation of inter- and intramolecular crosslinks (Ito et al. 1984, Burzio & Waite 2000, Bittner 2006, Mattinen et al. 2008).

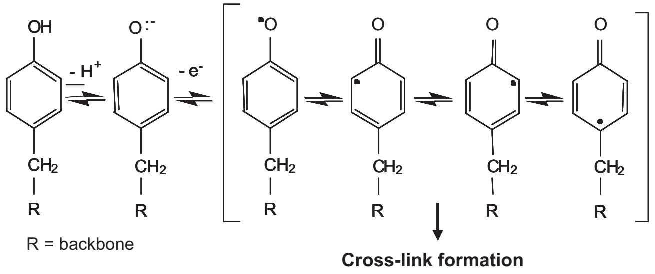
The chemical nature of various covalent bonds catalyzed by tyrosinases on proteins is not yet fully characterized. However, it is known that the oxidation products are very colorful, and the shade of these colors is varying from purple to black via various red and yellow shades, depending on the amino acid composition of the substrate (Sánchez-Ferrer et al. 1995, Bittner 2006, Monogioudi et al. 2009). The final color of the oxidation product is a very complex mixture of various shades resulting from the formation of different types of crosslinks. The shade of the color depends also on the pH of the sample. It has been assumed that the colors observed in crosslinked proteins can be attributed to the formation of localized conjugated double bond structures between the aromatic rings of tyrosine residues (Roberts et al. 1972), as well as to chemical bonds between aromatic rings and free amino groups (de Oliveira et al. 2006) as a result of the nonenzymatic chemical reactions of diquinones produced by tyrosinases (Raper & Wayne 1928, Ito et al. 1984). As a result of the color formation, the applicability of tyrosinases in certain food applications may be limited.

Laccases

Laccases (EC 1.10.3.2, benzenediol: oxygen oxidoreductase) are multi-copper enzymes catalyzing oxidation of a wide variety of phenolic compounds by a single-electron removal mechanism, which results in the formation of free radicals with concomitant reduction of molecular oxygen to water (Yaropolov et al. 1994, Thurston 1994) as shown in **Figure 3**.

Laccases are common enzymes in nature, occurring widely in fungi (for a review see Halaouli et al. 2006), whereas in higher plants their occurrence is more limited (Mayer & Stables 2002). The majority of laccases characterized so far have been derived from fungi, especially from white-rot fungi, in which their role is related to lignin degradation. In higher plants, laccases are involved in cell wall formation and lignin biosynthesis (Thurston 1994, Gianfrida et al. 1999) together with peroxidises. Laccases have been reported to be involved in cuticle sclerotization in insects (Kramer et al. 2001) and in the bacterial species of *Bacillus subtilis* in pigment production (Martins et al. 2002).

Many fungal laccases have their pH optima in an acidic pH range, although also neutral and alkaline laccases have been reported (Kiiskinen et al. 2002, Martins et al. 2002). The pH optima of laccases are not only affected by the enzyme but also by the substrate. The increasing pH decreases the redox potential of the phenolic substrates and makes the substrate more susceptible



$$4 \text{ Substrates (reduced)} + O_2 \xrightarrow{\text{laccase}} 4 \text{ Substrates (oxidized)} + 2\, H_2O$$

Figure 3

Oxidation of tyrosine side chain in proteins by laccase.

to oxidation. The stability of laccases is in general better in alkaline pH ranges (Xu 1996, Chefetz et al. 1998). Temperature stabilities of laccases vary considerably, depending on the source of the enzyme. In general, fungal laccases are stable at 30–60°C. The most thermostable laccases are from bacterial origin. For example, the half-life of the *Streptomyces lavendulae* enzyme is reported to be 100 min at 70°C (Suzuki et al. 2003), and the half-life of *Bacillus subtilis* CotA is 112 min at 80°C (Martins et al. 2002).

Laccases occur as monomeric proteins, and the molecular size typically varies between 40–100 kDa. All laccases have rather similar 3D structures containing three so-called cupredoxin-like domains, which are all needed for the catalytic activity. The copper atoms are organized into mononuclear and trinuclear centers. Presently, there are several complete laccase structures available (Bertrand et al. 2002, Piontek et al. 2002, Hakulinen et al. 2002).

Laccases have surprisingly broad substrate specificity: they are capable of oxidizing various phenolic compounds, e.g., diphenols, polyphenols, different substituted phenols, diamines, aromatic amines, benzenethiols, and even some inorganic compounds such as iodine (for a review see Thurston 1994). Laccases and tyrosinases are sometimes identified by the trivial name polyphenoloxidase (PPO). This is due to their partially overlapping substrate specificity. Laccases can, however, be distinguished from tyrosinases by the fact that they do not catalyze the hydroxylation reaction typical in tyrosinase catalyzed reactions (**Figure 2**). Compared with tyrosinases, the laccase-catalyzed crosslinking reactions are different: tyrosinase-catalyzed crosslinking is based on quinone formation, whereas laccase-catalyzed crosslinking is based on free radicals and their further reactions.

The phenolic moieties in proteins, i.e., tyrosine residues, can also be oxidized by laccase. As a result of laccase-catalyzed oxidation of phenolic components, free phenolic radicals are generated, which in turn can react further with other phenolic radicals or free aromatic and amino groups present in proteins (Xu 1996, Thurston 1994, Bollag et al. 1983). Subsequently, different covalent linkages can be formed between protein side chains as well as proteins and small phenolic compounds. *Trametes hirsuta* laccase has been reported to oxidize tyrosine, cysteine, and tryptophan (Mattinen et al. 2005). The covalent linkage connecting the model substrates was found to be an ether bond i.e., isodityrosine bond and only small amounts of dityrosine bonds could be detected.

Currently little data are available on the efficiency of the laccase-catalyzed crosslinking of proteins' functionality. Crosslinking of α-casein by ThL has been recently reported by Selinheimo et al. (2008). Some studies have also reported crosslinking abilities of laccases on proteinaceous food matrices (Minussi et al. 2002, Dickinson 1997, Færgemand et al. 1998a, Lantto et al. 2005). On the basis of these studies, it can be concluded that proteins, in general, are rather poor substrates for laccases, and small molecule mediators are needed for improved crosslinking (Lantto et al. 2004, Mattinen et al. 2005, Selinheimo et al. 2008).

Peroxidases

Peroxidases are a diverse group of oxidoreductases that use H_2O_2 as an electron acceptor to oxidize a variety of organic and inorganic substrates such as phenols (Yuang & Jiang 2003), aromatic phenols, phenolic acids, indoles, amines, sulfonates (Veitch 2004), thiols (Obinger et al. 1996), halides (Ekstrand & Björck 1986), and amino acids (Gross & Sizer 1959, Malencik & Anderson 1996). As a result of oxidation, a radical is formed that can react further with other substrates. Reactions catalyzed by peroxidases can be generally expressed by Equation 1:

$$H_2O_2 + 2AH_2 \rightarrow 2H_2O + 2AH^{\cdot}, \tag{1}$$

in which AH_2 represents a substrate and $AH^{\cdot}$ its free radical product. Concomitantly, H_2O_2 is reduced to water. The substrate specificity, pH, and temperature behavior of peroxidases vary with the origin.

Peroxidases are ubiquitous enzymes found from various plants and prokaryotic and eukaryotic microbes as well as from mammalian cells. Peroxidases can occur as intra- or extracellular enzymes. The best-characterized plant peroxidase is from horseradish, from which more than 40 isoenzymes have been isolated (Shih et al. 1971). The biological roles of plant peroxidases are related to biosynthesis and biodegradation of cell wall lignin, providing defense against pathogens and in regulation of growth (Yuang & Jiang 2003). In addition to the biological roles mentioned, horseradish peroxidase is involved in the indole-3-acetic acid metabolism (Krylov & Dunford 1996) that plays a role in tumor development (Veitch 2004).

Most peroxidases are heme proteins containing a ferric protoheme group. Some peroxidases have magnesium, vanadium, or selenium or the flavin group in the active site (Smith & Veitch 1998). The structure, reaction mechanism, and applications of peroxidases have been comprehensively reviewed by Veitch (2004).

It has been hypothesized that peroxidases affect the gluten network by the crosslinking of gluten proteins or by attaching arabinoxylans to gluten proteins via ferulic acid moieties and lysine, tyrosine, or cysteine residues (Hilhorst et al. 1999). On the other hand, peroxidases have been reported to be able to effectively crosslink selected proteins such as gelatin and casein (Matheis & Whitaker 1984). Given that peroxidases are common plant enzymes, they also work as endogenous enzymes, e.g., in flour.

Sulfhydryl Oxidases

Sulfhydryl oxidases (E.C. 1.8.3.3) catalyze the formation of de novo disulfide bonds from free thiol groups belonging to small molecules such as dithiothreitol (DTT), glutathione or to the cysteine residues of a polypeptide chain; the reaction requires the reduction of a molecule of oxygen to hydrogen peroxide per a new disulfide bond introduced following the stoichiometry in Equation 2:

$$2\text{R-SH} + O_2 \rightarrow \text{R-S-S-R} + H_2O_2. \tag{2}$$

An internal redox-active disulfide bond and a FAD moiety are the catalytic core of the most characterized sulfhydryl oxidases isolated from bacterial, fungal, plant, and mammalian sources. Metallo-sulfhydryl oxidases containing iron (Swaisgood & Abraham 1980) or copper (Yamada 1989) have also been reported. Representatives of both metallo- and flavo-sulfhydryl oxidases show activity on protein-associated thiol groups other than on small molecular weight thiols such as glutathione, cysteine, and DTT (Janolino et al. 1975, Jaje et al. 2007). Sulfhydryl oxidases are sometimes referred to as glutathione oxidases or erroneously associated to thiol oxidases (E.C. 1.8.3.2). Regardless, the fact is that these latter enzymes are by definition reducing molecular oxygen to water and not to hydrogen peroxide.

The crosslinking activity of sulfhydryl oxidases has so far not been investigated as extensively as for TGs or tyrosinases, although their application in the dairy industry dates back to the 1970s (Swaisgood 1977), and their effect on wheat dough was tested in 1987 (Kaufman & Fennema 1987). In wheat dough, sulfhydryl oxidases can act on the cysteine residues of gluten proteins and form additional disulfide bonds in the protein network, thus strengthening the matrix and secondly producing hydrogen peroxide, which can nonenzymatically crosslink wheat biopolymers.

Sulfhydryl oxidase activity was detected in milk in 1975, and it was attributed to an iron-containing enzyme (Janolino & Swaisgood 1975). This enzyme was the first sulfhydryl oxidase to be exploited by the food industry when Swaisgood (1977) proved its positive effect in the removal of the cooked flavor from ultra high temperature (UHT)-treated milk. It was also tested as a strengthening agent for wheat flour dough but with no significant effect (Kaufman & Fennema 1987).

In 1982, a fungal glutathione oxidase containing FAD and able to oxidize glutathione and several sulfhydryl compounds was reported in 1982 from *Penicillium* sp. K-6-5 but showed only negligible activity on the cysteine residues of a model protein such as RNAase A (Kusakabe et al. 1982). The first sulfhydryl oxidase active on protein-associated thiol groups was characterized in 1987 from *Aspergillus niger* (de la Motte & Wagner 1987), and its positive effect in combination with glucose oxidase on the rheological properties of wheat dough, even from low-protein content flours, has been shown, but the mechanism has not been fully clarified (Haarasilta & Vaisanen 1989).

A secreted FAD-containing sulfhydryl oxidase has been isolated from chicken egg white (Hoober et al. 1996) and shows higher activity on thiol groups associated with proteins and peptides than on those associated with small thiol compounds (Hoober et al. 1999), but no application has yet been tested. The first commercially available recombinant sulfhydryl oxidase has been recently released and is a recombinant form of Erv1p from baker's yeast (X-zyme, Dusseldorf, Germany). The suggested application is replacement of oxidizing agents such as ascorbic acid and promised gluten strengthening.

EFFECT OF PROTEIN STRUCTURE ON ENZYMATIC CROSSLINKING

Extent of crosslinking reactions is dependent on mainly two factors: introduction of optimum activity and stability conditions (temperature, pH, exclusion of inhibitors, etc.) for the related enzyme and the morphological state of the substrate molecule at the reaction conditions. The presence and accessibility of the target amino acid side chains depending on the conformation of the substrate molecules are the main factor affecting formation of inter- and/or intramolecular crosslinks in proteins.

A number of studies have shown that nonglobular proteins are more easily accessible to enzyme active sites than globular proteins. For example, caseins are reported to be very good substrates for TG because of their flexible and open tertiary structure (Færgemand et al. 1999, Lorenzen et al. 1998). Monogioudi et al. (2009) have recently shown the complete polymerization of β-casein by both TG and fungal tyrosinase. On the other hand, globular whey proteins in their native form are poor substrates for enzymatic catalysis (Sharma et al. 2001; Eissa et al. 2006; Mattinen et al. 2006, 2008).

The secondary and tertiary structures, as well as the size of globular proteins in their native state, may prevent the enzymatic reactions. Complete or partial denaturation of the molecule by chemical reduction of the disulfide bridges or by exposure to alkaline pH, high temperature, or high pressure treatments can, however, increase the extent of enzymatic crosslinking by TG (Færgemand et al. 1997b, 1998a; Eissa et al. 2004; Lee et al. 2002; Kang et al. 2003; Eissa & Khan 2005, 2006; Eissa et al. 2006). Tyrosinase, laccase, and peroxidase have also been reported to induce at least partial crosslinking of whey proteins (Thalmann & Lötzbeyer 2002, Færgemand et al. 1998), which was increased with protein denaturation upon addition of DTT (Færgemand et al. 1998). Thus, combination of nonenzymatic denaturation of proteins to subsequent enzymatic crosslinking may increase the applicability of enzymatic protein crosslinking in food processing.

EXPLOITATION OF PROTEIN CROSSLINKING IN CEREAL APPLICATIONS

Crosslinking of cereal proteins has major potential in tailoring technological and sensory quality of cereal products because proteins typically have an important role in structural characteristics of these products. Wheat proteins have unique properties, and therefore, wheat grain is the most common grain used in cereal food applications. Gliadin and glutenin proteins, i.e., the gluten proteins, account for approximately 80% of the wheat proteins, and their viscoelastic nature is the backbone for the formation of a protein network during dough mixing, determining bread quality with respect to crumb structure and loaf volume (Wrigley & Bietz 1988). The basis of wheat breadmaking is the optimal disulphide crosslinking of gluten proteins during dough mixing. In addition to wheat, rye, oat, and barley are used for production of cereal foods. The viscoelastic properties of rye, oat, and barley proteins are however different as compared with wheat proteins, and subsequently the applicability of rye, oat, and barley in breadmaking is limited.

Baking Applications

TG is the most studied crosslinking enzyme in breadmaking. It is reported to crosslink high molecular weight glutenins and gliadins to large insoluble polymers (Gerrard et al. 2000, 2001; Rosell et al. 2003). Moreover, water-extractable albumins and globulins have been reported to be crosslinked by TG (Gerrard et al. 2000, 2001). TG-mediated protein crosslinking has been shown to modify the rheological properties of gluten (Larré et al. 2000) by decreasing dough extensibility, increasing water absorption, and hindering the growth of air bubbles in the dough, which subsequently decreases the volume of bread (Gerrard et al. 1998, Autio et al. 2005). Addition of TG has been found to increase crumb firmness (Gerrard et al. 1998). However, several studies have shown that TG can improve the gluten networking properties and subsequently improve the baking quality of low-quality gluten (Autio et al. 2005). Gujrala & Rosell (2004) reported that TG improves dynamic rheological properties of rice flour doughs. In oat breadmaking, Salmenkallio-Marttila et al. (2004) reported that addition of TG made the oat breads harder and gummier than the breads baked without the added enzyme.

In frozen-dough baking, gluten quality is often reduced by the phenomena occurring in the freezing process. TG-catalyzed crosslinking of proteins has been reported to improve bread quality and freeze-thaw stability of the gluten network in frozen-dough processes. It has been shown that TG may significantly improve frozen dough bread quality, such as crumb strength of baked loaves and water absorption of the dough (Gerrard et al. 1998, 2000; Kim et al. 2008; Huang et al. 2008). TG has also been shown to have positive effects on the frozen storage stability of pastry and croissant doughs (Gerrard et al. 2000, 2001).

In addition to TG, several oxidative enzymes have been investigated in breadmaking applications. Peroxidase has also been suggested to affect both the thiol groups of gluten and the ferulic acid groups of arabinoxylans of dough. Use of peroxidase in breadmaking is reported to improve dough handling, dough tolerance, crumb structure, and loaf volume and to prevent staling (Dunnewind et al. 2002, Labat et al. 2001). Tyrosinase and laccase are rather novel experimental enzymes recently studied in breadmaking applications (Selinheimo et al. 2007a, Flander et al. 2008). Selinheimo et al. (2007a) reported that tyrosinase could effectively crosslink wheat gluten proteins with subsequent positive changes in the breadmaking quality. Laccase on the other hand can crosslink gluten proteins to some extent (Selinheimo et al. 2007a), although the main effect is crosslinking the arabinoxylans (Labat et al. 2000, Selinheimo et al. 2006b).

Pasta and Noodle Applications

The structure of pasta and noodles is largely determined by the well-organized protein network constituted mainly of gluten proteins. Crosslinking the gluten proteins by TG with subsequent reinforcement of the network is reported to result in increased resistance for thermal processing when compared with unmodified glutens (Larré et al. 2000, Babiker et al. 1996). According to Kuraishi et al. (2001), TG is rather widely used in pasta and noodle processing, especially in Japan. For instance, by using TG in noodles and pasta manufacturing, improved texture properties and prevention of texture deterioration upon cooking and storage have been reported (Sakamoto et al. 1996, Kuraishi et al. 2001, Yamazaki & Nishimura 2002, Wu & Corke 2005). As a result of the gluten crosslinking by TG, dough starch granules are entrapped in the gluten network, and the release of solid contents during boiling is reduced. This in turn reduces the stickiness of the product surface. Sakamoto et al. (1996) suggested that treatment of noodles and pasta with TG may develop good-quality pasta characteristics even when low-grade flours are used.

EXPLOITATION OF PROTEIN CROSSLINKING IN DAIRY APPLICATIONS

Utilization of milk protein products in food is dependent on their physicochemical and functional properties. In this sense, extensive knowledge on complex structure of various milk proteins is needed in order to be able to tailor them toward better functionality. Enzymes capable of modifying textural and water-binding properties of milk proteins by creating inter- or intramolecular covalent bonds may be exploited to fabricate milk products with acceptable texture in spite of low fat or protein content. Here, we review enzymatic modification of milk proteins and the effects on functional properties such as gelling, renneting, foaming, and emulsifying. Literature until 2005 on dairy applications of TG has been extensively covered in the review by Jaros et al. (2006a).

Yogurt Applications

Bovine milk is a complex system with approximately 3.5% w/w protein that is 80% composed of different casein fractions, 15% of heterogenous whey protein fractions, and 5% of nonprotein nitrogenous components. The lack of the well-defined secondary or tertiary structure makes caseins a good substrate for enzymatic reactions. In milk, caseins are preferred by crosslinking enzymes against whey proteins owing to the globular structure of β-lactoglobulin and α-lactalbumin and poor accessibility of target amino acid residues for enzymatic reactions (Sharma et al. 2001).

Enzyme-aided structure engineering of dairy products has attracted much interest since the introduction of TG. Introduction of covalent bonds by enzymatic catalysis into the gel network is known to increase the gel firmness leading to better structural and sensory properties of acid milk gels. In yogurt production, enzymatic crosslinking is mainly studied for replacement of added nonfat dry matter and stabilizers or reduction of dry matter content without changing textural properties of the final product. TG has been extensively studied in this respect, both with yogurt starter culture or chemical acidifier–induced milk gels. It has been reported that a small increase in the number of covalent crosslinks by TG treatment can lead to milk protein gels with increased gel strength (Anema et al. 2005, Lauber et al. 2000, Partanen et al. 2008, Lorenzen et al. 2002b, Ozer et al. 2007), increased elasticity (Jaros et al. 2006b), and less syneresis as a result of improved water holding capacity (Bönisch et al. 2007a, Myllärinen et al. 2007). TG-treated casein gels were reported to have a homogeneous microstructure with a finer network and smaller pores as compared with non-TG-treated gels (Myllärinen et al. 2007, Færgemand & Qvist 1997, Partanen et al. 2008). Introduction of covalent bonds into the casein gel network results in increased gel strength and elasticity. Extensive crosslinking was found to result in impaired gel firmness due to restriction of proper rearrangements during gel formation (Jaros et al. 2006b). In traditional yogurt production, effect of TG or TG-modified proteins on activity of starter bacteria has also been investigated. TG treatment of milk prior to starter culture addition results in prolonged fermentation times as small peptides needed for starter bacteria are not available as a result of crosslinking (Lorenzen et al. 2002b, Færgemand et al. 1999, Ozer et al. 2007). This was reported to have a negative effect on the aroma profile and acidity of yogurt prepared from TG-treated milk (Lorenzen et al. 2002b, Ozer et al. 2007). This could, however, be improved by concomitant addition of TG and starter bacteria. With optimized TG dosages nonfat yogurt with improved physical and sensory properties that are comparable to full-fat yogurt could be produced without need for additional protein or stabilizer (Ozer et al. 2007). Another promising application of protein crosslinking in milk gels could be substitution of milk by liquid milk whey in yogurt manufacture. Less syneresis was observed with TG treatment in yogurt samples, where milk was partially replaced by liquid milk whey (Gauche et al. 2009).

Oxidative enzymes (tyrosinase, laccase, peroxidase, etc.) are also interesting alternatives for crosslinking of different milk proteins. The mode of action of these enzymes has been studied using casein or whey protein compounds as substrates as reviewed by Buchert et al. (2007). Currently there are only a few published reports on possible effects of crosslinks attained by oxidative enzymes on the structural modification of acidified milk gels. Ercili-Cura et al. (2008) compared the effects of tyrosinase with two different origins, i.e., from *T. reesei* and from *A. bisporus*, on crosslinking ability of raw and heated milk as well as effects on acidified gels made after. *A. bisporus* tyrosinase was not able to induce crosslinks in either raw or heat-treated milk, whereas *T. reesei* tyrosinase was able to form crosslinks in both cases, resulting in increased gel stiffness in raw milk gels (Ercili Cura et al. 2008). Shotaro (2002) reported increased viscosity and improved gelling ability of milk proteins after laccase treatment. Recently, Hiller & Lorenzen (2009) reported increased viscosity of milk owing to laccase (together with chlorogenic acid) and glucose oxidase treatments leading to gelation depending on the incubation conditions. Ercili Cura et al. (2009) showed that laccase treatment alone does not affect the strength of acid-induced caseinate gels while in the presence of laccase and that with ferulic acid, significant increase in gel firmness was obtained.

Cheese Making and Quark Applications

Milk renneting during cheese manufacture is carried out by proteolytic disruption of hairy κ-casein layer in the casein micelle, which subsequently induces collapse of the micelles, aggregate formation, and finally creation of a protein network (Ennis & Mulvihill 2000, Bönisch et al. 2008). Effect of enzymatic crosslinking of the casein micelles on rennet gel formation has been studied mainly with TG. It was observed that TG treatment increased the required time for coagulation and/or flocculation of the casein micelles (O'Sullivan et al. 2002, Lorenzen 2000, Huppertz & de Kruif 2007a). Main discussion on those studies was to discover if the TG-induced impairment of rennet coagulation properties of caseins was caused by crosslinking on the primary stage, i.e., retarding the enzymatic hydrolysis of κ-casein, or in the secondary stage, i.e., retarding the aggregation of para-κ-casein micelles, leading to declined gel firmness. Recently, Bönisch et al. (2008) have shown that TG crosslinking affects both stages of rennet coagulation. From a technological point of view, the authors suggested use of TG and rennet simultaneously to prevent declined rennet coagulation properties. In this case, significant increase in the curd yield can be obtained as a result of enhanced serum, i.e. the remaining portion of milk after coagulation and separation of caseins, binding to the gel network (Bönisch et al. 2008) and thus increased whey protein incorporation (Cozzolino et al. 2003). TG is widely patented in cheese applications, mainly for incorporation of whey proteins into casein network with subsequent yield improvement. The patents differ mainly in the order in which TG is added to the process (Han & Spradlin 2000, Han et al. 2003, Kumazawa et al. 2002, Kumazawa & Miwa 2004, Kuraishi et al. 1996, Miwa et al. 2002, Cuksey et al. 2007). TG has also been patented for incorporation of whey without any stabilizers to obtain a firmer texture and less syneresis in cream cheese (Han et al. 2002). Lorenzen et al. (2002a) have reported use of TG treatment in manufacture of quark, which is a soft curd cheese mainly consumed in Europe. Quark prepared from TG-treated skim milk was smoother and creamier in consistency.

In cheese production, heating of milk to high temperatures is avoided as this impairs the renneting properties of milk as a result of the interactions of denatured whey proteins with κ-casein (Singh & Waungana 2001). However, in unheated or pasteurized milk, the efficiency of TG is limited because of the presence of an inhibitor. It has been reported that milk serum contains a low molecular weight TG inhibitor that forms a complex with cysteine in the active site, resulting in loss of enzyme activity. The inhibitor is heat-labile, but can be inactivated only at temperatures higher than pasteurization temperature (de Jong et al. 2003). Recently, it has been reported that addition of reducing agents such as glutathione to milk can also prevent the inhibition of TG (Miwa et al. 2002, Bönisch et al. 2007b, 2008). Currently, TG preparation containing yeast extract as a source of glutathione is commercially available for use of TG to crosslink raw or pasteurized milk in dairy food products.

According to current literature, enzymatic modification of milk prior to renneting for cheese manufacture does not seem to be promising, whereas textural and microstructural improvements can be achieved by crosslinking after or simultaneously with the rennet addition. Enzymatic crosslinking is also a promising technology to incorporate whey proteins into the network formed by caseins, thus decreasing the amount of waste whey and also possibly improving the nutritional value of the end products.

Others

The stability of milk against coagulation at sterilization temperatures has commercial importance in the manufacture of UHT milk and milk-containing products, such as concentrated milk. Increased heat stability of preheated and TG-treated milk at pH values of 6.6–7.3 compared with unheated TG-treated milk has been reported by O'Sullivan et al. (2002). The stability of casein micelles against high pressure and against heating in the presence of ethanol can also be increased by the TG (O'Sullivan et al. 2002, Smiddy et al. 2006). TG-induced crosslinking of skim milk increased ethanol stability against ethanol-induced coagulation (Huppertz & de Kruif 2007b).

Several experiments have also been carried out on quality improvements in frozen dairy desserts (Okada et al. 1993, Miyamoto & Kanbara 1994). Ice cream treated with TG has been reported to be smoother and easier to scoop, especially in the case of low-calorie, nonsugar ice cream, where icy texture often damages the quality (Okada et al. 1993).

EXPLOITATION OF PROTEIN CROSSLINKING IN MEAT AND FISH PROCESSING

By using crosslinking enzymes, functional properties such as texture and water holding of meat or fish products can be modified. In both products, the main target molecule for crosslinking is the myofibrillar protein myosin. Hitherto, TG has been the main crosslinking enzyme studied in meat applications, and it is also industrially applied in meat processing.

In the meat and fish industry, there is one distinct application in which crosslinking enzymes can markedly boost the manufacturing process and add value to meat and fish of poorer quality. This application is restructuring, i.e., stabile gluing of meat or fish pieces together. 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.

Restructuring of Meat

The demand for high quality, moderately priced meat products has been the driving force to develop methods of restructuring low-value cuts of poorer quality to improve their market value by making palatable steaks resembling intact muscle and to maximize the efficiency of carcass utilization. Traditionally, salt and phosphates in conjunction with heat treatment have been used to bind meat pieces together. Unheated comminuted products are usually frozen to enhance binding. Nowadays when consumers demand fresh, unfrozen meat, as well as lower salt content, technologies have been developed to eliminate the need for freezing and to enable the use of less salt. One of these technologies is the enzyme-aided restructuring (Kuraishi et al. 1997), which has been on a commercial scale for some time and is still the main TG application in the meat sector. TG has been found to improve the strength of restructured meat protein gels with or without added salt and phosphates (Wijngaards & Paardekoopper 1988, Nielsen et al. 1995, Kuraishi et al. 1997, Lee & Park 2003, Serrano et al. 2003). Kuraishi et al. (1997) reported that restructured meat products that are traditionally prepared using salt and phosphates can be prepared without added salts using TG. Lee & Park (2003) showed that hardness, chewiness, and springiness increased significantly by adding TG to the restructured ground lean pork. Water-holding capacity (WHC) of unheated samples decreased because of TG, but the enzyme had no effect on the cooking loss.

Restructuring of Fish

As with low-value meat cuts, low-value fish cuts and trimmings originating mainly from mechanical separation of fillets from various fish species are a nutritionally valuable side stream fraction. Enzyme-aided restructuring of these fish cuts and trimmings has become a feasible technology. TG as a binding agent as such or used together with proteins (Yetim & Ockerman 1995, Baker et al. 2000, Uresti et al. 2004, Ramirez et al. 2007a) and hydrocolloids (Ramirez et al. 2002, Uresti et al. 2003) has been used to restructure fish cuts, resulting in good textural properties. Currently, novel types of TG-aided restructured fish products containing pieces from different fish species and nonfish origin are spreading in the market.

Processed Meat Systems

In addition to binding fresh meat pieces together, positive effects of TG have also been observed in improving textural properties of processed meat systems. Tyrosinases and laccases are also known to be capable of crosslinking meat proteins and increasing the firmness of meat gels (Yamaguchi 2000; Lantto et al. 2005, 2006, 2007a,b). TG-catalyzed formation of covalent bonds in structural meat proteins leads, by definition, to firmer gel structures as has been clearly shown in many studies (Pietrasik 2003; Dondero et al. 2006; Jarmoluk & Pietrasik 2003; Pietrasik & Jarmoluk 2003; Pietrasik et al. 2007; Herrero et al. 2008; Lantto et al. 2007a; Trespalacios & Pla 2007a,b; Hammer 1998; Mugumura et al. 2003; Ahhmed et al. 2007). However, excessive increase in gel firmness owing to additional formation of covalent bonds in the myofibrillar protein network may cause constraint in protein mobility and flexibility of myofibrils, leading to an undesired decrease in WHC (Carballo et al. 2006). Decreased WHC is mostly an unwanted phenomenon and has been observed to take place in the presence of TG, particularly in heated comminuted meat systems when salt concentrations are low (Jiménez Colmenero et al. 2005, Carballo et al. 2006).

TG has not been shown unequivocally to be an adequate salt replacer from the water-holding viewpoint but, in high enough salt levels, TG is capable of improving both textural and water-holding properties (Hammer 1998, Dimitrakopoulou et al. 2005, Jiménez Colmenero et al. 2005, Carballo et al. 2006, Trespalacios & Pla 2007b). Improved water-holding caused by TG in low salt concentrations has, however, also been reported. TG treatment has also been reported to increase cooking yield of chicken meatballs (Tseng et al. 2000) and low-fat pork gels (Pietrasik et al. 2007).

Surimi Setting

Surimi, a translucent and elastic gel product made from minced saltwater fish muscle, is widely used as an ingredient in seafood analogs (An et al. 1996). Surimi is an (acto)myosin gel set in the presence of salt and calcium at an appropriate pH and elevated temperature. During the setting process, the temperature activates the endogenous enzymes naturally occurring in fish muscle. Endogenous Ca^{2+}-dependent TG (Tsukamasa et al. 1993) has a crucial role in the gel formation during the setting phase (0–40°C) of surimi preparation as a result of the polymerization of the myosin heavy chain molecules (Kumazawa et al. 1995). Readers interested in the role of Ca^{2+}-dependent mammalian or fish TGs in surimi preparation are encouraged to read the review by An et al. (1996).

The firmness of the gel is the main factor determining the quality of surimi. Ca^{2+}-independent microbial TGs have been studied and also applied successfully up to the industrial scale to improve the textural characteristics and mechanical properties of surimi of different fish origin and fish gel products. Microbial TG has been applied in surimi setting from such fish as striped mullet (Ramirez et al. 2007a,b), barred garfish (Benjakul et al. 2003), mackerel (Tsai et al. 1996, Dondero et al. 2002), Alaska pollock (Lee et al. 1997b), Atlantic croaker (Joseph et al. 1994), lizard fish (Yongsawatdigul & Piyadhammaviboon 2006), and golden threadfin (Jiang et al. 2000).

IMPACT OF CROSSLINKING ON PHYSIOLOGICAL FUNCTIONALITY OF PROTEINS

Enzymatic crosslinking of proteins is most generally applied to tailor the technological properties of the proteins and subsequent food structures. However, crosslinking of proteins may also affect the physiological properties of the end product when consumed by humans, for example, digestibility and allergenicity. A number of reports suggesting either positive or negative impacts of crosslinking on the physiological response of proteins exist, but these issues concerning the physiological functionality of crosslinked proteins are far from thoroughly studied and more research is needed.

Digestibility

The rate of protein digestion and amino acid absorption is known to have a major effect on the whole body protein anabolism (Boirie et al. 1997). Understanding the impacts of food processing on protein digestion is thus a prerequisite for predicting the effects of processing on protein anabolism. Heating during food processing has been reported to reduce the digestibility of food proteins (Duodu et al. 2003, Genovese & Lajolo 1996). Maillard reactions may also decrease in the nutritional properties of food products (Friedman 1996, Fayle et al. 2000, Miller & Gerrard 2005).

The wide use of TG on food processing has raised questions about the effect of the formed isopeptide crosslink on protein digestibility or bioavailability of lysine. According to Seguro et al. (1996b) and Hultsch et al. (2005), peptides containing an isopeptide bond can be cleaved by human peptidases such as pepsin and trypsin, and thus the use of TG in food processing would not cause health risks to consumers. Finot et al. (1978) reported that the lysine derivative of the isopeptide is absorbed unchanged in the intestine and is hydrolyzed in vivo (Seguro et al. 1995, 1996b). Experiments of Seguro et al. (1996a) showed that no significant differences in food intake and overall health were observed among rats fed by casein or TG-crosslinked casein diets, indicating that the isopeptide moiety in crosslinked caseins is digested. Crosslinking of the soy isolates by TG has, however, been found to decrease the in vitro digestibility of the protein (Tang et al. 2006). Similarly, Mariniello et al. (2007) reported that when phaseolin, a trimeric storage protein from *Phaseolus vulgaris*, was modified by microbial TG, the protein was less prone to proteolysis by pepsin and trypsin. Thus, TG-catalyzed protein crosslinking products are digested slower, but the nutritional value and bioavailability of the amino acid residues are similar as compared with those of untreated proteins. The studies in this area are rare, and more research is clearly needed to better understand the effect of crosslinking on the digestibility in vivo.

Allergenicity

Among the crosslinking enzymes, TG and peroxidase are two of the few studied in terms of their effects on allergenic properties of food proteins. Soy proteins have been reported to show decreased allergenicity after treatment by TG (Babiker et al. 1998). Similarly, TG was also found to reduce the immunoglobulin binding of peanut allergens in the peanut flour dispersion containing casein (Clare et al. 2008). Chung et al. (2004) showed that a significant decrease in the levels of the major allergens and also reduction in IgE binding was observed in roasted peanut proteins after the peroxidase treatment.

Enzymatic modification of cereal proteins in a view of physiological functionality has also been studied. Yong et al. (2006) concluded that the enzymatic deamidation could cause a decrease in the IgE binding efficiency of wheat gluten proteins. On the other hand, Palosuo et al. (2003) found that the ω-5 gliadin-derived peptides of wheat crosslinked by tissue TG caused a marked increase in IgE binding both in vitro and in vivo. Palosuo et al. (2003) hypothesized that the large allergen complexes resulting from the TG activity may cause allergenic reactions in sensitized individuals. However, crosslinking of proteins either by exogenous TG during food processing or by tissue TG in vivo can be expected to have different physiological impacts.

Stanic et al. (2009) have recently studied the effects of crosslinking of β-casein by TG on allergenicity. The authors reported that the isopeptide bonds formed after crosslinking by TG have little effect on modifying the IgE binding epitopes. However, when β-casein was crosslinked by *Trametes hirsuta* laccase and *Agaricus bisporus* tyrosinase with caffeic acid as co-substrate, the IgE binding was mitigated, and thus allergenicity of β-casein decreased approximately 16%.

Other Physiological Impacts

TG has also been associated to celiac disease, which is a chronic inflammatory intestinal disorder that is induced by wheat gluten and related proteins in barley and rye (Reif & Lerner 2004). The possibility of TG-mediated generation of the epitopes associated with the celiac response has arisen. Skovbjerg et al. (2004) suggested that the tissue TG in celiac patients has a role in deaminating gliadin proteins with subsequent generation of active epitopes involved in the autoimmune response of celiac patients. It has thus been speculated that microbial TG creates similar types of epitopes (Gerrard & Sutton 2005). So far, no experimental data have been produced supporting this assumption.

CONCLUSIONS AND FUTURE PROSPECTS

Food texture plays an important role in food stability and consumer perception. The significance of food texture has further been increased with the trend toward low fat, low calorie products. Protein crosslinking, especially by enzymatic means, has a lot of potential for providing specific and natural means for tailoring food structure.

Among potential enzymes for protein crosslinking are TG and various oxidative enzymes. The reaction mechanisms of these enzymes differ, which in turn results in different technological properties. The current commercial applications are based on TG. This microbial TG can be used in meat, dairy, fish, and baking applications. The research on other types of crosslinking enzymes is, however, very active, and therefore it can be anticipated that these new enzymes might be launched into the market in the future. Understanding of the chemical reaction mechanisms, as well as the technological and physiological impacts of the enzyme stages, is of utmost importance when these new enzyme concepts are being implemented.

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Errata

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