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Microbial Transglutaminase application in food industry

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Abstract: Modification of proteins by enzymes such as microbial transglutaminase (TG) has recently become of great interest to food industry.TG(EC 2.3.2.13) catalyses a reactions between peptide bound glutaminyl residues and primary amines and due this reaction it can be used to improve functional properties of some food products such as dairy products, meat products and cereal products.

[1] **Key Words:**Transglutaminase, Cross-linking, Dairy products, Meat products, Cereal products,Enzymes

1. Introduction

Enzymatic protein modifications have been reported in food processing industries (19)and it has become of great interest to food scientists (12) Various enzymes are used to meet different needs such as improving texture, flavor, or nutritional value, and simplyfing processing steps.(19)Enzymes are generally recognized as safe (GRAS)(3) Enzymatic modification has been suggested as a useful method due to high specificity of enzymatic reactions and thus the low risk of formation of toxic products(15) Microbial transglutaminase (MTGase,EC 2.3.2.13)(R-glutamyl –peptide:amine glutamyl transferase)(11)one of the enzymes widely used for protein modification in recent years(19).MTGase catalyzes the formation of inter and intra molecular ε-(λ-glutamyl)lysine crosslinks (Gg-L bonds)in several food proteins such as soy , milk . Egg and wheat proteins(19)It can catalyze conversion of soluble proteins to insoluble HMW protein polymers through formation of nonsulfide coavalent crosslinks . (12)

2. Sources

TG are widely distributed in animal tissues and body fluids(Aeschlimann&Paulsson 1994),plants, fishes(Araki&seki,1993) and microorganisms (Ando et al 1989;ZHU et al 1995;Seguro et al 1995)(2).Commercial TG was originally obtained only from animal tissues.(. calcium –dependent TG) recently ,studies on the production of TG by micro-organisms(calcium independent)have been started. The enzyme obtained from microbial fermentation has been applied in the treatment of food of different origins. Extracellulr TG was purified from cultural filtrate of *Streptoverticillium mobarens* (Dickinson 1997, Zhu et al ,1998;2000)from *Streptoverticillium sp.*(Ando et al.1989;Motoki et al.1990)from *Streptoverticillium ladakanum* (Tsai et al 1996;Jiang et al .2000)and from *Streptoverticillium lydicus* (Faregmand et al ,1998).Intracellular TG was also found in *bacillus subtilis* and in spherules of *physarum polycephalum*(Tsai et al .1996)(10, *18)*

3. Properties

Microbial enzyme is monomeric and simple protein with a molecular weight of about 38000, consisting of 331 amino acids (Isoelectric point 8.9)Schorsch et al.2000).(10). And has a single cysteine residue and

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two potential glycosylation sites(Thr-Xxx-Asn-)(21). The optimal temperature for enzyme reaction was 50C(Tsai e et al.1996)(10)The purified TG is strongly inhibited by pb ,Zn ,and Cu .because heavy metals bind the thiol group of the single cysteine residue, this strongly supports the idea that a cysteine residue could be part of active side of TG(15)**.**TG can be used in combination with high pressure treatment of food because it is capable of cross-linking protein under high pressure(15).

4. Mechanism

TG can modify proteins by the incorporation of amine ,by cross-linking,and by deamination .The affinity of TGase for different types of protein depends on the distribution of GLU residues as well on the secondary and tertiary structure of proteins(Matsumura, Chanyongvorakul et al.,1996).Casein , Soy proteins, conalbumin, rabbit and carp myosin, beef actin and myosin, ovomucin are examples of proteins which are suitable substrates for TGase(Christensen ,et al 1996(9)Sorensen et al . (1999) reported that TG can be used to introduce methionine into casein and soybean proteins and lysine into wheat gluten ,thereby showing that TG can also be utilized to improve the nutritional value of food proteins,(10). The formation of cross-linking does not reduce the nutritional quality of the food because the lysine residue remains available for digestion(Seguro,Kumazawa,Kuraishi et al. 1996)(4,7)

5. Application of TGase in food industry

5.1. Wheat products

Application of cross-linking to wheat gluten would be of particular interest because of their high glutamic content(approximately one-third of the total amino acids)(12) Lysine is essential to humans and is the first limiting amino acids in wheat products .hence its protection is critical. Crosslinking with glutamine using TG does not lead to loss of nutritional quality as lysine is still available for digestion(Seguro et al.,1996).Indeed ,cross-linking with glutamine may even protect lysine from various chemical reactions and proteolysis(11).

Iwami and Yasumoto (1986) introduced lysine into wheat gluten through cross-linking to improve nutritional value. (12)Gerrard et al (1998)indicated that TG may produce beneficial effects during bread making that are comparable to traditional oxidizing improvers(12)and its activity may lead to the spontaneous formation of disulfide bridges due to proximity of sulphure containing amino acids(**3**).Gliadin and glutenin are two major components of gluten in wheat flour dough that dominate viscoelasticity.The properties of gluten are dependent on wheat variety and growth condition and milling performance(19)MTGgase has the ability to modify wheat protein effectively as a result of the modification of some important physical properties of wheat flour dough ,including stickiness, extensibility ,and maximum resistance to extention.(19)Compared to L-ascorbic acid which has been used as a dough improver for decades, MTGase has proved to be a potential enhancer of baking properties because a very small dosage can cause obvious modification on dough properties.(19)Tg treatment increased the volum of puff pastry and croissants,conferring strength towards damage on freezing (Gerrard et al ,2000),moreover the addition of TG to bread doughs produced a significant increase in crumb strength and loaf volume(Basman et al.,2002;Grrard et al .,1998). TG has also been used to produce rice bread ,providing protein network capable of holding the gas produced during proofing , yielding a rice bread with an acceptable specific volum and crumb strength(Gujral and Rosell,2004)(**3**)Dough prepared from bug –damaged flour is runny and sticky and produces poor quality bread .hydrothermal and microwave treatment before milling, use of low levels of organic acids , and calcium chloride in dough formulation, and utilization of shorter fermentation periods in bread making have been used to counteract the negative effects of damage.Koksel,H et al (2000)investigated the possibility of mitigating the determined effects of bug damaged by using TG enzyme.the repairing effect of the TG enzyme is evident on dough samples hydrolyzed by wheat bug protease enzymes (12) The insect damaged doughs are characterized as beaing weak,sticky and difficult to handle and make up and show low consistency and mixing tolerance.bread baked with the insect – damaged flour has a much lower volum,poor crumb sensory quality and an irregular shape.formation of crosslinks between wheat proteins in insect damaged wheat flours could be a method for recovering strength of the gluten network.(**3**)

5.2. Meat products

MTGase can produce restricted meat by binding together small pieces of meat.Caseinate ,when reacted with MTGase ,becomes viscos, and functions as a glu to bind different foodstuffs together(21)Using this system ,large pieces of restructured meat such as beefsteaks or fish fillets can be produced from fragments(20)**.**pieces of meat ,can be bound together without any need for sodium chloride or phosphates,yielding healthy meat products.(21) Functional behavior of myofibrillar proteins is influenced by their ability to form viscous gels via protein-protein interaction to retain water and in the case of emulsions to form resistance film on the surface of the fat droplets. In order to improve the functional properties of meats ,processors are currently using a wide range of vegetable additives, especially soy protein derivates. often consumers reject these additives as being chemically modified or as being obtained from genetically modified plants(9)The use of enzymatically catalyzed reactions to modify protein structure is an important way of improving their functional .TGase catalyse the cross-linking of the myofirillar proteins from beef heart and improved their functional properties.he results suggest that .3 gr MTGase /100g proteins and a setting time of 60 min at 35 C are optimum to improve hydration and aggregation properties of myofibrillar proteins.(9) M;et al (1999)showed that the texture of chicken sausages was improved by the formation of ε-(γ-glutamyl)lysine crosslinks by the addition of TG in chicken sausages.(**13**)in another research the reported that crosslinking SPI and casein or WPI provided biopolymers with improved heat stability and emulsifying properties(2003)(13)

5.3. Casein

Casein ,the principle proteins in milk are Particulary good substrates for TG due principally to their flexible nature with little or no secondry structure in contrast to globular proteins and its flexibility, random-coil arrangement and the absence of any disulphide bonds in α- and β caseins,leaving the reactive groups exposed to the enzyme(10,15). Between the two main protein fractions of milk , the caseins can be easily cross-linked by mTGase , whereas the globular whey proteins are hardly susceptible to mTGase –induced reaction .(de jong and Koppelman,2002;Lorenzen 2002),with respect to isolated milk protein ,it was shown that the cross-linking decreased in the order of sodium caseinate >ultafiltrated skim milk powder> Skim milk powder >Whey protein isolate (Lorenzen,2002).Furthermore ,Lorenzen et al .(1998)found that ,for highly cross-linked caseinate ,the amount of free amino groups decreased around 5% ,indicatind that only a small number of cross-linking sites are necessary for complete oligomerization of casein. The susceptibility of all components of sodium caseinate for mTGase –induced reaction decreased in the order of κ- casein> α casein> β casein(Tang et al. 2005)(10).TG is a useful tool to change the rheological properties of caseins without damaging their special functional properties. and when applying TG in dairy products ,it is possible to increase gel strength ,surface viscosity, water –holding capacity, stability, rennetability and mechanical properties and decrease permeability**(**15**)**Effect of cross-linking on gel stiffness permeability and surface viscosity were highly dependent on enzyme dose(fargemand and qvist,1999)Nonaka et al(1992) found that gel made with a higher enzyme concentration was more viscoelastic. For skim milk gels ,the enzyme treatment in higher concentration caused substantial increase of breaking strength and hardness , while the strain and cohesiveness had little or no changes.(15).Dickinson (1997) reported that gels can be produced at a rather lower protein content by enzymic cross-linking than by thermal treatment,and the elastic moduli and breaking strengths of enzyme-cross-linked gels were greater than those of heat –set gels made under similar conditions.(15)The nitrogen solubility of individual caseins and sodium caseinate has been increased on incubation with a calcium–dependent TGase isolated from guinea pig liver (motoki, Nio, & Takinami ,1984;Motoki,Seguro,Nio,&Takinami,1986) and with a calcium –independent TGase isolated from a microbial source(Flanagan&FitzGerald,1996).Lorenzen (2000)reported an increase in the stability and viscosity of emulsions prepared with cross-linked NaCN compared to non cross-linked NaCN**(5).**Most studies on yogurt deal with set-style yogurt .Cross-linking of milk proteins might represent a considerable for improving texture and technofunctional properties of the final product without or by reduced dry matter enrichment.Some of the benefits for fermented dairy products including:increase gel strength;reducing synersis;producing a dry,smooth surface in case of set –style yogurt;and improving viscosity and creaminess for stirred yogurt.as Lorenzen et al.(2002) investigated the effects of mTGase treatment of the base milk on selected properties of set-style yogurt.as compared to a control sample,the incubation of milk with mTGase

prior to fermentation (declared activity:1000U/g,enzyme/substrate ratio:0.05%(W/W),incubation for 2h at 40C,followed by inactivation for 1 min at 80C)resulted in prolonged fermentation time.Faergement et al.(1999)also described a slight acidification delay in enzyme –treated milk to a cross-linkes induce,diminished availability of low molecular weight peptides,which are needed for the production of proteinases by starter lactobacilli**.(**10)

5.4. whey proteins

The globular whey proteins are hardly susceptible to mTGase,but the accessibility of β–Lactoglobulin and α -lactalbumin to mTGase-catalyzed reaction can be enhanced by treatment with DTT as a reducing agent .that cleave disulfide bonds ,leading to unfolding of the protein and resulting in an exposure of potential new cross-linking sites for mTGase (Coussons *et al.1992:de Jong and Koppelman 2002;Lee et al.2002).*Furthermore ,heat treatment (O Sullivan et al. 2002) and the application of high hydrostatic pressure (Lauber et al.2003) induce a denaturation of β- lactoglobulin, which results in a better accessibility for mTGase. mTGase is stable at high pressure ,indicating that cross-linking is possible(Lauber et al .2001)(17).

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