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Status of TG enzyme modified fish gelatin

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Abstract: TG enzyme can catalyze the ligation reaction (glycosylation reaction), crosslinking reaction and deamination reaction between or within proteins, and has great application potential in the food field. TG enzyme was used as coagulant to modify fish gelatin, and the effects of crosslinking temperature, pH, TG enzyme dosage, gelatin concentration and crosslinking time on the characteristics of fish gelatin gel were discussed.

Keywords: Fish gelatin; Salt-soluble protein; Protein; Gel strength.

1. Preface

Gelatin raw materials vary in source, processing and product quality, etc. Evaluation of different fish gelatin raw materials is a prerequisite for gelatin applications[1]. By cross-linking gelatin with TG enzyme and evaluating the physicochemical properties and application performance of gel products, the effect of TG enzyme on gelatin gel performance and application effect can be determined, so as to determine the process conditions of TG enzyme-catalyzed gelatin, enhance the application performance of gelatin and expand the application scope of gelatin[2].

2. Gelatin

Gelatin is a product obtained from the partial hydrolysis of natural collagen, which is traditionally obtained mainly from mammalian skin, bone, muscle and other connective tissues[3]. Because the raw materials are easier to obtain, the processing technology is mature, the cost is relatively low and other factors, making pig and cattle gelatin occupy more than 90% of the current gelatin market share, of which pig skin and cow skin account for 46% and 29.4%, and bone accounts for 23.1%. However, in recent years, with the emergence of some infectious diseases in mammals, such as "mad cow disease", "foot and mouth disease", etc., the safety of gelatin from mammals has been questioned, coupled with the differences in dietary habits of different religions, as well as vegetarian consumers In addition to the differences in dietary habits of different religions and the gradual expansion of the consumer market for vegetarians, the need to research different gelatine raw materials to meet different applications has made the development of a new type of gelatine different from mammalian gelatine a key issue in the market today.[4]

In recent years, researchers have considered fish-derived gelatine, avian gelatine, and polysaccharide thermo-reversible gels as alternatives to mammalian gelatine. The most researched gelatins are those extracted from the sea (skin, bones and fins from warm and cold waters)[5-7]. Compared to the bovine and porcine gelatins currently available in the market, fish-derived gelatins do not have the risk of outbreaks of infectious diseases such as bovine spongiform encephalopathy and hand, foot and mouth disease, and they also have the advantage of not being subject to vegetarianism and being acceptable to the followers of Islam. Many by-products are produced during the processing of fish, such as fish head, skin, scales, fins, skeleton, offal and eggs, etc. These fish-derived discards account for a large percentage, which can reach more than 60% of the total biomass, therefore, gelatin can be extracted from fish processing waste with a wide and easily available source of raw materials. Scales are an important fish industrial waste, accounting for about 5% of the material contained in fish collagen waste, and the use of fish scales to extract collagen or gelatin has been reported for a variety of fish species such as snapper and red tilapia, sardines, grass carp, deep-sea redfish, and Asian chub[8, 9].

TG enzymes are obtained from microorganisms (bacteria, fungi, and algae), animals, and plants by fermentation or extraction, and they have different degrees of differences in structure, properties, and functions and, therefore, application properties and areas of applicability[10].

Among animals, Clarke and other researchers first discovered the presence of TGases in mammals when studying guinea pigs; Wilhelm demonstrated the ability of TGases to catalyze the integration of polyamines into the γ-amide group of glutamines of protein molecules. Following the first studies in guinea pigs, TGases were found in the brain, thymus, and lungs of rodents, the liver of rabbits, and plasma cells of humans. TGase, which originates from animals, is less used in industrial production because of its complicated operations such as isolation and extraction and low yield. In plants, in 1987, Icekson et al. revealed the presence of TGase in plants by finding transaminase activity in yellowing pea seedlings. Subsequently, researchers have found a variety of plant-derived TGases in different parts of sunflower, begonia, potato, maize and many other plants, such as cell wall, chloroplast, mitochondrial and cytoplasm-derived TGases. However, plant TGases are not yet commonly used in commercial production in the market, and most of the plant-derived TGases are used in experimental investigations. In microorganisms, TGase was first isolated from a mutant strain of Streptomyces in 1989 by Ando et al. Subsequently, the presence of TGase was found in different strains (e.g. Streptomyces spp., Bacillus spp.) and other microorganisms one after another, and operations and studies on the isolation and purification, property identification, etc. of TGase derived from microorganisms were carried out.

Compared with TG enzymes from animals and plants, TG enzymes of microbial origin (MTG) have various excellent characteristics such as high catalytic capacity, high yield, low cost, and no need for Ca2+ catalytic activity. In addition, MTG has been widely used in food processing and manufacturing because it can be produced in large quantities

by fermentation process.

Glutamine transaminase (TGase) can catalyze linkage (glycosylation), cross-linking, and deamination reactions between or within proteins, resulting in the formation of network-like structures within and between molecules [4], which leads to changes in protein molecules for the purpose of protein modification. TG enzymes can modify the structural characteristics and physicochemical properties of proteins, such as improving the appearance, water holding capacity, hardness, viscosity and elasticity of food products, and have a large potential for application in food processing.

As catalysts, TGases can catalyze the following three types of biochemical reactions.

(a) Linkage reaction: TGase can catalyze the reaction of γ-carboxamido group with primary amine group to link proteins with amine compounds, using this reaction, some special amino acids can be introduced into protein molecules, thus increasing the nutrients and flavor of food.

(b) Cross-linking reaction: TG enzyme can catalyze the reaction of γ-carboxamido group with ε-acylamino group of lysine residue to produce ε-(γ-glutamine)-lysine isopeptide bond, this isopeptide bond makes the protein (or peptide) cross-linked, which in turn changes various physical properties of the protein.

(c) Deamidation reaction: When the ε-acylamino group of lysine residue and small molecule primary amine are not present, water will act as the acceptor of γ-amide group, and TGase can make the γ-amide group deamidate to produce glutamate residue, and the food containing glutamate can enhance the freshness, so adding TGase can make the food taste and flavor better.

As a green cross-linking agent, TGase is widely used in many fields such as food processing, health care and leather manufacturing, among which food processing has the greatest demand.

In food processing, TGase can catalyze the linkage reaction between (or within) proteins, which can change their gel properties, significantly enhance the product texture and improve the quality of the product. Through single-factor experiments, Wang Chunxiao [11] investigated and found that the gel strength of sodium caseinate reached the maximum when 140 U/g of TGase was added, with a maximum gel strength of 746.51 g-cm, when the temperature condition was set at 41°C and pH was set at 8.0, which was relatively effective when applied to meatballs. By studying the surimi of Siniperca chuatsi by thermal induction method,found that the addition of TGase to the surimi enhanced the three-dimensional mesh-like structure of surimi gelatin, in which the gel strength was found to be significantly increased when the amount of TGase added was 0.4 U/g and 0.6 U/g, respectively. Wang Le[12] et al. found through their study that brown rice cake had the maximum specific volume when 10 U/g and 2 U/g TGase was added to egg yolk paste and egg white, respectively, and the maximum value of specific volume was 6.0 mL/g. The addition of TGase to the sample increased the viscosity by 62.27%, its emulsification stability by 3.86%, and foam stability by 8.43%, which indicated that the addition of TGase could effectively improve the brown rice cake texture. In the application of dairy products, Lian Yanxiang et al. demonstrated through experimental research that adding appropriate amount of TGase in yogurt preparation could stabilize the structure of yogurt, reduce the amount of whey precipitation, make the yogurt thicker and smoother with delicate taste, improve the sensory evaluation and extend the shelf life of yogurt; in the processing of pasta products, Xia Mingjing[13] et al. found that TGase had an important role in pasta products, and the addition of TGase improved the water absorption of quinoa-wheat flour, the water absorption of quinoa-wheat flour, and the water stability of quinoa-wheat flour. The addition of TG enzyme increased the water absorption rate of quinoa-wheat flour, prolonged its stabilization time and reduced its weakening degree. At the same time, TGase increased the specific volume of bread, increased the sensory score, decreased the hardness of bread, increased the elasticity and cohesiveness, and significantly improved the quality of quinoa-wheat flour.

Besides, TG enzymes have a very important role in the pharmaceutical field, textile industry and leather processing[14].

Cross-linking refers to the process by which polymer chains are connected to each other (linear or branched chains) in the form of covalent bonds to form a three-dimensional network-like structure[15]. Gelatin gels are thermally reversible gels, which are formed by a mechanism in which protein molecules are unfolded by heat, exposing the reactive groups that need to react, which induces depolymerization and stretching between protein molecules, and upon cooling, hydrogen bonds are formed between peptide chains, which in turn further form gels.

The interaction between protein molecules is affected by the enzymatic cross-linking modification, and the gelling effect of cross-linked modified gelatin is higher than that of ordinary gelatin because more covalent bonds are generated in the enzymatically cross-linked modified gelatin, and the interaction between covalent bonds is stronger, thus the gel strength of enzymatically modified gelatin increases. The mechanism of cross-linking modification is to add cross-linking agent to the gelatin, so that the cross-linking agent group combines with the amino and carboxyl groups in the gelatin molecule to improve the mechanical properties and thermal stability of gelatin. Translated with Translator (free version)[16].

Cross-linking modified gelatin can improve the gelatin gelling effect and enhance the application performance of gelatin, the modification methods of gelatin are physical modification (blending, irradiation, etc.), and chemical modification (condensation, etherification, etc.), the commonly used cross-linking agents are TGase, kynepin, formaldehyde, diisocyanate, etc., of which, formaldehyde, diisocyanate have different degrees of toxicity, kynepin is less toxic, but its source is limited, the price is higher. TGase is a protein contained in human body without any toxicity, and TGase produced by microorganism is suitable and easy to obtain, and enzymatic cross-linking is safer and more efficient, meanwhile, TGase is still active when the temperature is low, and when gelatin forms a triple helix structure, the network structure formed by the cross-linking reaction catalyzed by TGase is more stable and the gel strength increases.

3. Conclusion

Compared with fish-derived gelatin, mammalian gelatin has relatively better physicochemical properties such as emulsification, gel strength, and film-forming ability, but the cross-linking reaction of protein molecules through the TGase-catalyzed reaction of γ-carboxamido groups with ε-acylamino groups of lysine residues to generate isopeptide bonds can make fish gelatin similar to mammalian gels in

terms of properties. Extensive covalent cross-linking during gel cooling and solidification may lead to an almost complete loss of thermal reversibility in synthetic gels, which is mainly influenced by whether the covalent cross-linking reaction occurs before or after the attachment region of the gelatin triple helix structure. Increasing the TG enzyme concentration was reported to increase the melting temperature and to increase the elasticity and cohesiveness of fish skin gelatin gels, but the gel strength and hardness decreased due to too rapid gel network formation.

After enzyme modification, gelatin not only has its original properties, but also has certain mechanical properties based on the original, which can be widely used in biofilm materials, medical fibers, biological coatings, industrial gelatin, etc. Therefore, the study of TG enzyme-catalyzed cross-linked gelatin research has some application significance.

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