**International Journal of Biology and Life Sciences**
ISSN: 2957-9511 | Vol. 2, No. 2, 2023

# Effect of microbial transglutaminase on thermal stability of milk protein

**Yunbin Dai*, Hongchen Li**

Heriot-Watt University, Edinburgh EH14 4AS, Scotland, UK
* **Corresponding author**: Yunbin Dai

**Abstract:** At present, Transglutaminase (abbreviated as TG, EC2.3.2.13) has been used in meat products, dairy products and baked goods in Japan and other countries. The stability of MP (Milk protein) colloid system is very important to the quality of dairy products. The effect of MTG (Microbial TG) on the thermal stability of MP was studied in this experiment. The change of HCT (Heat Coagulation Time) of MP emulsion before and after adding MTG was measured. The results show that the effect of MTG on MP leads to the decrease of its solubility in the region of pH < 4.0 or pH > 7.0, but it increases in the range near the isoelectric point of MP (pH 4.0 ~ 6.0). In the range of acidic pH value with low stability, the high calcium ion activity, low Zeta potential and micelle hydration make the milk have poor thermal stability and will solidify rapidly at 150°C. The hydrophobic interaction between MTG and MP changes the conformation of MP, which leads to the change of thermal stability.

**Keywords:** Microbial transglutaminase; Milk protein; Thermal stability.

## 1. Introduction

Transglutaminase (TG, EC2.3.2.13 for short) is a transferase that catalyzes acyl transfer reaction. By catalyzing acyl transfer reaction of glutamine, covalent cross-linking occurs between egg whites (or polypeptides), lysine containing e-N H 2 is cross-linked to egg whites, and protein molecules can also be deamidated. Studies have shown that deamidation of MP (Milk protein) by acid method can significantly improve its solubility [1-3], but hydrolysis of protein peptide chain and degradation of other amino acid residues are inevitable during acid treatment at high temperature [4]. In addition, their unique amino acid sequence and three-dimensional structure also give them a wide range of functional properties, all of which are very beneficial to food processors.

The application of TG involves medicine, industry, food and other fields. In recent years, the research on it in medical treatment has become more and more in-depth. It has been reported that TG is involved in the occurrence of abdominal diseases [5] and can even induce cancer [6]. The applicable pH of MTG (Microbial TG) is relatively wide, and it has good catalytic activity in the range of pH 4.0~pH 9.0 [7]; MTG has good physical and chemical properties and crosslinking ability, and is widely used in food field. It can improve the cross-linking, emulsifying, water-holding and foaming properties of food, improve the quality and flavor of food, and can play a significant role in different types of food [8]. MTG can also affect the precipitation of whey in yogurt. The research shows that MTG can make protein in yogurt produce cross-linking reaction, and then form a compact microstructure, which locks the free water and improves the water holding capacity, thus reducing the precipitation of whey [9]. It was found that adding 0.8% MTG to emulsified fat beef for 1 h, and treating the beef surface with 0.28% MTG, 0.4% NaCl and 0.09% polyphosphate as the optimal conditions, the best quality artificial fat beef could be obtained.

At present, TG has been used in meat products, dairy products and baked goods in Japan and other countries. However, the research in this field in China is still in its infancy, and its application in food industry is even less [10]. The stability of MP colloid system is very important to the quality of dairy products. The effect of MTG on the thermal stability of MP was studied in this experiment.

## 2. Materials and methods

### 2.1. Materials and reagents

Trihydroxymethyl aminomethane, low molecular weight standard protein, BioTek ELISA, steam sterilizer, Nanodrop photometer, TG, tabletop high-speed freezing centrifuge and other materials in clean bench are all food grade, biochemical grade or above AR grade.

### 2.2. Extraction of MP

In the experiment, the extraction temperature was room temperature, extraction time was 2 hours, and centrifugation time was 3000r/min for 15 minutes. After stirring for 30min, a certain amount of MTG (the ratio of enzyme to substrate is 1:5) is added for enzymolysis reaction, and the enzymolysis reaction temperature is 37°C and the reaction time is 0-48h. After 40min, take out the beaker, put it in the ice-water mixture and cool it for 1h, then transfer it to the refrigerator and store it at 4°C overnight. The next day, take out the MP made of different conditions, and let it stand at room temperature for 20min to be measured.

### 2.3. Thermal stability experiment

At room temperature, divide the emulsion into equal parts (10 mL each), adjust the pH value range from 6.4 to 7.4 with 0.1 mol/L hydrochloric acid or sodium hydroxide solution, let it stand for 1 h, and then readjust the pH value of the sample. Hct (heat coalescence time) is defined as the time from the time when the test tube is immersed in the oil bath to the time when the flocculation phenomenon is visible to the naked eye. The flocculation pH value is defined as: after the sample is flocculated, it is quickly taken out of the oil bath pot and cooled to 20°C with water, and the pH value of the sample is determined, which is the flocculation pH value.

The emulsion was diluted to a certain concentration with SMUF buffer with different pH values, and the Zeta potential of the emulsion at different pH values was measured by Zeta potential analyzer.

# 3. Result

## 3.1. Effect of MTG on solubility of MP

Solubility is an important functional characteristic of protein, because many other functional characteristics are often restricted by its solubility. In this experiment, the solubility of MP treated by MTG at different pH values was investigated. See Figure 1.

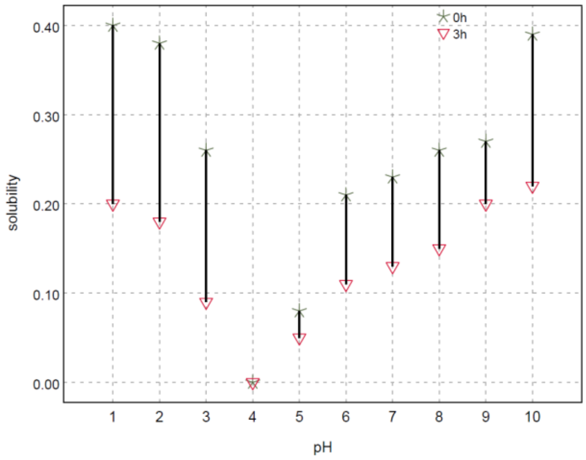


**Figure 1.** PH-solubility curve of MP

The effect of MTG on MP leads to the decrease of its solubility in the region of pH < 4.0 or pH > 7.0, but it increases in the region near the isoelectric point of MP (pH 4.0 ~ 6.0).

## 3.2. Emulsifiability and emulsion stability

The emulsion system is an unstable dispersion system in thermodynamics. protein is an amphoteric polymer with hydrophilic and lipophilic groups and regions, which can stabilize the emulsion system [11]. The experimental results showed that the emulsifying property of MP treated by enzyme was slightly improved in strong acid solution, but it was greatly reduced in weak acid solution (pH5), and it showed a trend of increasing first and then decreasing in neutral solution. In strong acidic and neutral solutions, the stability is better when the reaction time is less than 5 hours, and the stability becomes worse with the extension of reaction time, but the stability is the least ideal in weak acidic solutions. The emulsification stability of MP decreased after enzymolysis for 5 hours, which may be due to the further expansion of protein structure and the exposure of more hydrophobic groups, which led to the deterioration of hydrophilicity, thus affecting emulsification and stability.

## 3.3. Effect of MTG on thermal stability of MP

In order to study the influence of MTG on the thermal stability of MP emulsion, the HCT-pH curve of each emulsion was drawn when the addition amount of MTG was 1, 2.2 and 4.5g/L, the pH value was 6.3, 6.5, 7.0, 7.3 and 7.5, and the corresponding HCT was 150℃, and the results were shown in Figure 2.

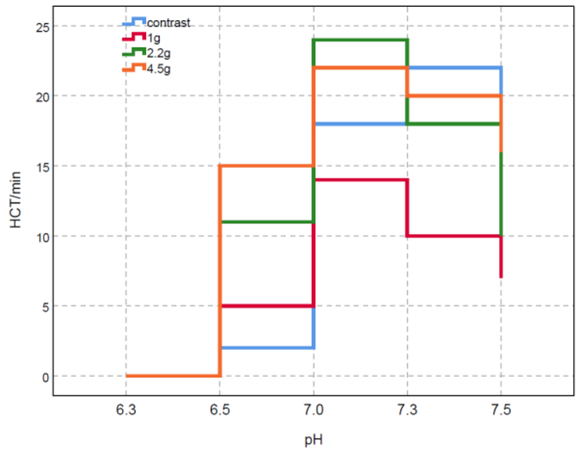


**Figure 2.** HCT-pH curve of MP emulsion

It can be seen that the control emulsion without MTG has the maximum HCT at pH 7.3, which is 22min. The addition of MTG can obviously improve the HCT of emulsion, and with the increase of the addition amount, the HCT corresponding to each pH value increases.

In the range of acidic pH value with low stability, the high calcium ion activity, low Zeta potential and micelle hydration make the milk have poor thermal stability and will solidify rapidly at 150℃. The hydrophobic interaction between MTG and protein changes the conformation of protein, which leads to the change of thermal stability.

# 4. Discussion

Whey protein is recovered from whey containing 93% water, which is one of the most comprehensive natural protein available. Therefore, it is necessary to make some changes in amino acid residues and polypeptide chains of whey protein by specific methods, so as to change the spatial structure and physical and chemical properties of protein macromolecules and improve their functional characteristics, which is of great significance for rational development and utilization of whey protein, environmental protection, effective use of cheap resources and expansion of its application fields. There are many methods to modify whey protein, such as chemical modification, physical modification, enzyme modification and mixed modification. Chemical modification and physical modification (non-enzymatic modification) are commonly used whey protein modification technologies at present, which are popular because of their simple reaction, wide application and remarkable effect. This is mainly because the formed cross-linking bonds make protein form a complex and tight spatial network structure, which enhances its ability to contain and bind water. A small amount of MTG can polymerize whey protein with a mass fraction of 15% to form a protein polymer with far better water holding capacity than that before polymerization.

The thermal stability of MP is related to the anti-coagulation ability of milk at sterilization temperature and the economic significance in the production of ultra-high temperature canned sterilized milk and milk-containing products, especially concentrated milk. The thermal stability of MP is very sensitive to different factors (pH, milk salt and milk protein) and treatment methods (preheating, concentration and homogenization). These factors will cause milk to partially or completely coagulate during processing or gel during storage. Many studies show that TG can improve the thermal stability of MP [5]. This study shows that the high calcium ion activity, low Zeta potential and micelle hydration make MP have poor thermal stability and will solidify rapidly at 150℃ The hydrophobic interaction between MTG and protein changes the conformation of protein, which leads to the change of thermal stability.

The emulsion must have sufficient thermal stability before it can be sterilized by ultra-high temperature heating, and the fat globules in the emulsion must be small enough and stable to ensure the stability of the product during storage. When MP emulsion is heated, protein denaturation after heating increases the surface hydrophobicity and promotes the coagulation of heat-sensitive whey protein, and cross-linking between free protein (with relatively high surface hydrophobicity) and interfacial adsorption protein (with relatively low surface hydrophobicity) in water phase is easy to occur [6]. The results of this study showed that the emulsifying property of MP after enzyme treatment was slightly improved in strong acid solution, but it was greatly reduced in weak acid solution (pH5), and it showed a trend of increasing first and then decreasing in neutral solution. In strong acidic and neutral solutions, the stability is better when the reaction time is less than 5 hours, and the stability becomes worse with the extension of reaction time, but the stability is the least ideal in weak acidic solutions. The emulsification stability of MP decreased after enzymolysis for 5 hours, which may be due to the further expansion of protein structure and the exposure of more hydrophobic groups, which led to the deterioration of hydrophilicity, thus affecting emulsification and stability.

# 5. Conclusions

The effect of MTG on MP leads to the decrease of its solubility in the region of pH < 4.0 or pH > 7.0, but it increases in the region near the isoelectric point of MP (pH 4.0 ~ 6.0). In the range of acidic pH with low stability, the high calcium ion activity, low Zeta potential and micelle hydration make MP have poor thermal stability and will solidify rapidly at 150℃. The hydrophobic interaction between MTG and protein changes the conformation of protein, which leads to the change of thermal stability. Emulsifiability of MP after enzyme treatment was slightly improved in strong acid solution, but it was greatly reduced in weak acid solution (pH5), and it first increased and then decreased in neutral solution. In strong acidic and neutral solutions, the stability is better when the reaction time is less than 5 hours, and the stability becomes worse with the extension of reaction time, but the stability is the least ideal in weak acidic solutions.

# References


[1] Chia J,Mcrae J L,Kukuljan S,et al. A1 beta-casein milk protein and other environmental pre-disposing factors for type 1 diabetes[J]. Nutrition & Diabetes, 2017, 7(5):274.

[2] Nra B,Gf A,Aa A,et al. Individualized target fortification of breast milk with protein, carbohydrates, and fat for preterm infants: A double-blind randomized controlled trial[J]. Clinical Nutrition, 2021, 40(1):54-63.

[3] Cowley F C,Barber D G,Houlihan A V,et al. Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism[J]. Journal of Dairy Science, 2015, 98(4):2356-2368.

[4] Crowley S V,Desautel B,Gazi I,et al. Rehydration characteristics of milk protein concentrate powders[J]. Journal of Food Engineering, 2015, 149(10):105-113.

[5] Crowley, Shane V,Gazi, et al. Rehydration characteristics of milk protein concentrate powders[J]. Journal of food engineering, 2015.

[6] Thijs H,Molenberghs G,Verbeke G. The Milk Protein Trial: Influence Analysis of the Dropout Process[J]. Biometrical Journal, 2015, 42(5):617-646.

[7] Chronakis I S,Kasapis S,Richardson R K. Small deformation rheological properties of single and mixed Maltodextrin - Milk protein systems[J]. Carbohydrate Polymers, 2015, 29(2):137-148.

[8] Domagala J,Pluta-Kubica A,Wieteska-Sliwa I,et al. The influence of milk protein cross-linking by transglutaminase on technology, composition and quality properties of Gouda-type cheese[J]. International Dairy Journal, 2022(130):130.

[9] Gharibzahedi S,Chronakis I S. Crosslinking of milk proteins by microbial transglutaminase: Utilization in functional yogurt products[J]. Food Chemistry, 2018, 245(15):620-632.

[10] Darnay L. Determination of mTG Activity in Low-Fat Semi-Hard Cheese Using Fluorescent Labelling[J]. Journal of Fluorescence, 2016, 27(2):1-5.

[11] Salinas-Valdes A,Millan J,Serna-Saldivar S O,et al. Yield and Textural Characteristics of Panela Cheeses Produced with Dairy-Vegetable Protein (Soybean or Peanut) Blends Supplemented with Transglutaminase[J]. Journal of Food Science, 2015, 80(10-12):2950-2956.