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Research Article

Effect of microbial transglutaminase on physicochemical properties of ostrich meat ball

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Abstract

The physicochemical properties of ostrich (*Struthio camelus*) meat ball added with different levels of microbial transglutaminase (MTGase) and subjected to heat at 40°C for 30 min (one step heating) or 40°C for 30 min followed by 80°C for 20 min (two step heating) were studied. The lowest cooking loss was found in one step heating sample added with 1% (w/w) MTGase. Breaking force of ostrich meat ball increased with the increasing of MTGase concentration (P<0.05). However, no significant differences in deformation of all treated samples were observed (P>0.05). Addition of MTGase affected in decreasing of expressible moisture, especially in two step heating sample with 1% (w/w) MTGase addition. Two steps heating provided the highest whiteness value compared to that of one step heating process. Solubility of protein in ostrich meat balls were decreased with the increasing of MTGase concentrations. SDS-PAGE analysis revealed that the myosin heavy chain (MHC) content decreased and that the cross-linked protein amount, apparently increased with MTGase addition, especially in two step heating sample added with 0.7 and 1% (w/w). These results suggest that it is possible to improve physicochemical properties of protein gels by adding MTGase, thus increasing their potential utilization in mince based products.

Keywords: cross-linking, *Struthio camelus*, poultry, MTGase, Thailand.

Introduction

Ostrich production in Thailand has received increasing attention during the last few years. Meat from the ostrich is gaining in importance in recent years and is presented and marketed as a new red meat alternative. In general the ostrich meat is similar in protein, amino acid and mineral contents to other red meat sources [1] but has a low intramuscular fat content and high content of polyunsaturated fatty acids [2]. Several ostrich meat products (fermented sausage, ham, steak, pressurized ostrich meats, hot deboned ostrich and Thai style sausages) are already found in the market. In addition, due to increased health awareness among consumers, ostrich meat and related products will have good market potential in the near future. Ostrich meat ball produced from trimmed meat, a by-product of steak and/or chunk meat production is interesting as a value added product. In addition, meat-balls are a popular Chinese-style emulsion meat product in Asian countries.

Muscle protein gelation contributes to desirable texture and fat-water emulsion stabilization in processed meat. Poultry meat is now being used to manufacture many processed meat products that traditionally have been made from pork or fish. The texture is an important characteristic that significantly influences product palatability and consumer acceptance. Typically, meat balls made from poultry meat do not provide desirable gel strength compared to that of the mammal species. Enzymatic methods are frequently used to control technological properties of products, including gelling and water holding capacity.

Transglutaminase (TGase; protein-glutamine g-glutamyltransferase, EC 2.3.2.13) is a well known cross-linking enzyme that can catalyze the formation of δ-(γ-glutamyl) lysyl crosslinks among food proteins. This enzyme has been used to improve rheological properties of food [3]. Ahhmed et al. [4] reported that the texture of sausages was improved by the formation of δ-(γ-glutamyl) lysine crosslinks by the addition of TGase in chicken and beef sausages. Tseng et al. [5] also reported that both the yield and gel strength of the chicken meat-balls increased as TGase supplement increased.

The objectives of this study were to improve the textural properties of trimmed ostrich meat ball by addition of microbial transglutaminase (MTGase) and to determine the extent of such improvement by measuring the physical and chemical properties of the ostrich meat ball.

Materials and Methods

Chemicals and raw materials

Sodium dodecyl sulphate (SDS) and bovine serum albumin were obtained from Fluka (Buchs,Switzerland). Beta-mercaptoethanol (*β*ME) and Coomassie Brilliant Blue G-250 were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). *N,N,N',N'*-tetramethyl ethylene diamine (TEMED) was purchased from Bio-Rad Laboratories (Hercules, CA, USA). Hydrochloric acid, tris (hydroxymethyl) aminomethane and other chemicals of analytical grade were obtained from Merck (Darmstadt, Germany).

Trimmed ostrich meat (*Struthio camelus*) was obtained from a farm in Chiang Rai, Thailand. MTGase (Activa TG-AK: 50-84 units/g) was obtained from Ajitrade (Thailand) Co., LTD (Bangkok, Thailand). Salt, sugar, garlic, pepper and other food grade ingredients were purchased from Makro superstore, Chiang Rai, Thailand.

Minced ostrich preparation

The ostrich meat obtained from the store was washed with tap water and drained for 5 minutes. The fat, connective tissue and other undesired parts were removed. The trimmed ostrich meat was cut into small pieces and then minced with a meat mincer. The resultant product was referred to as "minced ostrich" and kept in the freezer (-18°C) until used.

Preparation of ostrich meat ball

The minced ostrich was used as the main raw material for ostrich meat ball production. The recipe of ostrich meat ball consists of 250g of the minced meat, 5g of salt, 0.75g of phosphate, 1.0g of sugar, 12.5g of tapioca flour, 2g of garlic, 2g of pepper and 50g of ice were prepared. The different concentrations of MTGase (0, 0.3, 0.5, 0.7, and 1% w/w) were added into the mixture and then subjected to chopping until the sol (paste) was obtained. The sol was divided into two parts. The first part was subjected to cooking at 40^{o}C for 30 min. Cooked ostrich meat ball from this process was called "one-step heating". Remaining sol was also subjected to cooking at 40^{o}C for 30 min and then heated at 80^{o}C for 20 min. This sample was called "two-step heating". Cooked ostrich meat ball was cooled down in ice-water for 30 min and then kept in a refrigerator overnight prior to property analysis.

Physical properties of ostrich meat ball determinations

Cooking loss

The ostrich meat balls (20g) were placed in a polyethylene bag and heated in water bath at 80°C for 5 min. The drip was drained from the sample. The weight loss was calculated by the difference between the sample weights measured before and after cooking and expressed as a percentage of the weight before cooking.

Texture analysis

Texture analysis of ostrich meat ball was carried out using a Model Texture Analyzer (TAXT2. plus, Surrey, UK). Ostrich meat ball was equilibrated at room temperature (28–30°C) before analysis. Five cylindrical samples (2.5cm in length) prepared and placed in the texture analyzer equipped with a spherical plunger (5mm diameter, 60mm/min depression speed). Breaking force and deformation were recorded.

Expressible moisture

Expressible moisture was measured according to the method of Ng [6]. Ostrich meat balls were cut into a thickness of 5mm, weighed and placed between three pieces of Whatman paper No. 1. The standard weight (5kg) was placed on the top of the sample for 2 min and then removed the sample to reweigh. Expressible moisture was calculated and expressed as a percentage of the initial sample weight.

Whiteness

Three samples from each treatment were subjected to whiteness measurement using a colorimeter (Hunterlab colorquestEX, Virginia, U.S.A.) equipped with a 10 mm port size, illuminant D^{65} and 10^{0} observers. CIE L* (lightness), a* (redness/greenness), and b* (yellowness/biueness) values were recorded. Whiteness was calculated using the following equation:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^* + b^*]^{1/2}$$

Chemical properties of ostrich meat ball determination

Protein solubility

Protein solubility was determined according to the procedure of Tang *et al.* [7]. The protein in the sample (2g) was extracted with cold 0.25M potassium phosphate buffer (pH 7.2). The mixture was homogenized and kept at 4°C overnight with shaking. The obtained sample was centrifuged at 1500xg for 20 min. The protein content in supernatant was determined by Biuret method. Total protein was extracted by using 0.5M NaOH. Protein solubility was calculated by the difference between the total and the proteins solubilized in the supernatant.

Electrophoresis

Protein pattern of the sample was determined according to the method of Laemmli [8]. Two grams of sample were added with 18 ml of 5%SDS solution, homogenized and then incubated at 85°C in a temperature-controlled water bath for 1 h to solubilize the proteins. Solubilized protein was mixed at the ratio of 1:1 with sample buffer (0.5M Tris-HCl pH 6.8, 4%SDS, 20%glycerol, 10%βME) and then boiled for 3 min. The treated sample (20µg protein) was loaded into the gel made of 4% stacking and 10% separating gels, and then subjected to electrophoresis at a constant current of 15mA/gel. After electrophoresis, the gels were stained with Coomassie brilliant blue R-250 in the mixture of methanol-acetic solution.

Statistical analysis

Analysis of Variance (ANOVA) was used to analyze the data from triplicate measurements. Differences between means were evaluated by Duncan's Multiple Range Test by using the SPSS statistic program (Version 11.5).

Results and Discussion

Physical properties of ostrich meat ball

Cooking loss

Cooking loss of ostrich meat ball added with different concentrations of MTGase (0, 0.3, 0.5, 0.7 and 1%, w/w) and two cooking conditions is shown in Figure 1. The highest cooking loss was found in the control samples (without MTGase addition) when compared with the treatment samples. The cooking loss of ostrich meat balls was decreased when the concentration of MTGase was increased from 0.3 to 1.0% (w/w) ($P<0.05$). The results also showed that higher cooking loss value was found in all samples cooked by two step heating than the one step heating, except in the control. For one step heating, decreasing of 77% cooking loss was found when the sample was added with 0.3% (w/w) MTGase compared with the control. However, the lowest cooking loss was obtained when MTGase concentration was added up to 1% (w/w) and also one step heating provided the highest water holding capacity as an adverse effect to the cooking loss. This result was probably due to the strong interaction between protein molecules by the function of MTGase addition. In addition, samples incubated for long times always presented significantly higher cooking loss values, than did samples incubated for shorter periods. Tseng *et al.* [5] reported that the yield of low-salt chicken meat balls increased with higher percentage of MTGase addition, suggesting the MTGase could improve emulsion stability and hydration properties. Pietrasik and Li-Chan [9] observed that MTGase addition favourably reduced the cooking loss of pork batter gels.

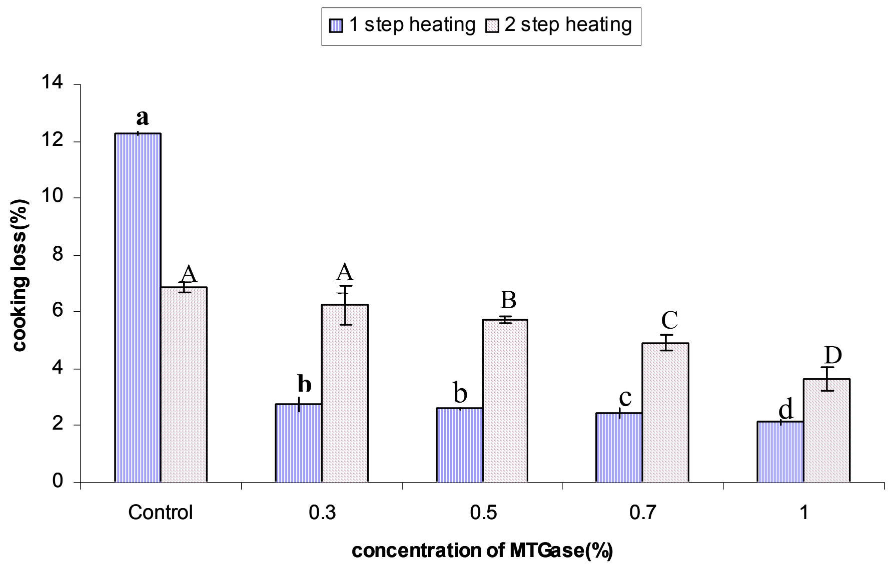

Figure 1. **Effect of MTGase and heating conditions on cooking loss of ostrich meat ball.** Bars represent the standard deviation from triplicate determinations. Different letters in each heating condition indicate significant differences ($P<0.05$).

Textural properties

The breaking force and deformation of ostrich meat ball with and without added MTGase cooked with different heating conditions are depicted in Figure 2. The highest breaking force was found in the sample added with 1% (w/w) MTGase and heated with 2-steps heating process (7,764.40g), while the lowest value was found in the control sample of 1-step and 2-steps heating condition. A slight increase in the breaking force was found in all samples when the concentration of MTGase increased ($P<0.05$). Addition of 1% MTGase increased the breaking force of the ostrich samples by 73% and 23% in 1-step heating and 2-steps heating, respectively. In general, 2-step heating provided higher breaking strength than that of 1-step heating. From the result, the breaking force of the control sample of 2-step heating was higher than that of one step heating 92%. Treatment with MTGase enhances the texture and gel strength of meat and meat proteins in many products by forming a bond between Gln and Lys, which improves the rigidity and gel elasticity of meat products, avoiding some undesirable attributes such as stickiness, high viscosity and excessive meat adhesiveness [10]. Muguruma *et al*. [11] reported that the texture of chicken sausage was improved by the formation of ε-(γ-glutamyl) lysine crosslink by the addition of MTGase. Herrero *et al*. [12] also reported that addition of MTGase increased in b-sheet and turns structure, accompanied by increases in hardness, springiness and cohesiveness and decreases in adhesiveness of meat systems.

The highest deformation was found in the ostrich meat ball treated with no addition of MTGase and heated by 1-step heating (14.75 mm). While the lowest value was found in the sample added with 1% MTGase. However, the result showed that addition of MTGase up to 1% could not significantly increase the deformation of all samples ($P>0.05$). MTGase was a good proteinbinding agent and could be a good additive to help the functionality of proteins, in order to improve the texture of meat.

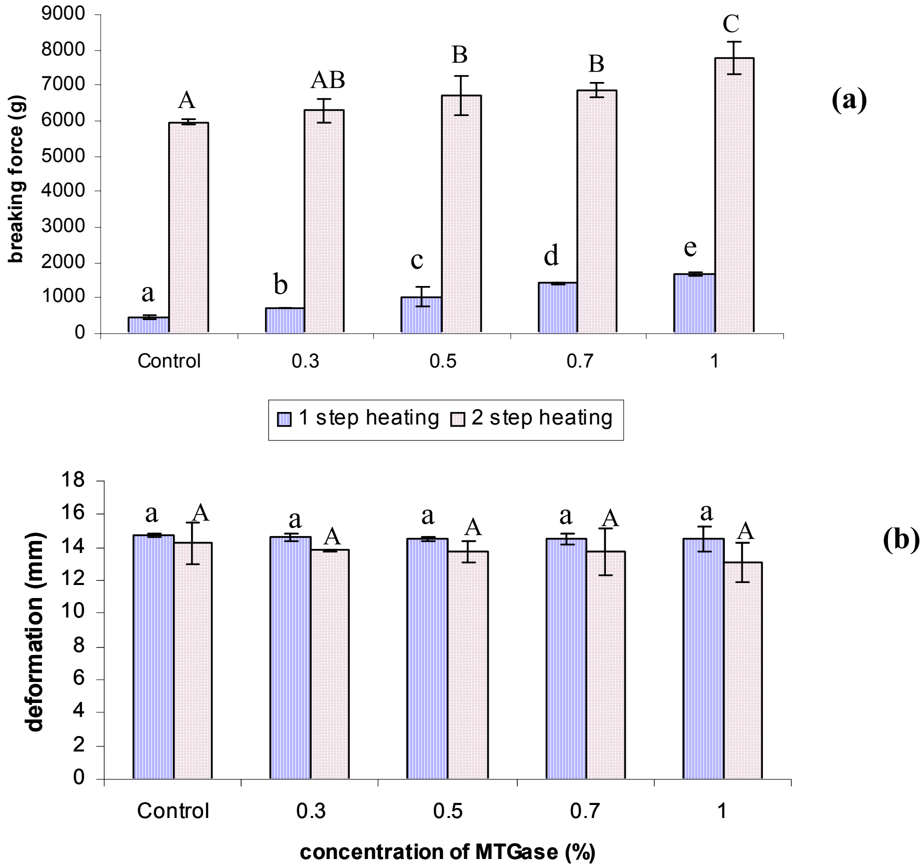

Figure 2. Breaking force (a) and deformation (b) of ostrich meat ball added with different concentrations of MTGase.

Bars represent the standard deviation from triplicate determinations. Different letters in the same heating condition indicate significant differences (*P*<0.05).

Expressible moisture

The expressible moisture of ostrich meat balls was expressed as the weight loss of the sample pressed with the standard weight (5kg) for a specific of time. It related to the water holding capacity of the muscle sample. The results are shown in Table 1. An increasing in expressible moisture was found when the concentration of MTGase increased in both 1-step and 2-steps heating. However, the result also showed that no significant differences of expressible moisture were observed in 2-steps heating samples when the MTGase addition was 0.3% (*P*>0.05). The lowest expressible moisture was found in ostrich meat ball treated with 1% MTGase in both 1-step and 2-steps heating. Decreasing of the expressible moisture (57%) was found in the 1-step heating control sample compared to the sample added with 1% MTGase (*P*<0.05). Low expressible moisture of the protein gels samples suggested more water retained in the gel network [13].

Tammatinna *et al*. [14] reported that addition of MTGase in combination with the appropriate setting condition could be used to improve gel forming ability via non-disulphide covalent bonds.

Table 1. Expressible moisture of ostrich meat ball treated with different conditions.

MTGase concentration (%)	Expressible moisture (%)	
	1 step heating	2 steps heating
0.0	13.9 ± 0.20^{a}	6.98 ± 1.85^{a}
0.3	8.82 ± 0.42^{b}	5.40 ± 0.05^{ab}
0.5	6.70 ± 0.11^{c}	5.33 ± 0.26^{ab}
0.7	6.33 ± 0.28^{d}	4.78 ± 0.72^{b}
1.0	5.91 ± 0.07^{d}	3.96 ± 0.52^{b}

*Values are given as mean ± SD from triplicate determinations.
**Different superscripts in the same column indicate significant differences (P<0.05).

Whiteness

Whiteness of ostrich meat balls added with different concentrations of MTGase and different cooking conditions is shown in Figure 3. The highest whiteness value was found in the control sample (without MTGase) of both 1- and 2-steps heating. However, it was slightly decreased when the MTGase concentrations were increased (P>0.05). There was no significant differences in whiteness of one step heating samples (P>0.05). For 2-steps heating, the whiteness of the sample was decreased 6% from the control when 1% (w/w) of MTGase was added. The higher whiteness values were obtained in two step heating because of high temperature was affected to denaturation of myoglobin caused of reduction the a* (redness) value. Uresti *et al*. [15] reported that there were no significant differences between fish gels containing 0% (control) and 1% MTGase. Tseng *et al*. [5] also reported that there were no significant differences of colour parameters between chicken meat balls added with MTGase at the concentration of 0 to 1% (w/w).

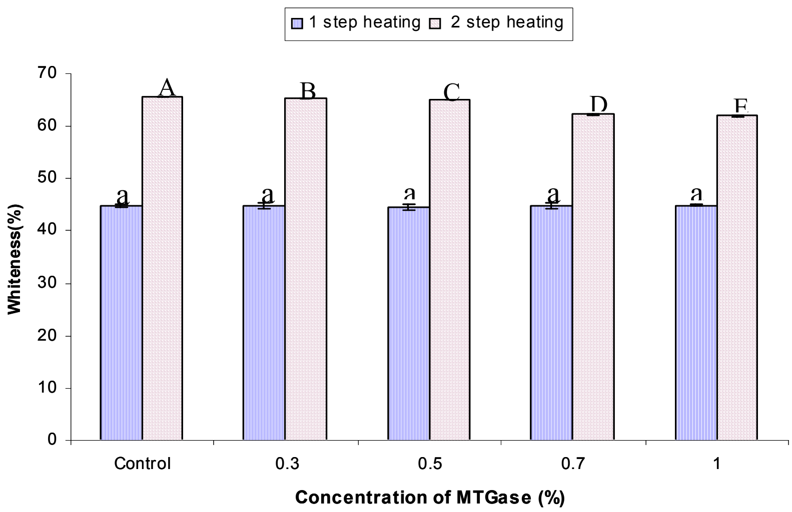

Figure 3. Whiteness of ostrich meat ball added with various concentrations of MTGase and different heating conditions.

Bars represent the standard deviation from triplicate determinations. Different letters in the same heating condition indicate significant differences (P<0.05).

Determination of chemical properties of ostrich meat ball

Protein solubility

The solubility of protein in ostrich meat ball treated with different concentrations of MTGase (0-1%, w/w) and different heating conditions is shown in Table 2. The highest protein solubility was found in the control sample (without MTGase addition) of 1-step heating, while the lowest value was found in the sample treated with 1% (w/w) MTGase from 2-steps heating. Increasing of MTGase levels resulted in decreased protein solubility, especially at the high level of addition for 1-step heating sample (P<0.05). However, no significant difference in protein solubility was found in two step heating samples (P>0.05). The solubility of protein decreased from 10.09 to 1.49% by using 2-steps heating process. As expected, MTGase treatment resulted in gradual and marked decreases in protein solubility, especially at high enzyme concentration (1%, w/w) with 2-steps heating process. It can be mentioned that the addition of MTGase led to firmer gel networks through the formation of intermolecular glutamyl-lysine crosslinks resulting in decreased solubility of proteins. Decreases in protein solubility were related to the extent of cross-linking or incubation time, and were attributed to conformational changes of proteins and the formation of high Mw insoluble biopolymers [7]. Tseng *et al*. [5] reported that the formation of isopeptide bonds is irreversible and contributes to strong protein–protein interactions that stabilize the network.

Table 2. Effect of MTGase and heating conditions on protein solubility of ostrich meat ball.

MTGase concentration (%)	Protein solubility (%)*	
	1 step heating	2 steps heating
0.0	10.09 ± 0.55[a]**	1.49 ± 0.20[a]
0.3	9.69 ± 0.55[a]	1.45 ± 0.07[a]
0.5	9.79 ± 0.24[a]	1.44 ± 0.16[a]
0.7	6.90 ± 0.42[b]	1.43 ± 0.18[a]
1.0	6.77 ± 0.58[b]	1.42 ± 0.19[a]

*Values are given as mean ± SD from triplicate determinations.

**Different superscripts in the same column indicate significant differences (P< 0.05).

Protein pattern

Protein pattern of ostrich meat ball added with different concentration of MTGase and heating by 1- and 2- steps process is shown in Figure 4. The patterns of protein were the same in all treatments that showed the major protein components of myofibrillar proteins (MHC and AC). A slightly decrease of MHC was observed in both 1-step and 2-steps heating when the concentration of MTGase was increased. No changes in AC band were observed in any sample treated with MTGase. However, increasing of the cross-linked polymer was observed on the top of the gel when the enzyme concentrations were increased. Higher cross-linked polymer of ostrich protein was found in 2-steps heating than that found in 1-step heating process.

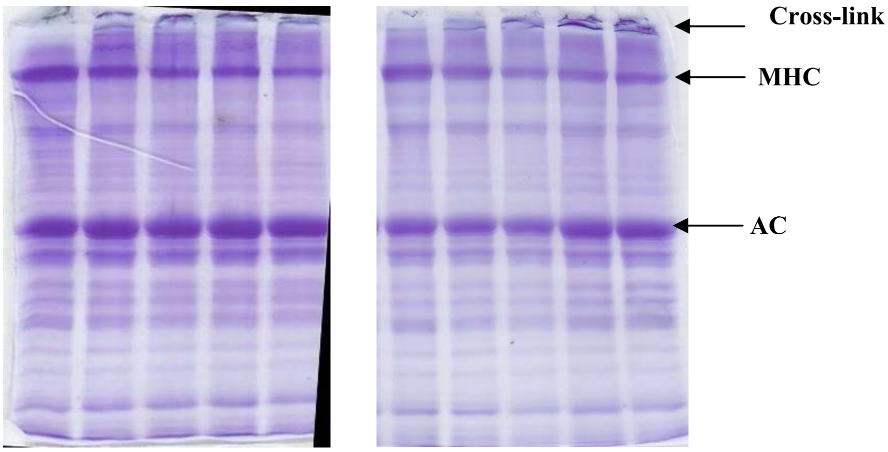

Figure 4. Protein patterns of ostrich meat ball treated with different concentrations of MTGase.
MHC, myosin heavy chain; AC, actin. Numbers represent the MTGase concentration (%).

Kumazawa *et al*. [16] reported that the decrease in the MHC content on SDS-PAGE may have been due to crosslinking of MHC. MTGase was very powerful for induction of MHC cross-linking [14]. The decrease of MHC band intensity was concomitant with the increased breaking force (Figure 2). MTGase can act as a combining agent, facilitating the aggregation of certain proteins via bonding between specific amino acids (Gln and Lys), to generate numerous polymers, which are considered to be important residual bio-products in meat [10]. The formation of δ-(γ-glutamyl) lysine isopeptide bonds results in both intra- and intermolecular crosslinking of proteins, leading to polymerization [3].

Conclusion

Addition of MTGase for ostrich meat ball production resulted in decreased cooking loss, expressible moisture, protein solubility and whiteness of the samples. One percentage of MTGase addition could improve the textural properties of ostrich meat ball, especially in 2-steps heating. MHC band intensity decreased with appearance of cross-linked proteins when the MTGase concentrations increased.

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