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Effect of NaCl, Gum Arabic and Microbial Transglutaminase on the Gel and Emulsion Characteristics of Porcine Myofibrillar Proteins

Munkhtugs Davaatseren[1] and Geun-Pyo Hong*

Department of Food Bioengineering, Jeju National University, Jeju 690-756, Korea
[1]*Department of Bioresources and Food Science, Konkuk University, Seoul 143-701, Korea*

Abstract

This study investigated the effect of gum arabic (GA) combined with microbial transglutaminase (TG) on the functional properties of porcine myofibrillar protein (MP). As an indicator of functional property, heat-set gel and emulsion characteristics of MP treated with GA and/or TG were explored under varying NaCl concentrations (0.1-0.6 M). The GA improved thermal gelling ability of MP during thermal processing and after cooling, and concomitantly added TG assisted the formation of viscoelastic MP gel formation. Meanwhile, the addition of GA decreased cooking yield of MP gel at 0.6 M NaCl concentration, and the yield was further decreased by TG addition, mainly attributed by enhancement of protein-protein interactions. Emulsion characteristics indicated that GA had emulsifying ability and the addition of GA increased the emulsification activity index (EAI) of MP-stabilized emulsion. However, GA showed a negative effect on emulsion stability, particularly great drop in the emulsion stability index (ESI) was found in GA treatment at 0.6 M NaCl. Consequently, the results indicated that GA had a potential advantage to form a viscoelastic MP gel. For the practical aspect, the application of GA in meat processing had to be limited to the purposes of texture enhancer such as restructured products, but not low-salt products and emulsion-type meat products.

Keywords: myofibrillar, gum arabic, transglutaminase, ionic strength, functional properties

Introduction

Myofibrillar protein (MP) is a key protein responsible for functional properties of whole meat and processed meat (Xiong, 2004). Solubility of MP is the most important factor affecting the functional properties of MP, and the MP is highly labile depending on pH and ionic strength. MP is soluble at ionic strength more than 0.5 M NaCl under neutral pH (Xiong, 2004), therefore, it is hard to expect advantageous functional properties of MP either at a low ionic strength or at an acidic pH. Some techniques have been introduced to enhance the solubility and the functional properties of MP. Among them, polysaccharide conjugation is a promising technique to improve the availability of MP by enhancing the MP solubility (Sato *et al*., 2003). To conjugate protein-polysaccharides, Maillard reaction has been widely investigated, and the resultant conjugates had high solubility under wide pH

ranges even at the isoelectric point of its origin (Sato *et al*., 2000). Meanwhile, the protein-polysaccharide conjugation by Maillard reaction is limited its application because of an elongated reaction time and a relatively high reacting temperature (Flanagan and Singh, 2006). An enzymatic conjugation has been attempted, and microbial transglutaminase (TG) has a potential to conjugate proteins to polysaccharides which contain either small amount of glycoprotein (Flanagan and Singh, 2006) or amino group (Sang *et al*., 2009) by cross-linking each other.

Gum arabic (GA), which is an exudate of *Acacia Senegal*, has wide uses in food, textile, pottery, lithography, cosmetics and pharmaceutical industries (Ali *et al*., 2009). GA contains small amount of proteins linked by covalently in its structure and has a good emulsifying and film-forming properties (Belitz *et al*., 2009). It is very soluble in water, and the solution viscosity starts to rise steeply only at high concentrations unlike to many other polysaccharides (Belitz *et al*., 2009). Flanagan and Singh (2006) observed a notable conjugation of sodium caseinate and GA by TG reaction using a multiangle laser light scattering. Cloas *et al*. (1993) also reported high solubility profiles of vegetable proteins conjugated to GA at iso-

*Corresponding author: Geun-Pyo Hong, Department of Food Bioengineering, Jeju National University, Jeju 690-756, Korea. Tel: +82-64-754-3615, Fax: +82-64-755-3601, E-mail: gphong @jejunu.ac.kr

electric point of the proteins. Excluding the above study, however, no information either on glycosylation of MP or on functional properties of their conjugates was available. In overall, high polysaccharide to protein ratio (10:1) was applied for the conjugation (Flanagan and Singh, 2006), because the protein content of GA was very low (ca. 2%) and there was a possibility of intra-molecular cross-linking of protein. However, thermodynamic incompatibility between protein and polysaccharide limits the usage of high amount of polysaccharide in protein based foods (Tolstoguzov, 2003). Meanwhile, gums have been reported to have various functional properties (Chanamai and Mc-Clements, 2002; Djordjevic et al., 2008; Shang and Xiong, 2010). Therefore, this study aimed to evaluate the effects of GA and TG on functional properties of MP under various NaCl concentrations.

Materials and Methods

Materials

Porcine *m. longissimus dorsi* with pH 5.5-5.7 was selected randomly 24 h post mortem from both sides of 3 carcasses. The meat was trimmed of all visible fat and connective tissue and cut into 1 cm cubes. The meat cubes were divided into approximately 200 g portions, vacuum packaged in polyethylene pouches, and frozen at -30°C prior to use (within 30 d). The TG (Activa-TI, 1% enzyme and 99% maltodextrin) was donated by Ajinomoto Inc. (USA). The GA (spray dried) was purchase from Sigma-Aldrich Co. (USA). All chemicals used in this study were reagent grade.

MP extraction

The frozen meat cubes were thawed at 4°C overnight. The MP was extracted by the method of Xiong (1993) with minor modifications (Chin et al., 2009). The minced meat was washed three times using 4 vol. (w/v) of 0.1 M NaCl in 50 mM sodium phosphate buffer (pH 6.5) followed by washing with 8 vol. (w/v) of 0.1 M NaCl (pH 6.5). The final MP suspension was filtered through two layers of gauze and adjusted to pH 6.5. The washed MP was precipitated by centrifugation at 1,500 g for 15 min under refrigerated condition. The protein concentration of the MP was determined by the Biuret method (Gornall et al., 1949) and kept in ice prior to use (within 3 d).

Sample preparation

The MP suspended samples containing GA and/or TG were formulated based on the previous investigation

Table 1. Sample formulation and experimental design

Treatment	Ingredients (%, w/w)[1]			
	MP	GA	TG	Oil
Gelation				
MP	4	-	-	-
MP+GA	4	2	-	-
MP+TG	4	-	0.3	-
MP+TG+GA	4	2	0.3	-
Emulsion				
MP	1	-	-	10
MP+GA	1	1	-	10
MP+TG	1	-	0.2	10
MP+TG+GA	1	1	0.2	10

[1]MP, myofibrillar protein; GA, gum arabic, TG, microbial transglutaminase.

(Hong et al., 2012) as shown in Table 1. Each ingredients (MP, GA and TG) were dissolved individually in a 50 mM sodium phosphate buffer (pH 6.5) containing 0.1, 0.3 and 0.6 M NaCl (final concentration basis), respectively. For gelation characteristics, 4% (w/w) MP was mixed with 2% (w/w) GA and/or 0.3% (w/w) TG. In the case of emulsion characteristics, 10% (w/w) corn oil-in-water emulsion stabilized by 1% (w/w) MP was prepared with 1% (w/w) GA and/or 0.2% (w/w) TG. Emulsion mixture was homogenized for 1 min at 17,000 rpm using an ultra-turrex (T8, IKA Laboratories, Germany) and used as an emulsion dispersed sample.

Gel characteristics

To estimate the MP gel characteristics, 5 g aliquots were transferred into glass test tubes (15 mm diameter) and incubated at 4°C for 24 h. After incubation, MP samples were heated in a programmable water bath by linearly increasing temperature from 4 to 80°C by the rate of 1°C/min, cooled in an ice for 5 min, and then tempered at ambient for 2 h. Exudates in test tubes were carefully discarded, and the weights of gels were measured to determine the yield of MP gel. The yield was expressed as a percentage of initial sample weight. After measuring the yield, samples were compressed with an Instron testing machine (Model 3340, Instron, USA) with a 9 mm diameter of plunge and 50 mm/min head speed. The force at failure (the first peak) was expressed as gel strength.

For thermal gelling behavior, the sample mixtures prepared freshly were subjected to dynamic rheological test using an oscillatory rheometer (AR-1000, TA Instruments, Inc., USA) equipped with 40 mm parallel plates with 1 mm gap. The sample was loaded onto the sample plate and covered with paraffin oil to prevent evaporative loss

during heating. Before oscillation, sample was equilibrated at 20°C for 3 min and sheared with heating from 20°C to 80°C at a rate of 1°C/min at a fixed frequency of 0.1 Hz with a maximum strain of 0.02. The storage modulus (G') was recorded throughout the thermal scanning.

Emulsion characteristics

The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by the methods of Pearce and Kinsella (1978). The emulsion was incubated at ambient temperature for 2 h with stirring gently and the dispersions (30 μL) were diluted in 6 mL of 0.1% (w/v) SDS solution. The absorbance at 500 nm was measured at 0 h and 2 h, respectively. The EAI was expressed as total surface dimension of MP-coated oil droplets per MP concentration (Pearce and Kinsella, 1978). The ESI was calculated by percentage of turbidity change at 2 h over its initial (0 h).

For creaming index, 10 g of emulsion dispersions were transferred to a glass test tube (1 cm diameter) and stored at ambient temperature for 6 h. The creaming index was expressed as percentage height of serum over total height of emulsion.

To evaluate microstructure of the emulsions, one drop of emulsions incubated at ambient temperature for 6 h was placed on a slide glass and covered with a cover slip. The microstructures of the emulsions were observed using an optical microscope (Nikon microscope Eclipse E400, Nikon Corp., Japan) connected to a CCD camera (CCD-300 T-RC, DAGE-MTI, USA).

Statistical analysis

The completely randomized design was adopted in the present study. The data were analyzed by the general linear model using the SAS (ver. 9.0). Analysis of variance (ANOVA) was performed, and the means were separated by Duncan's multiple range test when the main effects were significant ($p<0.05$).

Results and Discussion

Gel characteristics

At 0.1 M NaCl concentration, thermal gelling behavior of MP was distinguished by 3-step including steady increase in G' up to 56.4°C, steep increase up to 72.4°C and decrease in G' at higher than 72.4°C (Fig. 1A). The pattern of G' was identical when GA and/or TG were added into MP suspension. The maximum G' of GA treatment was higher than that of control, still the maximum G' of GA treatment was lower than those TG treatments. Since the steep increase in G' indicated network formation of MP (Xiong, 1993), TG contributed to thermal gelation of MP better than GA. However, the maximum G' of the treatments was lower than 0.24 kPa, indicating weak thermal gel formation during thermal scan. Decrease in G' at higher than 72.4°C would be resulted from the moisture exudation caused by partial MP aggregation. Similar results were also obtained at 0.3 M NaCl concentration (Fig. 1B). The MP exhibited the maximum G' at 72.2°C thereafter showing the decrease in the G'. The addition of GA and/or TG increased the G' of MP during thermal scan. In addition, the G' did not show a peak but slightly increased up to 80°C, reflecting that GA and TG assisted a uniform MP gel formation. Nevertheless, the maximum G of TG/GA treatment was lower than 0.6 kPa, reflecting less elastic MP gel formation at 0.3 M NaCl concentration. The impacts of GA and TG on thermal MP gel formation were identical at 0.6 M NaCl concentration (Fig. 1C). TG increased the G' and the addition of GA further increased the G' of MP during heating. The results indicated that both GA and TG had a positive effect on elastic MP gel formation. Consequently, TG and GA had a po-
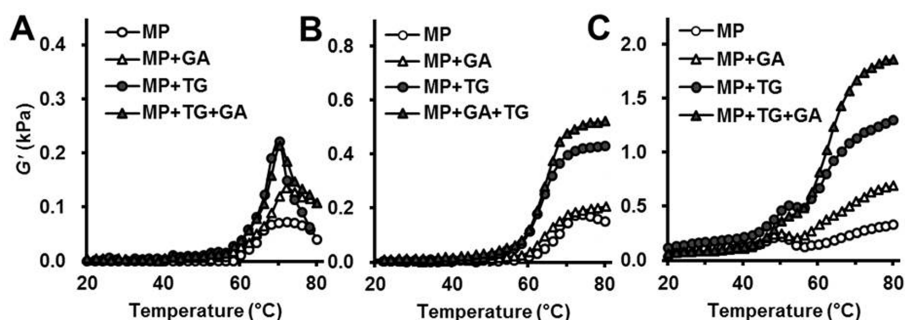

Fig. 1. Effects of microbial transglutaminase (TG) and gum arabic (GA) on the storage modulus (G') of myofibrillar protein (MP) gel prepared at varying ionic strengths.

tential advantage to form the elastic MP gelation, whereas high ionic strength (or NaCl concentration) was essential to elastic MP gelation.

As depicted in Fig. 2A, both ingredients (GA and/or TG) and NaCl concentrations affected the gel strength of MP gels ($p<0.05$). For gel strength, the addition of GA showed a positive effect while the impact was lower than that of TG ($p<0.05$). The maximum gel strength was obtained in GA combined with TG treatment regardless of ionic strength ($p<0.05$). The effect of TG on MP gel strength was already reported in previous studies (Chin *et al*., 2009; Kuraish *et al*., 1997). Although there was no information on the effect of GA on texturization of gel-type foods, the present study demonstrated that GA had a good MP gel forming ability and the effect was more considerable when TG was combined. For ionic strength, the gel strength of each treatment did not differ at 0.1 M and 0.3 M NaCl concentration, while the gel strength increased at 0.6 M NaCl concentration ($p<0.05$). Since MP was a key ingredient in the gel formation, the solubility of MP was crucial to form an elastic gel. The MP gels formed at low ionic strength was weak, hence the simultaneous addition of GA and TG could not provide an elasticity of heat-set

MP gel.

Alternately, GA attributed a negative effect on cooking yield of MP gel (Fig. 2B). For control (MP treatment), increasing ionic strength increased the cooking yield of MP gel from 53% at 0.1 M NaCl to 94% at 0.6 M NaCl concentration ($p<0.05$). The pattern was still maintained in TG treatment of which yield was proportional to ionic strength, although the yields of TG treatment were lower than those of control ($p<0.05$). Hong *et al*. (2010) reported that the addition of TG decreased cooking yield of MP gel and the usage of hydrocolloids such as alginate was required to diminish the TG-mediated moisture loss in MP gel. Chin *et al*. (2009) noted that TG-catalyzed moisture loss of MP gel was caused by enhancing protein-protein interactions with reducing protein-water interaction. Previously GA was expected to retain moisture in MP gel matrix. In the current study, cooking yield of GA treatment did not differ from that of control at 0.1 M NaCl concentration. Meanwhile, the cooking yields of GA treatment were lower than those of control at 0.3 M and 0.6 M NaCl concentrations ($p<0.05$). The results were also in accordance with the comparison of TG and GA/TG treatments. Furthermore, cooking yield of GA/TG treatment was lower at 0.6 M NaCl than that at 0.3 M NaCl ($p<0.05$). Todd *et al*. (1989) postulated that cooking loss of restructured pork increased when GA was also added. The author suggested that the presence of NaCl affected the hydrogen bonds which accounted for the cooking loss of restructured pork prepared with GA (Todd *et al*., 1989). Consequently, the results reflected that GA had a potential application to form an elastic heat-set MP gel, whereas water-binding ingredients had to be considered to prevent moisture loss caused by the addition of GA.

Emulsion characteristics

Emulsifying ability of MP-stabilized emulsions are depicted in Fig. 3A. The EAI of MP was 8.61 m^2/g at 0.1 M NaCl and increased with increasing NaCl concentration ($p<0.05$). It is identified that the EAI is directly affected by amount or solubility of proteins (Pearce and Kinsella, 1978). Increase of NaCl provided more soluble characteristics of MP and the MP adsorbed effectively on the surface of emulsion droplets, resulting in higher EAI (Chobert *et al*., 1988). The addition of GA further increased the EAI of MP-stabilized emulsion at all tested NaCl concentrations ($p<0.05$). The high EAI of GA treatment comparing to that of control (MP) was not understood in this study. Our previous study indicated that the addition of polysaccharides such as sodium alginate did not imp-
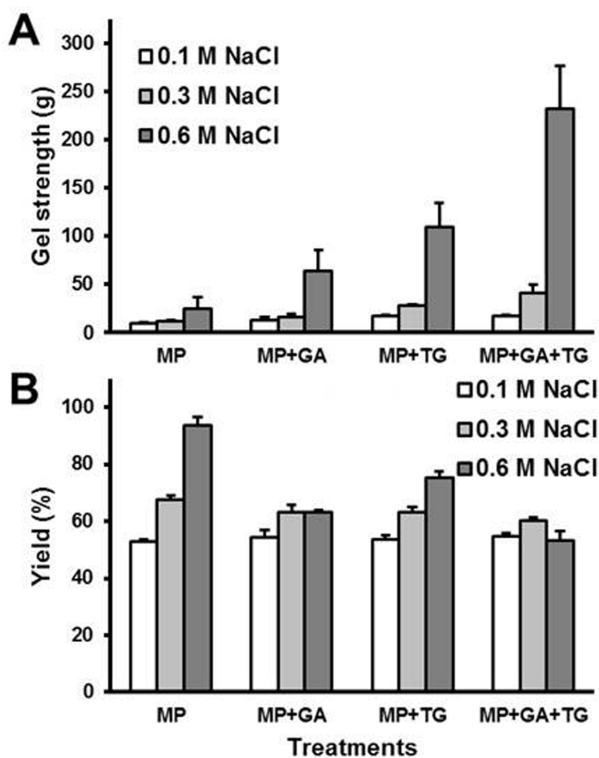

Fig. 2. Effects of microbial transglutaminase (TG) and gum arabic (GA) on (a) gel strength and (b) yield of myofibrillar protein (MP) at various NaCl concentrations (n=3).
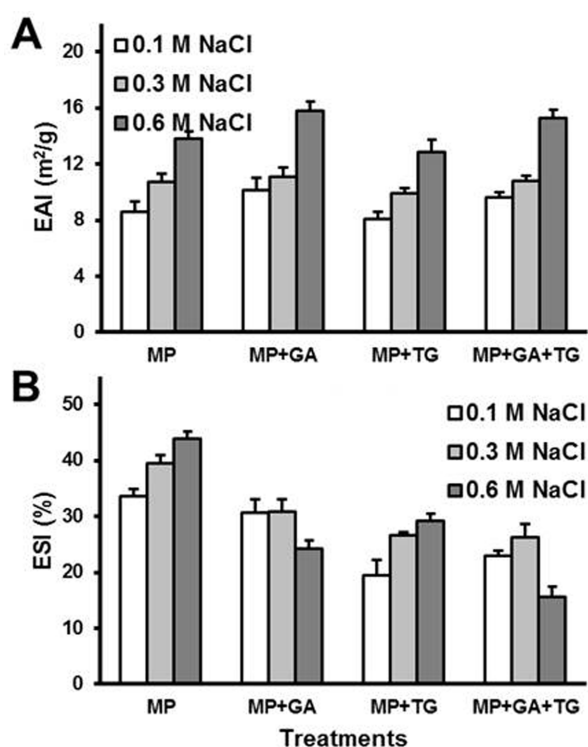
Fig. 3. Effects of microbial transglutaminase (TG) and gum arabic (GA) on emulsification characteristics of myofibrillar protein (MP) stabilized O/W emulsion at various NaCl concentrations (n=3).

rove the EAI of MP-stabilized emulsions or even attributed negative effects on the emulsifying capacity due to their high viscosity (Hong *et al*., 2012). Meanwhile, the effect of GA as an emulsifier has been reported by Montenegro *et al*. (2012). Yadav *et al*. (2007) suggested that protein fractions in GA would be involved in emulsification activity of GA, which warranted further exploration. Alternately, the addition of TG slightly decreased the EAI of MP-stabilized emulsion, however, the differences in EAI between controls (MP) and TG treatments were not significantly different at all tested NaCl concentrations. The results were not in consistency with our previous observation (Hong *et al*., 2012) where TG showed negative effect on emulsifying capacity but improved emulsion stability of MP-stabilized emulsion. The results reflected that TG did not attribute emulsifying capacity of MP. It should be noted that TG-mediated crosslinking reaction was not as intensive as modifying the emulsifying ability of MP, because of short reaction time (2 h). GA/TG treatment exhibited better emulsifying capacity than that of control ($p<0.05$), nevertheless the EAI of GA/TG treatment did not differ from that of GA treatment. According to the review of Kato (2002), protein-polysac-

charide conjugates exhibited greater emulsifying ability than the protein alone. In the present study, however, TG-mediated MP-GA conjugation was not found based on the comparison of GA and GA/TG treatments as shown in the comparison of control and TG treatment.

The ESI was affected by both treatments and NaCl concentrations (Fig. 3B). The MP-stabilized emulsion showed higher ESI with increasing NaCl concentration ($p<0.05$). MP was completely solubilized at high NaCl concentration and the MP sol exhibited high viscosity (Ionescu *et al*., 2007). Overall emulsion stability was related to the rheological property of continuous phase, hence the higher the viscosity of continuous phase, the better the emulsion stability (Yadav *et al*., 2007). On the other hand, the addition of GA decreased the ESI of MP stabilized emulsion comparing to control ($p<0.05$). In particular, the ESI of GA treatment was significantly lower at 0.6 M NaCl than those at < 0.3 M NaCl ($p<0.05$). The result was not in agreement with Tadav *et al*. (2007) where GA improved the emulsion stability. The different results would be explained by amount of GA in the emulsion formulation. In the present study, 1% (w/w) GA was added in the formulation. Rheological property of GA was distinct from other polysaccharides so that the addition of GA up to 20% did not affect the viscosity of the solution (Belitz *et al*., 2009). It was hardly expected that 1% GA would improve emulsion stability in this study. However, it was unclear why GA caused emulsion instability at high NaCl concentration. It was thought that MP might have a thermodynamic incompatibility with GA that was also observed in low cooking yield. For microstructure, emulsion droplets of GA treatment at 0.6 M NaCl showed higher size comparing to that at 0.3 M NaCl (Fig. 4). Consequently, GA participated in emulsification, while low viscosity of GA could not maintain the emulsion stability. The incompatibility of MP and GA would manifest droplet coalescence thereby resulting in low emulsion stability. For TG treatment, significantly lower ESI was found at all NaCl concentrations comparing to those of control ($p<0.05$). The microstructure confirmed the TG-induced instability of MP-stabilized emulsion. Based on larger droplet sizes, droplet coalescence was shown in TG treatment at 0.1 M NaCl which would be due to low emulsifying capacity. Meanwhile, TG treatment at 0.6 M NaCl showed intensive aggregation among emulsion droplets which caused excessive droplet flocculation. Although, droplet sizes of emulsions prepared at 0.6 M NaCl were relatively small comparing to those at 0.1 M NaCl, flocculated emulsion droplets indicated the low emulsion sta-
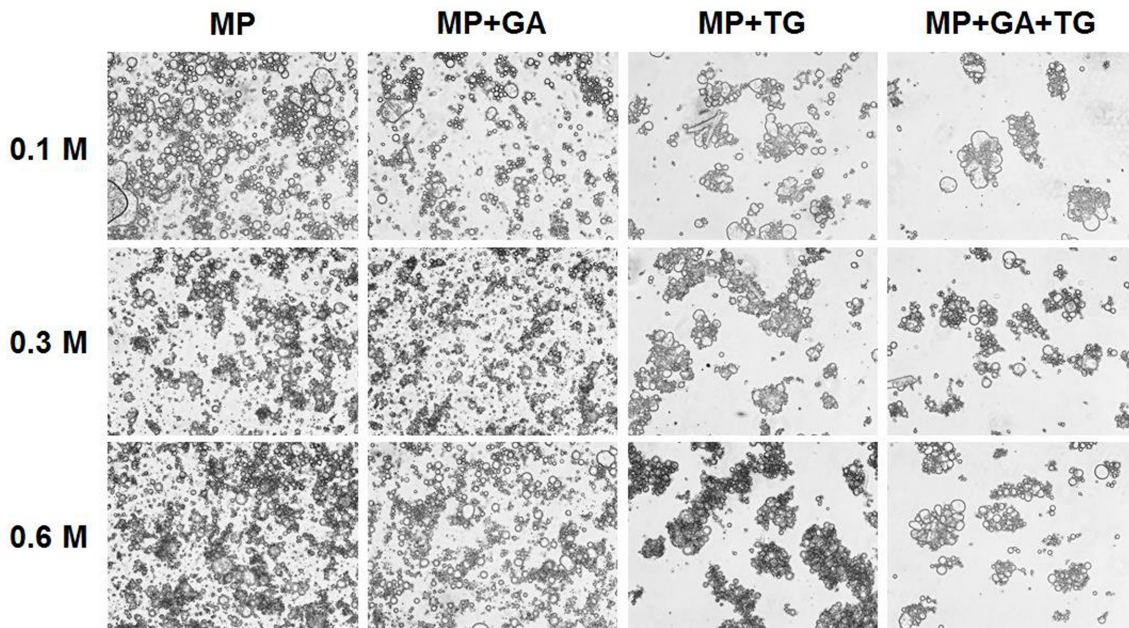
Fig. 4. Optical microscopy of myofibrillar protein (MP) stabilized O/W emulsion as affected by gum arabic (GA), microbial transglutaminase (TG) and ionic strength.

bility. Eventually, protein aggregation caused by TG-catalyzed polymerization would destabilize the MP-stabilized emulsion (Hong *et al*., 2012). The results indicated that both TG and GA had negative attribute to emulsion stability. The GA/TG treatment showed intermediate emulsion stability between GA and TG treatment, while the lowest stability among all treatments was shown in GA/TG treatment at 0.6 M NaCl. These phenomena would be resulted from TG-mediated MP aggregation as well as incompatible nature of GA and soluble MP.

Conclusion

Based on the result, GA was good texturizing and emulsifying agent in meat processing. Nevertheless, GA also manifested drawback in water retention and emulsion stability of model meat products. There was no evidence of TG-mediated MP-GA conjugation. Although, low cooking yield and emulsion stability of GA treated MP were not completely understood in the present study, this study demonstrated different functional properties of GA comparing to other polysaccharide-based additives in meat formulation. Additional exploration was still required to confirm the incompatibility of MP and GA.

Acknowledgements

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