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COMPARINGTHE EFFECT OF MICROBIAL TRANSGLUTAMINASE ON RHEOLOGICAL PROPERTIES OF DIFFERENT FISH PASTE

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ABSTRACT

Microbial transglutaminase (MTG) effects on dynamic rheological properties of three kinds of fish (silver carp, common carp and rainbow) and chicken pasteswas investigated. Temperature sweep (25-90°^{C}), time sweep (45 min at 40°^{C}), and SDS-PAGE was carried out for each samples. At the final temperature (90 °^{C}), the sample of silver carp containing active MTG had the greatest G' among others. However, MTG was less effective on chicken paste, but anegotiable difference has been observed among various kind of meats, mainly due to differences in the physiological and biochemical properties.

Keywords: Chicken, Fish, Microbial Trans glutaminase, Rheology properties.

INTRODUCTION

Nowadays, the demand for high quality meat products s raising in the world. The mechanical properties offinal product is one of quality parameters that directly affects its consumer preference.Gelation process in meat product affects the overall quality andacceptability of that, andthe structural properties of the matrix and the type of intermolecular interactions occurring under different processing conditions determine the functional properties and texture of gels (Alvarez et.al., 1999).

Transglutaminase (TGase; protein-glutamine -glutamyltransferase, EC 2.3.2.13) is an enzyme can catalyzes acyl-transfer reaction between the γ-carboxyamide group of peptide bound glutamine residues (acyl donors) and a primary amines (acyl acceptors), including the ε -amino group of lysine residues in proteins. Using of transglutaminase- catalyzed reactions can modify the functional properties of food proteins (Benjakul *et.al.*, 2003 and Zhu *et.al.*, 1995). Microbial transglutaminase (MTGase) is commonly used by industry to improve the mechanical properties of meat and fish products (Uresti *et.al.*, 2006). MTG may have different effects on various types of meat (Ahhmed *et.al.*, 2007). Rheological studies involving oscillatory shear flow are commonly used to characterize the mechanical properties of gels.We can understand MTG effectson gel properties with this type of experiment. Myofibrillar proteins are very important because contribute to meat gelation system[5]. The functional and textural properties of meat depend on gel-forming ability. However, some factors influence the gelling properties of muscle protein, including meat species, death condition, maturation (Benjakul et.al., 2003), myofibrillar protein concentration, state and amount of water, pH, time/temperature of comminution and interactions between myofibrillar proteins and added ingredients (Belibagli et.al., 2003).

There are many researches that investigated the effects of MTGase on vary species meat,such as pork (Hong and Chin, 2012 and Pietrasik and Li-chan, 2002), beef (Castroet.al., 2009, Martínez et.al., 2010 and Pietrasik, 2002), chicken (Abdulatef et.al., 2009, Tseng, 2000 and Sun, 2011), and fish (Uresti et.al., 2006, Cardoso, 2007, Cardoso et.al., 2010 and Moreno et.al., 2010). The objective of the present study is to compare the effect of MTG on rheological properties between three types of fish meat, rainbow trout (*Oncorhynchusmykiss*) which is a species of salmonid native to tributaries of the Pacific Ocean in Asia and North America, common carp (*Cyprinuscarpio*) which is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia, and silver carp (*Hipophthalmichthysmolitri*) which is an abundant warm water fish, that its fillets present an attractive white colour, and chicken meat.

MATERIALS AND METHODS

MATERIALS

Fresh chicken breasts, common carp (*Cyprinuscarpio*), silver carp*(Hipophthalmichthysmolitrix)* and rainbow trout (*Oncorhynchusmykiss)* were purchased from local marketthen transported on ice to the laboratory. Transglutaminase (Activa WM – 99% maltodextrine and 1% MTGase) was gifted fromAjinomoto, Europe S.A.S Co, France.

SAMPLE PREPARATION

Hand deboned skinless chicken breasts and fishes were removed of frills and ground in a chopper

(Mt1200,Pars KhazarCo, Iran) equipped with a 5mm opening disc. The meats paste kept in plastic bag at -18 °C until analyzed.

SDS-PAGE

Frozen minced meat was partially thawed at 4°C for 2 h, cutinto small piecesand chopped by a mini chopper (Bosch, model MMR08R1, UK). NaCl at 1.5% then after 2 min MTG at the level of 1.5% were added and mixed for 3 min. The MTG was inactivated in 100°C for 5 min. The samples were preparedaccording to table 1.They were incubated at 40°C for 45 min. Myofibrilar proteins were extracted with 1M NaCl.SDS–PAGE analysis was performed according to the method of Laemmli (19).A 12% acrylamide separation gel and a 4% acrylamide stacking gel were used.The pattern of proteinas polymer, myosin heavy chain (MHC) and actin (AC) were evaluated.

Table 1- Samples formulation

Samples	Meat type	Salt (%)	MTG (%)
C	chicken	1.5	
Ca_1	chicken	1.5	1.5 active
Ca_2	chicken	1.5	1.5 active
K	common carp	1.5	-
Ka_1	common carp	1.5	1.5 active
Ka_2	common carp	1.5	1.5 active
F	Silver carp	1.5	-
Fa_1	Silver carp	1.5	1.5 active
Fa_2	Silver carp	1.5	1.5 active
G	Rainbow	1.5	-
Ga_1	Rainbow	1.5	1.5 active
Ga_2	Rainbow	1.5	1.5 active

MEASUREMENT OF RHEOLOGICAL PROPERTIES

Samples were prepared as mentioned above without incubation step. Rheological properties of samples were analyzed using a MCR301rheometer (Anton Paar, Austria) equipped with a 25 mm-parallel plate geometry at a gap of1.0 mm. The space between the parallel plate geometry and sample table was covered with mineral oil to prevent dehydration. Temperature sweep analysis to measure thechanges in dynamic rheological parameter including storage moduli (G'), during heating were performed at a constant frequency of 0.1 Hz and amplitude strain of 0.5%, which was within the linear viscoelastic region, from 25to 90°C at an increasing rate of 1 °C/min.Time sweep analysis for G'was also performed for 45 min at 40°C, a constant frequency of 0.1 Hz and amplitude strain of 0.5%.

RESULT AND DISCUSSION

SDS-PAGE

SDS-PAGEprofiles (Figs. 1-3) showed high molecular weight biopolymers in theborderline between the stacking gel and the separating gel, in sample common carp with active MTG (Ka_1). The results were also confirmedby Chin et al. (Chin and Xiong, 2009), Hong et al. (2012), Kilic (2003) and Chin *et al.* (2009), that is due to MTG reaction (2010). Myosin bands were less dense in samples treated with active MTGase. There are some cross-linking reactions betweenmyosin and other meat proteins that formed the additionalband at the top of the gel. Also, electrophoretic study showed that HMW proteins were mainly affected cross-linking action of MTGase, which formed aggregates insoluble in SDS-PAGE gel. As a result, in samples silver carp and rainbow trout with active MTG (Fa_1, Ga_1), these proteins werepartially absent from the soluble protein fraction used in the electrophoresis. This result was inagree with some other researches (Ahhmed et.al., 2007, Sun , 2011 and Cardoso et.al., 210). Bands representing samples treated with MTG stained less intensely than the controls.There was a decrease in the myosin heavy chain band while MTGase added (Ahhmed et.al., 2007 and Ahhmed et.al., 2009).

Chicken samples with active MTG (Ca_1) showed no significant changes in SDS-PAGE pattern, the intensity of the bandsin chicken sample was not reduced considerably too. As a result of current study, the reaction of MTG is not the same infish and chicken paste. It is known that MTG activity depends on the number of amino acidresidualon the surface of myosin in the proteins. These differences were assumed to be due to differing susceptibility of MTG to myosin proteins between species (Ahhmed et.al., 2009).proteins in chicken are folded into a strand shape that tightly encases a considerable number of glutamineand lysine residues, whereas MTG substrate cannot couple glutamine and lysine (Ahhmed et.al., 2009).

RHEOLOGICAL PROPERTIES

Heat-induced rheological changes in chicken and fish pastes catalysed by the MTGase were studied by evaluating the storage modulus (G') of the meat samples over a temperature range of 25–90 °C.At 40–50 °C, the G' of the chicken samples remained constant. The control curves (no added enzymes, Fig. 2) had a transition phase over the temperature range of 50–60 °C reaching a minimum slightly above 50 °C. .It is a consequence of light meromyosin denaturation, which is seen as a weakening of interactions between myosin molecules. The decline of G' at 50–60 °C has also been seen in beef myofibrils (Egelandsdal and Mitchell, 1987).However the decrease of G'was not observed in the samples with added MTG.This might be due to the presence of maltodextrinin MTG commercial formulation. Moreover G' increase was more greater in the samples containing active MTG. Thisis probablybecause of MTG counteracted thedenaturation of myosin molecules and thus forming isopeptide of (g-glutamyl) lysinebondsin myosin molecules prevented from the G' drop.On further heating, G' increased rapidly above 60 °C due to gelation of myosin and the chicken meat samples reached the final gelation stage.

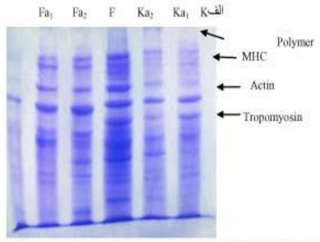

Fig 1.The SDS–PAGE pattern. Silver carp samples. (F: meat, Fa_1: meat+ active MTG, Fa_2: meat+ inactive MTG), common carp samples (K: meat, Ka_1: meat+ active MTG, Ka_2: meat+ inactive MTG)

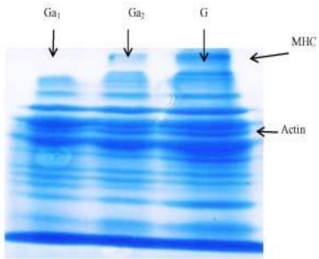

Fig 2.The SDS–PAGE pattern.Rainbow trout samples. (G: meat, Ga_1: meat+ active MTG, Ga_2: meat+ inactive MTG)

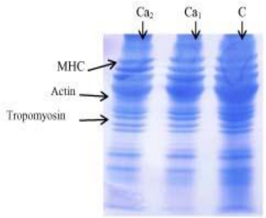

Fig 3.The SDS–PAGE pattern. Chicken samples. (C: meat, Ca_1: meat+ active MTG, Ca_2: meat+ inactive MTG)

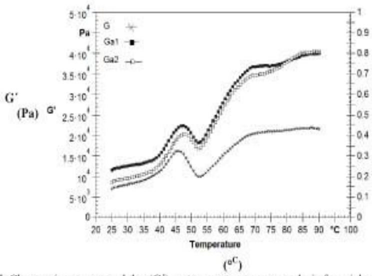

Fig 7: Changes in storage modulus (G') on temperature sweep analysis for rainbow trout meat paste. (G: meat, Ga_1: meat+ active MTG, Ga_2: meat+ inactive MTG)

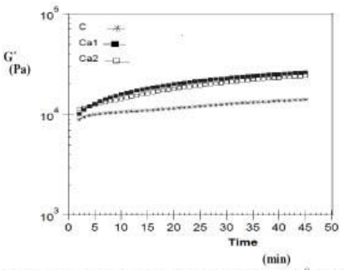

Fig 8: Changes in storage modulus (G') on time sweep analysis (at 40°C) for chicken meat paste. (C: meat, Ca_1: meat+ active MTG, Ca_2: meat+ inactive MTG)

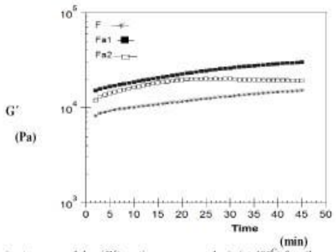

Fig 9: Changes in storage modulus (G') on time sweep analysis (at 40°C) for silver carp meat paste. (F: meat, Fa_1: meat+ active MTG, Fa_2: meat+ inactive MTG)

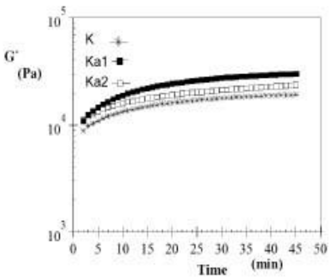

Fig 10: Changes in storage modulus (G') on time sweep analysis (at 40°C) for common carp meat paste. (K: meat, Ka_1: meat+ active MTG, Ka_2: meat+ inactive MTG)

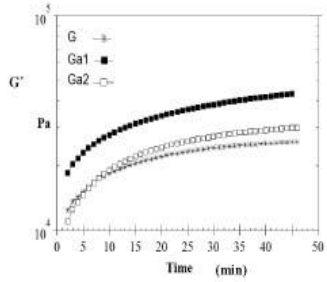

Fig 11: Changes in storage modulus (G') on time sweep analysis (at 40°C) for rainbow trout meat paste. (G: meat, Ga_1: meat+ active MTG, Ga_2: meat+ inactive MTG)

Fish myofibrillar protein (Mf) is thermally less stable than that ofother type of meats and protein denaturation easily damages its functional properties (Saeki, 1998).This may because of lack of constant phase in fish samples. In common carp (K, Ka_1, Ka_2) and rainbow trout (G, Ga_1, Ga_2) samples,there was the decline of G' at 45-50°C, then above 50 °C, the myosin denaturation was started and G' increased.HoweverG' decreasing wasn't seen in silver carp samples(F,Fa_1,Fa_2).Adding active MTG in silver carp was more effective than other samples. Lantto *et al.* (2006) indicated that Microbial TG increased the storage modulusofthe pork meat, at temperature 40 °C-75 °C.

In compare with control, at final heating temperature (90°C), the greatest increase of G' was related to silver carp contained active MTG and, the lowest one belonged to chicken.

According to time sweep (Fig. 3) after 45 min at 40°C, G' of Ca_1 didn't change significantly in compare with control samples. Other samples showed an increase in G' moduli. Proteins in chicken are folded into a string shape that strongly encases a great number of glutamineand lysine residues, whereas MTG substrate cannot connect glutamine and lysine (Ahhmed et.al., 2009). Ahhmed *et.al*, (2008) and Lan et al. (1995) have reported differences in gelation properties between pork, beef, fish, chicken and turkey breast and thigh muscles.

CONCLUSION

MTG had various effects on different kinds of meat. It had the greatest effect on fish samples and, chicken myofibrilar proteins was not affected significantly by MTG. MTG was the most effective on silver carp myofibrilar proteins among four sources of meat studied in this research. The result of this study confirms Abdulatef *et al*. (2009) who indicated that access of MTG to chicken and beef myofibrils is different because it depends on physiological (muscles and their fibre types), biological (substrates) and biochemical (inhibitors and amino acids) parameters (Abdulatef *et.al.,* 2009).

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