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Article

Gel-Forming Ability of Mackerel (*Rastrelliger Branchysoma*) Protein Isolate as Affected by Microbial Transglutaminase

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Abstract

The properties of gel from short-bodied mackerel (*Rastrelliger branchysoma*) alkaline-solubilized protein isolate with different levels (0, 0.25, 0.5, 1.0 and 1.5 unit/g sample) of microbial transglutaminase added (MTGase) and subjected to setting at 40 °C for 30 min, prior to heating at 90 °C for 20 min were investigated. The breaking force of the protein isolate gels with MTGase added at all levels was higher than that of the control gel (without the addition of MTGase) ($p < 0.05$). However, the increase in the amount of MTGase added had no effect on the breaking force of protein isolate gels ($p > 0.05$). From the result, no changes in deformation in all gels treated with and without MTGase were noticeable ($p > 0.05$). The lowest expressible moisture was found in the gel with 0.25 unit/g of MTGase ($p < 0.05$). Generally, the whiteness of all gels incorporating MTGase were lower than that without MTGase ($p < 0.05$). Therefore, the addition of MTGase at 0.25 unit/g sample in combination with an appropriate heating regime was a promising means to improve the gel properties, especially strength and water holding capacity, of short-bodied mackerel protein isolate prepared by alkaline solubilization.

Keywords: Short-bodied mackerel, protein isolate, gel-forming ability, microbial transglutaminase

Introduction

The gel-forming ability of under-utilized small pelagic fish meat has been known to be lower than that of white muscle fish. This limitation is mainly due to the large quantity of lipids and sarcoplasmic proteins, especially myoglobin, in the muscle tissue associated with higher proteolytic activity [1]. Generally, high-quality surimi with improved gel strength and whiteness can be obtained when as much dark muscle as possible is removed [2]. As more dark muscle is removed, some light muscle is obtained but the yields decrease [3]. Abundant dark muscle located along the lateral line near the skin in some red-fleshed fish species such as mackerel and sardine is difficult to remove with a meat separator [1-2,4-5]. Generally, the washing process is necessary for color improvement and gel strengthening of surimi produced from the whole muscle.

Alkaline-aided solubilization, invented by Hultin and Kelleher [3], has shown significant potential as a new method for maximal protein recovery from muscle foods especially dark muscle fish. The extraction mechanism of this process is to solubilize the muscle protein at a high pH to separate soluble proteins, bone, skin, connective tissue, cellular membranes, and neutral storage lipids through centrifugation [6]. The solubilized proteins are collected and recovered by an isoelectric precipitation to give a highly functional and stable protein isolate [7-10]. However, in some cases, proteins recovered by this process have poor gel forming properties which are possibly associated with the low setting phenomenon or inappropriate protein structure for cross-linking or gelation [11-13]. The presence of sarcoplasmic proteins co-precipitated with myofibrillar proteins during the isoelectric precipitation has been cited as one of the reasons for the poorer gelation characteristics of sardine, mackerel and tilapia proteins recovered by

alkaline-aided processes compared with conventional surimi [11,13]. Hultin and Kelleher [3], Haard *et al* [14] and Sikorski [15] reported that small quantities of sarcoplasmic proteins had an adverse effect on the strength and deformability of myofibrillar protein gels. These proteins may interfere with myosin cross-linking during gel matrix formation because they did not form gels and had poorer water holding capacity. Furthermore, Perez-Mateos and Lanier [12] reported that the Atlantic menhaden gels made from alkaline-aided processes gave generally lower punch force and deformation values because the gels exhibited no apparent transglutaminase/ setting activity.

Enzyme-induced gelation refers to cross-linking between protein chains resulting in the formation of a gel structure. The most widely used enzyme to form food gels is transglutaminase (TGase), a protein-glutamine γ-glutamyltransferase (EC 2.3.2.13) enzyme capable of catalyzing acyl transfer reactions, introducing covalent cross-links between proteins [16-17]. When the ε-amino groups of lysine residues act as acyl receptors, intra- and intermolecular ε-(γ-glutamine)-lysine bonds are formed [18]. TGase has been used in restructured and extended meat products [19-21] and for the gelation of fish proteins and surimi products [18,22].

TGase from microorganisms such as *Streptoverticillium mobaraense*, *Streptomyces lydicus* and *Streptomyces ladakanum*, referred to as microbial transglutaminase (MTGase), has been used to increase the gel strength by inducing the polymerization of proteins [18,23-27]. Though MTGase has been successfully used for improving the gel property of various proteins including surimi [18,28-30], no information regarding the use of MTGase in protein isolate of short-bodied mackerel has been reported. Therefore, the objective of this study was to investigate the effect of MTGase at different levels on the gel properties of short-bodied mackerel proteins recovered by using an alkaline-aided process.

Materials and methods

Chemicals

Sodium chloride was obtained from Merck (Darmstadt, Germany). MTGase powder from *Streptoverticillium mobaraense* (TG-B) was supplied by Ajinomoto (Thailand) Co., Ltd. (Bangkok, Thailand). The activity of MTGase in TG-B powder was 54.98 unit/g as determined by Folk's method [31]. Sodium hydroxide and hydrochloric acid were obtained from Fluka (Buchs, Switzerland).

Fish Samples

Short-bodied mackerel (*Rastrelliger branchysoma*) with an average weight of 70 - 80 g were caught at Thasala, Nakhon Si Thammarat Coast along the Gulf of Thailand during June, 2009. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Division of Food Technology, School of Agricultural Technology, Walailak University within 30 min. The fish were immediately washed and filleted. The muscle was kept on ice during preparation and analysis. The pH value of fish muscle was 6.52 - 6.55.

Protein Isolation by Alkaline-Aided Process

To isolate the protein by alkaline solubilization process, the method described by Undeland *et al* [6] was used. The mince (250 g) was homogenized for 1 min at a speed of 13,500 rpm with 2,250 ml of cold distilled water (4 °C) using an IKA homogenizer (Selangor, Malaysia). The homogenate was adjusted to pH 10.8 using 2 N NaOH. The soluble proteins were then precipitated by adjusting the pH to 5.5 using 2 N HCl. Precipitated proteins were collected and their pH was adjusted to 7.0 using 2 N NaOH. Protein isolate was added with 4 % sucrose and 4 % sorbitol, mixed well with a mixer (Moulinex Masterchef 350, Paris, France) and frozen using an air-blast freezer. The frozen samples were kept at −18 °C until used. The storage time was not more than 1 month.

Gel Preparation

To prepare the gel, the frozen sample was thawed at 4 °C until the core temperature reached 0 °C. The sample was then cut into small pieces and the moisture content was adjusted to 80 %. The sample was placed in the mixer (Moulinex Masterchef 350, Paris, France), with 2.5 % (w/w) NaCl added and chopped for 5 min in a walk-in cold room at 4 °C to obtain the homogeneous sol. During chopping, different levels of MTGase

powder were added to obtain samples with MTGase concentrations of 0, 0.25, 0.5, 1.0 and 1.5 unit/g. The sol was then stuffed into a 2.5 cm diameter polyvinylidene casing before both ends of the casing were sealed tightly. The sol was then incubated at 40 °C for 30 min, followed by heating at 90 °C for 20 min in a temperature controlled water bath (Memmert, Schwabach, Germany) [5]. The gels were immediately cooled in iced water and stored for 24 h at 4 °C prior to analysis.

Measurement of Gel Properties

Texture Analysis

Texture analysis of gels was carried out using a Model TA-XT2 texture analyzer (Stable Micro System, Surrey, UK). Gels were equilibrated at room temperature (25 - 27 °C) before analysis. Three cylindrical samples (2.5 cm in length) were prepared and tested. Breaking force (strength) and deformation (cohesiveness/ elasticity) were measured by the texture analyzer equipped with a spherical plunger (5 mm diameter) with a depression speed of 60 mm/min.

Determination of Whiteness

Three gel samples from each treatment were subjected to whiteness measurement using a HunterLab (Color QuestXE, Virginia, U.S.A). Illuminant C was used as the light source of measurement. CIE L*, a* and b* values were measured. Whiteness was calculated using the following equation [32]:

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

Determination of Expressible Moisture

Expressible moisture was measured according to the method of Ng [33]. Cylindrical gel samples were cut to a thickness of 5 mm, weighed (X) and placed between two pieces of Whatman paper No.1 at the bottom and one piece of paper on the top. A standard weight (5 kg) was placed on the top of the sample for 2 min, and then the sample was removed from the papers and weighed again (Y). Expressible drip was calculated and expressed as a percentage of the sample weight as follows:

$$\text{Expressible drip (\%)} = [(X-Y)/X] \times 100 \quad (2)$$

Statistical Analysis

All analyzes were preformed in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test [34]. Analysis was performed using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and discussion

Effect of MTGase on Strength and Deformability of Gels from Short-Bodied Mackerel Protein Isolate

The breaking force of gels incorporated with different levels of MTGase is shown in **Figure 1**. Generally, the breaking force of the gel was positively correlated with gel strength. Higher gel strength was found in gels with MTGase added at all concentrations when compared with that without MTGase ($p < 0.05$). The unfolding of muscle protein molecules after alkaline treatment might cause the exposure of the reactive groups for cross-linking induced by MTGase. Chaijan *et al* [11] reported that alkaline pH could modify the charge of proteins, resulting in the repulsion of molecules with subsequent conformational changes. Increases in the level of MTGase from 0.25 to 1.5 unit/g sample had no significant effect on the gel strength of protein isolates ($p > 0.05$). Therefore, the use of MTGase at 0.25 unit/g sample for gel strength improvement could be an economical means for industry. From the result, the strength of the gel with MTGase at a concentration of 0.25 unit/g increased by 37.1 % when compared to that of the gel without MTGase. The improved gel properties are the result of polymerization of myosin heavy chains (MHC) induced by MTGase [35]. MTGase might induce the formation of non-disulfide covalent bonds to a greater extent [36]. MTGase was found to mediate the formation of myosin cross linking via the ε-(γ-glutamyl-lysine) linkage [37]. As a result, the strength of the gel matrix was enhanced. However, the increase in MTGase concentration from 0.25 - 1.50 unit/g sample had no effect on the strength of protein isolate gels ($p > 0.05$). Jiang *et al* [38] reported that gel strength of surimi from threadfin bream and Alaska pollack increased when MTGase was added up to 0.3 and 0.2 unit/g surimi, respectively. Excessive amounts of MTGase resulted in a decrease in breaking force of

surimi gels [39]. The optimal level of MTGase for minced mackerel was 0.34 unit/g [40]. Tammatinna *et al* [36] reported that the highest breaking force of white shrimp (*Penaeus vannamei*) gel was found when the MTGase level was 0.8 unit/g. The amount of MTGase needed depends on the types of fish as well as other factors such as freshness, protein quality and harvesting season [41]. The result suggests that the substrate in mackerel protein isolate might be limited or an inappropriate protein structure might form during the alkaline treatment. Matsumura *et al* [42] reported that the conformation of proteins also enhances the TGase reaction. Therefore, the increase in the amount of enzyme added had no effect on cross-linking ability. Thus, the conformation of muscle proteins, especially MHC, possibly affected the cross-linking efficacy of MTGase. The deformation of gels with different levels of MTGase is illustrated in **Figure 2**. No changes in deformation in all gels treated with and without MTGase were noticeable ($p > 0.05$). The result suggests that MTGase can enhance the gel strength of the protein isolate but not the elasticity. The results indicate that protein-protein interactions with different degrees were established in all gels but the bonds stabilizing these interactions might restrict rotation to the same extent. Although the addition of MTGase resulted in improved gel strength via covalent modification the rotational characteristics as well as the flexibility of the molecules might not be different. Therefore, the deformability of the gels enhanced by MTGase was comparable to that of the control gel (without MTGase).

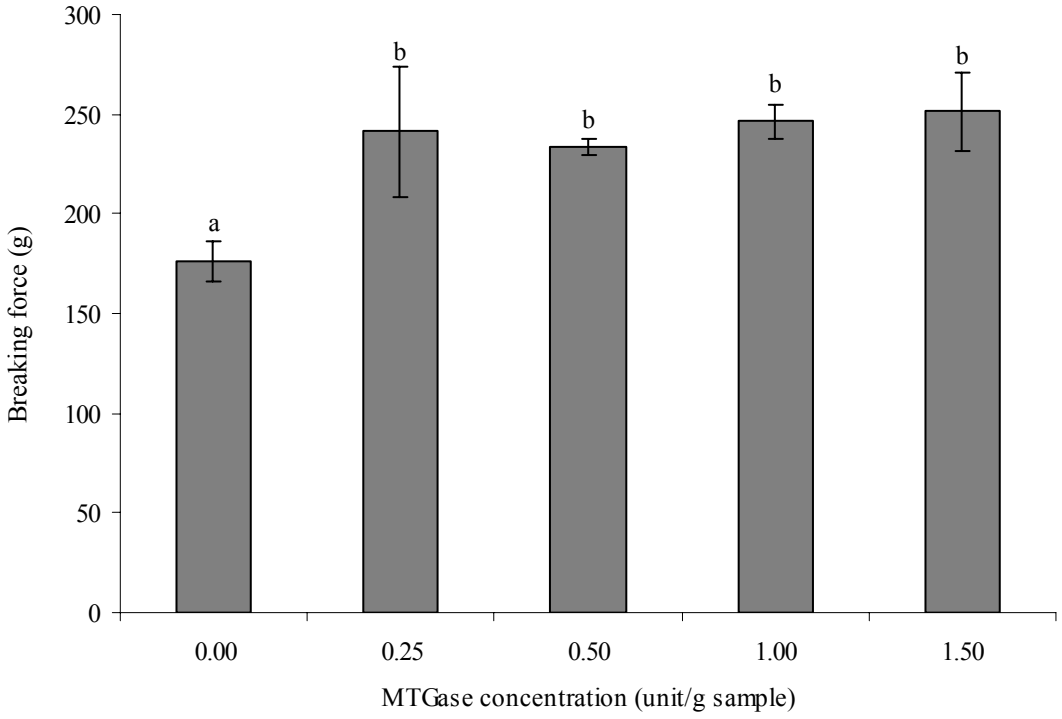

Figure 1 Breaking force of gels from short-bodied mackerel protein isolate with various levels of MTGase. Bars indicate standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

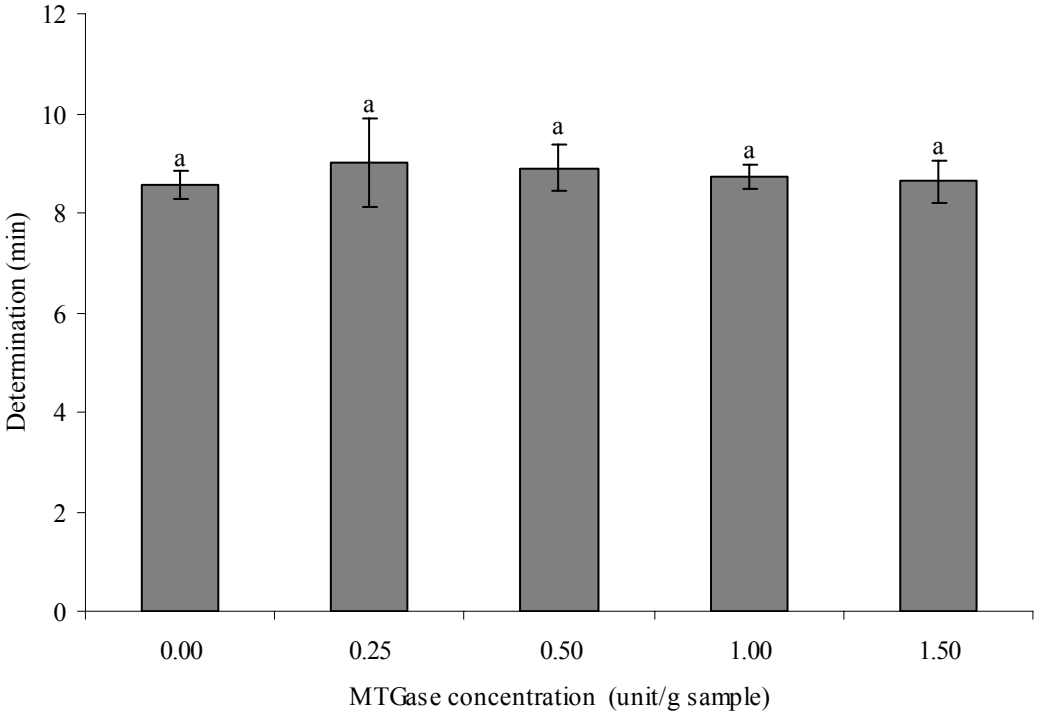

Figure 2 Deformation of gels from short-bodied mackerel protein isolate with various levels of MTGase. Bars indicate standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

Effect of MTGase on Expressible Moisture and Whiteness of Gels from Short-Bodied Mackerel Protein Isolate

The effect of MTGase on expressible moisture of protein isolate gels is shown in **Figure 3**. Different expressible moisture content suggested the differences in water holding capacity of gel network. The lower expressible moisture concomitance with a higher breaking force (**Figure 1**) was found in the gels with MTGase added when compared with the control gel ($p < 0.05$). The addition of MTGase at a level of 0.25 unit/g sample seemed to improve the water holding capacity of the protein isolate gel effectively (**Figure 3**). The expressible moisture content of gels decreased by approximately 17, 13, 15 and 11 % in the presence of MTGase at levels of 0.25, 0.5, 1.0 and 1.5 unit/g, respectively, when compared with that of the control gel. The lowest expressible moisture indicates the highest water holding capacity of the gel matrix [11]. Thus, low expressible moisture content of the gels suggests more water is retained in the gel network [43]. Therefore, the addition of MTGase could be used to improve gel-forming ability via covalent bonds. The stronger gel network was possibly associated with its capacity to hold water.

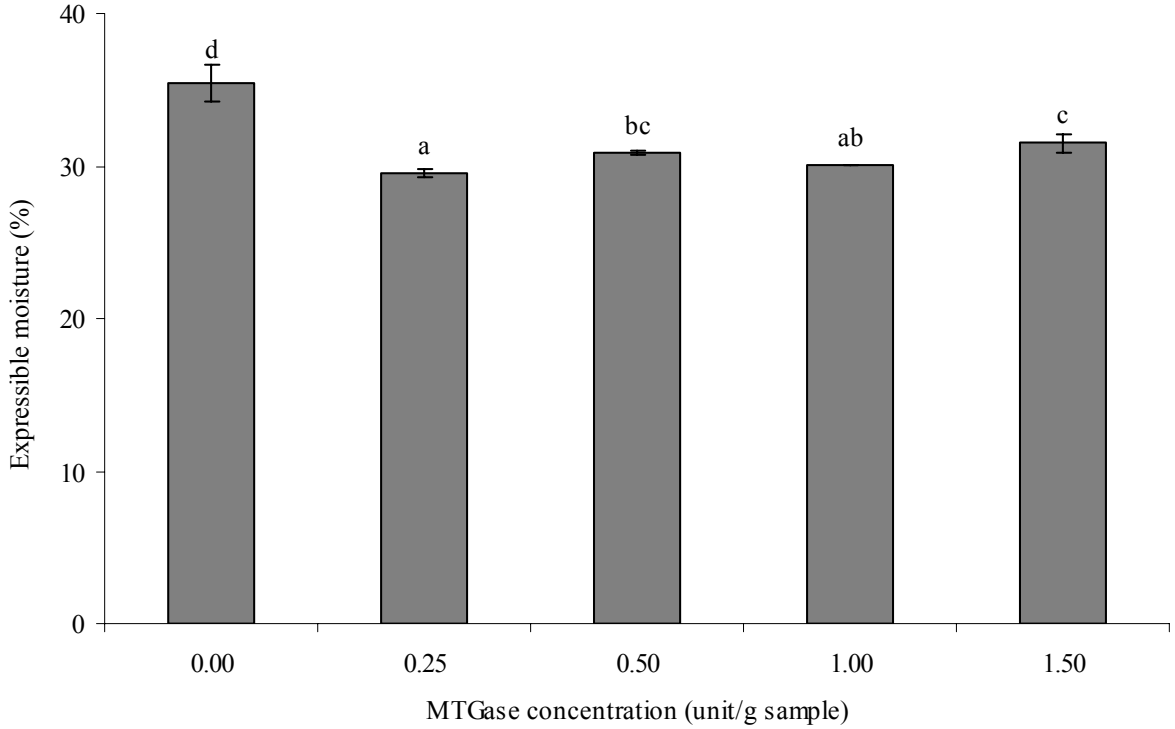

Figure 3 Expressible moisture of gels from short-bodied mackerel protein isolate with various levels of MTGase. Bars indicate standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

The whiteness of the protein isolate gels is depicted in **Figure 4**. The addition of MTGase resulted in a decrease in the whiteness of gels when compared with the control gel (without MTGase added) ($p < 0.05$). This effect tended to be more pronounced with an increase in the MTGase level. The composition of the MTGase powder used might affect the color of the gels. If salts or metal ions were present in the enzyme powder, these compounds could enhance the discoloration of the resulting gels to some extent. The Maillard reaction and the oxidation of lipids and myoglobin might participate in the coloration reaction of the protein isolate gels. It can be postulated that oxidation of myoglobin and lipids during heating might be induced by some prooxidants found in MTGase powder. Therefore, the colored compounds might occur to a greater extent when the MTGase level was increased. During heating, not only metmyoglobin formation caused a decrease in the whiteness of the gels but the Maillard browning reaction also affected the color of the gels. Aldehydes generated from lipid oxidation can participate in the Maillard reaction. Hence, the brown pigment can be formed to a greater extent and cause decreased whiteness in the gels. Chaijan *et al* [44] reported that both myoglobin and lipid oxidations caused the discoloration of dark-fleshed fish meat during frozen storage. Aldehydes or carbonyl compounds produced from lipid oxidation can interact with protein amino groups via Maillard reactions [44]. As a consequence, any resulting colored reaction products could lower the whiteness of the protein isolate gels.

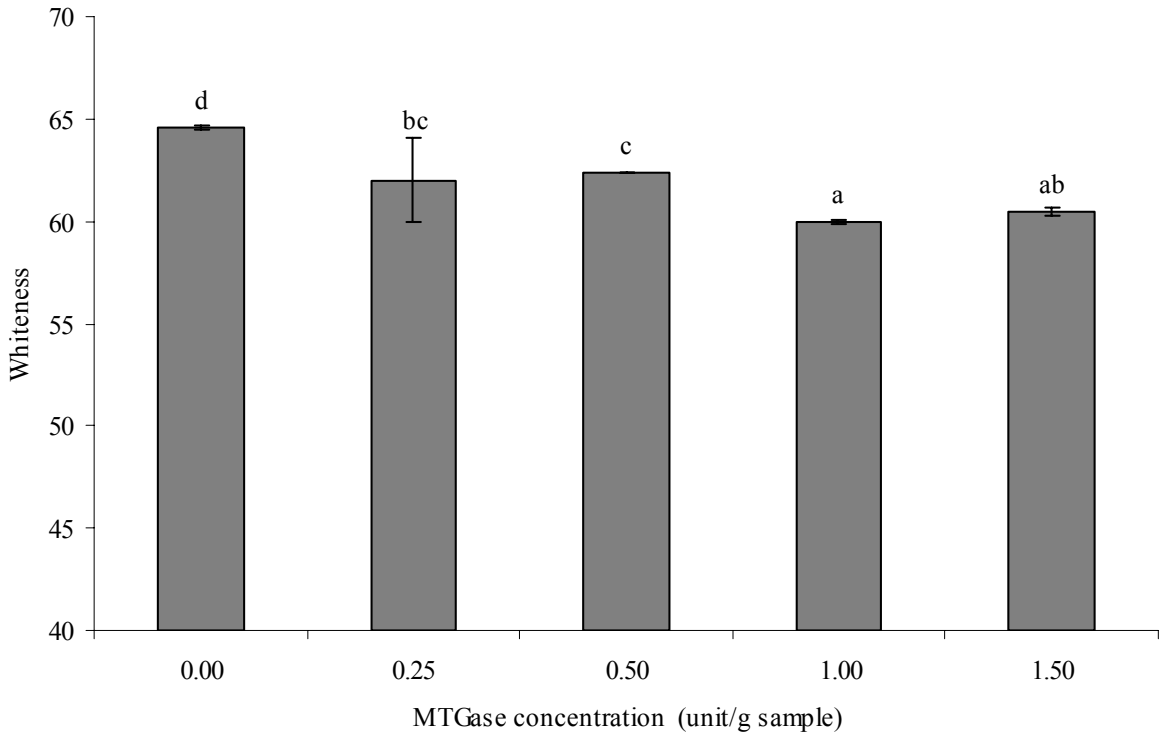

Figure 4 Whiteness of gels from short-bodied mackerel protein isolate with various levels of MTGase. Bars indicate standard deviation from triplicate determinations. Different letters indicate significant differences ($p < 0.05$).

Conclusions

The addition of MTGase, especially at a concentration of 0.25 unit/g, can improve the textural properties of alkaline-isolated protein gels. The presence of MTGase potently enhanced the strength and water holding capacity of the protein isolate gel but not for the deformation (elasticity). However, the addition of MTGase rendered the gels with the lower whiteness when compared to that without MTGase added.

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