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Food Chemistry 80 (2003) 551–556

Food Chemistry

www.elsevier.com/locate/foodchem

Negative effect of combining microbial transglutaminase with low methoxyl pectins on the mechanical properties and colour attributes of fish gels

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Received 22 March 2002; received in revised form 2 July 2002; accepted 2 July 2002

Abstract

The objective of this work was to determine the effect of combining different concentrations of microbial transglutaminase (MTGase) and types of low methoxyl (LM) pectin on the mechanical properties (textural profile analysis, puncture test) and the colour attributes of fish restructured products. A disruptive effect was observed when LM pectin and MTGase were added to fish gels. Adding 1% MTGase with LM32 pectin significantly decreased the hardness (3.85 kg), springiness (0.631) and chewiness (0.434 kg). When LM32 pectin and 0.1% MTGase were added together, significant decreases of the three puncture test parameters were observed. The chroma of fish gels decreased significantly when 0.3% MTGase was added. Fish gels containing the LM35 pectin had higher chroma values than all other fish gels with the same level of MTGase. Hue value was increased in all fish gels after increasing the MTGase level. The results obtained showed that LM pectin is not suitable for use in products containing MTGase.

Keywords: Surimi; Transglutaminase; Pectin; Fish

1. Introduction

The shrimp by-catch is an abundant resource that is widely misused in many countries. Many fish are caught during the catching of shrimp. In the Gulf of Mexico, 75,000 tons of shrimp are caught annually by Mexican fishing boats. An average of 83% in volume of shrimp catching is by-catch. Thus at least 355,000 tons of by-catch are being wasted. It is estimated that 80% of this resource is fish (Southeastern Fisheries Association, 2001). Several factors influence the wasting of this natural resource: small size, diversity of species, presence of non-commercial or non-edible species, and the absence of processing alternatives.

Mexican flounder (*Cyclopsetta chittendenni*), Northern kingfish (*Menticirrhus saxatilis*), Atlantic croaker (*Micropogon undulatus*), and Barred grunt (*Conodon nobilis*) are some of the most abundant fish species caught as shrimp by-catch in the Tamaulipas coast, Mexico. The biochemical and mechanical properties of such species have been previously reported (Morales, Ramírez, Vivanco, & Vázquez, 2000; Ramos-Martínez, Morales-González, Ramírez, García-Carreño, & Montejano-Gaitán, 1999). Most of them show too poor gelling properties for processing as surimi products. Therefore, an alternative processing technique could be restructuring fish products using microbial transglutaminase (Ramírez, Uresti, Téllez, & Vázquez, 2002; Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002).

The biochemistry of fish muscle is distinct from that of mammals and birds, and so fish products must be processed in a different way from red meat and poultry. Particular phenomena in fish products are Modori and Suwari (setting). Modori is a deteriorative phenomenon that takes place at temperatures near 60 °C. It has been associated with the presence and activity of endogenous serine and cysteine muscle proteases (An, Peters, & Seymour, 1996; Ramírez, García-Carreño, Morales, &

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0308-8146/03/$ - see front matter
PII: S0308-8146(02)00343-6

Sánchez, 2002; Ramos-Martínez et al., 1999; Sánchez, Ramírez, Morales, & Montejano, 1998). Setting or suwari is the name for the gelling phenomenon of muscle protein at temperatures in the range 0–40 °C. It has been associated with a calcium dependent muscle endogenous transglutaminase. Transglutaminase catalyses the formation of covalent bonds between adjacent proteins, thereby improving the gel structure. This enzyme catalyses an acyl transfer reaction between γ-carboxyamide groups of glutaminyl residues in proteins. When the primary amine is the ϵ-amino group of lysine and lysyl residues, ε-(γ-glutamyl)lysine cross-linking takes place (Kumazawa, Seguro, Takamura, & Motoki, 1993). This enzyme is recognized as the one responsible for the setting phenomenon. Nowadays, a commercial microbial transglutaminase (MTGase) has been employed to improve the mechanical and textural properties of different protein foods, including surimi products. MTGase catalyzes the same reaction as endogenous transglutaminase, but the first is non-calcium dependent and shows lower deamidation affinity than endogenous transglutaminase of fish or pig (Ohtsuka, Umezawa, Nio, & Kubota, 2001). Several studies have been conducted to determine the optimal conditions for using MTGase in surimi gels (Lee, Lanier, Hamann, & Knopp, 1997; Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2000; Ramírez, Santos, Morales, Morrissey, & Vázquez, 2000) and restructured fish products (Ramírez, Uresti et al., 2002; Téllez-Luis et al., 2002). But the effect of combining MTGase with other additives has been scarcely studied. Only the effect of combining MTGase with UV irradiation has been studied (Shann-Tzong, Shan-Zen, & Guo-Jane, 1998).

Different additives, such as non-meat proteins or hydrocolloids (Gómez-Guillén & Montero, 1996; Gómez-Guillén, Borderías, & Montero, 1997) are being applied to improve the mechanical and functional properties of surimi and fish restructured gels. Carragenan, konjak and starch are the hydrocolloids that are mainly used (Borderías & Pérez-Mateos, 1996; Park, 2000). Xanthan and locust bean at a ratio of 1:3, have been shown to be compatible with surimi gels (Ramírez, Barrera, Morales, & Vázquez, 2002). Other hydrocolloids, such as high methoxyl pectins, have shown low compatibility with surimi gels (Barrera, Ramírez, González-Cabriales, & Vázquez, 2002). Recently, the feasibility of improving the mechanical properties of surimi and restructured fish gels, by using amidated low methoxyl pectin (ALM pectin), has been reported (Barrera et al., 2002). ALM pectin is used to improve the mechanical properties and water holding capacity of fish gels, just slightly modifying the colour attributes.

The objective of this work was to determine the effect of combining MTGase with low methoxyl (LM) pectins on the mechanical properties and colour attributes of fish restructured products.

2. Materials and methods

2.1. Frozen raw fish paste

Mexican flounder or sole (*Cyclopsetta chittendeni*) was obtained from a fish market in Tampico, Tamaulipas, Mexico. Fifty kilograms of whole fresh fish (ca. 130 fishes) were washed and kept in ice until processing. The fish were processed about 6 h after being caught. Mexican flounder was headed, gutted and washed. The skin and bones were removed with a Bibun deboning machine (Model NF2DX, Fujiyama, Japan), with a drum having 5 mm diameter perforations. The whole fish paste was mixed with the cryoprotectant sucrose (8%), using a Hobart mixer (model VCM. Troy, Ohio) and then packed into polyethylene bags (2 kg), frozen within 5 h at −30 °C in a Crepaco plate freezer (Model B-5854-AM12, Crepaco, Inc. Chicago, IL) and stored at −20 °C until needed. Only one batch of whole paste containing 78(±2)% of water was used.

2.2. Restructured fish product preparation

Samples of 250 g of whole paste were selected from a 2 kg bag, partially thawed at room temperature, cut into small pieces and chopped in a 5.5 l capacity Hobart cutter (Model 84145, Troy, Ohio) for 3 min with 2.5% salt to solubilize the myofibrillar proteins. Microbial transglutaminase and ALM pectin were added as described in the next section. The final chopping temperature was maintained below 15 °C. The paste was stuffed into stainless tubes (diameter = 1.87 cm; length = 17.75 cm) and sprayed with commercial vegetable oil to prevent sticking. The tubes were capped before the thermal treatments: 40 °C for 60 min, followed by 90 °C for 15 min. After cooking, tubes were immediately removed, placed in a cold water bath and cooled at 4–5 °C for 30 min. All gels were removed from the tubes and stored overnight at 4 °C in polystyrene bags, prior to testing.

2.3. Transglutaminase and pectin addition

MTGase, Active TG-TI, was supplied by Ajinomoto USA, Inc. (Teaneck, NJ). The composition of the product was maltodextrin 99% and transglutaminase 1%. The MTGase activity was ca. 100 units/g. In this work, the enzyme concentration was reported as commercial concentration.

Low methoxyl pectin LM 32 Powder (27–35% DE, Tic Pretested Pectin; Lot 0004894), and ALM pectin LM 35 Powder (27–33% DE, Tic Pretested Pectin; Lot 0003655) were provided by Tic Gums (Belcamp, MD).

MTGase (0.1 or 0.3% w/w) and pectins (1% w/w) were added directly to whole fish pastes in a dry form after the myofibrillar proteins were solubilized. Control gels were obtained without the addition (0%) of MTGase.

2.4. Texture profile analysis (TPA)

Samples of restructured fish products of 3 cm length were equilibrated to room temperature for 30 min into a plastic bag to avoid dehydration before textural analysis. Textural analysis was determined using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England). Textural profile analysis (TPA) was performed using a cylinder probe (P50) with a diameter of 50 mm. Samples were compressed to 50% of initial height using a compression speed of 2 mm s^{-1}. Hardness, springiness, cohesiveness and chewiness were reported. Six samples were analysed for each treatment with two replicates.

2.5. Puncture test

A puncture test was performed compressing samples to 75% of the initial height using a compression speed of 60 mm min^{-1} and a spherical probe (model P/20) with 1.2 cm diameter. The breaking force (kg), deformation (mm) and work of penetration (gel strength) (kg cm) for each treatment were measured. Samples were placed on the base of the texturometer, taking care that the spherical probe reached the sample at the centre. Six samples were analysed for each treatment. Each treatment was obtained with two replicates.

2.6. Expressible water

The amount of expressible water (EW) for each treatment was measured. Samples of 3 g (±0.2 g) of fish restructured gels were weighed and put between two layers of filter paper. Samples were placed at the bottom of 50 ml centrifuge tubes and centrifuged at 1000 *g* for 15 min at 15 °C. Immediately after centrifugation, the fish gel samples were weighed and the EW was calculated as follows:

$$\mathrm{EW} = \frac{W_i - W_f}{W_i} \cdot 100$$

where: W_i = initial weight of fish gel; W_f = final weight of fish gel. Three samples were analyzed for each treatment and averages are reported. Each treatment was obtained with two replicates.

2.7. Colour attributes

The spectral reflectance of restructured fish gels was determined using a HunterLab MiniScan XE Plus spectrocolorimeter (model 45/0-L; Hunter Assoc., Reston, VA, USA) calibrated against black and white tiles. CIE L^*, a^*, and b^* values, chroma ($[a^{*2} + b^{*2}]^{1/2}$), and hue angle (arc tan b^*/a^*) were calculated, based on illuminant C and the 2° standard observer.

2.8. Statistical analysis

Statistical analysis was performed using a Statgraphics 5.0 (Software Publishing Corporation, Bitstream Inc.). LSD's multiple range tests were used to determine significantly difference ($P < 0.05$) among treatments.

3. Results and discussion

3.1. General conditions

Fish gels were obtained incubating fish pastes at 40 °C by 1 h using different concentrations of MTGase and LM pectins. Such conditions were selected because they have been found as optimum for the enzyme activity in surimi gels from warm-water fish species (Ramírez, Rodríguez-Sosa et al., 2000; Ramírez, Santos et al., 2000).

3.2. Changes on textural profile analysis (TPA)

Fig. 1 shows the results for hardness and chewiness and Fig. 2 for springiness and cohesiveness. TPA parameters of fish gels were improved by adding MTGase alone. Hardness varied from 4.25 to 5.56 kg, chewiness from 0.611 to 1.091 kg, springiness from 0.740 to 0.837 and cohesiveness from 0.193 to 0.233. The lowest values corresponded to control fish gels without MTGase. Only hardness and chewiness were improved significantly

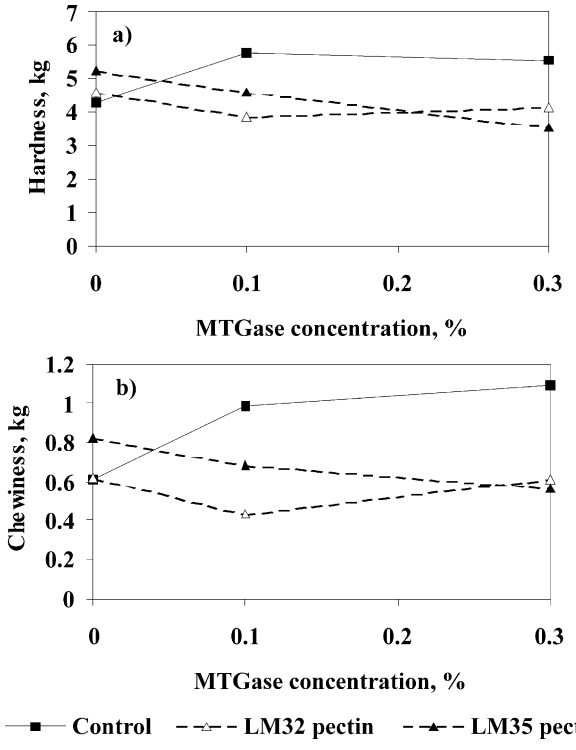

Fig. 1. Effect of microbial transglutaminase and low methoxyl pectins on the hardness and chewiness of fish restructured products.

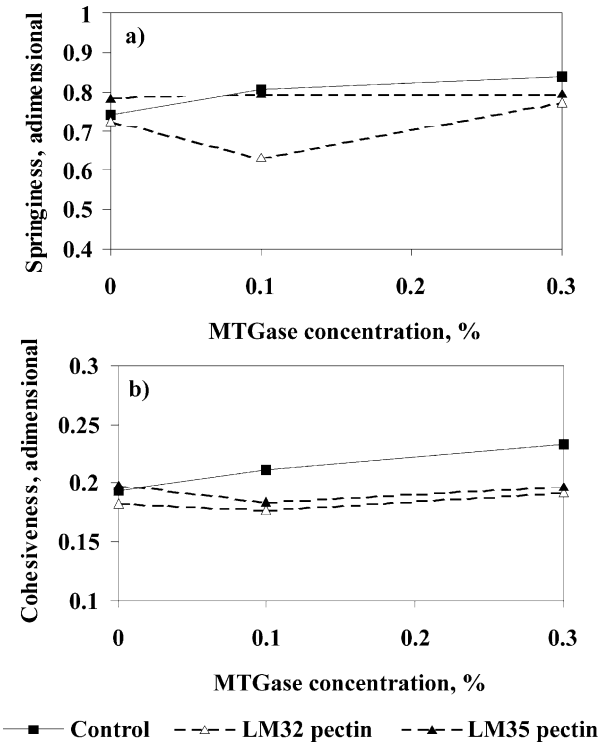

Fig. 2. Effect of microbial transglutaminase and low methoxyl pectins on the springiness and cohesiveness of fish restructured products.

($P<0.05$) by adding 0.1 and 0.3% MTGase (Fig. 1). Cohesiveness and springiness were improved significantly only when 0.3% MTGase was added. Despite the improving effect of MTGase, the cohesiveness of fish gels remained very low. The beneficial effect of MTGase on the mechanical and textural properties of protein gels (including surimi and fish gels) has been extensively reported and the effect was associated with formation of covalent bonds between adjacent proteins (Kumazawa et al., 1993).

TPA parameters of fish gels were not improved significantly ($P<0.05$) by adding 1% LM32 pectin (non-amidated pectin). This result is in accordance with a previous study (Barrera et al., 2002). The TPA values were: hardness, 4.60 kg; springiness, 0.727; cohesiveness, 0.183 and chewiness, 0.620 kg.

A disruptive effect was observed when LM pectin and MTGase were added to fish gels. Adding 1% MTGase with LM32 pectin significantly decreased the hardness (3.85 kg), springiness (0.631) and chewiness (0.434 kg). Such values were not modified significantly when 0.3% MTGase was added. These results showed that LM pectin, added to surimi gels at 1%, had no beneficial effect on textural properties of fish gels, but had an incompatible effect with MTGase when added together to fish gels.

The fish gels obtained with 1% ALM pectin (LM35 pectin) showed the next textural properties: hardness, 5.22 kg; springiness, 0.785; cohesiveness, 0.198 and chewiness, 0.821 kg. Only hardness and chewiness were modified significantly ($P<0.05$) with an enhanced effect by adding the ALM pectin. When MTGase was added with ALM pectin to fish gels, a disruptive effect was observed. The highest level of MTGase gave the lowest values of hardness (3.54 kg) and chewiness (0.562). Cohesiveness and springiness were not modified by adding MTGase to fish gels containing ALM pectin. The results obtained confirm that ALM pectin improves the textural properties of fish gels (Barrera et al., 2002). However, ALM pectin seems to have a disruptive effect on fish gels when combined with MTGase. The origin of such incompatibility remains unclear.

3.3. Puncture test

Fig. 3 shows the results for the puncture test. Control fish gels, obtained without pectin or MTGase, had a breaking force of 1.276 kg, deformation of 13.6 mm and gel strength of 17.427 kg mm. These values were improved significantly ($P<0.05$) by adding MTGase. However, differences between adding 0.1 or 0.3% MTGase were not significant. The improvement of these mechanical parameters by adding MTGase to restructured fish products obtained from Silver carp (*Hypophthalmichthys molitrix*), using massaging techniques, was also recently reported (Téllez-Luis et al., 2002).

Fish gels treated with LM32 pectin (non-amidated pectin) did not show a difference ($P<0.05$) in breaking force (1.359 kg) or gel strength values (15.179 kg mm) as compared with control fish gels obtained without additives. However, the deformation value (11.2 mm) was significantly decreased by adding the LM32 pectin. When LM32 pectin and 0.1% MTGase were added together, significant decreases of the three puncture test parameters were observed. These results confirm that LM32 pectin and MTGase were incompatible when added to fish gels. The detrimental effect of this combination might be associated with a disruptive effect on the gel structure.

A slight improvement of mechanical properties was observed when 0.3% MTGase was added, reaching similar values to control fish gels containing LM32 pectin. However, values obtained by adding LM32 pectin and 0.3% MTGase were lower than values obtained by fish gels with 0.3% MTGase alone. This behaviour could be explained by a low disruptive effect of LM32 pectin, which can be partially buried by increasing the MTGase level. This means that MTGase increases the covalent bonds between adjacent proteins and that this effect is enough to partially avoid the disruptive effect of adding LM32 pectin.

The addition of 1% LM35 pectin (amidated pectin) to fish gels significantly improved the breaking force (1.606 kg) and the gel strength (20.253 kg mm). Deformation (12.57 mm) was not affected by adding the hydrocolloid. Both the breaking force and the gel strength were affected significantly by the concentration of MTGase added

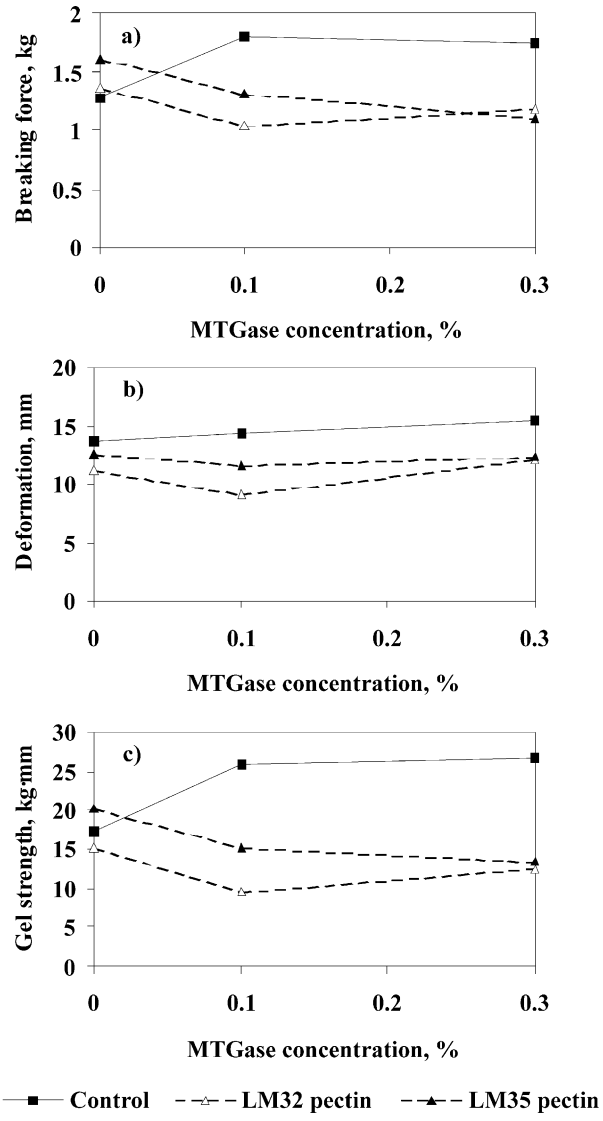

Fig. 3. Effect of microbial transglutaminase and low methoxyl pectins on the puncture test parameters of fish restructured products.

with LM35 pectin. The values of mechanical properties decreased when the concentration of MTGase was increased. The deformation parameter was not affected by adding MTGase with LM35 pectin. The fish gels containing just 0.1% MTGase and 1% LM35 pectin did not show a significant difference ($P<0.05$) in the breaking force (1.312 kg), deformation (11.58 mm) or gel strength (15.237 kg mm) compared with control fish gels without pectin. These results confirm a previous study (Barrera et al., 2002) that LM35 pectin improves the mechanical properties of surimi gels. However, a disruptive effect was observed when both LM35 pectin and MTGase were added together.

Solutions of 1% LM35 pectin did not form gels when 0.1% MTGase was added and incubated at 40 °C for 1 h. Therefore the feasibility of having carbohydrate-MTGase interactions did not occur. It is feasible that protein–carbohydrate interactions formed are incompatible with covalent crosslinking of proteins induced by MTGase. However, it is also feasible that MTGase induced a more rigid gel, which affects or is affected by the protein–carbohydrate and/or carbohydrate–water interactions. More studies are needed to elucidate the mechanism involved in the incompatibility shown by these additives.

3.4. Colour attributes

The effect of MTGase and LM pectin on colour attributes L^*, a^*, b^* chroma and hue of fish gels is shown in Table 1. The L^* parameter varied from 53.3 to 83.9. This parameter was affected only by the concentration of MTGase without a significant effect ($P<0.05$) by the pectin addition. There were no significant differences between fish gels containing 0% (control) and 1% MTGase, regardless of the kind of LM pectin added. However, the L^* parameter decreased significantly in all fish gels when 0.3% MTGase was added. Significant differences of L^* parameters between fish gels containing 0.3% MTGase and LM pectin were not observed. MTGase induces covalent crosslinking between adjacent proteins, promoting the formation of stronger gels and, according to these results, modifying the lightness of fish gels.

The a^* attribute was modified for both factors, MTGase level and type of pectin (Table 1). The a^* attribute was increased significantly ($P<0.05$) when the concentration of MTGase was increased. Both pectins used, LM32 and LM35, increased the a^* attribute of all fish gels. This means that both pectins increased the redness of fish gels. However, despite all these significant changes, the a^* parameter of all fish gels remained in the grayish zone (near zero).

The b^* attribute was modified significantly by both factors, MTGase level and the type of LM pectin, but the behaviour was not equal to that observed for the a^* attribute. The b^* value was higher in fish gels containing LM35 pectin than the control or fish gels with LM32 pectin. Fish gels containing 0.1% MTGase showed higher values of the b^* attribute than the control. Adding 0.3% MTGase significantly decreased the b^* parameter for all fish gels.

The chroma of fish gels decreased significantly when 0.3% MTGase was added. Fish gels containing the LM35 pectin had higher chroma values than all other fish gels with the same level of MTGase. Hue value was increased in all fish gels after increasing the MTGase level. This means that the fish gel colour changed slightly from yellowish to greenish due to MTGase. Fish gels containing LM35 pectin showed higher values of hue, indicating a more greenish colour. However, all fish gels visually remained in gray colours, accordingly to the low values obtained for a^* and b^* and high levels of L^*.

Table 1
Effect of combining 1% low methoxyl pectins and MTGase on colour attributes of fish gels

Kind of pectin	MTGase conc. (%)	L^*	a^*	b^*	Chroma	Hue
Control	0	80.9a (5.6)	−2.91a (0.2)	12.0a (1.1)	12.4a (1.2)	103.61a (0.59)
Control	0.1	83.2a (3.1)	−2.54b (0.1)	13.53b (0.7)	13.8b (0.7)	100.64b (0.60)
Control	0.3	53.3b (1.4)	−1.2c (0.1)	9.1c (0.5)	9.2c (0.5)	97.34c (0.72)
LM32	0	82.5a (4.7)	−1.7a (0.1)	13.0a (1.0)	13.1a (0.5)	97.41a (0.01)
LM32	0.1	82.2a (4.7)	−1.54b (0.1)	13.6a (1.0)	13.7a (0.9)	96.49b (0.72)
LM32	0.3	54.9b (1.5)	−0.8c (0.1)	9.0 b (0.8)	9.1b (0.8)	94.97c (0.45)
LM35	0	83.9a (3.9)	−2.0a (01)	13.9a (1.2)	14.0a (1.1)	98.10a (0.82)
LM35	0.1	82.8a (2.4)	−1.5b (0.2)	15.1b (0.6)	15.1a (0.6)	95.49b (0.66)
LM35	0.3	56.0b (2.3)	−0.9c (0.1)	10.2c (0.9)	10.3b (0.9)	94.88b (0.61)

Different letters in the same row indicate significant differences ($P<0.05$) between levels of MTGase for each kind of pectin added. Values in parentheses indicate the standard deviations of the mean.

4. Conclusions

The amidated low methoxyl pectin significantly increased the hardness and gel strength parameters of fish gels. However, it showed a disruptive effect when added together with MTGase. The disruptive effect might be associated with interferences in forming the three-dimensional structures of the gels. More studies are needed to elucidate the origin of this behaviour. Both MTGase and gel strength affected the colour attributes. The effect of LM pectins was associated with the colour of both hydrocolloids. The effect of the MTGase was associated with a more structured system, which modifies the reflectance of the light. These results show that ALM pectin is suitable for improving the mechanical properties of fish gels. However, under the conditions studied, ALM pectin is not suitable for use in products containing MTGase.

Acknowledgements

The authors are grateful to CONACYT and OMNILIFE for the financial support of this work (Project 35951 B).

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