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Low-salt restructured products from striped mullet (*Mugil cephalus*) using microbial transglutaminase or whey protein concentrate as additives

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Abstract

Striped mullet (*Mugil cephalus*) is an abundant fish species in the north side of the Gulf of Mexico. Despite its high volume of capture for the commercialization of roe, the flesh of striped mullet is underutilized because of its dark colour and unpleasant odour. The objective of this work was to determine the feasibility of obtaining restructured products from striped mullet aided by additives. Microbial transglutaminase (MTGase) or whey protein concentrate (WPC) were used as binders. Two concentrations of salt (10 or 20 g/kg) were tested to evaluate the effects on the mechanical properties (texture profile analysis and puncture test), expressible water and colour attributes. Minced fish from striped mullet yielded good gels at both salt concentrations with MTGase. The results showed that MTGase (3 g/kg) and low-salt concentration (10 g/kg) were appropriate for improving the properties of restructured products obtained from striped mullet.

Keywords: Striped mullet; Transglutaminase; Whey; Low-salt; Restructured fish products

1. Introduction

Mugil cephalus (striped mullet or sea mullet) is an abundant and extremely widespread fish species found in temperate and tropical waters through the world. This fish is not considered as a threatened or endangered species and is the object of both commercial fishery and game angling. The mullet caught near ocean shores are mostly spawning run fish and the catch has increased as a result of a growing market for sea mullet roe, a highly popular delicatessen fish product. However, the mullet has tasty fat flesh which can absorb undesired odorous compounds from its diet (Collins, 1985; Murdy, Birdsong, & Musick, 1997). For this reason, in many countries, such as Mexico, it is mainly caught to commercialize its roe and the flesh is considered as a by-product.

The increasing demand for striped mullet roe requires the development of technologies to process its flesh, which has low commercial value due to its dark-red colour and because it collects odorous compounds from its diet.

Several studies dealing with the feasibility of using the mullet flesh in commercial processes to obtain fish products have been reported: cold marinades of the roll-mop type (Wootton & Chuah, 1981); fish protein isolates and hydrolysates (Morales de León, Gálvez-Mariscal, & Téllez-Sill, 1990); salted fish products (El-Sahn, El-Sharnouby, & Moharram, 1997), cold-smoked fillets (Antoine, Marshall, Sims, O'Keefe, & Wei, 2000) and surimi (Morales, Ramírez, Vivanco, & Vázquez, 2001; Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2000).

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An alternative process involves obtaining a fish paste by mechanical separation of the flesh, using a debonning machine, and then preparing restructured fish products. Restructuring yields fish products with high commercial value from different sources: non-commercial fish species, fish with size smaller than commercial (such as shrimp by-catch) and trimmings from fillets of commercial fish species. Although several methods of restructuring have been developed, the most commonly used include cutting, tumbling and massaging (with or without vacuum).

All these techniques use salt (20–30 g/kg) to solubilize and extract myofibrillar proteins which form a sticky exudate, responsible for the binding in these products (Borderías & Pérez-Mateos, 1996; Zimmerman, Bissel, & McIntosh, 1996). It was demonstrated, in other fish species, that it is not feasible to obtain restructured products in the absence of salt (Uresti, Téllez-Luis, Ramírez, & Vázquez, 2004). On the other hand, consumers are demanding healthier foods and there is a great interest in lightly-salted products to prevent and control adverse blood pressure levels in humans.

Proteins have been used as binding agents or as additive to improve mechanical and functional properties of fish products, e.g. egg white (Yetim & Ockerman, 1995), casein and beef plasma-thrombin (Baker, Lanier, & Green, 2000), casein, whey protein concentrate (WPC) and microbial transglutaminase (MTGase) (Uresti et al., 2004).

The objective of this work was to determine the feasibility of using microbial transglutaminase (MTGase) or whey protein concentrate (WPC) as binding agents to prepare low-salted restructured fish products from striped mullet.

2. Material and methods

2.1. Raw material

Fresh striped mullet (*Mugil cephalus*) was obtained directly from fishermen in Tamaulipas, Mexico. Fish were eviscerated and filleted at facilities of the fish processor, thoroughly rinsed with cold tap water, stored in ice, and carried to the food technology laboratory (Department of Food Technology, UAMRA-UAT). Fish were stored in ice and processed approximately 24 h after being caught.

2.2. Preparation of restructured fish products

Fillets (15 kg) were ground in a meat grinder (Momat, Model 10022. México, D.F.) using a 5 mm plate. Restructured products were obtained from homogenized fish mince. For each treatment, fish mince samples (0.5 kg) were chopped in a 5.5 l capacity Hobart cutter (Model 84145, Troy, OH, USA.) for 3 min with salt. Two levels of salt were assayed: 10 g/kg (low-salt concentration) or 20 g/kg (regular-salt concentration). Then 3 g/kg of MTGase, Active TG-TI (Ajinomoto USA, Inc., Teaneck, NJ), or 10 g/kg of WPC were dispersed individually into the fish paste in a dry form. A control, containing no additives, was also prepared. The temperature of the fish paste remained below 15 °C throughout the chopping operation for all treatments studied. The soluble paste was stuffed into stainless-steel tubes (1.87 cm ID and 17.75 cm length) and sprayed with commercial vegetable oil to prevent sticking. Tubes were capped before immersion in water at 40 °C for 1 h, followed by immersion in water at 90 °C for 15 min. After cooking, the tubes were immediately removed, placed in a refrigerated water bath, and cooled at 4–5 °C for 30 min. The gels were removed from the tubes and stored overnight at 4 °C in polystyrene bags before testing.

2.3. Mechanical properties

Mechanical properties were measured using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England). Cylindrical samples (1.87×3 cm) of restructured fish product were cut and equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before testing.

Textural profile analysis (TPA) was performed using an aluminium cylindrical probe (P/50) of 50 mm diameter. Samples were compressed to 75% of the initial height using a compression speed of 60 mm/min. Fracturability, hardness, springiness, cohesiveness and chewiness were reported for each treatment. Six samples were also analyzed for each treatment.

A puncture test was performed, compressing samples to 75% of the initial height, using a compression speed of 1 mm/s and a spherical probe (P/20) with 1.2 cm diameter. The breaking force (kg), deformation (cm) and work of penetration (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 analyzed for each treatment.

2.4. Expressible water

The amount of expressible water for each treatment was measured. Samples of 3 ± 0.1 g of cooked gels were weighed and put between two layers of filter paper (Whatman No. 1). Samples were placed at the bottom of 50 ml centrifuge tubes and centrifuged at 1500 g for 5 min at 15 °C. Immediately after centrifugation, the fish sample was removed and weighed. The amount of expressible water was calculated as follows:

$$E_w = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

where E_w is the percentage of expressible water (%), W_i is the initial weight (g) and W_f is the final weight (g). Three samples were analyzed for each treatment and averages were recorded.

2.5. Colour attributes

Spectral reflectance of fish pastes (before cooking) and gels (after cooking) 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. The colour measurements were done before the textural analysis; thus the samples had the same size as before mentioned.

2.6. Statistical analysis

Data were analyzed using Statgraphics 5 software (Manugistics, Inc., Rockville, MD). A multifactorial analysis of variance was carried out. Differences among mean values were established using the least significant difference (LSD) multiple range test and were considered significant when $P < 0.05$.

3. Results and discussion

3.1. Texture profile analysis of fish gels

Fig. 1 shows the results of the TPA analysis. For low-salt gels, fracturability increased from 4.68 to 10.01 kg and hardness from 4.91 to 10.88 kg. Both parameters, fracturability and hardness, were increased significantly ($P < 0.05$) by adding MTGase, but WPC showed no effect. Control gel samples, with a regular-salt level, showed higher values of hardness, than did low-salt gels, but fracturability was not increased significant ($P < 0.05$). The hardness and fracturability of regular-salt gels were increased significantly by adding MTGase and WPC. No significant difference ($P < 0.05$) was found between low-salt and regular-salt gels containing MTGase. WPC had a better effect in improving fracturability and hardness of fish gels when 20 g/kg of salt was added.

The increase in mechanical properties of low-salt restructured products, by adding 3 g/kg of MTGase, has been also reported for silver carp (*Hypophthalmichthys molitrix*) (Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002). However, WPC failed to improve mechanical properties of low-salt restructured products from silver carp (*H. molitrix*) (Uresti et al., 2004). The improving of mechanical properties of surimi gels from striped mullet at regular-salt levels by using MTGase (3 g/kg), or endogenous transglutaminase has been reported (Ramírez et al., 2000; Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2003).

Low-salt gels varied in springiness from 0.79 to 0.90, cohesiveness varied from 0.19 to 0.28 and chewiness varied from 0.72 to 2.73 kg. All three parameters were increased by adding MTGase. The use of WPC did not affect the cohesiveness and chewiness, but significantly decreased the springiness (Fig. 2).

In regular-salt gels, springiness varied from 0.72 to 0.86; cohesiveness varied from 0.26 to 0.27 and chewiness varied from 1.83 to 3.62 kg. Samples containing MTGase and WPC showed higher values of springiness and chewiness

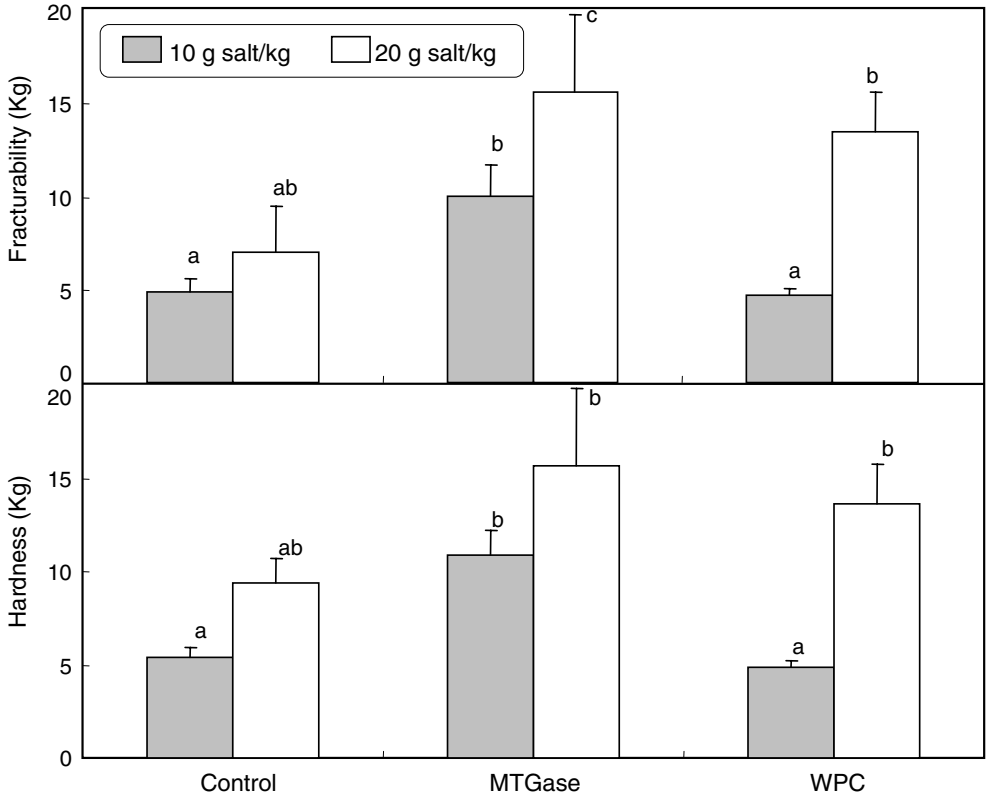

Fig. 1. Effect of microbial transglutaminase (MTGase) or whey protein concentrate (WPC) on hardness and fracturability of gels at two different levels of salt. Mean values of three replicates. Bars indicate the standard error.

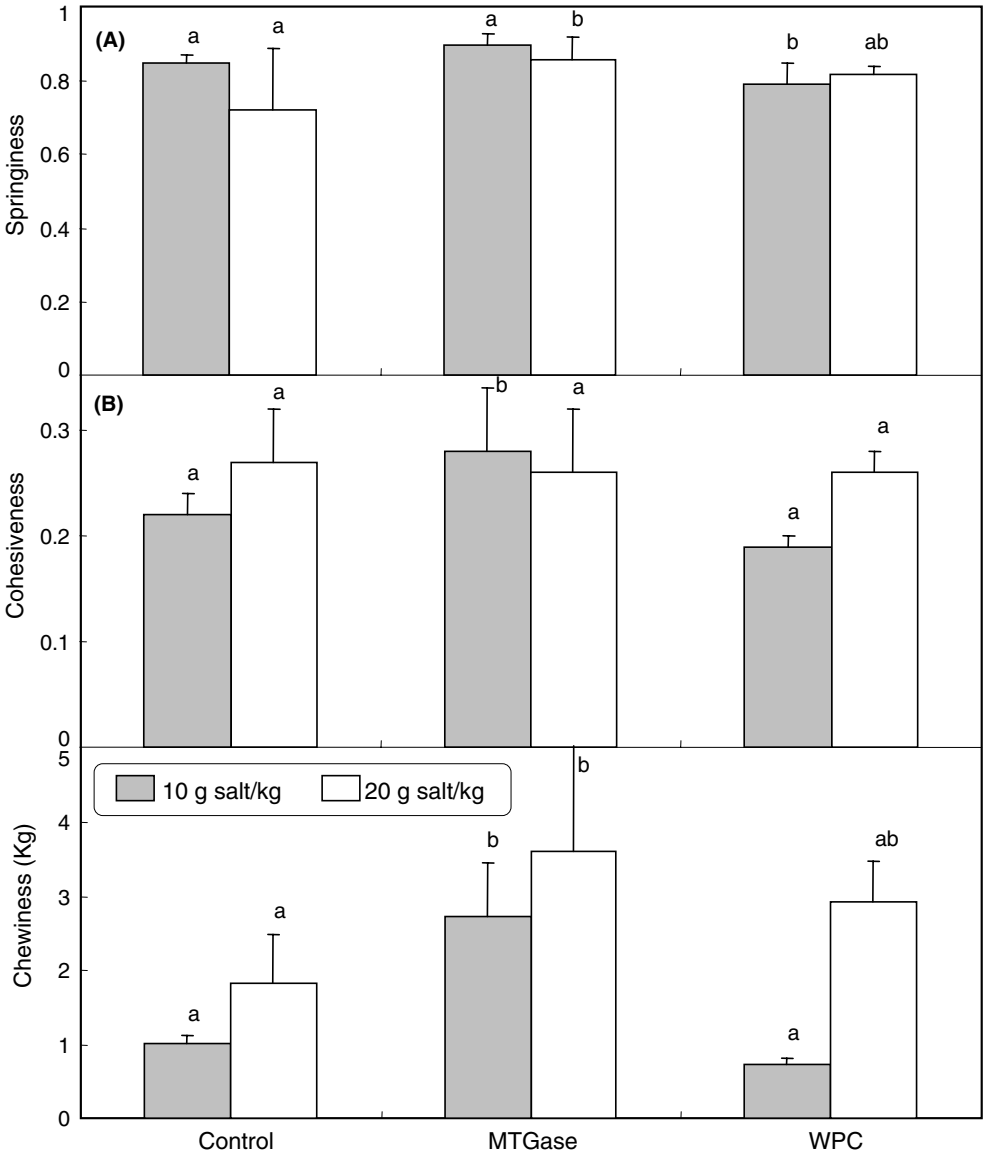

Fig. 2. Effect of microbial transglutaminase (MTGase) or whey protein concentrate (WPC) on springiness, cohesiveness and chewiness of gels at two different levels of salt. Mean values of three replicates. Bars indicate the standard error.

than did control samples. Cohesiveness was not affected and remained at a low value. Springiness was not affected by the added salt in any treatment. Cohesiveness showed a higher value in regular-salt gels than in low-salt gels when WPC was added. Chewiness was higher in samples containing regular-salt than in low-salt gels, when MTGase or WPC were added.

3.2. Puncture test

The changes in puncture test parameters for all treatments are shown in Fig. 3. The breaking force of low-salt gels varied from 1.587 to 2.779 kg; deformation varied from 13 to 16.8 cm and gel strength varied from 21.27 to 46.84 kg cm. Deformation, breaking force and gel strength increased significantly ($P < 0.05$) after adding MTGase, compared with the control. WPC did not affect the breaking force and gel strength parameters, but it had a negative effect on deformation values.

The breaking force of gels containing regular-salt concentration varied from 2.37 to 3.18 kg; deformation varied from 16.9 to 19.2 cm, and gel strength varied from 40.21 to 61.09 kg cm. Also, breaking force, deformation and gel strength increased significantly ($P < 0.5$) after adding MTGase compared with the control sample. WPC increased the breaking force and gel strength, without affecting deformation.

The control gels with regular-salt levels showed higher values of breaking force and gel strength than did low-salt controls. There were no differences between low-salt and regular-salt samples after adding MTGase. The gels containing WPC and regular-salt showed higher values of breaking force, deformation and gel strength than did gels containing WPC and low-salt levels.

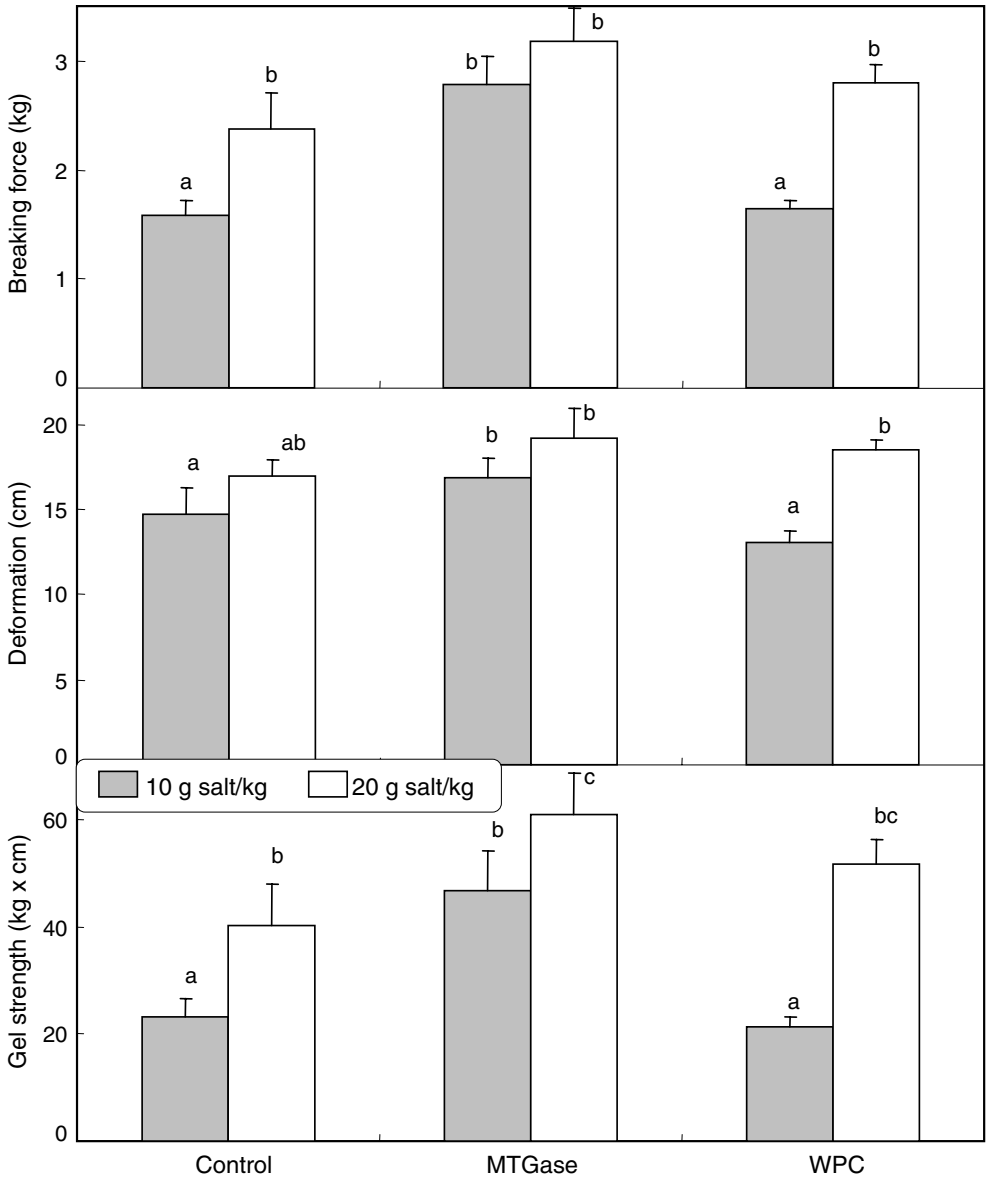

Fig. 3. Effect of microbial transglutaminase (MTGase) or whey protein concentrate (WPC) on breaking force, deformation and gel strength of gels at two different levels of salt. Mean values of three replicates. Bars indicate the standard error.

The results obtained from mechanical properties, indicate that it is feasible to obtain restructured fish products from striped mullet. Low-salt gels showed appropriate mechanical properties, although higher mechanical properties were obtained by using regular-salt levels (20 g/kg). The increasing effect on mechanical properties of using MTGase in restructured fish products with low-salt level, has previously been reported for fish gels from other species, such as silver carp (*H. molitrix*) (Ramírez, Uresti, Téllez, & Vázquez, 2002; Téllez-Luis et al., 2002).

3.3. Changes in colour attributes

Colour attributes of striped mullet restructured fish products are shown in Table 1. The L^* parameter in low-salt gels was not affected by adding neither MTGase nor WPC. The a^* parameter was significantly increased ($P < 0.05$) after adding WPC, indicating an increase in redness. The b^* parameter and the c^* attribute were increased by MTGase and WPC, indicating an increase in yellowness and chromaticity, respectively. The h^* attribute was decreased slightly but significantly ($P < 0.05$) after adding WPC, indicating a change from a yellow hue to a slightly more reddish hue.

The L^* attribute of regular-salt gels was not affected by adding MTGase or WPC. The a^* parameter was higher in samples containing MTGase, indicating an increase in redness. The b^* parameter, and the c^* and h^* attributes were not affected after adding MTGase or WPC compared with the control sample.

There were no significant changes in L^*, c^* and h^* attributes between low-salt and regular-salt control gels, indicating that the level of salt did not affect the colour in

Table 1
Effect of microbial transglutaminase (MTGase) or whey protein concentrate (WPC) on colour attributes of fish restructured products with 10 or 20 g NaCl/kg

Additive	Salt level (g/kg)									
	10	20	10	20	10	20	10	20	10	20
	L^*		a^*		b^*		c^*		h^*	
Control	64.0^{aA}	62.2^{aA}	0.94^{aA}	1.09^{aB}	14.8^{aA}	14.8^{aA}	14.8^{aA}	14.9^{aA}	86.2^{aA}	85.8^{aA}
	(1.5)	(1.1)	(0.08)	(0.05)	(0.1)	(0.3)	(0.1)	(0.3)	(0.4)	(0.1)
MTGase	63.7^{aA}	59.7^{aB}	1.10^{aA}	1.21^{bA}	16.2^{bA}	14.9^{aB}	16.3^{bA}	14.9^{aA}	86.1^{aA}	85.3^{aA}
	(1.2)	(1.3)	(0.23)	(0.16)	(0.4)	(0.2)	(0.4)	(0.1)	(0.8)	(0.6)
WPC	65.3^{aA}	61.0^{aB}	1.70^{bA}	1.13^{abB}	16.4^{bA}	15.2^{aB}	16.5^{bA}	15.3^{aB}	84.1^{bA}	85.7^{aB}
	(1.7)	(0.9)	(0.12)	(0.09)	(0.3)	(0.1)	(0.3)	(0.1)	(0.4)	(0.3)

$^{A–B}$ Different capital letters indicate differences ($P < 0.05$) between levels of salt (rows) for each treatment.
$^{a–b}$ Different letters indicate differences ($P < 0.05$) between treatments at the same salt level (columns) for each colour parameter. Values in parentheses indicate the standard deviations of the means.

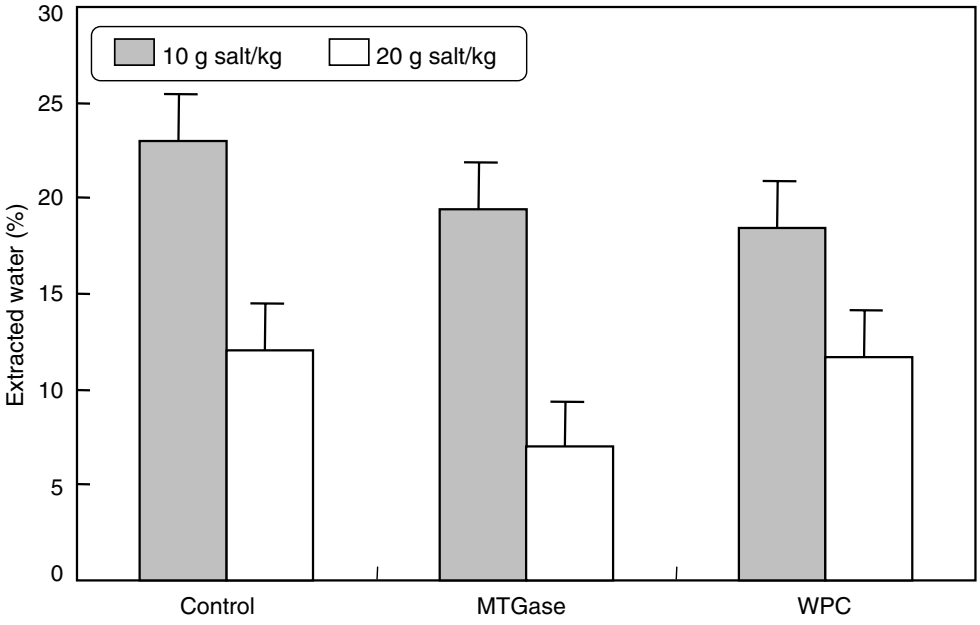

Fig. 4. Effect of microbial transglutaminase (MTGase) or whey protein concentrate (WPC) on extracted water of gels at two different levels of salt. Mean values of three replicates. Bars indicate the standard error.

restructured products. Gels obtained with regular-salt and MTGase were slightly darker (lower L^* than were low-salt gels treated with MTGase. WPC decreased the L^* in gels containing regular-salt, compared with low-salt gels, but decreased the chromaticity and increased the h^* attribute. This behaviour suggests that the effect of WPC was not perceptible.

3.4. Extracted water

The amount of extracted water is an indirect way of measuring water holding capacity (WHC). The higher the amount of water extracted, the lower is the WHC. The amount of extracted water in low-salt gels varied from 18.5% to 23% (Fig. 4). Adding of WPC induced a significant decrease in the amount of extracted water. Regular-salt gels showed lower amount of extracted water than did low-salt gels. The amount of extracted water in regular-salt gels varied from 6.96% to 12.1%. Gels containing MTGase showed the lowest amount of extracted water.

4. Conclusions

Restructured fish products from striped mullet showed appropriate mechanical properties at 10 and 20 g NaCl/kg. The mechanical properties of gels were higher at regular-salt level (20 g/kg) than at low-salt level (10 g/kg). The use of MTGase improved the mechanical properties of the restructured product at both salt levels, but WPC only improved the mechanical properties when a regular-salt level was used.

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