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Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*)

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Abstract

Restructured fish products from the filleting waste from silver carp (*Hypophthalmichthys molitrix*) were obtained using sodium caseinate (1%), whey protein concentrate (WPC) (1%) or MTGase (0.3%) at different levels of salt (0%, 1% or 2%). The restructured products were obtained by incubating at 40 °C for 60 min and then at 90 °C for 20 min. Changes in the mechanical properties were studied by measuring changes in the textural profile analyses, punch test and torsional test analyses and expressible water. The mechanical properties increased when the salt level was also increased. The mechanical properties of non-salted and low-salt products were increased by using dairy proteins. Sodium caseinate had a greater effect in improving mechanical properties than WPC. MTGase increased expressible water. The results obtained showed that the mechanical properties of low-salt restructured fish products can be improved by using dairy proteins combined with MTGase, with a slight increase in expressible water.

Keywords: Fish; Restructured; Transglutaminase; Salt; Texture

1. Introduction

The techniques most used for restructuring and extending low-value fish cuts and trimmings depend on solubilizing and extracting myofibrillar proteins with 2–3% salt by cutting, tumbling or blending to obtain sticky exudates. These are then used to bind meat pieces or to obtain gels (Kuraishi et al., 1997). Nowadays consumers are demanding healthier foods and there is a great interest in lightly salted products. To meet this public health demand, the food industry should increase the supply of low-salt foods because reducing salt intake can prevent and control adverse blood pressure levels (Narhinen et al., 1998). However, the level of salt employed during blending is important because it influences the amount of exuded protein obtained. The exuded protein acts as a binding agent and affects the protein aggregation mechanism and overall quality of the final product. A different mechanism of temperature-induced aggregation has been reported for myosin, influenced by the ionic strength (Boyer, Joandel, Ouali, & Culioli, 1996; Ramírez, Martín-Polo, & Bandman, 2000).

Several additives have been used to improve the mechanical and functional properties of restructured fish products, including proteinaceous additives, such as egg white (Yetim & Ockerman, 1995) casein and beef plasma-thrombin (Baker, Lanier, & Green, 2000) as well as several food hydrocolloids, such as xanthan, guar, kappa and iota carrageenan and pectins (Ramírez, Barrera, Morales, & Vázquez, 2002a; Ramírez, Uresti, Téllez-Luis, & Vázquez, 2002b; Pérez-Mateos & Montero, 2002; Pérez-Mateos, Solas, & Montero, 2002;

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Microbial transglutaminase (MTGase) is widely used in the food industry to induce covalent cross-linking of proteins. The use of MTGase to reduce the salt needed in restructured fish products has been previously reported (Ramírez et al., 2002a, 2002b; Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002). Addition of 0.3% MTGase gave rise to low-salt fish restructured products containing only 1% salt. However, it was not feasible to obtain restructured products by adding just MTGase in the absence of salt. The objective of this work was to determine the feasibility of using dairy proteins and MTGase as binding agents, for obtaining unsalted or lightly salted restructured fish products, using low value cuts from filleting of silver carp (*Hypophthalmichthys molitrix*), an abundant fish species in Mexican waters.

2. Material and methods

2.1. Frozen fish paste

Fresh low value cuts from filleting of silver carp (*H. molitrix*) were obtained in Tampico, Tamaulipas, Mexico. The fish wastes were stored in ice and transported to the laboratory (less than 1 h) and thoroughly rinsed with cold tap water. Skin and bones were removed with a Bibun deboning machine (Model NF2DX, Hiroshima, Japan) with a drum having 5 mm diameter perforations. The fish paste obtained was mixed with 80 g sucrose kg^{-1} as cryoprotectant, using a Hobart mixer (model VCM. Troy, Ohio) and then packed into polyethylene bags (2 kg) and frozen within 5 h at −30 °C in a Crepaco plate freezer (Model B-5854-AM12, Crepaco, Inc., Chicago, IL). The frozen paste was then stored at −20 °C until needed (3 weeks).

2.2. Production of fish gels

Restructured products were prepared from frozen fish paste. Bags containing fish paste were thawed during 24 h at 4 °C. For each treatment, 250 g of fish paste were chopped in a 5.5 l capacity cutter model 84145 (Hobart, Troy, OH) for 3 min. Microbial transglutaminase, Active TG-TI (AJINOMOTO USA, Inc., Teaneck, NJ), whey protein and sodium caseinate were dispersed into the fish paste in a dry form after the first 3 min and the paste was mixed for 2 min more. The temperature of the fish paste remained below 15 °C throughout the chopping operation for all treatments studied. Sodium caseinate and whey protein were added at 10 g kg^{-1}, MTGase was added at (3 g kg^{-1}) and three levels of NaCl (0 g kg^{-1} control, 10 g kg^{-1} or 20 g kg^{-1}) were assayed. The paste was stuffed into stainless steel tubes (diameter = 1.87 cm; length = 17.75 cm) and sprayed with commercial regular vegetable oil to prevent sticking. The tubes were capped before being immersed in water at 40 °C for 1 h, followed by immersion in water at 90 °C for 20 min. After cooking, the tubes were immediately removed, placed in a water bath and cooled at 4–5 °C for 30 min. All restructured fish products were removed from the tubes and stored overnight at 4 °C in polystyrene bags, prior to testing.

2.3. Mechanical properties

Cylindrical samples of restructured fish products of 1.87 cm diameter and 3 cm length were obtained. Samples were equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before the mechanical properties were measured. The mechanical properties were determined using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England).

The puncture test was performed, compressing samples to 75% of the initial height using a compression speed of 60 mm min^{-1} and a cylinder probe (P/20) with 1.2 cm diameter. The breaking force (g), deformation (cm) and work of penetration (g × 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.

Textural profile analysis (TPA) was performed using an aluminium cylindrical probe (P/50) with 50 mm diameter. Samples were compressed to 75% of the initial height using a compression speed of 60 mm min^{-1}. Hardness, springiness and cohesiveness were reported for each treatment. Six samples were also analysed for each treatment.

2.4. Expressible water

The amount of expressible water for each treatment was measured. Samples of 3 g (±0.1 g) of cooked gels were weighed and put onto 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 samples were removed and reweighed and the amount of expressible water was calculated as follows:

$$E_w = \frac{W_i - W_f}{W_i} \cdot 100,$$

where E_w is the percentage of expressible water, W_i is the initial weight and W_f is the final weight. Three samples were analysed for each treatment and averages are reported.

2.5. Statistical analysis

Data were analysed 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. Effects of dairy proteins at different salt levels on the properties of fish gels

Fish gels were obtained at three different levels of salt: 0% (unsalted), 1% (low-salt) and 2% (regular-salt). Both dairy proteins, sodium caseinate (Na-caseinate) and whey protein concentrate (WPC), were added at 1% (w/w). For comparative purposes, control gels without dairy proteins were also obtained. Mechanical properties and water-holding capacity were measured in all treatments.

3.1.1. Puncture test

The mechanical properties of the fish gels as measured by the puncture test, increased with increasing salt level from 0% to 2%, in all products, containing or not containing dairy proteins (Table 1). The breaking force (BF) increased from 490.8 g in the control sample without salt to 729.4 and 1064.9 g by adding 1% and 2% salt, respectively. The values of BF of unsalted fish gels increased by adding 1% of WPC or Na-caseinate. Low-salt fish gels obtained without dairy proteins showed lower values than samples containing 1% salt and 1% WPC or Na-caseinate. In fish gels obtained with 2% salt, only those samples containing Na-caseinate showed higher values of BF than control samples without dairy proteins. According to these results the samples containing 1% Na-caseinate showed higher BF values than samples containing 1% WPC or control samples without dairy proteins at each salt level (0%, 1% or 2%). Deformation values of unsalted and low-salt control gels without dairy proteins showed no significant differences ($P < 0.05$) (Table 1) and both were lower than deformation values of gels obtained with 2% salt. Low-salt gels containing WPC and Na-caseinate showed higher values of deformation than control samples. Deformation values were not significantly different in all gels obtained by adding 2% salt ($P < 0.05$). Gel strength and BF values showed similar behaviour.

3.1.2. Textural profile analysis

Hardness and springiness TPA parameters were affected for both factors: salt level and dairy proteins (Table 2). Hardness values increased with increasing salt level from 0% to 2% in all treatments for control and

Table 1
Effects of dairy proteins in the puncture test parameters of fish gels with different levels of salt

Salt	Breaking force (g)			Deformation (cm)			Gel strength (g × cm)		
	0%	1%	2%	0%	1%	2%	0%	1%	2%
Control	490.8^{aA}	729.4^{bA}	1064.9^{cA}	9.4^{aA}	9.7^{aA}	12.0^{bA}	4600^{aA}	7092^{bA}	12797^{cA}
	(12.0)	(9.8)	(26.0)	(0.8)	(0.5)	(0.9)	(454)	(368)	(1098)
Na-caseinate	740.8^{aC}	1025.6^{bC}	1224.0^{cB}	10.0^{aB}	12.3^{bB}	11.8^{bA}	7444^{aC}	12695^{bC}	14418^{bA}
	(20.4)	(71.0)	(77.5)	(0.8)	(0.8)	(0.4)	(691)	(1560)	(848)
WPC	677.3^{aB}	896.7^{bB}	997.3^{cA}	9.3^{aA}	11.2^{bA}	12.6^{bA}	6315^{aB}	10010^{bB}	12638^{bA}
	(22.3)	(31.7)	(49.0)	(0.5)	(1.2)	(2.5)	(487)	(1104)	(3032)

$^{A–C}$Different capital letters indicate differences ($P < 0.05$) between treatments (columns).
$^{a–c}$Different letters indicate differences ($P < 0.05$) between levels of salt for each measured mechanical property. Values in parentheses indicate the standard deviations of the means.

Table 2
Effects of the dairy proteins on the TPA parameters of fish gels with different levels of salt

	Hardness (kg)			Springiness			Cohesiveness		
	0%	1%	2%	0%	1%	2%	0%	1%	2%
Control	1.75^{aA}	2.39^{bA}	3.03^{cA}	0.449^{aA}	0.488^{aA}	0.651^{bA}	0.140^{aA}	0.142^{aA}	0.147^{aA}
	(0.07)	(0.09)	(0.36)	(0.05)	(0.08)	(0.07)	(0.01)	(0.01)	(0.02)
Sodium caseinate	2.61^{aC}	3.01^{bC}	3.98^{cC}	0.499^{aB}	0.537^{aB}	0.737^{bB}	0.129^{aA}	0.109^{aA}	0.157^{bA}
	(0.12)	(0.02)	(0.13)	(0.07)	(0.11)	(0.14)	(0.01)	(0.03)	(0.01)
Whey protein concentrate	2.40^{aB}	2.70^{bB}	3.40^{cB}	0.437^{aA}	0.466^{aA}	0.647^{bA}	0.120^{aA}	0.109^{aA}	0.129^{aA}
	(0.09)	(0.04)	(0.16)	(0.07)	(0.06)	(0.08)	(0.01)	(0.01)	(0.02)

$^{A–C}$Different capital letters indicate differences ($P < 0.05$) between treatments (columns).
$^{a–c}$Different letters indicate differences ($P < 0.05$) between levels of salt for each measured mechanical property. Values in parentheses indicate the standard deviations of the means.

gels containing dairy proteins. Samples containing Na-caseinate showed higher values of hardness at all salt levels. Springiness values increased significantly ($P < 0.05$) only in samples containing 2% salt as compared with samples without salt in products containing (or not containing) dairy proteins. Samples containing Na-caseinate showed higher values of springiness. Cohesiveness values were very low in all samples, ranging from 0.109 to 0.157 and showed no significant difference ($P < 0.05$).

3.1.3. Torsion test parameters

The torsion test has been widely used to determine functionality of surimi gels. Shear stress seems to be more accurate for measuring changes in mechanical properties caused by differences in processing or formulation additives. Shear strain values higher than 1.9 have been associated with gels showing elastic texture, such as the texture of crustaceans (shrimps, crab, lobster) (Hamann & MacDonald, 1992). In this study, shear stress values increased from 11.7 kPa in unsalted gels to 19.2 and 26.6 in low-salted gels and gels containing 2% salt, respectively (Fig. 1). Unsalted gels containing WPC and Na-caseinate showed significantly higher values of shear stress (17.1 and 22.4 kPa, respectively) than control unsalted gels. Low-salted gels

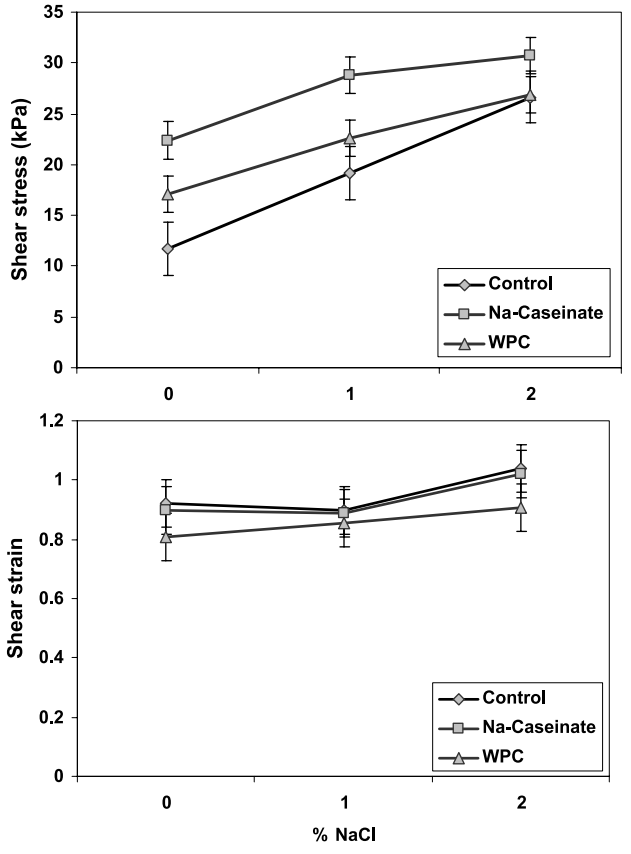

Fig. 1. Torsion analysis of restructured products obtained with dairy proteins at different salt levels. Bars show standard deviations.

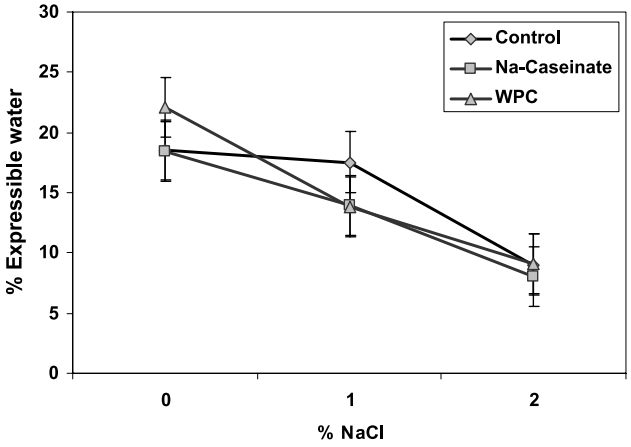

Fig. 2. Expressible water of restructured products obtained with dairy proteins at different salt levels. Bars show standard deviations.

containing dairy proteins showed higher values of shear stress than gels obtained without dairy proteins. In products containing 2% salt, only samples to which Na-caseinate had been added showed higher values of shear stress than the control samples. Fish gels showed low values of shear strain, ranging from 0.81 to 1.04. No significant differences were found among treatments.

3.1.4. Expressible water

The amount of expressible water decreased from 18.4–22.1% in samples without salt to 8.0–9.1% in samples containing 2% NaCl (Fig. 2). There were no significant differences between control samples and samples with WHC or Na-caseinate at the same level of salt addition (0%, 1% or 2% NaCl).

Results obtained indicate that both dairy proteins increased the mechanical properties of unsalted and low-salt fish gels when added at 1%, with any measurable effect in WHC. Low-salt products containing 1% sodium caseinate showed similar mechanical properties to the control samples obtained with 2% salt and no dairy proteins and the difference in expressible water between samples was 5%.

3.2. Effect of microbial transglutaminase and dairy proteins at different salt levels on properties of fish gels

A second set of experiments was conducted to determine the presence of a combined effect of MTGase and dairy proteins on the mechanical properties and water-holding capacity of fish gels.

3.2.1. Textural profile analysis

As previously reported (Ramírez et al., 2002a, 2002b; Téllez-Luis et al., 2002), MTGase required that myofibrillar proteins be solube to induce covalent protein cross-linking. Although 1% salt was enough to allow MTGase to induce protein interactions increasing the hardness, a higher value of hardness was obtained by

Table 3
Effects of MTGase and dairy proteins on the TPA parameters of fish gels with different levels of salt

	Hardness (kg)			Springiness			Cohesiveness		
	0%	1%	2%	0%	1%	2%	0%	1%	2%
MTGase	2.01^{aA}	2.83^{bA}	5.18^{cA}	0.471^{aA}	0.539^{abA}	0.745^{cA}	0.148^{aA}	0.134^{aA}	0.208^{bA}
	(0.08)	(0.08)	(0.29)	(0.04)	(0.07)	(0.06)	(0.05)	(0.01)	(0.02)
MTGase + Na-Caseinate	2.91^{aB}	3.52^{bB}	4.31^{cA}	0.570^{aA}	0.680^{abA}	0.766^{bA}	0.140^{aA}	0.131^{aA}	0.170^{aA}
	(0.13)	(0.19)	(0.69)	(0.07)	(0.12)	(0.10)	(0.08)	(0.07)	(0.05)
MTGase + WPC	2.87^{aB}	3.77^{bB}	4.75^{cA}	0.481^{aA}	0.622^{abA}	0.678^{bA}	0.145^{aA}	0.134^{aA}	0.151^{aA}
	(0.31)	(0.19)	(0.15)	(0.07)	(0.08)	(0.11)	(0.02)	(0.01)	(0.02)

$^{A–C}$Different capital letters indicate differences ($P < 0.05$) between treatments (columns).
$^{a–c}$Different letters indicate differences ($P < 0.05$) between levels of salt for each measured mechanical property. Values in parentheses indicate the standard deviations of the means.

adding 2% salt (Table 3). In restructured products without salt, adding dairy proteins and MTGase improved the hardness near to 50% compared with the control (2.01 kg). In the low-salt (1% NaCl) product, higher values of hardness were obtained by adding MTGase and dairy proteins than by adding only MTGase, suggesting a positive interactive effect between meat proteins, dairy proteins and MTGase. In products containing 2% NaCl, higher values of hardness were obtained in samples containing only MTGase than in samples containing MTGase and dairy proteins, suggesting a slight negative effect. Springiness values were increased significantly ($P < 0.05$) by adding 2% salt. There was no significant difference between treatments at each salt level. Cohesiveness was increased by adding 2% NaCl only in samples containing MTGase without dairy proteins. However this parameter was very low in all samples, ranging from 0.131 to 0.208.

3.2.2. Torsion test

Shear stress values show similar behaviour to hardness values. Shear stress values were increased by adding salt (Fig. 3), the higher the level of salt, the higher the shear stress in all samples. However, samples containing WPC showed lower values of shear stress than the control and samples containing dairy proteins when 2% salt was used. In samples without salt and low-salt products (1% NaCl), products containing dairy proteins and MTGase showed higher values than products containing only MTGase. The shear strain increased from 0.75–0.86 in samples without salt to 1.02–1.09 in products with 2% NaCl. No significant differences ($P < 0.05$) were found between the treatments.

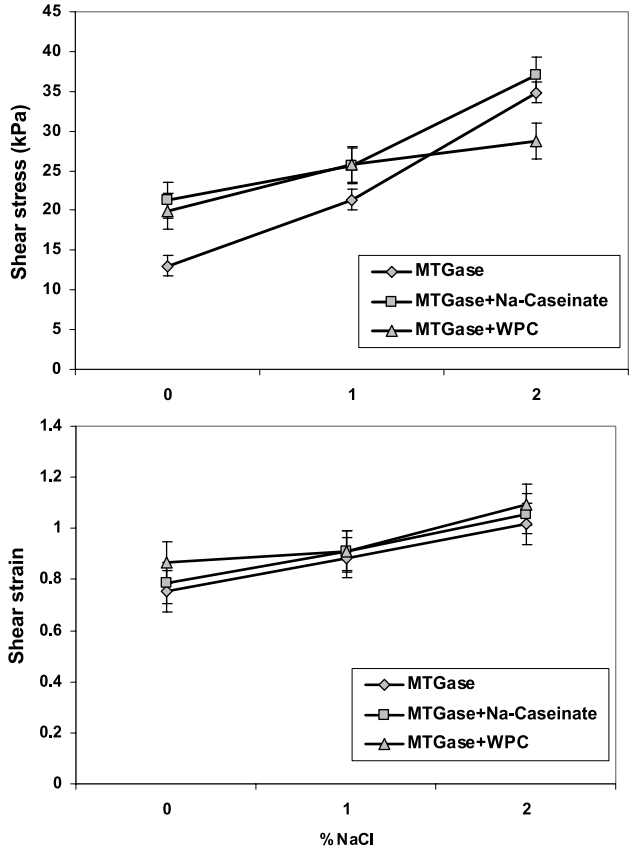

Fig. 3. Torsion analysis of restructured products obtained with MTGase and dairy proteins at different salt levels. Bars show standard deviations.

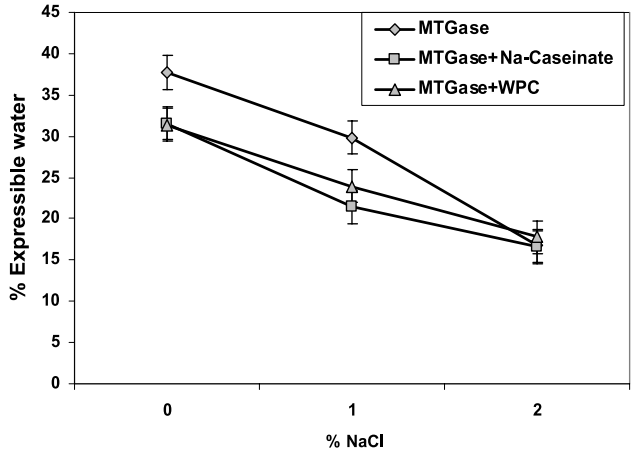

Fig. 4. Expressible water of restructured products obtained with MTGase and dairy proteins at different salt levels. Bars show standard deviations.

3.2.3. Expressible water

The amount of expressible water decreased with increasing concentration of salt (Fig. 4). Dairy proteins decreased the expressible water more than MTGase in unsalted and low-salt products, suggesting a more structured product. Recently an increase in expressible water in unsalted and low-salt restructured fish products has been reported (Ramírez et al., 2002a, 2002b;Téllez-Luis et al., 2002).

4. Conclusions

A decrease in salt level has a negative effect on the protein extractability and solubility and consequently on the mechanical properties. The protein solubilized and extracted during the blending also serves as a substrate for crosslinking reactions by MTGase. The results obtained showed that MTGase required the addition of salt to fish paste to improve the mechanical properties. The use of dairy proteins and MTGase had an improving effect on the mechanical properties of low salt products and decreased the amount of expressible water obtained by using MTGase alone.

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