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Restructured Fish Product from White Croacker (*Micropogonias furnieri*) Mince Using Microbial Transglutaminase

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ABSTRACT

*This study aimed at determining the influence of three concentrations of commercial transglutaminase enzyme in restructured fillet of minced fish from white croacker (*Micropogonias furnieri*), one of the four marine species with notability in Brazil. The restructured fillet developed had advantages when compared to traditional fillet, such as absence of spine and less flavour intensity (washes cycles). Washing process for white croacker mince was compared with five clarification agents: water (control), phosphoric acid (H3PO4), sodium chloride (NaCl), calcium carbonate (CaCO3) and sodium bicarbonate (NaHCO3). The higher quality product (whiteness) was obtained with calcium carbonate washes. Three concentrations (1.5, 1.0 and 0.5%) of microbial transglutaminase MGTase (Active TG-B %v/v and Active TG-BP %w/w) were compared, in order to produce fish restructured product (boneless fillet). The concentration of 1.5% (both enzymes), produced better results. The restructured products were compared by sensory analysis and showed better sensory parameters (appearance, odour, flavour and texture) samples treated with Active TG-B (solution form).*

Key words: Seafood, minced fish, white croacker, transglutaminase, restructured fish

INTRODUCTION

Fish is an important dietary constituent of several population groups and it has significant nutritional value, such as high quality proteins, vitamins, minerals and lipids, besides being the largest source of ω-3 series polyunsaturated fatty acids (especially the EPA and DHA), which bring several benefits to human health (Belda and Pourchet-Campos, 1991; Badolato et al., 1994; Kim and Lall, 2000; Connor, 2000; Hardman, 2002; Limin, Feng and Jing, 2006; Visentainer et al., 2007).

The white croacker (*Micropogonias furnieri*) is a migratory euryhaline teleostean demersal fish found in the Atlantic Ocean from Northern Venezuela (20°N) to the Gulf of St. Mathias (41°S), Argentina (Vasconcellos and Haimovici, 2006; Berois et al., 2007). Over 50% of the total fisheries production in the region (south of Brazil) is supported by sciaenid species and the white croacker *Micropogonias furnieri* (Fig. 1) is one of the most abundant and important for local fisheries (Vasconcellos and Haimovici, 2006).

The composition of edible fish portion varies as a function of many factors, such as specie, sex,

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sexual maturity degree, size, place of capture, water temperature, type of feeding and season (Soccol and Oetterer, 2003). White croacker and minced showed the same variation during season (Table 1), as demonstrated by Badolato et al. (1994) and Luzia et al. (2003).

White croacker is captured more intensively in spring months (October to January) with medium size of 30 cm (Vasconcellos and Haimovici, 2006; Haimovici and Umpierre, 1996), but the fillet yield is low (31-36%) and by-products with 10% of meat with high quality is not commercially used for mince production.

Figure 1 - White croacker (*Micropogonias furnieri*).

Table 1 – Chemical composition of white croacker during season

Chemical composition (g/100g) of white croacker fillet				
Season	**Moisture**	**Ash**	**Lipid**	**Protein**
SU	79.5	0.9	0.5	18.9
AU	77.2	1.4	0.8	20.7
WI	77.9	1.0	0.7	19.8
SP	83.8	1.0	0.5	14.5
Chemical composition (g/100g)of white croacker mince				
Season	**Moisture**	**Ash**	**Lipid**	**Protein**
SU	79.7	1.4	1.4	16.7
AU	77.0	1.3	1.3	20.1
WI	81.6	0.7	1.1	16.2
SP	83.1	0.1	0.6	16.5

SU (Summer: December 21 to March 21); AU (Autumn: March 21 to June 21); WI (Winter: June 21 to September 21), SP (Spring: Sept. 21 to December 21).

In seafood processing, it is of great interest to maximize the yield of marketable products, including the developments of methods for restructuring low-value cuts and trimmings to improve their appearance, flavour and texture and to enhance the market value. Then an alternative process involves obtaining a fish paste by mechanical separation of the flesh and then preparing restructured fish products with high economic and nutritive value (Ramírez et al., 2007).

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 (Bruschi, 2001; Mira and Lanfer-Marquez, 2005; Ramírez et al., 2007).

The production of transglutaminase by microorganisms makes it possible to apply this enzyme in a variety of food processes. Transglutaminase is an enzyme capable of catalyzing acyl-transfer reactions introducing covalent cross-links between the proteins as well as peptides and various primary amines, thereby improving the gel structure (Zhu et al., 1995; Jiang, Lev and Tsai, 1998; Uresti et al. 2003; 2004). Table 2 shows the application of microbial transglutaminase (MTGase) in seafood processing (Zhu et al., 1995; Piccolo, 2006).

Table 2 - Overview of application of MTGase in seafood processing.

Source	Product	Effect
Fish	Fish paste	Improved texture and appearance
Krill	Krill paste	Improved texture
Fish	Fishburguer	Improved elasticity, texture, taste and flavour
Collagens	Shark-fin imitation	Imitation of delicious food

Nowadays, a commercial microbial transglutaminase (MTGase) is employed to improve the mechanical and textural properties of different protein foods, including surimi products (Barrera et al., 2002). 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 (Ohtsuka et al., 2001; Sato et al., 2001). Several studies have been conducted to determine the optimal conditions for using MTGase in restructured fish products (Zhu et al., 1995; Téllez-Luis et al., 2002; Uresti et al., 2004; Ramírez et al., 2007).

The objective of this work was to determine the feasibility of using microbial transglutaminase (MTGase) as binding agent to prepare a restructured fish products from white croacker mince with acceptable sensorial characteristics.

MATERIAL AND METHODS

Raw material

Fresh white croacker (*Micropogonias furnieri*) was obtained directly from the market, thoroughly rinsed with cold tap water, stored in ice and transported to the laboratory immediately in less than one hour, where it was washed, weighed, headed, gutted and filleted manually. Skin and bone were removed manually and fillets were washed and weighed. Fillets were grounded with 4 mm diameter perforations to obtain a homogeneous mince, according to Jesus et al (2001). The obtained mince was maintained at refrigeration temperature until the clarification process.

Minced fish clarification process

Mince washing process was compared with five clarification agents: water (control), phosphoric acid (H_3PO_4), sodium chloride (NaCl), calcium carbonate ($CaCO_3$) and sodium bicarbonate ($NaHCO_3$). For each treatment, fish mince samples (50 g) were mixed with 150 ml of clarification solution (Table 3) in a mince/clarification solutions ratio of 1:3, with constant agitation (Gomes et al., 1994; Cândido; Nogueira; Sgarbieri, 1998; Barreto and Beirão, 1999; Tenuta and Jesus, 2003). For each step, the concentration diminished and the third step samples were washed only with distilled water.

After three washing cycle at 10°C for 10 minutes for each treatment, all the samples were followed by manually dewatering employing cheesecloth as filtering material (Barrera et al., 2002) and pressed to remove the excess water, weighed, packed and stored at 4°C (Simões et al., 1998; Monterrey-Quintero and Sobral, 2000; Mira and Lanfer-Marquez, 2005).

Table 3 – Washing cycles.

Clarification agents	STEP I	STEP II	STEP III
Water	150mL	150mL	150mL
H_3PO_4	150mL (0.05%)	150mL (0.025%)	150mL (0%)
$CaCO_3$	150mL (0.5%)	150mL (0.25%)	150mL (0%)
NaCl	150mL (0.5%)	150mL (0.25%)	150mL (0%)
$NaHCO_3$	150mL (0.5%)	150mL (0.25%)	150mL (0%)

Preparation of restructured product

The clarified minced was examined to make sure that it was free of bones and then "glued" together using a food-grade enzyme produced by Ajinomoto Co (Ajinomoto, 2007). The binding agent used was a commercial transglutaminase (MTGase), Activa TG-B diluted in distilled water (1:5, w/v) and TG-BP (dry form), which was separated from a culture of *Streptoverticillium mobaraense*.

All enzymes were tested in three concentrations reported as a commercial concentration (0.5, 1.0

and 1.5%) dissolved in distilled water prior to being mixed into the mince and compared with a control sample (without enzyme), according to Piccolo (2006). Control product was obtained without the addition of MTGase.
The minced fish treated by mTGase was then formatted in a cylindrical sample of 3.5 cm diameter and 1 cm length by pressure and stored at 10°C for 160 minutes previously calculated in preliminary experiments, considering the temperature profile of the MTGase activity (Ajinomoto, 2007).

Sensorial analysis
Quantitative descriptive analysis (QDA) was performed with the presence of a staff of 30 non-trained panellists who registered the sensory attributes: appearance, flavour, taste and texture (unstructured line scale of 9 cm) according to Dutcosky (1996). The test was accomplished in a sensory panel room where each person received three samples of hot fried restructured products for evaluation.
Statistical analysis was performed using Statistica for Windows (StatSoft Release 6.0) and ANOVA test were used to determine significantly differences ($p<0.05$) among the treatments.

RESULTS AND DISCUSSION

Minced fish production
The filleting yield was 32.1%, which was lower than yields of 36% obtained by Bruschi (2001) for the same specie. This filleting yield variability could be a function of several aspects, such as fish size, filleting system (mechanical or manual), practice of fillet workers, which contributed to an increase and/or decrease in the percentage of meat withholding carcass (Bykowski and Dutkiewicz, 1996; Souza, 2002). The process could be optimized using Meat Recovery and Meat Separation Machinery, reaching a percentage around 50% as obtained by Bykowski and Dutkiewicz (1996) and Mira and Lanfer-Marques (2005).

Minced fish clarification process
The most important step of mince and/or surimi processing to ensure the maximum gelling, as well as colorless and odourless is efficient washing (Park and Morrissey, 2000). According to Hultin and Kelleher (2000), the addition of alkali in the surimi wash water produced a higher quality product than just using water. Various concentrations of sodium bicarbonate may be added in one or more of the wash steps to increase pH. Sodium chloride is also sometimes added. Some authors considered that mince washing was significant in reducing the water soluble components (heme-pigments), improving the textural quality (Lin and Park, 1996; Lee et al, 1998; Tenuta and Jesus, 2003).
Several studies have demonstrated chlorine water as a better agent for clarifying the minced fish and to improve its safety (Monterrey-Quintero and Sobral, 2000; Jesus et al., 2001; Mira and Lanfer-Marquez, 2005). In this study (Fig. 2) all the clarification agents used showed positive results, i.e., there was bleaching (whiteness) when compared to non-washed minced. Moreover, phosphoric acid and calcium carbonate had higher whiteness results.
The result of calcium carbonate was considered better because the washed mince was more homogeneous (doughy) when compared with phosphoric acid (mass with granular appearance). Another reason that contributed to this choice was the lower price of $CaCO_3$ when compared to H_3PO_4 as well to avoid the contact with acids that would always be a factor of risk to personal safety and equipment.
The washing process has the secondary goal, i.e., removing the natural components of the minced fish, such as proteins soluble in water, blood and other components (heme pigments), which could accelerate the deterioration (lipid oxidation and microorganisms) during the storage of low temperatures. Another interesting result was that washed minced fish presented an intense characteristic aroma even after freezing and thawing. The yield reached in this process was 98.13%, which was considered satisfactory.

Preparation of restructured product
The pH result after wash cycles with CaCO3 was higher (pH 10) than the range of MTGase activity (pH 5-8), but in preliminary study, the restructuring was found. It has been suggested that gelation is improved after this type of washing process because the solubility of the sarcoplasmic proteins is increased and there is a decreased rate of denaturation as the muscle pH is increased (Hultin and Kelleher, 2000). The restructuring probably can be optimized with a simple reduction in concentrations of $CaCO_3$ or with the addition of

another washing cycle with water, though these suggestions would need to be proven through new trials and future studies.

According to Lee et al (1998), washed mince must be titrated at pH 8.0 for the best condition of MTGase activity. The adjustment of pH in the range of high enzyme activity (pH 5-8) probably might entail a reduction in the cost production, because the efficiency of the restructuring would be related directly to enzyme activity.

Figure 3 shows the production steps of restructured products using the enzymes (MTGase), Activa TG-B (solution form) and TG-BP (dry form).

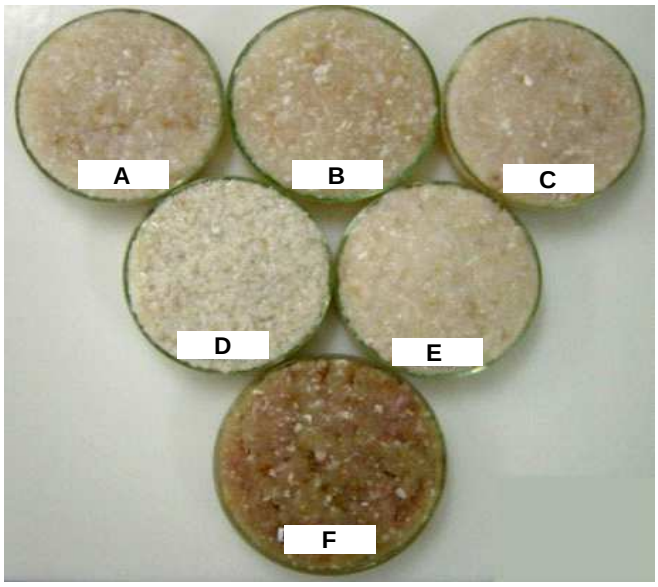

Figure 2 -Washing steps in minced fish clarification process (A: NaCl; B: $NaHCO_3$, C: Water (control); D: H_3PO_4, E: $CaCO_3$ and F: non-washed mince).

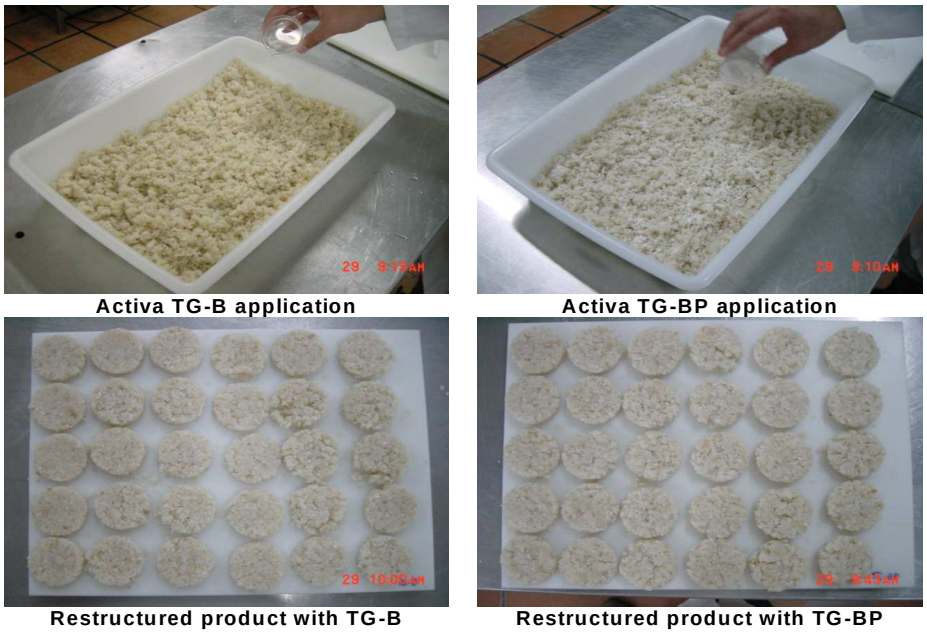

Figure 3 - Restructured product steps.

The structure formed by pressure during fish product forming with MTGase showed the importance of this procedure during MTGase activity. When pressure is released, the protein is restructured initially by hydrogen bonding and later by MTGase activity (Montero, 2005).

Figure 4 shows the comparison of the MTGase concentrations used before and after frying samples. The 1.5% concentrations achieved the best results before and after frying, showing a improvement of restructuring with increasing MTGase concentration, as obtained by Uresti et al. (2003); however, other concentrations also resulted a small restructuring. The MTGase (TG-B and TGBP) concentration of 1.5% was chosen the for sensory analysis. Uresti et al. (2004) and Ramírez et al. (2007) obtained restructuring using MTGase associated with milk protein proving the property of restructuring in meat products with low salt concentration.

As mentioned previously, the fish characteristic aroma was intense even after thawing (before the use of the transglutaminase) and the mince color remained attractive what was considered satisfactory.

The results of this stage through visual examination showed that MTGase Activa TG-BP sample had superior characteristics on the restructuring when compared to MTGase Activa TG-B, which should be supported by sensory analysis. A problem encountered in this stage was an excessive drip loss after thawing (lower water holding capacity), which led difficulties for final product formatting. To improve this process, a mixture of phosphates could be used (Hultin and Kelleher, 2000).

Several studies have been used to improve the mechanical and functional properties of restructured fish products, dairy proteins, salt at low concentrations, phosphate, starch and hydrocolloids, such as carrageenan (Barreto and Beirão, 1999; Uresti et al, 2004; Gonçalves, 2005; Ramírez et al. 2007).

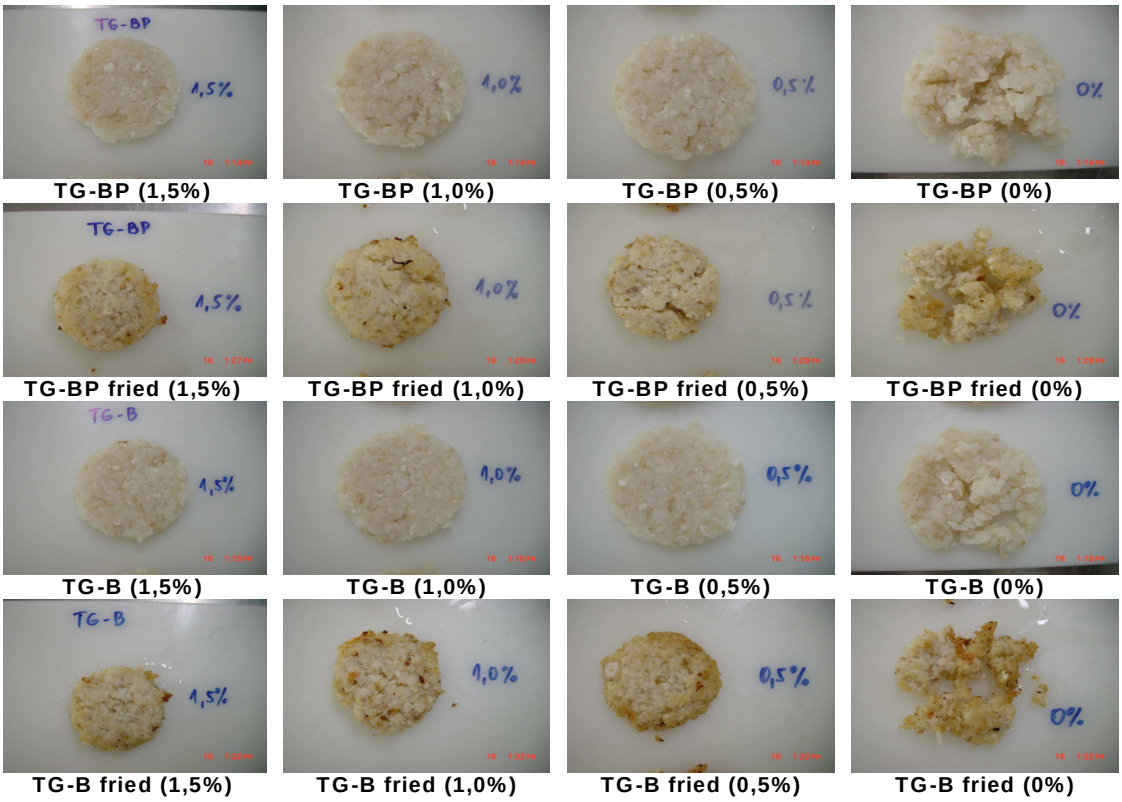

Figure 4 - Different concentrations of MTGase before and after frying.

Sensorial analysis

Both the samples were approved by panelists and significant difference between the samples MTGase Activa TG-B (solution form) and TG-BP (dry form) (Table 4) were observed.

It was interesting note that in all the sensorial parameters evaluated the sample treated with MTGase in solution form (TG-B) was superior to powder form (TAG-BP), which indicated its use to make a better restructured product.

Table 4 - Sensorial parameters of restructured product (mean ± Std.Dv).

Sensorial Parameters	MTGase TG-B	MTGase TG-BP
APPEARANCE	6,33 ± 1,15a	5,82 ± 0,84b
FLAVOUR	5,74 ± 1,24a	5,24 ± 0,83b
TASTE	5,13 ± 0,83a	4,22 ± 0,34 b
TEXTURE	5,78 ± 0,69a	4,81 ± 0,31b

Different letters in the line mean significantly different ($p < 0,05$); n = 30.

CONCLUSIONS

It could be concluded that the five substances tested as clarification agents (H_2O, H_3PO_4, NaCl, $CaCO_3$, $NaHCO_3$) achieved positive results when compared to non-washed mince, with further clarification (more white mince) by $CaCO_3$ and H_2PO_4, but the $CaCO_3$ was chosen as the best agent for clarification to present better uniformity, lower cost and better environmental security. Probably pH values of washed mince with $CaCO_3$ (pH 10) remained above the range of optimal enzyme activity (5-8). However, more studies are needed to determine the effect of level of $CaCO_3$ addition on the restructuring properties of MTGase.

In developing the restructured product, results were satisfactory for all the concentrations of transglutaminase (1.5, 1.0 and 0.5%) in both the treatments (MTGase TAG-B and TAG-BP). However, the samples with higher concentration (1.5%) showed best restructuring. The results of the comparison of sensory analysis for the treatment MTGase Active TG-B and TG-BP showed that both the restructured products were approved but TAG-B was better than TAG-BP.

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RESUMO

Este estudo teve como objetivo determinar a influência de três concentrações de enzima transglutaminase comercial em filés reestruturado a partir de polpa de corvina (*Micropogonias furnieri*), uma das quatro espécies marinhas notáveis no Brasil. O filé reestruturado desenvolvido tem vantagens quando comparado aos filés tradicionais, tais como, a ausência da espinhas e sabor menos intenso (ciclos de lavagens). O processo de lavagem da polpa de corvina foi comparado com cinco agentes clarificantes: água (controle), ácido fosfórico (H_3PO_4), cloreto de sódio (NaCl), carbonato de cálcio ($CaCO_3$) e bicarbonato de sódio ($NaHCO_3$). O produto de qualidade superior (mais branco) foi obtido com a lavagem com carbonato de cálcio. Três concentrações (1,5%, 1,0% e 0,5%) de transglutaminase microbiana (Activa TG-B % v/v e Activa TG-BP % p/p) foram comparadas a fim de produzir o produto reestruturado (filé sem espinha). A concentração de 1,5% (ambas as enzimas) produziu melhores resultados. Os produtos reestruturados foram comparados através de análise sensorial, e apresentaram melhores parâmetros sensoriais (aparência, odor, sabor e textura), as amostras tratadas com Activa TG-B (forma de solução).

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