Reduced-salt restructured European hake (*Merluccius merluccius*) obtained using microbial transglutaminase

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ABSTRACT

Restructured products of European hake (*Merluccius merluccius*) could provide new functional products increasing their commercial value. Restructuring allows to obtain free-fish-bone products adequate for children and elder people. The aim of this work was to develop restructured hake using reduced-salt and microbial transglutaminase. The products are also adequate for people with blood pressure disorders. Textural profile analysis, expressible water, dry matter, water activity and colour parameters were measured. The temperature effect of the setting phenomenon was evaluated at three conditions: 7ºC/12 h, 25ºC/2 h or 40ºC/15 min using 15 g/kg of NaCl and 300 U/kg of transglutaminase. The results showed that the parameters were improved at 25ºC/2 h. In a second step, salt was reduced to 10 g/kg and the setting was fixed at 25ºC/2 h. Transglutaminase concentration was studied at 150, 300, 600 or 900 U/kg. The models showed that hardness similar than that of turkey breast can be obtained using 377 U/kg of transglutaminase. Slices can be made for sandwiches, opening new markets for the European hake. The results demonstrated that the restructuring of European hake is feasible.

Keywords: transglutaminase; *Merluccius merluccius;* restructured fish products; setting phenomenon.

1. Introduction

Fish products are an important source of beneficial nutrients such as proteins, aminoacids, minerals and polyunsaturated fatty acids (PUFA), principally omega-3 such as eicosapentaenoic (EPA) acid and docosahexaenoic (DHA) acid (Palmeira, Mársico, Monteiro, Lemos, & Conte Junior, 2016) . The nutritional benefits and high digestibility of fish consumption were confirmed by a high number of studies (Visciano, Perugini, Manera, Salese, Martino, & Amorena, 2014) . Last studies associated the consumption of fish products with a decreased risk of cardiovascular diseases as well as vasodilatatory and anti-inflammatory properties (Roncarati et al., 2012; Afonso et al., 2013; Visciano, Perugini, Manera, Salese, Martino, & Amorena, 2014) .

Scientific advances and new methods in food industry, such as restructuring technology or new additives, can provide healthy options to increase the consumption of beneficial foods such as fish products (Martelo-Vidal, Mesas, & Vazquez, 2012) . The use of the enzyme transglutaminase allows to reduce the salt levels and to improve quality parameters (Cardoso, Ribeiro, & Mendes, 2014) . The restructuring technology allows to obtain fish products without fish-bone adequate for children and elder people. The salt reduction makes the products also adequate for people with high blood pressure disorders. Therefore the health of people is improved (Kunnath, Lekshmi, Chouksey, Kannuchamy, & Gudipati, 2015; Cardoso, Ribeiro, & Mendes, 2014) .

Restructuring process was used on several meats such as chicken (Yin, Reed, & Park, 2014) and hen meat (Gupta, Sharma, & Mendiratta, 2015) , pork ham (Terjung et al., 2016), poultry (Cofrades, López-López, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2011) or exotic meat as caiman (Canto et al., 2014). Moreover it was used to restructure muscle from marine species such as blue crab (Martinez, Robledo, Velazquez, Ramirez, Vazquez, & Uresti, 2014) , blue fish such as white tuna (*Thunnus alalunga*) (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016), Atlantic mackerel (*Scomber scombrus*) (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) , ribbonfish (Wu, Yuan, Chen, Liu, Ye, & Hu, 2015) , nile tipalia (Wongthahan & Thawornchinsombut, 2015; Oliveira Filho, Oliveira, Sobral, Balieiro, Natori, & Viegas, 2015) or striped mullet (*Mugil cephalus*) (Ramirez, del Angel, Uresti, Velazquez, & Vazquez, 2007; Ramirez, Del Angel, Uresti, Velazquez, & Vazquez, 2007) .

European hake (*Merluccius merluccius*) is a species with high commercial value. Hake is usually consumed fresh or frozen as breaded fillets and bars. There are a few processed products from hake like sausage, cooked jam and other cold cuts. Therefore, restructured products of hake could provide new products increasing more their commercial value.

Mains steps to obtain restructured fish products are: myofibrillar proteins extraction and solubilization using NaCl (20–30 g/kg recommended) following by a setting phenomenon at 0 - 40ºC and denaturation process of protein structure by heat at 90ºC for at least 40 min (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016; Ramirez, Uresti, Velazquez, & Vazquez, 2011; Martinez, Robledo, Velazquez, Ramirez, Vazquez, & Uresti, 2014) .

Microbial transglutaminase (MTG) is a protein-glutamine gamma-glutamyltransferase (EC 2.3.2.13) which improves textural properties, heat stability, emulsifying properties and gelation without change on the nutritional quality of products rich in proteins such as fish muscle (Gaspar & De Góes-Favoni, 2015; Hinz, Huppertz, Kulozik, & Kelly, 2007; Moreno, Carballo, & Borderías, 2010) . It is an extracellular enzyme obtained by fermentation of microorganism like *Streptoverticillium mobaraense* (Martelo-Vidal, Mesas, & Vazquez, 2012) . This enzyme catalyzes the covalent bond formation between proteins providing better structure to gels (Ramirez, Uresti, Velazquez, & Vazquez, 2011; Kunnath, Lekshmi, Chouksey, Kannuchamy, & Gudipati, 2015) . MTG no require Ca^{+2} to act. Its optimal range of pH is 6-7 and optimal temperature range is 45–55ºC. The structure performed is stable after thermic treatment. The enzyme loses the activity at 70ºC for 5 min (Tellez-Luis, Ramirez, & Vazquez, 2004; Yokoyama, Nio, & Kikuchi, 2004) .

There are not mathematical models that describe the effect of MTG on textural parameters of the final restructured product. Therefore the aim of this work was to develop a novel reduced-salt restructured product from European hake (*Merluccius merluccius*) muscle using transglutaminase and to obtain mathematical models for the effect of MTG on textural parameters of the final products. The development of a new product resembling turkey breast was tested as a case study.

2. Materials and methods

2.1. *Raw Materials*

European hake (*Merluccius merluccius*) were obtained from local market of Lugo (Northwest of Spain). Hakes were transported to laboratory into polystyrene boxes with ice. Skin and bones were removed manually (Martelo-Vidal, Mesas, & Vazquez, 2012; Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) . Microbial transglutaminase was obtained in our laboratory following the manufacture process described in our Spanish patent (Delfino et al., 2011). A colorimetric procedure was used to determine enzyme transglutaminase activity before use (Grossowicz, Wainfan, Borek, & Waelsch, 1950) . Briefly, N-α-CBZ-gln-gly (Sigma-Aldrich Corp, St. Louis, MO, USA) was used as substrate. A calibration curve was made using L-glutamic acid γ-monohydroxamate (Sigma-Aldrich Corp, St. Louis, MO, USA. One unit of transglutaminase is defined as the formation of 1 micromol l-glutamic acid γ-monohydroxamate in 1 min at 37ºC.

2.2. Methods

2.2.1. Restructured hake products

A first set of experiments was assayed using 15 g/kg of NaCl and 300 U of MTG per kg of hake muscle to select the best setting temperature. These concentrations were based on previous studies (Martelo-Vidal, Mesas, & Vazquez, 2012; Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016; Tzikas, Soultos, Ambrosiadis, Lazaridou, & Georgakis, 2015) .

Hake muscle was minced and washed with distillate water on a ratio 1:3 of muscle:water at a temperature lower than 15ºC for 5 min. The resulting paste was mixed with NaCl and MTG for 5 min and it was stuffed in stainless moulds. Moulds with paste were immersed in water bath for setting processing at three different conditions of temperature and time (7ºC for 12 h, 25 ºC for 2 h and 40ºC for 15 min). After setting process, molds were heated in water bath at 90ºC for 45 min. Following they were cooled in ice-water bath for 5-7 min and stored at 4ºC for 24 h before analysis (Martelo-Vidal, Mesas, & Vazquez, 2012; Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) . Controls were obtained with the same setting conditions but without MTG.

In a second set of experiments MTG concentration using a reduced NaCl concentration (10 g/kg) was modeled and optimized. Therefore, five MTG concentrations were assayed (0, 150, 300, 600, 900 U/kg) using the best setting conditions determined previously in the first set of experiments. The reduced NaCl concentration (10 g/kg) was selected based on preliminary studies (Ramirez, del Angel, Uresti, Velazquez, & Vazquez, 2007). The restructured European hake products obtained were equilibrated in plastic bags to avoid dehydration at room temperature for 30 min before analysis.

2.2.2. Expressible water, water activity and dry matter

Expressible water (Ew) was analysed as an indirect measure to evaluate the water holding capacity. Samples were weighed (2 ± 0.2 g) and put in two layers of filter paper disk. Following, they were centrifuged at 1000 *g* and 4ºC for 15 min. Then wet disk filter papers were removed and samples were weighed. The percentage of expressible water was determine as Equation (1):

$$Ew = \frac{G_0 - G}{G_0} \cdot 100 \qquad (1)$$

Where Ew is the percentage of expressible water, G_0 is the initial weight (g) and G is the final weight. For each treatment, four samples were measured (Martelo-Vidal, Mesas, & Vazquez, 2012; Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

Water activity (A_w) was determined for each treatment using AquaLab meter (Pullman, USA). Three replicates were analysed for each treatment (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

Percentage of dry matter was determined by triplicate for each treatment. Samples were weighed (5 ± 0.2 g) and put into a preweighed crucible. Then the samples were dried for 24 h at 98ºC. Following, the samples were reweighed and dry matter was determined as Equation (2)

$$DM = \frac{P_d}{P_w} \cdot 100 \qquad (2)$$

Where DM is the percentage of dry matter, P_d is the dry weight (g) and P_w is the wet weight (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

2.2.3. Colour determinations

Colour of samples was measured using a ColorStriker meter (Mathai, Hannover, Germany). Values of L* (Lightness), a* (redness) and b* (yellowness) were calculated based on illuminant C and the 2º standard observer. Nine samples were measured for each treatment (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016; Uresti, Lopez-Arias, Ramirez, & Vazquez, 2003) .

2.2.4. Mechanical properties

Mechanical properties were determined using a TA-XTplus (Stable Micro System, Viena Court, UK). Samples were cut into cubes of 2 x 2 x 2 cm. Texture Profile Analysis (TPA) was performed using cylindrical aluminium probe of diameter 50 mm (P/50). Compression speed was 60 mm/min until a compression of 75% of the original height (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011; Martinez, Robledo, Velazquez, Ramirez, Vazquez, & Uresti, 2014) . Five textural parameters (harness, adhesiveness, springiness, cohesiveness and chewiness) were determined for each experiment and ten samples were analysed for each experiment (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013; Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016; Torres, Saraiva, Guerra-Rodríguez, Aubourg, & Vázquez, 2014) .

2.2.5. Data and Statistical analysis

Duncan's multiple-range test at a significance level of 0.05 was used for the comparison of the mean values between treatments (Statistica, Dell Statistica, Tulsa, OK, USA).

Adjustment of experimental data to model was performed using the solver utility (Solver, Microsoft Excel 2013, Microsoft Corporation, Redmond, WA, USA). The models obtained were statistically validated by regression coefficients (r^2) and Fisher test probability. The 95% confidence intervals were determined in Microsoft Excel by the procedure of Brown (2001).

3. Results and discussion

3.1. Selection of setting temperature

Fish proteins can gelling after a setting phenomenon in the range 0-40ºC. For a cold-water fish, the setting process at 0ºC for 12-24 h or 25ºC for 2 h is recommended. However, for a warm-water fish a temperature of 40ºC for 20 min is recommended (Ramirez, Uresti, Velazquez, & Vazquez, 2011) . European hake is a cold-water fish but it can be caught in warm-water zones. For this reason, in a first study, restructured products were obtained using three setting conditions: 7ºC for 12 h, 25ºC for 2 h or 40ºC for 15 min. Results are shown in the next sections.

3.1.1. Expressible water, water activity and dry matter

Table 1 shows the changes of expressible water (Ew), water activity (Aw) and dry matter (DM) of restructured fish products with and without MTG at the three setting conditions studied. Ew affects the juiciness and tenderness of restructured fish products and it is opposite to the concept of water holding capacity. Therefore low values of Ew are related with high values of water holding capacity of fish mince (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013; Ramirez, Rodriguez-Sosa, Morales, & Vazquez, 2003) . Hence low values of Ew have a positive effect on the sensory perception of texture of the final products.

Values of Ew without MTG varied from 47.34% to 51.64%, meanwhile with MTG they varied from 35.12% to 51.11%. When similar conditions were applied in restructured white tuna (*Thunnus alalunga*), values of Ew were 36.37% to 43.71% without MTG and 32.77% to 37.00% with MTG. In white tuna products lower Ew values were obtained using 4ºC for 12 h with or without MTG (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) . The lowest Ew value in restructured hake without MTG was obtained performing the setting at 7ºC for 12 h. No significant differences were found between the treatments at 25ºC and 40ºC.

However, when MTG was added, a significant low Ew value (35.12%) was obtained performing the setting at 25ºC for 2 h. This value of Ew was in the range of those determined in commercial products of turkey breast (23-36%) (Martelo-Vidal, Mesas, & Vazquez, 2012) . However, it was very higher than those determined by other authors in gilthead sea bream restructured, using MTG and normal and low-salt contents, with values from 4.3% to 4.5% for restructured with regular NaCl contents and 7% to 10% for low-salt restructured (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) . These differences can be due to variations in the analytical method (1500 *g* vs 1000 *g*) that make the results cannot be comparable.

Water activity (Aw) ranged from 0.977 to 0.986 without MTG and from 0.978 to 0.981 with MTG (Table 1). Low values of Aw are desirable because they improve the preservation of the products, decreasing microbial growth and slowing enzymatic and chemical reactions. Without MTG, the lowest value of Aw (0.977 ± 0.008) was obtained at 7ºC for 12 h. In the restructured products with MTG and under the same setting conditions, the value increased slightly (0.978 ± 0.002). Moreover, at 25ºC for 2 h without MTG the value of Aw was 0.979 ± 0.001 and with MTG 0.981 ± 0.005. Only using 40ºC for 15 min, the Aw value decreased from 0.986 ± 0.008 to 0.979 ± 0.004 when MTG was used. However, no significant differences were found between the treatments. These Aw values were according with those determined for restructured white tuna products (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) and for restructured gilthead sea bream (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) . The value of Aw was lower (0.962) in restructured tuna obtained with 450 U/kg of MTG and the setting for 18 h. The values of Aw in restructured gilthead sea bream varied from 0.983 to 0.985 using MTG and 10 g/kg of salt and from 0.973 to 0.978 for samples containing 20 g/kg of NaCl. The decrease in Aw can be due to the salt effect that reduces Aw (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) .

Dry matter (DM) results varied in the range 15.27–16.85 % for products without MTG (Table 1). In the restructured hake with MTG the DM range was 15.27–18.10%. For both case, the highest value was obtained in the setting at 25 ºC for 2 h. Small differences between restructured hake products with and without MTG were obtained. A significant high value (18.10%) was obtained when MTG was used at 25ºC for 2 h. However, these values were considerably lower than those determined in restructured white tuna (*Thunnus alalunga*) where the DM values were in the range 23.74–25.72 % (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

3.1.2. Colour determinations

Table 2 shows the results of colour L*, a* and b* parameters. Lightness (L*) values varied in range from 79.16 to 81.21 in restructured hake without MTG and from 77.15 to 79.62 in the products with MTG. The lowest L* value was obtained at 7ºC for 12 h with MTG (77.15). This value was significant different that the obtained without MTG at 7ºC for 12 h.

Values of a* varied from -1.51 to -2.83 in restructured hake without MTG and from -1.94 to -2.64 in restructured hake with MTG. Significant differences were found at 25ºC for 2 h due to the effect of MTG. No significant differences were found in the treatment when the setting was performed at 7ºC or 40ºC.

Moreover, b* values varied from 3.23 to 5.01 in restructured hake without MTG and from 3.07 to 4.74 in restructured hake with MTG. Using 7ºC or 40ºC no significant difference was observed due to the use of MTG. However, using 25ºC, a significant difference in b* values was observed due to MTG, reducing the value from 5.01 to 3.07.

Overall, although some significant differences were observed, results indicate that temperature and MTG have a slight effect on colour parameters.

The lightness obtained in these restructured products was whiter than those obtained in other fish products such as restructured white tuna, gilthead sea bream or mackerel products. For example, L* for white tuna using similar setting conditions varied from 66.04 to 69.82 (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) , for gilthead sea bream was from 68.7 to 72.6 (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) , for mackerel products was from 45.87 to 76.55 (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013) and for restructured sole was from 40.14 to 53.70 (Uresti, Lopez-Arias, Gonzalez-Cabriales, Ramirez, & Vazquez, 2003) . Values of L* from restructured hake were according with those determined in other fish products with white mince such as Alaska Pollack with values of L* in range of 82.60 - 86.30 (Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014) or Mexican flounder with values from 65.65 to 82.45 (Tellez-Luis, Ramirez, & Vazquez, 2004) .

Values of redness (a*) and yellowness (b*) were lower than values determined in other restructured fish. In the aforementioned studies for restructured white tuna, the a* value varied from 3.32 to 4.42 and the b* value from 11.02 to 12.82 (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

Furthermore, values of a* in mackerel varied in a higher range than hake, from -0.09 to 6.77 and b* from 7.91 to 15.96 (Aubourg, Torres, Saraiva, Guerra-Rodríguez, & Vázquez, 2013) . Nevertheless, the redness determined in restructured hake was comparable with values obtained in gilthead sea bream, Mexican flounder or Alaska Pollack with values from -0.9 to -2.0 for Gilthead sea bream (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) , -0.22 to -1.47 for Mexican flounder (Tellez-Luis, Ramirez, & Vazquez, 2004) and 0.3 to -2.8 for Alaska Pollack (Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014) . The b* value determined in restructured hake was lower than that of other similar products. Values of 11.1 to 14.7 were measured in Gilthead sea bream (Andres-Bello, Garcia-Segovia, Ramirez, & Martinez-Monzo, 2011) , 9.49 to 10.88 in Mexican flounder and 5.4 to 9.5 in Alaska Pollack (Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014; Tellez-Luis, Ramirez, & Vazquez, 2004) . This could suggest a lower tendency to rancidity of hake mince than other fish species.

3.1.3. Texture parameters

Results of texture profile analysis (hardness, adhesiveness, springiness, cohesiveness and chewiness) with and without MTG at the setting conditions studied are shown in Figure 1. Figure 1a shows changes on hardness. Values of hardness varied in the wide range 3755–10723 g. Adhesiveness varied in the range -40.96 to -6.02 g·s (Figure 1b), springiness varied in the range 0.550–0.889 (Figure 1c) and cohesiveness varied from 0.262 to 0.452 (Figure 1d). The range of chewiness (Figure 1e) was from 542 to 4045 g.

It was observed that some textural parameters in restructured hake were improved by using MTG. In hardness, significant differences were observed due to the effect of MTG in the products obtained with the setting at 25ºC for 2 h and 40ºC for 15 min. The differences are bigger at 40ºC than 25ºC. The low hardness at 25ºC without MTG can be related with the effect of endogenous proteases with an optimal temperature close 45ºC (Ramirez, Rodriguez-Sosa, Morales, & Vazquez, 2003).

Adhesiveness showed a great deviation in some treatments. This can be due to the effect of variables that are not under control in the samples. For adhesiveness, a significant effect of MTG at 7ºC or 25ºC was not observed meanwhile the effect at 40ºC was significant. For springiness, a significant effect of the MTG was observed for all the setting conditions studied, with the highest value obtained when setting at 25ºC for 2 h. Cohesiveness showed a significant effect of the MTG only at 40ºC for 15 min.

Chewiness is a variable derived from the previous cited. It measures globally the texture of the product. At 7ºC for 12 h, the value of chewiness was low and the effect of MTG was not significant. However, the effect of MTG was significant at 25º for 2 h and at 40ºC for 15 min. The highest value of chewiness was obtained at 40ºC, but it was not significantly different than the value obtained at 25ºC. Therefore, setting conditions at 25ºC for 2 h showed the best textural properties in the restructured hake.

These textural parameters of restructured hake compare very well with those of other fish products. In restructured white tuna applying similar setting conditions, values of hardness and adhesiveness varied in higher ranges (from 11485 to 20981 g and from -67.29 to -2.15 g·s, respectively). Values of springiness and cohesiveness of restructured hake were similar to those obtained in restructured white tuna (0.443–0.793 for springiness and 0.285–0.416 for cohesiveness). Values of chewiness for restructured hake were lower than those determined for restructured white tuna (1480–6930 g) (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) .

Moreover, the texture of restructured hake was very different to that of restructured silver carp products. The hardness value was 2400 g for restructured silver carp obtained with 25 g/kg of salt and without MTG (Wang et al., 2013), a value lower than that obtained for restructured hake. However, springiness and cohesiveness values for silver carp were higher than those values obtained in restructured hake (0.980 and 0.850, respectively). Chewiness in silver carp products (2350 g) was into the range of values determined for the restructured hake of this study.

Furthermore, restructured pangasius performed with different concentrations of salt, MTG, sodium caseinate and egg white using different setting process (cold and hot process), provided hardness values similar to those obtained for restructured hake (3410 - 12550 g using hot process of setting). However, springiness, cohesiveness and chewiness values were higher than values determined in restructured hake. Springiness values were 0.840–0.950 (cold setting process) and 0.830–1.160 (hot setting temperatures), cohesiveness values were 0.580–0.660 (in cold setting process) and 0.570–0.660 (in hot temperatures of setting treatment) and chewiness values were 360–1290 g using cold setting temperatures and 2490–6900 g using hot setting temperatures (Kunnath, Lekshmi, Chouksey, Kannuchamy, & Gudipati, 2015) .

3.2. Optimization of setting process

Considering the results of first part of this study, setting treatment at 25 ºC for 2 h was selected as the best treatment for obtain restructured European hake products. The average daily dietary salt intake is more than double the recommended level (Zandstra, Lion, & Newson, 2016) . Therefore in a second study, levels of NaCl were reduced to 10 g/kg to obtain reduced-salt products that can be consumed by people with blood pressure disorders. Different levels of MTG were evaluated (0, 150, 300, 600, 900 U/kg) and their effect on texture parameters was modelled and optimized.

3.2.1. Mechanical properties

The experimental dependence of MTG concentration at the setting conditions of 25ºC for 2 h using 10 g/kg of NaCl on texture parameters (hardness, adhesiveness, springiness, cohesiveness and chewiness) is shown in Figure 2. Hardness increased with MTG concentration. The lowest value of hardness (7004 g) was obtained using 0 U/kg of MTG. Using 900 U/kg, the hardness reached up to a maximum value of 16204 g (Figure 2a). The adhesiveness values decreased using MTG compared with the control without MTG (-88.92 g·s) (Figure 2b). The value of springiness (Figure 2c) without MTG was 0.556. Using MTG the springiness was increased with the MTG concentration up to 0.844.

The behaviour of the cohesiveness values was similar, increasing with the MTG concentration (Figure 2d). Values of chewiness (Figure 2e) showed also an increase with MTG concentration in the restructured hake.

Springiness, cohesiveness and chewiness values of restructured hake were comparable with those values of restructured white tuna which showed values in the range 0.632–0.802 for springiness, 0.352–0.416 for cohesiveness and 4167–7308 g for chewiness. The maximum hardness value obtained for restructured white tuna was 23978 g and the highest value of adhesiveness was -12.12 g.s (Martelo-Vidal, Fernández-No, Guerra-Rodríguez, & Vázquez, 2016) . Texture properties of restructured white tuna were affected by MTG concentration like restructured hake.

No models to predict the effect of MTG on texture parameters were found in the literature. Therefore, a model was proposed and fitted to describe the effect of the MTG concentration on the mechanical properties of the restructured hake. In this study, we have proposed to adapt a model used for the kinetic modelling of hydrolysis reactions (Rodriguez-Chong, Ramirez, Garrote, & Vazquez, 2004) , changing the time as independent variable for the MTG concentration. This novel empirical approach allows, solving the differential equations, to predict the textural properties using the following model (3):

$$Y = \frac{k_1 \cdot Y_m}{k_2 - k_1}\left(e^{-k_1 TG} - e^{-k_2 TG}\right) + Y_0 \cdot e^{-k_2 TG} \quad (3)$$

Where Y is the dependent variable (hardness, adhesiveness, springiness, cohesiveness or chewiness), Y_0 is the initial value of Y, Y_m is the maximum value of Y, TG is transglutaminase concentration (U/kg), k_1 is the rate of the positive effect of MTG and k_2 is the rate of negative effect of proteases and other negative factors.

The model of Eq. (3) has been fitted to model all the textural variables. Table 3 shows the fitting results of the models and statistical parameters to evaluate the models (determination coefficient r^2 and F-test probability).

The statistical value of F-test probability showed that the models were accurate in describing the experimental data. Values of r^2 and F-test probability showed a good agreement between experimental and predicted data for hardness (r^2 0.9613 and F-test 0.9677), adhesiveness (r^2 0.9954 and F-test 0.9298), springiness (r^2 0.9694 and F-test 0.9895) and chewiness (r^2 0.9611 and F-test 0.9640). Cohesiveness model showed the worst fitting with r^2 0.5028 and F-test 0.5194.

The values of k_2 obtained suggest that the negative effect of proteases and other negative factors are only important on the adhesiveness and springiness properties. The limitations of the models obtained are related with the fish species. A new model should be obtained for each species of interest. The equations proposed in our work can be adjusted for any fish species. Therefore the models obtained can be used to predict the MTG concentration needed to obtain restructured hake with a desirable texture.

For comparative purposes and as example of application, texture parameters of commercial turkey breast products were measured (Table 4) since a similar product can be obtained with restructured hake.

The hardness values of turkey breast products varied from 9163 to 14377 g and adhesiveness values varied in the range from -35.69 to -10.66 g·s. These products showed springiness values from 0.800 to 0.864, cohesiveness from 0.216 to 0.292 and chewiness values from 1504 to 3519 g.

Therefore, as application example, the models obtained for restructured European hake were used to determine the optimal MTG concentration to obtain restructured European hake products with values of texture similar to those of commercial turkey breast products. The optimal MTG concentrations predicted for each mechanical property are showed in Table 5. Hence to obtain hardness of 10491 g, the model predicted that 377 U/kg of MTG must be used. For obtain value of -21.56 g·s for adhesiveness, it should be used 251 U of MTG per kg of hake mince. Moreover, when value of 0.835 for springiness is desirable, 589 U of MTG per kg should be used and to obtain values of 2242 g for chewiness, concentration of MTG must be decrease until 172 U/kg. In this example, it was selected the value of MTG to obtain hardness similar than that of turkey breast products. A new production of restructured hake was performed using different shapes for the moulds. The new products can be made slices for sandwiches, opening new markets for the European hake as a functional food for children, elders and people with blood pressure disorders. This example states the feasibility of modifying the texture of restructured European hake, using the fitted models to determine the optimal MTG concentration.

4. Conclusions

The results showed that is feasible to obtain restructured products from European hake using transglutaminase and reduced-salt concentration (10 g/kg). The setting phenomenon at 25ºC for 2 h is recommended in the restructuring process for restructured hake. This is the first time that a mathematical model is proposed for modelling the effect of transglutaminase on restructured fish. The developed mathematical models are very useful to produce hake restructured products with a desirable texture. Using these models, it was showed that it is feasible to obtain European hake restructured products with texture similar than that of commercial turkey breast obtaining a functional food.

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Table 1

Changes of expressible water (Ew), water activity (Aw), dry matter (DM) of restructured fish products with microbial transglutaminase (MTG) and controls without MTG. Means and standard deviation of 3 replicates.

Setting Conditions	**Ew (%)**	**Aw**	**DM (%)**
7ºC/12 h (No MTG)	47.34 ± 1.43^{b}	0.977 ± 0.008^{a}	16.58 ± 0.073^{b}
25ºC/2 h (No MTG)	51.64 ± 1.24^{c}	0.979 ± 0.001^{a}	16.85 ± 0.177^{b}
40ºC/15 min (No MTG)	49.59 ± 3.48^{c}	0.986 ± 0.008^{a}	15.27 ± 0.493^{a}
7ºC/12 h (MTG)	46.99 ± 0.61^{b}	0.978 ± 0.002^{a}	15.27 ± 0.568^{a}
25ºC/2 h (MTG)	35.12 ± 3.82^{a}	0.981 ± 0.005^{a}	18.10 ± 0.076^{c}
40ºC/15 min (MTG)	51.11 ± 1.92^{c}	0.979 ± 0.004^{a}	16.46 ± 0.601^{b}

Note: Different letters for the same parameter indicate a significant difference ($p < 0.05$).

Table 2

Changes of colour parameters (L*, a*, b*) of restructured fish products with microbial transglutaminase (MTG) and controls without MTG. Means and standard deviation of 3 replicates.

Setting Conditions	**L***	**a***	**b***
7ºC/12 h (No MTG)	80.54 ± 0.871^{b}	-1.75 ± 0.169^{ab}	4.71 ± 0.883^{b}
25ºC/2 h (No MTG)	81.21 ± 1.350^{b}	-1.51 ± 0.127^{a}	5.01 ± 0.694^{b}
40ºC/15 min (No MTG)	79.16 ± 2.527^{a}	-2.83 ± 0.494^{c}	3.23 ± 1.139^{ab}
7ºC/12 h (MTG)	77.15 ± 1.048^{a}	-1.94 ± 0.174^{b}	4.28 ± 0.973^{b}
25ºC/2 h (MTG)	78.70 ± 0.631^{ab}	-2.64 ± 0.157^{c}	3.07 ± 0.631^{a}
40ºC/15 min (MTG)	79.62 ± 0.512^{ab}	-2.44 ± 0.107^{c}	4.74 ± 0.479^{b}

Note: Different letters for the same parameter indicate a significant difference ($p < 0.05$).

Table 3

Model and statistical parameters of restructured hake with microbial transglutaminase (MTG) and the setting at 25ºC for 2 h.

	Hardness	**Adhesiveness**	**Springiness**	**Chewiness**
k_1 (U^{-1})	0.000098	0.692727	0.001661	0.000106
k_2 (U^{-1})	0.000000	0.006104	0.000490	0.000000
Y_0	6077	-90	0.557	1230
Y_m	121557	-10	0.787	55714
r^2	0.13	0.54	0.97	0.96
F-prob	0.97	0.93	0.99	0.96

Table 4

Textural parameters measured in commercial products of turkey breast.

	Range	**Mean**	**Standard deviation**
Hardness (g)	9163-14377	10491	3420
Adhesiveness (g·s)	(-35.69) – (-10.66)	-21.57	12.82
Springiness	0.800-0.864	0.835	0.032
Cohesiveness	0.216-0.292	0.248	0.039
Chewiness (g)	1504-3519	2242	1110

Table 5

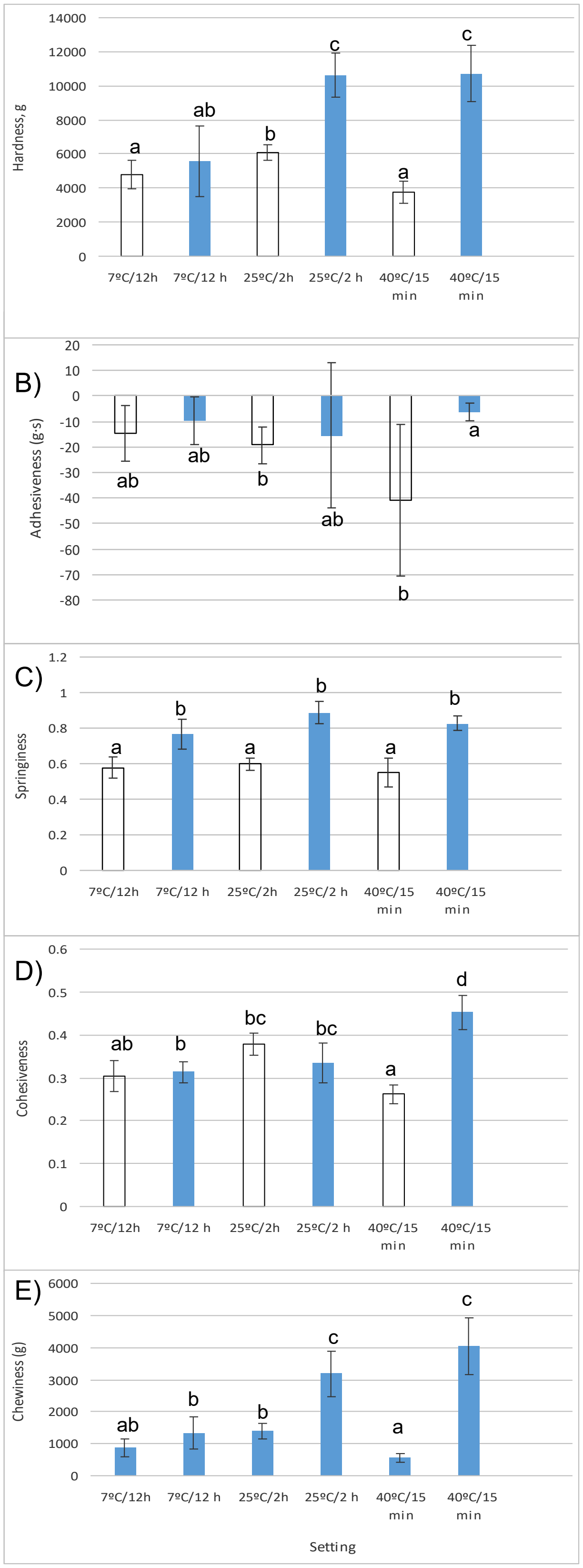
Values of transglutaminase predicted by the models in the optimal texture conditions.

	Optimal textural conditions	**Optimized transglutaminase, U/kg**
Hardness (g)	10491	377
Adhesiveness (g·s)	-21.57	251
Springiness	0.835	589
Chewiness (g)	2242	172

Figure Legends

Figure 1. Textural parameters of restructured European hake (*Merluccius merluccius*) performed using different setting conditions without MTG (white bars) and with MTG (blue bars). Lines show standard deviations. Different letters for the same parameter indicate a significant difference ($p < 0.05$).

Figure 2. Experimental and predicted dependence of transglutaminase concentration on the texture parameters of restructured European hake (*Merluccius merluccius*) using the setting phenomenon at 25ºC for 2 h. Bars show standard deviations. Dashed lines show 95% confidence intervals.



Hardness, g
14000
12000
10000
8000
6000
4000
2000
0
a
ab
b
c
a
c
7ºC/12h
7ºC/12 h
25ºC/2h
25ºC/2 h
40ºC/15 min
40ºC/15 min
B)
Adhesiveness (g·s)
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
ab
ab
b
ab
b
a
C)
Springiness
1.2
1
0.8
0.6
0.4
0.2
0
a
b
a
b
a
b
7ºC/12h
7ºC/12 h
25ºC/2h
25ºC/2 h
40ºC/15 min
40ºC/15 min
D)
Cohesiveness
0.6
0.5
0.4
0.3
0.2
0.1
0
ab
b
bc
bc
a
d
7ºC/12h
7ºC/12 h
25ºC/2h
25ºC/2 h
40ºC/15 min
40ºC/15 min
E)
Chewiness (g)
6000
5000
4000
3000
2000
1000
0
ab
b
b
c
a
c
7ºC/12h
7ºC/12 h
25ºC/2h
25ºC/2 h
40ºC/15 min
40ºC/15 min
Setting

Figure 1

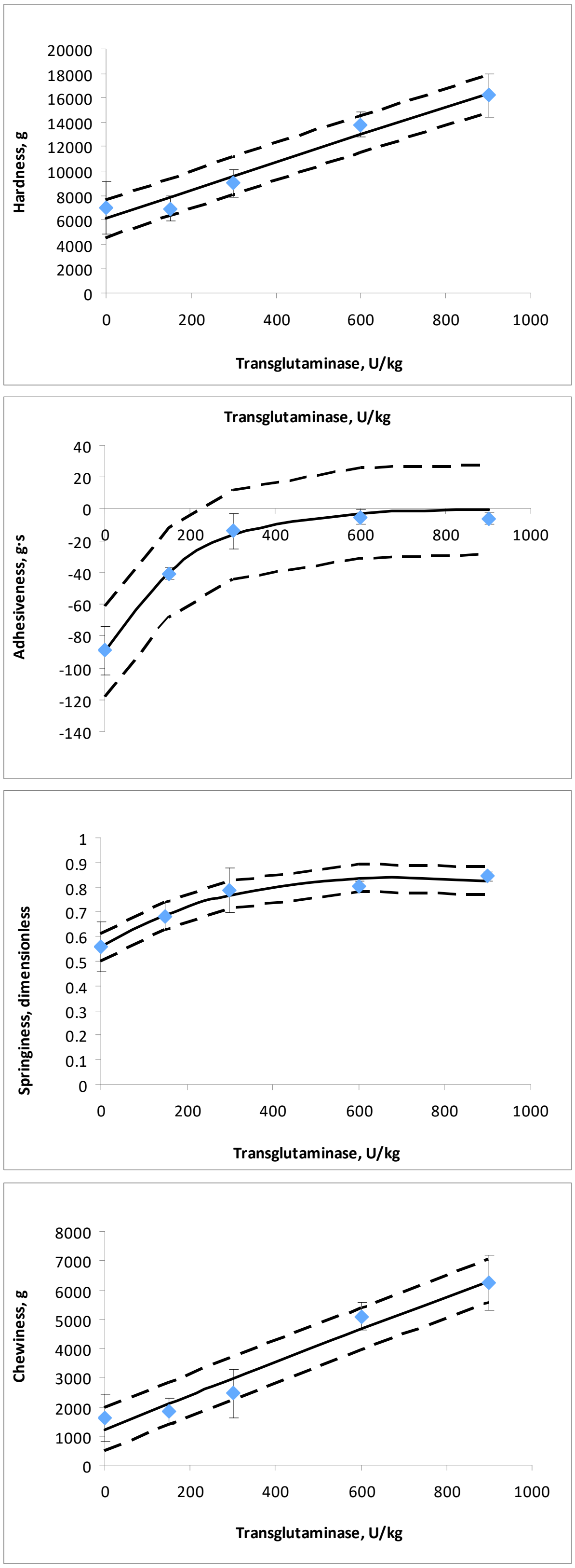

Hardness, g
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
200
400
600
800
1000
Transglutaminase, U/kg
Transglutaminase, U/kg
Adhesiveness, g·s
40
20
0
-20
-40
-60
-80
-100
-120
-140
0
200
400
600
800
1000
Springiness, dimensionless
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
800
1000
Transglutaminase, U/kg
Chewiness, g
8000
7000
6000
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
Transglutaminase, U/kg

Figure 2