LWT - Food Science and Technology 96 (2018) 296–306

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effects of high pressure processing on the physical properties of fish ham prepared with farmed meagre (*Argyrosomus regius*) with reduced use of microbial transglutaminase

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ARTICLE INFO

Keywords:
Seafood
Thermal processing
High pressure processing
Gel products
Texture
Restructured products

ABSTRACT

Marketing issues of small-sized meagre can be overcome with the development of fish hams. This study aimed to test high pressure processing (HPP) to promote gelation of meagre hams, as an alternative to the heat processing. It was also aimed to reduce microbial transglutaminase (MTGase) from the formulation of HPP hams.

Meagre hams were subjected to HPP varying different pressure parameters. The water holding capacity (WHC) and folding properties of hams were not affected by HPP, compared with heat processed hams. Whiteness was lower in HPP hams, and values increased with pressure level. The best results were obtained at 350 and 500 MPa at 30 °C, which also enhanced the textural properties of hams.

Meagre hams prepared with different contents of MTGase (0–5.0 g/kg) were subjected to HPP. This enzyme did not affect the WHC and the folding properties of hams within each condition tested. HPP hams can be prepared with lower levels of MTGase (2.5 g/kg), without compromising the textural properties of hams.

The results showed that it is possible to produce meagre hams with good textural properties and to reduce the MTGase content using HPP, validating the use of this technology as an alternative to the heat-induced gelation.

1. Introduction

Meagre (*Argyrosomus regius*) is a marine fish species common in the Mediterranean area where farming has gained economic importance, especially for specimens that attained at least 1 kg. According to Monfort (2010), portion-sized meagre are not considered suited for marketing as this size fish have a large head, large bones, little flesh, and are not very tasty, and thus few farmed fish are sold at a size below 1 kg. A solution to overcome the marketing problems of small-sized fish could be the development of innovative functional foods. The potential of small-sized meagre for the preparation of heat-induced gels with dietary fibers (Cardoso, Ribeiro, & Mendes, 2014, 2015) and fish sausages (Ribeiro et al., 2013) has been demonstrated. Within the variety of traditional meat products in marketplaces, cooked ham is particularly appropriated, on account of its broad public acceptance supported by suitable organoleptic and technological characteristics, for essaying meagre as a novel raw material. Apart from the fish hams developed by Cardoso, Mendes, and Nunes (2013) with hake or gilthead sea bream and incorporation of dietary fibers, which evidenced the potential of this new product as a healthier alternative to the traditional pork ham, the preparation of fish hams with meagre incorporating dietary fibers would be a novelty since no other similar product is known. Among dietary fibers, chicory root fiber, inner pea fiber, and carrageenan have been identified as promising ingredients for the formulation of restructured fish products (Nunes, Batista, Raymundo, Alves, & Sousa, 2003; Ortiz & Aguilera, 2004; Tolstoguzov, 1991). Chicory root fiber is a low caloric ingredient and can be used as a fat mimetic additive, as in fish sausages, ensuring smoothness, creaminess, and an oily mouth feel (Cardoso, Mendes, & Nunes, 2008; Ribeiro et al., 2013). Inner pea fiber can be used to increase gel strength and hardness, as in Cape hake gel

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https://doi.org/10.1016/j.lwt.2018.05.044
Received 23 February 2018; Received in revised form 17 May 2018; Accepted 18 May 2018
Available online 21 May 2018
0023-6438/

products (Cardoso et al., 2008). Carrageenan also has gelling ability and its incorporation can increase hardness, springiness, and water holding capacity (WHC), as in sea bass gel products (Cardoso, Mendes, Vaz-Pires, & Nunes, 2011). It is also known that the effects caused by the incorporation of fibers can differ between fish species (Cardoso et al., 2008, 2009).

Quality and texture of gelified products are affected by thermal treatment usually applied to promote gelation, due to protein degradation and lipid oxidation (Cardoso, Mendes, Saraiva, Vaz-Pires, & Nunes, 2010). Therefore, alternative gelation techniques to the traditional thermal treatments are being investigated. Among gelation promoting additives, microbial transglutaminase (MTGase) has been widely used in the food industry for cross-linking of proteins (Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002). Previous studies on cold gelation showed that MTGase was able to generate gels, prepared with trout and hake mince, with good properties (Moreno, Javier Borderías, & Baron, 2010).

The procedure for preparation of cooked fish ham is partially based on the process typically used in the production of pork hams in Southern Europe (Barat, Grau, Ibáñez, & Fito, 2005) and involves a cooking step. Nevertheless the thermal processing used to promote gelation induces a certain degree of protein degradation and, particularly, myosin, the myofibrillar protein responsible for functional and mechanical properties, is subjected to proteolytic degradation leading to the loss of textural quality (Ramírez, García-Carreño, Morales, & Sánchez, 2002), thus warranting the need for an alternative processing technology.

High pressure processing (HPP) is a technology of growing research interest for the processing and preservation of food. This technology is well known for its potential to better retain the food's nutritional and organoleptic characteristics, and also for having the ability to inactivate spoilage and pathogenic microorganisms, thus extending food shelf life (Patterson, Linton, & Doona, 2007). Moreover, HPP can be used to create new product textures since it induces modifications on food functional properties (Chapleau & de Lamballerie-Anton, 2003).

Research on the use of HPP to induce gelation of fish products, as an alternative to thermal treatments, has recently increased (Cando et al., 2015, 2016; Ma, Yi, Yu, Li, & Chen, 2015; Moreno et al., 2015). Studies indicate that HPP promotes an aggregation characterized by side-to-side interactions of proteins with a low degree of denaturation, instead of aggregation of proteins with large changes in molecular conformation as occurs in thermal gelation (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2006). Also, HPP has the potential to produce fish gels with better textural properties than heat-induced gels (Cardoso et al., 2010; Ma et al., 2015).

To the best of authors' knowledge, the development of fish hams prepared with meagre and dietary fibers has not been studied. Moreover, the influence of other pressure parameters, besides pressure level, in the gelification of fish products has not been explored. The positive results obtained regarding the gelifying quality of meagre (Cardoso, Ribeiro, & Mendes, 2015), in contrast to other fish species, justify the processing of such raw material with HPP to develop gel products. In this context, the aims of this work were: to determine suitable HPP conditions (pressure level, pressurization time, and temperature) in the preparation of meagre hams as an alternative to the thermal treatment for the gelation process; to evaluate if the effects of HPP on the texture of fish hams can overcome the need for MTGase addition.

Texture is the main drawback in the mimicking of the traditional pork ham, justified by significant differences at the connective tissue level, and also in view of the detrimental effect of the traditional heat process on the fish ham textural parameters. Thus, a particular emphasis was given to the study of the physical properties of the new meagre ham in order to deliver to consumers a food with the expected textural characteristics.

2. Material and methods

2.1. Experimental treatments

In order to optimize the preparation of meagre hams two experiments were conducted. As starting point, three meagre hams were subjected to each experimental condition (thermal processing or HPP). Different pressure levels (200, 350, and 500 MPa), pressurization times (10 and 20 min), and temperatures (10 and 30 °C) were combined in a total of 12 HPP conditions according to a full factorial design experiment and tested to evaluate the effect of HPP on physical properties of fish hams, identifying the best HPP conditions to obtain a fish ham comparable to heat processed fish ham.

In a second experiment, fish hams were prepared with different amounts (0, 2.5, and 5.0 g/kg) of microbial transglutaminase (MTGase) in order to test if HPP could be used as an alternative to MTGase in its effects on physical properties. Three meagre hams were subjected to each experimental condition (thermal processing or HPP). Fish hams were subjected to those HPP conditions that provided the best results in the first experiment (350MPa/10min/30 °C, 350MPa/20min/30 °C, and 500MPa/10min/30 °C).

2.1.1. Raw material and ingredients

Farmed meagre (*Argyrosomus regius*) was captured from IPMA Aquaculture Research Station at Olhão, Portugal (EPPO) and slaughtered by immersion on an ice and sea water (1 kg:1 L) bath. The fish were kept in ice and transported to the laboratory within 24 h. The individual fish weight was 376 ± 111 g. Fish was processed (headed, tailed, gutted, and filleted) at low temperature (below 10 °C) and, after filleting, meagre was minced in a 694 BAADER meat deboner (BAADER, Lübeck, Germany) equipped with a 3 mm diameter hole rotating cylinder.

The remaining ingredients used for the preparation of fish hams were all of food grade materials manufactured by different companies, as specified in Table 1. Sodium triphosphate was of analytical grade from Merck (Darmstadt, Germany). Sodium triphosphate was used as fat/protein stabilizer acting on protein crosslinking, controls pH and removes traces of iron and reduces sensitization to discoloration. Sodium chloride was used to increase the solubility of the proteins and crosslinking. Sodium nitrite was used for color fixation, imparting flavor and characteristic aromas, reduces lipid oxidation and inhibits growth of *Clostridium botulinum*. Casings and packages used were of food grade.

2.1.2. Preparation of meagre hams

The preparation of meagre hams was done according to the procedure described by Cardoso et al. (2013), as shown in the flow sheet of Fig. 1. The ingredients amount of fish hams with 5.0 g/kg MTGase are presented in Table 1. Water was added in the form of ice to adjust the moisture content to 80 g/100 g in the final fish ham. The mixture of meagre mince and the remaining ingredients was done in a refrigerated vacuum homogenizer (UM12, Stephan and Söhne, Hameln, Germany), being the whole mixing process performed under vacuum at refrigerated temperatures (below 7 °C). In a first step, meagre mince was mixed (1420 rpm, 1 min) with sodium triphosphate, salt (NaCl), and nitrified salt. Then, ice (70 g/100 g of the total amount of added water, taking into account the final moisture level of 80 g/100 g), MTGase, and sucrose were added and the same mixing conditions were applied. Finally, the carrageenan, chicory fibre (previously hydrated with 30 g/100 g of the total amount of added water taking into account the final moisture level of 80 g/100 g), pea protein, and the ham flavour were added and mixed (2800 rpm, 2 min).

The final mixture was packed in 25 mm diameter cellulose casings with a hydraulic filler (EB-12, Mainca Equipamientos Carnicos, S.L., Granollers, Spain), followed by manual twisting and knotting. Fish hams were vacuum packed. The setting of fish hams was performed by

Table 1
Ingredients information and amounts used for the formulation of meagre hams adjusted to 80 g/100 g final moisture.

Ingredients	Amount (g/kg)	Ingredients specifications	Suppliers
Meagre mince	546		
Water/ice	381		
Sodium triphosphate	7.0	CAS number: 7758-29-4; Food Additive Code: E451	Merck (Darmstadt, Germany)
Salt (NaCl)	12.5		VATEL (Alverca, Portugal)
Nitrified salt	2.5	Palatinata cure® is a curing salt composed of sodium nitrite (CAS number: 7632-00-0; E250), potassium nitrate (CAS number: 7757-79-1; E252), and sodium chloride (CAS number: 7647-14-5).	BK Giulini (Ladenburg, Switzerland)
Microbial transglutaminase (MTGase)	5.0	ACTIVA® GS is a transglutaminase preparation which also contains sodium chloride, gelatin, trisodium phosphate, maltodextrin, and safflower oil. It presents an activity of about 100 U/g. EC 2.3.2.13	Ajinomoto (Tokyo, Japan)
Sucrose	5.0		SIDUL (Santa Iria de Azóia, Portugal)
Carrageenan	20.0	VISCARIN ME 3525 is composed by carrageenan (carrageenan, Irish moss, Condrous extract) and potassium chloride (monopotassium chloride and potassium monochloride).	FMC Biopolymer (Philadelphia, USA)
Chicory fibre	10.0	Fibrulose® (oligofructose) are soluble fibers obtained from chicory root.	cosucra (Warcoing, Belgium)
Pea protein	10.0	PISANE® is a protein isolate extracted in a natural process from the yellow pea.	cosucra (Warcoing, Belgium)
Ham flavour	1.0	Givaudan Ham (Polish) flavour. KQ-340-053-9.	BK Giulini (Ladenburg, Switzerland)

immersion in water at 30 °C for 30 min. A total of 3 batches, each one with about 4 kg, were prepared for each experiment. Three fish hams with about 25 cm long and 300 g were used for each treatment condition. After the setting, fish hams were subjected to the traditional thermal treatment or to HPP.

2.1.2.1. Thermal processing. Thermal processing of meagre hams was performed in a water bath circulator at 82 °C for 1 h 50 min, to achieve an internal temperature of 72 °C, followed by a rapid cooling in an ice-water bath and then storage in refrigerated conditions (5 °C) overnight. The fish hams obtained after thermal processing were designated by control samples.

2.1.2.2. High pressure processing (HPP). The HPP treatments were carried out with industrial high pressure equipment (Hiperbaric 55, Hiperbaric, Burgos, Spain). This equipment has a pressure vessel of 20 cm inner diameter, 2 m length, and a maximum operation pressure of 600 MPa, connected to a refrigeration unit (RMA KH 40 LT, Ferroli, San Bonifacio, Italy) for temperature control of the inlet water used as the pressurizing fluid. Pressure build-up took place at a compression rate of approximately 200 MPa/min and adiabatic heating caused an increase in temperature of, approximately, 3 °C for each 100 MPa applied, while the decompression occurred almost instantaneously. Different pressure levels (200, 350, and 500 MPa), pressurization times (10 and 20 min), and temperatures (10 and 30 °C) were combined and applied in meagre hams. The pressurization time does not include the come-up time. A rapid cooling was also performed to fish hams subjected to high pressure processing, and all were kept in refrigerated conditions (5 °C) overnight.

2.2. Physical properties

2.2.1. Color

Color measurements of fish ham mince were assessed with a colorimeter (CR-410, Konica Minolta Camera, Co, Japan). The colorimeter was calibrated against a white standard plate (CIE $L^*a^*b^*$ system: $L^* = 97.79$; $a^* = -0.02$; $b^* = 1.84$) and the illuminant setting D65 was used. Lightness (L^*), red-green value (a^*), and yellow-blue value (b^*) were measured. For the assessment of color, whiteness and chroma were estimated accordingly to the following equations (Sahin & Sumnu, 2006).

$$Whiteness = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2}$$

Color was also expressed as the difference of color (ΔE^*) in the different parameters, accordingly to the equation (Sahin & Sumnu, 2006):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^*, and Δb^* correspond to the variation between the color parameter of fish ham treated with HPP and that of the control fish ham. For each replicate, ΔE^* was determined using the average L^*, a^*, and b^* values obtained for the control. All determinations were performed in triplicate.

2.2.2. Water holding capacity (WHC)

The water holding capacity (WHC) was measured with the method described by Sánchez-González et al. (2008). A cubic piece of fish ham (*ca.* 2 g; *ca.* 1.7 cm^3; W_s) with two folded filter papers (also weighted, W_i) in the bottom of a centrifuge tube were submitted to 3000 × *g* for 10 min at 20 °C (3K30, Sigma, Osterode, Germany). After centrifugation, the sample was removed and the filter papers were weighed (W_f). WHC was expressed as g of water in sample after centrifugation per 100 g of water initially present in sample:

$$WHC = \frac{W_s \times \frac{H}{100} - (W_f - W_i)}{W_s \times \frac{H}{100}} \times 100$$

where H is the moisture (g/100 g). All determinations were performed in triplicate.

2.2.3. Texture

Prior to texture measurements, meagre ham samples were taken out from casings and tempered to room temperature (22 °C).

2.2.3.1. Folding test. Folding test was done according to a previous work (Mendes, Gómez-Guillén, & Montero, 1997). Fish ham samples were cut into 2 mm slices with 25 mm diameter. The evaluation was performed manually in accordance with a five-point grade system as follows: Grade 5, no crack when folded into quadrants; Grade 4, no cracks when folded in half; Grade 3, crack develops gradually when folded in half; Grade 2, crack develops immediately when folded in half; and Grade 1, crumbles when pressed by finger. All determinations were performed in triplicate.

2.2.3.2. Instrumental texture measurements. Instrumental texture measurements were carried out using a model Instron 4301

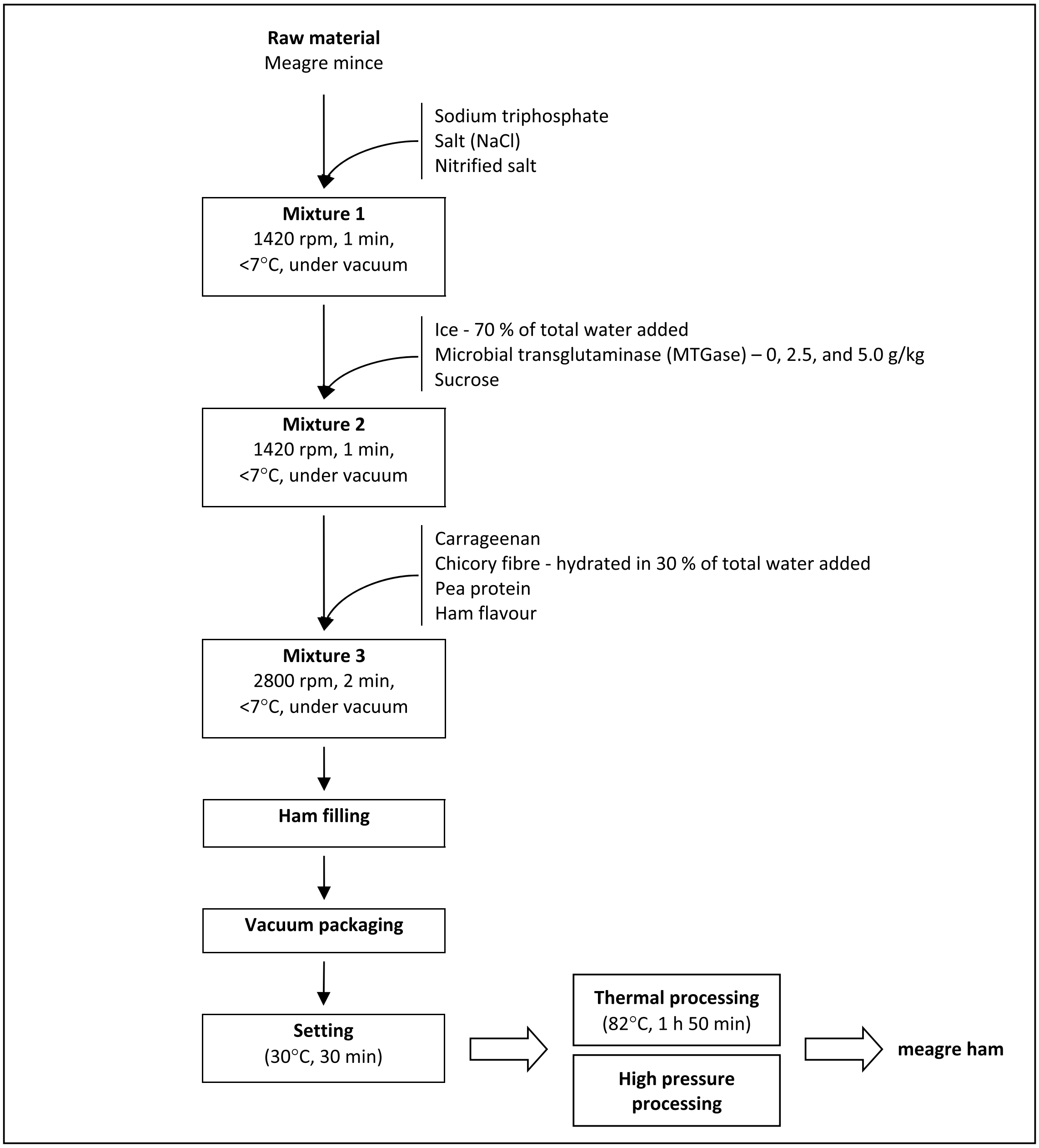

Fig. 1. Flowsheet for the preparation of meagre hams. High pressure processing was applied varying pressure level (200, 350, and 500 MPa), pressurization time (10 and 20 min), and temperature (10 and 30 °C).

texturometer (Instron Engineering Corp., Norwood, MA USA) following the procedures described by Cardoso et al. (2008). Fish ham samples were cut into pieces of 25 mm height and 25 mm diameter.

For the puncture test, each sample was penetrated to the breaking point using a metal probe with a 5 mm diameter spherical head. The cross speed head was 10 mm/min with a 1000 N load cell. The breaking force (N) and breaking deformation (mm) were measured, while the gel strength (N.mm) was determined by multiplying these two parameters. Rupture work (J), the area bellow the force-deformation curve until the maximum breaking force is reached, was determined and it also reflects the strength of the gel.

For the texture profile analysis, each sample was compressed twice, by means of a 60% compression level test, with a cylindrical plunger (50 mm diameter) attached to a 1000 N load cell at a deformation rate of 50 mm/min. The following parameters were determined: hardness (N), maximum height of first peak on first compression; cohesiveness, ratio of second-compression to first-compression positive areas; gumminess (N), product of hardness and cohesiveness; chewiness (N), product of springiness (ratio of the height of the sample on the second-compression to the original compression distance) and gumminess.

For the compression-relaxation test, the procedure was as for the texture profile analysis, except that the sample was compressed only once and held for 1 min. Elasticity was calculated as:

$$Elasticity\ (\%) = 100 - \frac{F_0 - F_1}{F_0} \times 100$$

where F_0 is the force registered at the onset of relaxation immediately after sample compression and F_1 is the force registered after 1 min of relaxation. All determinations were performed in quadriplicate.

2.3. Statistical analysis

For each experiment, the effects of treatments were tested with a one-way analysis of variance, followed by a multiple comparisons test

Table 2
Water holding capacity and puncture test results of meagre hams (5.0 g/kg MTGase) submitted to heat processing or high pressure processing.

Processing conditions				Water holding capacity (g/100 g)	Breaking force (N)	Breaking deformation (mm)	Gel strength (N.mm)	Rupture work (× 10^{-3} J)
Heat processing	82 °C, 1 h 50 min			97.3 ± 1.1^{ab}	2.00 ± 0.08^{bc}	8.72 ± 0.46^{e}	17.47 ± 1.49^{cde}	7.65 ± 0.47^{def}
High pressure processing	200 MPa	10 min	10 °C	97.2 ± 0.9^{ab}	0.95 ± 0.10^{e}	14.20 ± 0.82^{a}	13.55 ± 2.11^{f}	5.40 ± 0.91^{g}
			30 °C	97.6 ± 0.6^{a}	2.20 ± 0.14^{ab}	13.24 ± 0.58^{ab}	29.15 ± 2.82^{ab}	11.55 ± 1.16^{ab}
		20 min	10 °C	97.2 ± 0.3^{ab}	1.18 ± 0.05^{e}	12.08 ± 0.18^{bc}	14.19 ± 0.57^{def}	6.05 ± 0.57^{fg}
			30 °C	97.3 ± 0.6^{ab}	2.35 ± 0.17^{a}	13.43 ± 0.96^{ab}	31.63 ± 4.17^{a}	12.28 ± 1.52^{a}
	350 MPa	10 min	10 °C	97.1 ± 0.7^{ab}	1.70 ± 0.00^{cd}	12.05 ± 0.49^{bc}	20.49 ± 0.83^{def}	7.68 ± 0.33^{def}
			30 °C	96.4 ± 1.3^{ab}	2.28 ± 0.10^{ab}	11.03 ± 0.30^{cd}	25.08 ± 0.93^{bc}	9.90 ± 0.47^{bc}
		20 min	10 °C	96.0 ± 0.1^{ab}	1.75 ± 0.19^{cd}	10.68 ± 0.70^{cd}	18.79 ± 3.34^{def}	7.55 ± 0.96^{ef}
			30 °C	95.9 ± 1.1^{ab}	2.25 ± 0.13^{ab}	10.66 ± 0.29^{cd}	23.99 ± 1.79^{bc}	9.65 ± 0.65^{bcd}
	500 MPa	10 min	10 °C	96.8 ± 0.9^{ab}	1.58 ± 0.10^{d}	10.68 ± 0.27^{cd}	16.84 ± 1.36^{ef}	6.45 ± 0.44^{fg}
			30 °C	94.6 ± 2.3^{ab}	2.23 ± 0.15^{ab}	9.96 ± 1.35^{de}	22.28 ± 4.27^{bcd}	9.05 ± 1.31^{cde}
		20 min	10 °C	96.3 ± 0.5^{ab}	1.60 ± 0.08^{d}	10.61 ± 0.21^{cd}	16.98 ± 1.04^{ef}	6.70 ± 0.29^{fg}
			30 °C	94.3 ± 1.6^{b}	2.30 ± 0.14^{ab}	10.83 ± 0.46^{cd}	24.92 ± 1.98^{ab}	10.05 ± 0.65^{bc}

Values are presented as average ± standard deviation. Different superscript letters denote significant differences ($p < 0.05$) among fish hams subjected to different treatments. Three meagre hams were analyzed for each processing condition.

(Tukey's honestly significant difference, HSD) to identify the differences between treatments.

The results of WHC and texture analysis of fish hams were evaluated together by factor analysis, using the principal components method (PCA), to identify the HPP treatments comparable to control (first experiment) and to evaluate if MTGase could be reduced in the formulation of fish hams (second experiment). Only principal components with eigenvalues higher than one were considered in the analyses. The WHC and texture variables were also tested for correlation.

All statistical analyses were tested at a 0.05 level of probability with the software STATISTICA™ 6.1 (Statsoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Optimization of HPP conditions to develop a meagre ham

3.1.1. Water holding capacity

The WHC of control meagre hams was high (97.3 ± 1.1 g/100 g) and no significant differences were found when comparing with HPP hams (Table 2), indicating that HPP induced gelification. Still, pressure level affected the WHC of fish hams, as those subjected to 500MPa/20min/30 °C presented significant lower values (94.3 ± 1.6 g/100 g) than those treated at 200MPa/10min/30 °C (97.6 ± 0.6 g/100 g). Ma et al. (2015) suggested that the decrease of WHC in surimi gels at pressure levels above 400 MPa could be related with the rupture of the gels 3D network structure because the excessive high pressure may destroy some proteins cross-linking. The fracture of the gel network structure, would then result in more free water being transferred from the internal to the external gel network structure (Ma et al., 2015). Furthermore, it was also reported that HPP surimi gels subjected to 650 MPa revealed a more compact structure retaining less water, than gels subjected to 400 MPa that revealed a fibrous structure (Tabilo-Munizaga & Barbosa-Cánovas, 2005). Since HPP resulted in WHC values comparable to control fish hams, it seems feasible to replace the thermal treatment with HPP to produce meagre hams. Previous studies on heat-induced meagre gel products prepared with different dietary fibers reported lower WHC values (*ca.* 60–85 g/100 g) (Cardoso & Mendes, 2015; Cardoso et al., 2013, 2015).

The effect of HPP on the WHC of fish gels, in comparison with heat-induced gels, seems also to be dependent not only on the pressure intensity, but furthermore to vary according to the fish species and gels formulation/preparation. Cape hake protein gels had lower WHC (46–56 g/100 g in heat-induced gels), and HPP (100–300 MPa, 5–15 min, 1–5 cycles, 30 °C) caused an increase in the WHC to 55–70 g/100 g (Cardoso et al., 2010). In golden threadfin bream gels, HPP (100–600 MPa, 15 min, 25 °C) also affected the WHC, except at 600 MPa, in comparison with heat-induced gels (Ma et al., 2015). In contrast, similar results were obtained for Alaska pollock and Pacific whiting surimi gels, as these products presented high WHC values (over 90 g/100 g), though little affected by HPP (400–650 MPa, 10 min, 22 °C) (Tabilo-Munizaga & Barbosa-Cánovas, 2005). Also, water binding capacity of Alaska Pollock surimi gels was not affected by HPP in gels incorporated with MTGase (Cando et al., 2016).

3.1.2. Texture

Regarding the texture evaluation of meagre hams, no cracks were observed when folded into quadrants, presenting the maximum point grade in the folding test (5.0 ± 0.0), independently of the treatment applied. Comparable folding test values were reported for Cape hake protein gels subjected to pressure levels up to 300 MPa (5–15 min, 1–5 cycles, 30 °C), although heat processed ones had lower values (Cardoso et al., 2010).

The results obtained after subjecting fish hams to puncture test are presented in Table 2. Fish hams treated at 200 MPa presented the highest breaking deformation values. In contrast, products treated at 350 and 500 MPa were closer to control fish hams, especially in terms of breaking deformation and gel strength. HPP at 30 °C resulted in significantly higher values of breaking force, gel strength, and rupture work, compared with HPP at 10 °C for all pressure levels. In fish gels prepared with flying fish surimi, HPP treatments (40–200 MPa, 10 min, −15 °C) decreased breaking force, in comparison with heat processed gels (Moreno et al., 2015). Cardoso et al. (2010) reported that in Cape hake protein gels, HPP (100–300 MPa, 5–15 min, 1–5 cycles, 30 °C) did not improve the gel strength, in comparison with that of heat processed gels. On the other hand, in golden threadfin bream surimi gels, HPP increased rupture work at pressure levels of 300–600 MPa (15 min, 25 °C), in comparison with heat-induced gels (Ma et al., 2015). In particular, rupture work was affected by pressure level, increasing with the increase of pressure level up to 400 MPa and then declining for higher pressure levels (Ma et al., 2015). HPP (200 MPa, 60 min, 4 °C) increased the gel strength of gels prepared with round tilapia (Hwang, Lai, & Hsu, 2007).

In what concerns the TPA and compression-relaxation tests (Table 3), the HPP fish hams had higher cohesiveness than control samples. In terms of hardness, gumminess, and chewiness fish hams treated at 350 and 500 MPa at 30 °C were, in general, comparable to heat processed fish hams. Temperature also affected the hardness, gumminess, and chewiness, being only significant in the mildest treatments. In general, better elasticity was observed in fish hams treated at 350 and 500 MPa. HPP meagre hams presented higher hardness, gumminess, and chewiness than HPP Cape hake gels (100–300 MPa, 5–15 min, 1–5 cycles, 30 °C), while similar cohesiveness

Table 3
Compression and compression-relaxation test results of meagre hams (5.0 g/kg MTGase) submitted to heat processing or high pressure processing.

Processing conditions				Hardness (N)	Cohesiveness	Gumminess (N)	Elasticity (%)	Chewiness (N)
Heat processing	82 °C, 1 h 50 min			64.23 ± 10.75^{a}	0.50 ± 0.01^{c}	34.95 ± 1.26^{ab}	65.19 ± 0.74^{c}	29.55 ± 0.87^{abc}
High pressure processing	200 MPa	10 min	10 °C	19.28 ± 2.14^{e}	0.68 ± 0.03^{ab}	13.05 ± 1.08^{d}	48.53 ± 1.02^{g}	11.06 ± 0.89^{f}
			30 °C	34.35 ± 2.01^{cd}	0.70 ± 0.02^{ab}	23.96 ± 0.92^{c}	58.12 ± 0.19^{e}	20.31 ± 0.92^{de}
		20 min	10 °C	23.55 ± 2.76^{de}	0.69 ± 0.02^{ab}	16.25 ± 1.47^{d}	53.36 ± 2.80^{f}	13.56 ± 1.08^{ef}
			30 °C	40.30 ± 0.17^{bc}	0.69 ± 0.03^{ab}	28.36 ± 1.16^{bc}	62.11 ± 0.56^{d}	24.16 ± 1.12^{bcd}
	350 MPa	10 min	10 °C	34.88 ± 4.49^{cd}	0.71 ± 0.01^{a}	24.69 ± 3.22^{c}	69.79 ± 0.54^{b}	21.57 ± 2.75^{d}
			30 °C	49.83 ± 3.27^{ab}	0.68 ± 0.01^{ab}	34.02 ± 2.05^{ab}	73.45 ± 0.26^{a}	30.03 ± 1.86^{abc}
		20 min	10 °C	44.40 ± 4.99^{bc}	0.70 ± 0.03^{ab}	30.94 ± 3.54^{abc}	70.03 ± 0.16^{b}	26.80 ± 2.98^{abcd}
			30 °C	53.35 ± 4.57^{ab}	0.71 ± 0.03^{a}	37.56 ± 1.82^{a}	73.23 ± 0.75^{a}	32.96 ± 1.98^{a}
	500 MPa	10 min	10 °C	45.70 ± 8.80^{bc}	0.64 ± 0.03^{b}	29.19 ± 4.43^{bc}	69.83 ± 0.20^{b}	26.09 ± 4.31^{abcd}
			30 °C	51.83 ± 2.62^{ab}	0.67 ± 0.02^{ab}	34.50 ± 1.99^{ab}	71.28 ± 0.90^{ab}	31.05 ± 2.90^{ab}
		20 min	10 °C	39.95 ± 11.80^{bc}	0.67 ± 0.03^{ab}	26.35 ± 6.38^{c}	69.68 ± 0.62^{b}	23.02 ± 5.72^{cd}
			30 °C	46.07 ± 3.37^{bc}	0.68 ± 0.02^{ab}	31.61 ± 2.13^{abc}	73.04 ± 0.53^{a}	27.61 ± 2.17^{abcd}

Values are presented as average ± standard deviation. Different superscript letters denote significant differences ($p < 0.05$) among fish hams subjected to different treatments. Three meagre hams were analyzed for each processing condition.

was reported (Cardoso et al., 2010).

A previous study, linked the increase of hardness, gumminess, chewiness, and springiness to the denaturation of proteins and cellular damage in fillets processed at 150–300 MPa (15 min, room temperature) (Yagiz et al., 2009). In particular for minced muscle, HPP induced denaturation of muscle proteins, increasing hardness as pressure (275–310 MPa) and pressurization time (2–6 min) increased, which was associated with the formation of heavy molecular weight polypeptides and reduction of myosin heavy chain (among other proteins), most likely through disulfide bonding (Ramirez-Suarez & Morrissey, 2006). In surimi gels subjected to HPP at 400 MPa, the increase of rupture work corresponded to a denser, micromesh-like, more uniform, and hierarchical network structure, which possibly facilitated the orderly unfolding and cross-linking of proteins and the formation of an organized 3D network induced by covalent bond and non-covalent cross-linking (Ma et al., 2015). Similar mechanisms may be responsible for the changes in texture observed in the current study.

In what concerns the HPP effect on transglutaminase action, both endogenous and added, the results showed that HPP treatments performed at 10 °C restricted the activity of such enzymes, as expected, while processing at 30 °C possibly extended their action, at least during the processing, and resulted in hams with better textural properties. Moreover, it was demonstrated that transglutaminase remains active, although with lower values, in gels prepared from different fish species after HPP (300 MPa) (Gilleland, Lanier, & Hamann, 1997; Montero, López-Caballero, Pérez-Mateos, Solas, & Gómez-Guillén, 2005) and its activity is enhanced by the fact that the pressure facilitates the denaturation and breakdown of myosin, the principal substrate of transglutaminase (Gilleland et al., 1997).

HPP at 350 and 500 MPa at 30 °C can be used in alternative to the traditional heat-induced gelation, as several textural parameters of meagre hams were similar. Additionally, these HPP conditions improved elasticity and cohesiveness of fish hams.

In the industry, the costs involved in the production of a new product or in the use of a different technology are an important issue that might determine its applicability, and thus it is implicit a preference, in the case of HPP, for less expensive treatments with lower pressure levels and/or pressurization times. In this sense, for the second experiment fish hams were limited to the following conditions 350MPa/10min/30 °C, 350MPa/20min/30 °C, and 500MPa/10min/30 °C.

3.1.3. Color

To evaluate if HPP affects color, fish hams were prepared without adding any food colorants, being only added nitrified salt for color fixation. Whiteness presented the highest value in heat processed fish hams (whiteness = 67.0 ± 0.1), and this parameter decreased in fish hams treated with HPP (Fig. 2). Among fish hams treated with HPP, it was evident that the increase of the pressure level caused an enhancement of the whiteness of the fish hams. Pressurization time and temperature also influenced the whiteness, but not in a clear trend. In a previous work, the increase of whiteness was linked to the degree of protein denaturation, in particular to myoglobin denaturation, as happens with cooking process (Hwang et al., 2007). The use of MTGase also affects the microstructure of HPP gels, becoming more uniform, with smaller and evenly distributed pores, increasing opaqueness leading to smaller light absorption and, hence, to higher whiteness (Cardoso et al., 2010). Moreover, it is possible that HPP increases the oxidizing potential of the medium, and consequently myoglobin oxidation occurs, as well as other oxidative processes such as lipid and protein oxidation, affecting color (Oliveira, Neto, Santos, Ferreira, & Rosenthal, 2017).

The highest red-green value (a^*) was observed in heat processed fish hams ($a^* = 4.5 \pm 0.0$), and similarly to whiteness, HPP decreased the red components of the color and thus induced lower a^* values in the fish hams (Fig. 2). Among the pressure variables tested, the effect of pressure level in a^* values of fish hams was more pronounced, being denoted a decrease of this parameter with the increase of pressure level. The red-green value was also slightly changed with pressurization time and temperature, but such variations were dependent on the HPP condition.

The yellow-blue (b^*) and chroma values presented similar variations (Fig. 2). Meagre hams subjected to 200 MPa had closer yellow components and chroma values compared to heat processed fish hams. In contrast, HPP at 350 and 500 MPa decreased b^* and chroma values of fish hams. In general, the increase of temperature also increased these color parameters, while pressurization time had almost no effect.

In comparison with other fish gels, HPP affected the color differently, which might be due to differences in the muscle color of the species used, HPP conditions, as well as to the ingredients used for the preparation of gels. In particular, in the case of suwari gels prepared with flying fish surimi previously subjected to HPP (40–200 MPa, 10 min, −15 °C), L^* was not affected by pressure level (Moreno et al., 2015). In its turn, suwari gels prepared with Alaska Pollock surimi experienced an increase in lightness with the increase of pressure level (150–300 MPa, 10 min, 10 °C) (Cando et al., 2015). Cardoso et al. (2010) observed a decrease in whiteness, a^*, and b^* values with the HPP in gels prepared with Cape hake, while the pressure level (100–300 MPa, 15 min, 30 °C) caused an increase of whiteness and b^* values.

Moreover, fish hams prepared in this study, without colorants, presented comparable lightness and yellow-blue values as those hams prepared with pork ($L^* = 60.6$–69.0; $a^* = 8.7$–14.6; $b^* = 5.8$–9.7) (Casiraghi, Alamprese, & Pompei, 2007; Válková, Saláková, Buchtová, & Tremlová, 2007), while the red-green value was more similar to that of hams prepared with turkey ($L^* = 72.3$–74.6; $a^* = 4.3$–5.5;

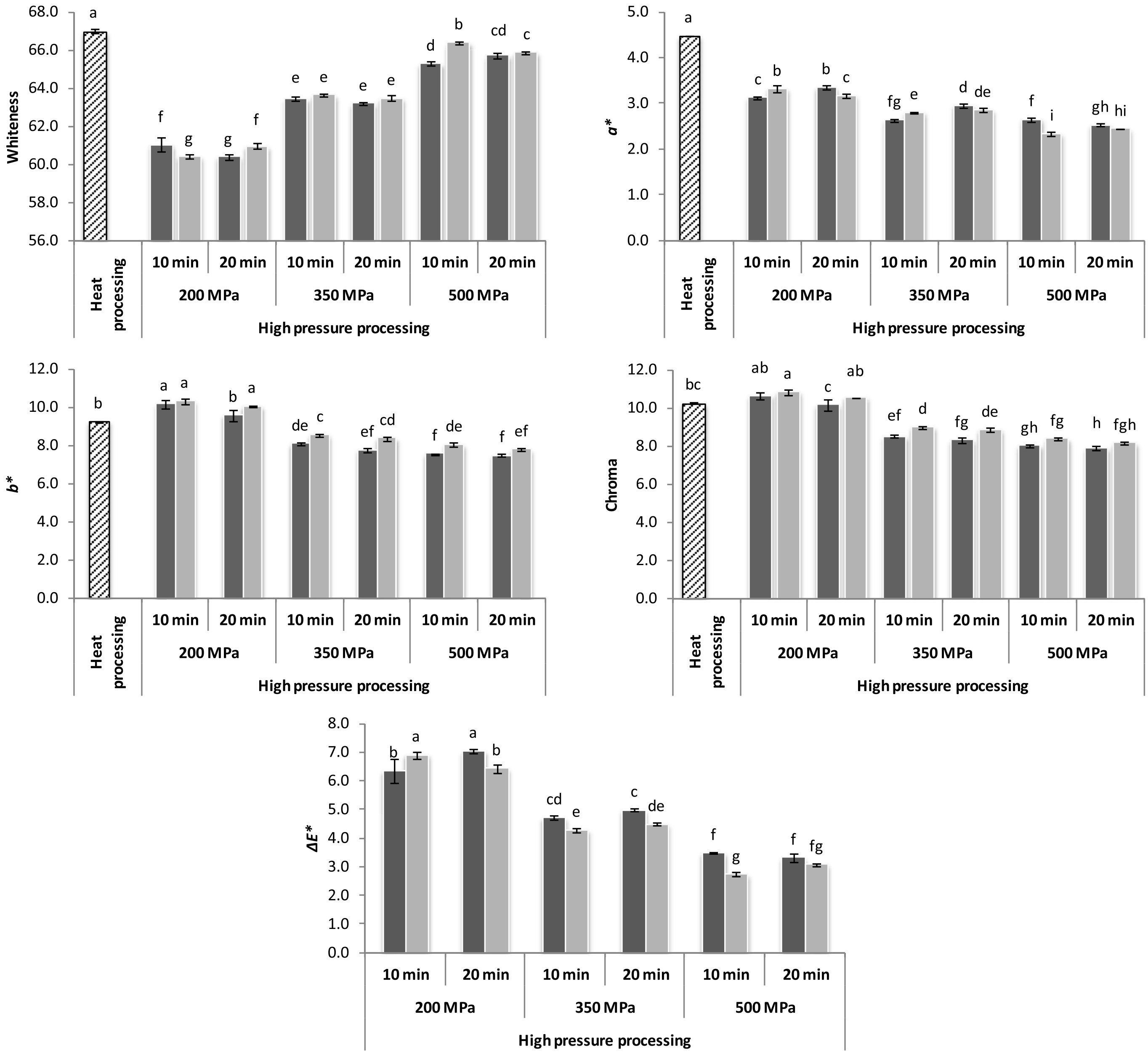

Fig. 2. Color parameters of meagre hams (5.0 g/kg MTGase) submitted to heat processing (control) or high pressure processing. High pressure processing was applied at 10 °C (■) and 30 °C (■). Values are presented as average ± standard deviation. The difference of color (ΔE^*) was determined by comparison with control fish ham. Different letters denote significant differences ($p < 0.05$) among fish hams subjected to different treatments. Three meagre hams were analyzed for each processing condition.

b^* = 5.5–8.1) (Iqbal, Sun, & Allen, 2013). Considering all color parameters, the HPP treatments at 500 MPa presented the lowest difference of color (ΔE^*) in relation to heat processed fish hams (Fig. 2). Although HPP affects the color of fish hams, such changes should not compromise the acceptance of the hams.

3.1.4. Principal components analysis

A multivariate analysis was performed to all data obtained from WHC and texture evaluation of meagre hams in order to detect groups of samples according to the treatments applied. Fig. 3 shows the first two PCs plotted against each other for the different fish hams. The first PC is strongly correlated with elasticity, hardness, gumminess, and chewiness (loadings were −0.85, −0.90, −0.97, and −0.97, respectively), while the second PC is correlated with rupture work, gel strength, and cohesiveness (loadings were −0.84, −0.88, and −0.62, respectively). Breaking deformation and breaking force are correlated with both PC1 (loadings were 0.69 and −0.83, respectively) and PC2 (loadings were −0.64 and −0.52, respectively), while the WHC is correlated with the third PC but it was not shown in the plot as it did not add information for the study.

The plot shows a separation of fish hams treated at 200 MPa from the remaining fish hams (350–500 MPa and control), based on differences in the breaking deformation. The temperature applied in the HPP treatments, 10 and 30 °C, also affected the distribution of fish ham samples in the plot, for all fish hams treated with HPP, based on differences in rupture work, gel strength, and breaking force. In general, samples treated at 350 MPa overlapped those treated at 500 MPa especially at 30 °C. The position of these samples in the plot is associated with high values of hardness, gumminess, elasticity, and chewiness. The findings suggested from the principal component analysis

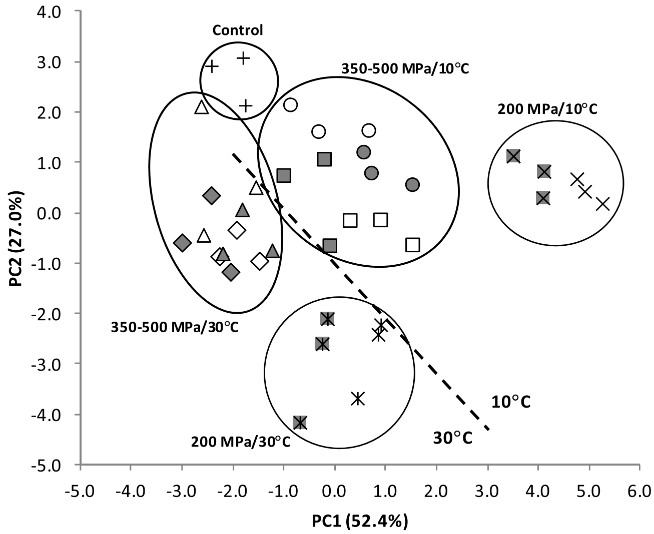

Fig. 3. Principal components (PC) analysis of WHC and texture evaluation on meagre hams (5.0 g/kg MTGase) submitted to heat processing (control) or high pressure processing. PC3 (not shown) explained 11.9% of the variation of the original variables. Three meagre hams were analyzed for each processing condition. + Control; × 200MPa/10min/10 °C; ⊠ 200MPa/20min/10 °C; ⋇ 200MPa/10min/30 °C; ⊠ 200MPa/20min/30 °C; □ 350MPa/10min/10 °C; ■ 350MPa/20min/10 °C; ◇ 350MPa/10min/30 °C; ◆ 350MPa/20min/30 °C; ○ 500MPa/10min/10 °C; ● 500MPa/20min/10 °C; △ 500MPa/10min/30 °C; ▲ 500MPa/20min/30 °C.

were confirmed by ANOVA.

Moreover, the proximity of the projection of the variables in the plot of principal components (results not shown) suggests a strong correlation between rupture work and gel strength, and also between the variables chewiness, hardness, elasticity, and gumminess, which was confirmed by correlation tests (r > 0.0.84). Strong correlations were also determined between breaking force and rupture work (r = 0.88), and breaking force and gel strength (r = 0.85).

3.2. Effect of different levels of MTGase in meagre hams treated with HPP

3.2.1. Water holding capacity

In the second experiment and in order to produce a food product with fewer additives, the effect of HPP on the reduction/elimination of MTGase from meagre hams formulation was studied. MTGase catalyzes the intra- and intermolecular transverse cross-linking of proteins by an acyl transfer reaction between γ-carboxamide groups of glutamine residues of proteins and primary amines, such as lysine, making it possible to create large polymeric protein structures (Moreno et al., 2010). WHC highest value was recorded for the control sample with 5.0 g/kg MTGase (98.0 ± 0.5 g/100 g), and some treatments presented significant lower values of WHC, being the lowest values observed for 500MPa/10min/30 °C with 2.5 g/kg MTGase (94.4 ± 1.0 g/100 g) (Table 4). Nevertheless, the content of MTGase (0, 2.5, and 5.0 g/kg) did not affect the WHC within each condition tested.

In order to obtain WHC comparable to control ham (5.0 g/kg MTGase) MTGase in hams processed at 350MPa/20min/30 °C should be reduced to 2.5 g/kg and in hams processed at 350MPa/10min/30 °C it can be eliminated. Still, the differences in the WHC of fish hams from other processing conditions are not very important, and thus any formulation is viable. In comparison, the effect of MTGase (5.0 g/kg) in WHC was not conclusive in a study with Cape hake protein gels subjected to HPP (Cardoso et al., 2010). It is known that MTGase induces the formation of cross-links, resulting in the formation of a porous protein matrix with a high capacity to soak up and retain water (Gaspar & de Góes-Favoni, 2015). However, this effect cannot be clearly noticed in meagre hams, possibly because HPP in its turn might have destroyed the cross-linking, particularly ionic bonds, between proteins and counteracted MTGase action, and also due to remarkable high WHC in meagre hams even without MTGase.

3.2.2. Texture

Changes in the MTGase content of ham's formulation showed no effect on the folding test results as no cracks were formed in samples when folded into quadrants, independently of the treatment (5.0 ± 0.0). Cardoso et al. (2010) reported also that MTGase did not affect the folding test results of hake protein gels, although such an outcome was not evaluated for each HPP condition tested, but was considered all together.

The results of puncture test (Table 4) show that meagre hams prepared without MTGase and subjected to HPP presented the lowest values of breaking force, gel strength, and rupture work. Considering the conditions tested, HPP *per se* cannot produce a meagre ham with the desired gel textural properties. In contrast, thermal processing without MTGase resulted in hams with better textural properties. This clearly shows that heating is more efficient in the proteins unfolding and formation of intermolecular bonds (mainly disulfide bonds), which produce the three-dimensional network structure of the gel (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). Moreover, the activity of endogenous transglutaminase might have also contributed to the characteristics of control fish hams, since it is also possible that HPP affected the activity of this enzyme. Previous studies showed that HPP (250–300 MPa, 15 min, 4 °C) does not affect the activity of endogenous transglutaminase in Alaska pollock surimi gels (Ashie & Lanier, 1999), but results showing that the enzyme is pressure sensitive were reported for other fish gels, like those prepared with horse mackerel (300 MPa, 15 min, 25 °C) (Montero et al., 2005).

MTGase cannot be eliminated from gels if HPP is used, but a reduction in MTGase is possible since HPP fish hams with 2.5 and 5.0 g/kg of MTGase had comparable breaking force, gel strength, and rupture work to control hams with 5.0 g/kg MTGase, independently of the HPP condition applied. In Cape hake protein gels subjected to HPP, the use MTGase (5.0 g/kg) also increased gel strength, particularly the breaking force (Cardoso et al., 2010).

In relation to the compression tests (Table 5), HPP fish hams without MTGase had significantly lower values of hardness and gumminess, while HPP fish hams with MTGase (2.5 and 5.0 g/kg) had comparable or higher hardness and gumminess values to heat processed fish hams with 5.0 g/kg MTGase. Cohesiveness, elasticity, and chewiness in HPP fish hams were affected by the content of MTGase, increasing with the increase of MTGase. The results obtained for these textural parameters also support the need for MTGase in the formulation of HPP fish hams, but a reduction to 2.5 g/kg does not compromise the textural properties of fish hams. The effect of MTGase (5.0 g/kg) in Cape hake protein gels subjected to HPP was analogous to meagre hams, improving hardness, gumminess, cohesiveness, and chewiness (Cardoso et al., 2010). This study also showed that MTGase promoted the formation of a network structure, that become more uniform, with smaller and evenly distributed pores with HPP (Cardoso et al., 2010).

In some HPP conditions, fish hams presented better textural properties than heat processed ones with 5.0 g/kg MTGase: all HPP fish hams with 5.0 g/kg MTGase presented higher cohesiveness; all HPP fish hams with 5.0 g/kg MTGase and fish hams with 2.5 g/kg MTGase subjected to 350 MPa (10–20 min) exhibited higher elasticity; fish hams with 5.0 g/kg MTGase subjected to 350 MPa (10–20 min) had higher gumminess and chewiness.

3.2.3. Principal components analysis

As for the first experiment, a multivariate analysis was performed with the data of WHC and texture evaluation in order to evaluate if MTGase could be reduced/eliminated from meagre hams formulation (Fig. 4 shows the first two PCs). In this analysis, the second PC is correlated with breaking deformation and WHC (loadings were 0.61 and

Table 4
Effect of microbial transglutaminase (MTGase) on the water holding capacity and puncture test results of meagre hams submitted to heat processing or high pressure processing.

Processing conditions		Water holding capacity (g/100 g)	Breaking force (N)	Breaking deformation (mm)	Gel strength (N.mm)	Rupture work ($\times 10^{-3}$ J)
Heat processing	0 g/kg MTGase	96.2 ± 0.5^{abcd}	2.05 ± 0.10^{b}	6.70 ± 0.20^{f}	13.74 ± 1.01^{d}	7.58 ± 0.50^{d}
82 °C, 1 h 50min	2.5 g/kg MTGase	96.8 ± 0.2^{ab}	2.73 ± 0.13^{a}	9.83 ± 0.07^{a}	25.93 ± 0.79^{a}	13.38 ± 0.25^{a}
	5.0 g/kg MTGase	98.0 ± 0.5^{a}	2.28 ± 0.10^{ab}	8.72 ± 0.11^{b}	19.83 ± 0.96^{bc}	10.65 ± 0.42^{abc}
High pressure processing	0 g/kg MTGase	96.4 ± 0.5^{abcd}	0.90 ± 0.12^{c}	8.62 ± 0.13^{bc}	7.51 ± 1.36^{e}	4.20 ± 0.50^{e}
350MPa/10min/30 °C	2.5 g/kg MTGase	96.4 ± 1.2^{abcd}	2.13 ± 0.51^{ab}	8.71 ± 0.28^{b}	18.43 ± 3.95^{bcd}	10.23 ± 2.05^{bcd}
	5.0 g/kg MTGase	95.5 ± 0.7^{bcd}	2.25 ± 0.38^{ab}	8.26 ± 0.32^{bcd}	18.65 ± 3.68^{bcd}	10.15 ± 2.20^{bcd}
High pressure processing	0 g/kg MTGase	95.7 ± 0.6^{bcd}	0.85 ± 0.06^{c}	7.62 ± 0.29^{de}	6.47 ± 0.39^{e}	3.45 ± 0.25^{e}
350MPa/20min/30 °C	2.5 g/kg MTGase	96.5 ± 0.6^{abc}	2.75 ± 0.06^{a}	8.32 ± 0.39^{bcd}	22.87 ± 1.01^{ab}	13.00 ± 0.45^{ab}
	5.0 g/kg MTGase	94.9 ± 0.2^{bcd}	2.05 ± 0.33^{b}	7.67 ± 0.04^{cde}	16.06 ± 2.73^{cd}	8.70 ± 1.14^{cd}
High pressure processing	0 g/kg MTGase	94.6 ± 1.0^{cd}	0.85 ± 0.17^{c}	7.08 ± 0.48^{ef}	6.06 ± 1.43^{e}	3.18 ± 0.73^{e}
500MPa/10min/30 °C	2.5 g/kg MTGase	94.4 ± 1.0^{d}	1.98 ± 0.38^{b}	7.60 ± 0.54^{e}	14.93 ± 2.38^{cd}	8.43 ± 1.31^{cd}
	5.0 g/kg MTGase	94.6 ± 0.6^{cd}	2.75 ± 0.30^{a}	8.14 ± 0.47^{bcd}	22.40 ± 2.93^{ab}	11.95 ± 1.95^{ab}

Values are presented as average ± standard deviation. Different superscript letters denote significant differences ($p < 0.05$) among fish hams subjected to different treatments. Three meagre hams were analyzed for each processing condition.

0.86, respectively), while the first one is correlated with the remaining variables (loadings varied between 0.69 and 0.95).

The PCA plot shows a clear separation of fish hams without MTGase processed with HPP from the remaining fish hams (control included), on the basis of PC1, due to differences in breaking force, gel strength, rupture work, hardness, gumminess, and chewiness. Meagre hams treated at 350 MPa (10 or 20 min) or heat processed, all prepared with 2.5 g/kg MTGase, are closer to control with 5.0 g/kg MTGase, associated with higher values of breaking deformation and WHC. On the other hand, HPP fish hams with 5.0 g/kg of MTGase are also grouped together and somehow apart linked to higher values of cohesiveness and elasticity and lower WHC. The findings suggested from the PCA were confirmed by ANOVA.

Correlation was also evaluated with the data of the second experiment, being the most relevant correlations between cohesiveness and elasticity (r = 0.83), among the variables chewiness, hardness, and gumminess (r > 0.89), and among breaking force, rupture work, and gel strength (r > 0.97).

Gel strength [peak force (N) × distance at the peak (cm)] or rupture work (energy consumed until peak force (joules)] have been used as an alternative for the characterization of the gel-forming characteristics (strength) of protein gels, mainly in restructured fish products. Regarding the more typical use of rupture work or gel strength to characterize gels, as point out in previous studies, for the case of meagre hams, the results show that these two parameters are strongly correlated (r = 0.882, $p < 0.01$; data from both experiments) thus indicating also that either of them can be used separately for the strength characterization of meagre hams.

4. Conclusions

Meagre hams with good textural properties were prepared using HPP. HPP did not change the WHC and the folding properties of meagre hams, in comparison with control hams (heat processed). HPP affected the color of meagre hams, and although it can be manipulated, those effects need to be taken into consideration when producing a ham with pre-defined characteristics. HPP at 350 and 500 MPa at 30 °C enhanced the textural properties (regarding puncture, compression, and compression-relaxation tests) of meagre hams.

The HPP conditions 350MPa/10min/30 °C, 350MPa/20min/30 °C, and 500MPa/10min/30 °C tested with different levels of MTGase (0, 2.5, and 5.0 g/kg) showed that this enzyme did not affect the WHC and the folding properties of meagre hams within each tested conditions. The reduction of MTGase to 2.5 g/kg in meagre hams subjected to HPP does not compromise the textural properties, but it cannot be eliminated from the formulation. MTGase had an effect as a gelation promoting additive, even when used at a reduced concentration in the fish ham, with an optimum temperature of activity at 30 °C (during setting and HPP), along with the other parameters applied concomitant to the high pressure treatment.

Table 5
Effect of microbial transglutaminase (MTGase) on compression and compression-relaxation test results of meagre hams submitted to heat processing or high pressure processing.

Processing conditions		Hardness (N)	Cohesiveness	Gumminess (N)	Elasticity (%)	Chewiness (N)
Heat processing	0 g/kg MTGase	52.83 ± 3.51^{a}	0.48 ± 0.02^{f}	25.48 ± 0.57^{cd}	64.28 ± 0.14^{e}	21.40 ± 0.86^{de}
82 °C, 1 h 50min	2.5 g/kg MTGase	54.18 ± 1.28^{a}	0.54 ± 0.01^{def}	29.52 ± 0.92^{abc}	66.88 ± 0.35^{d}	24.76 ± 1.10^{bcde}
	5.0 g/kg MTGase	41.85 ± 2.01^{bc}	0.60 ± 0.01^{cde}	25.19 ± 0.99^{cd}	69.02 ± 0.97^{c}	21.43 ± 1.06^{de}
High pressure processing	0 g/kg MTGase	29.50 ± 3.31^{de}	0.54 ± 0.06^{def}	15.71 ± 1.62^{e}	60.33 ± 0.48^{f}	13.28 ± 1.52^{fg}
350MPa/10min/30 °C	2.5 g/kg MTGase	40.50 ± 4.83^{bc}	0.62 ± 0.07^{bcd}	25.37 ± 5.18^{cd}	70.55 ± 0.55^{b}	22.07 ± 5.16^{cde}
	5.0 g/kg MTGase	49.60 ± 4.05^{ab}	0.72 ± 0.02^{a}	35.48 ± 2.31^{a}	76.49 ± 0.38^{a}	32.09 ± 2.21^{a}
High pressure processing	0 g/kg MTGase	19.80 ± 0.94^{ef}	0.51 ± 0.01^{f}	10.14 ± 0.51^{e}	58.62 ± 0.47^{g}	8.20 ± 0.44^{g}
350MPa/20min/30 °C	2.5 g/kg MTGase	50.15 ± 4.80^{ab}	0.66 ± 0.02^{abc}	33.14 ± 2.95^{a}	70.60 ± 0.40^{b}	29.09 ± 2.94^{ab}
	5.0 g/kg MTGase	45.78 ± 4.26^{ab}	0.69 ± 0.02^{abc}	31.45 ± 3.27^{ab}	76.13 ± 0.44^{a}	28.33 ± 3.35^{abc}
High pressure processing	0 g/kg MTGase	18.98 ± 1.25^{f}	0.52 ± 0.03^{ef}	9.93 ± 0.42^{e}	58.22 ± 0.71^{g}	8.05 ± 0.35^{g}
500MPa/10min/30 °C	2.5 g/kg MTGase	33.93 ± 4.19^{cd}	0.68 ± 0.05^{abc}	23.00 ± 1.36^{d}	69.35 ± 0.89^{bc}	19.48 ± 1.59^{ef}
	5.0 g/kg MTGase	41.28 ± 8.16^{bc}	0.70 ± 0.05^{ab}	28.93 ± 5.67^{abc}	75.64 ± 0.13^{a}	26.49 ± 5.83^{abcd}

Values are presented as average ± standard deviation. Different superscript letters denote significant differences ($p < 0.05$) among fish hams subjected to different treatments. Three meagre hams were analyzed for each processing condition.

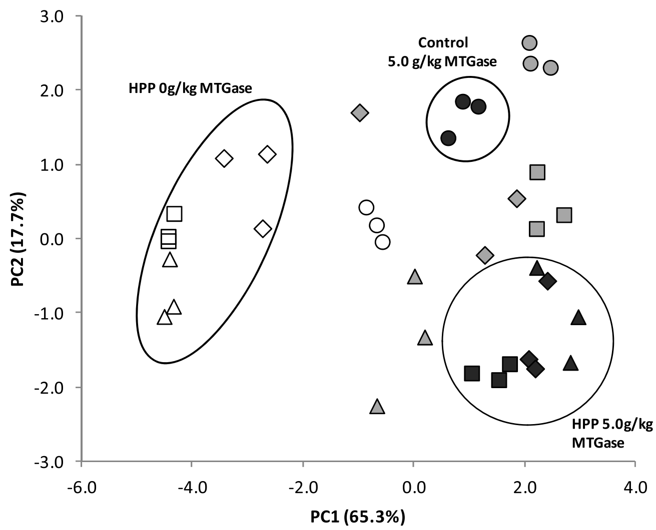

Fig. 4. Principal components (PC) analysis of WHC and texture evaluation on meagre fish hams prepared with different levels of microbial transglutaminase (MTGase) and submitted to heat processing (control) or high pressure processing (HPP). Three meagre hams were analyzed for each processing condition. ○ Control 0 g/kg MTGase; ● (grey) Control 2.5 g/kg MTGase; ● Control 5.0 g/kg MTGase; ◇ 350MPa/10min/30 °C 0 g/kg MTGase; ◆ (grey) 350MPa/10min/30 °C 2.5 g/kg MTGase; ◆ 350MPa/10min/30 °C 5.0 g/kg MTGase; □ 350MPa/20min/30 °C 0 g/kg MTGase; ■ (grey) 350MPa/20min/30 °C 2.5 g/kg MTGase; ■ 350MPa/20min/30 °C 5.0 g/kg MTGase; △ 500MPa/10min/30 °C 0 g/kg MTGase; ▲ (grey) 500MPa/10min/30 °C 2.5 g/kg MTGase; ▲ 500MPa/10min/30 °C 5.0 g/kg MTGase.

The results obtained confirmed the potential of HPP to produce meagre hams with good textural properties and to reduce the MTGase content in the formulations, thus validating the use of this technology as an alternative to the traditional heat-induced gelation. Regarding the potential of HPP to better retain food's nutritional characteristics, studies need to be carried out to evaluate changes in the nutritional quality (*e.g.* amino acids and fatty acids) and safety of meagre hams prepared with HPP, along with evaluating consumer preferences by sensory analysis.

Acknowledgments

This study was supported by the National Project AQUACOR “Pilot Project on the farming of meagre (*Argyrosomus regius*) in different production systems” (ref: PROMAR 31-03-05FEP-003) and by FCT/MEC through the financial support to the QOPNA research unit (ref: FCT UID/QUI/00062/2013), through national funds and were applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. B. Teixeira acknowledges the Portuguese Foundation for Science and Technology (FCT), the European Social Fund (FSE) and the Ministry of Education and Science for supporting a grant (ref. SFRH/BPD/92929/2013).

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