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Chemical reagents as probes: Application to fish protein gels and detection of a cysteine TGase in hake

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ABSTRACT

A new concept was applied to fish products. Chemical reagents targeting specific chemical bonds were incorporated in to gel products for assessing the importance of non-covalent (sodium dodecylsulphate, 1.0%, w/w), disulphide (dithiothreitol, 0.2%, w/w) or transglutaminase-catalyzed (N-ethylmaleimide, 0.2%, w/w) bonding. These reagents acting as chemical probes were applied to the study of sea bass and hake protein gels and the effect of MTGase (0.5%, w/w).

These reagents were valuable for reaching conclusions. The action of an endogenous cysteine TGase in hake products was detected. It was found that frozen storage and protein denaturation are fundamental not only for explaining differences between raw materials, but also seem to favour a different action mode of MTGase in each raw material. Moreover, this study may help to improve processed products, for instance, the positive interaction between MTGase and the disruption of disulphide bonds in hake gels may find a useful application through incorporation of cysteine + MTGase.

1. Introduction

Development of restructured fish products and application of additives have been used to attain a better gelation and textural quality (Sánchez, Pérez-Mateos, & Borderías, 2004).

For this purpose, a deeper knowledge of the underlying chemical phenomena associated with the effect of ingredients or the type of raw material on gelation must be achieved in order to improve food properties. This demands new concepts, such that applied to whey protein gels (Havea, Carr, & Creamer, 2004; Havea, Watkinson, & Kuhn-Sherlock, 2009), chemical reagents as protein bonding probes. According to this concept, reagents targeting specific chemical bonds were incorporated in to products for the sole purpose of assessing the relative importance of each type of protein bonding. This enables to link the properties of gel products to each type of chemical bond and to reach a deeper knowledge of the functionality of muscle proteins (Asghar, Samejima, & Yasui, 1985). The acquired knowledge of the protein bonding phenomena may point to new ways of improving the quality of fish products.

In this work, the non-covalent, disulphide and transglutaminase-catalyzed bonding in heat-induced fish gels were addressed as these are important in these products (Lee & Lanier, 1995; Niwa, 1992). Choice of the reagents targeting these bonds was based on studies on whey protein gels (Havea et al., 2009). Sodium dodecylsulphate (preventing non-covalent bonding), dithiothreitol (disulphide) and N-ethylmaleimide (transglutaminase-catalyzed) were chosen. Concentrations of these reagents in the final product, took into account protein contents in the products and amino acid composition (cysteine) of fish proteins (Havea et al., 2009; Liu & Hsieh, 2008).

MTGase is an additive used to improve texture, being used in the food industry for promoting protein cross-linking (Téllez-Luis, Uresti, Ramírez, & Vázquez, 2002). It has effectively improved textural properties under certain conditions (Cardoso, Mendes, & Nunes, 2007; Ramírez, Rodríguez-Sosa, Morales, & Vázquez, 2000). A level of 0.5% (w/w) MTGase enhanced the texture of minced mackerel (*Scomber scombrus* and *Scomber japonicus*) products (Cardoso, Mendes, Vaz-Pires, & Nunes, 2009). Moreover, a study on MTGase addition to giant squid (*Dosidicus gigas*) surimi showed that 0.5% (w/w) MTGase enabled good texture (Moreno, Cardoso, Solas, & Borderías, 2009).

Great variability in the properties of gel products made from different raw materials and of the MTGase action on them has been reported. Some works found an effect of MTGase mainly upon deformability (Moreno et al., 2009), others reported an increase in shear stress, for instance, in Pacific whiting (Lee & Park, 1998) or carp

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(Tsukamasa, Miyake, Ando, & Makinodan, 2000) gels. The choice of fresh sea bass and frozen hake in this work was prompted by the observed differences in the quality of gel products from these raw materials (Cardoso et al., 2007; Cardoso, Mendes, Vaz-Pires, & Nunes, 2010). With the same processing conditions, fresh fish products had a better texture. Thus, a deeper understanding of the gelation process in such raw material is important.

The aim of this work was to apply a new concept to fish protein gels: chemical reagents as probes in order to study the protein reactions that underlie the effects of MTGase addition and of the type of raw material on the properties of heat-induced gels.

2. Materials and methods

2.1. Materials

Fresh farmed sea bass (*Dicentrarchus labrax*) from Greece were bought in a supermarket and headed, tailed, gutted and filleted at < 10 °C. Fish weight varied between 300 and 400 g. Freshness level assessment pointed to less than one week of chilled storage.

Frozen South African hake (*Merluccius capensis*) was bought already headed and gutted from a local frozen fish processor after two months storage. Each fish batch was kept frozen at −28 °C and processed within one week after its arrival at the laboratory.

Microbial transglutaminase TG-K (MTGase) ACTIVA® GS was supplied by Ajinomoto (Tokyo, Japan) and presented an activity of 100 $U.g^{-1}$.ACTIVA® GS also contained: sodium chloride, gelatin, trisodium phosphate, maltodextrin, and safflower oil.

Sodium dodecylsulphate (SDS), dithiothreitol (DTT), N-ethylmaleimide (NEM) and other chemicals were of analytical grade and from Merck (Darmstadt, Germany).

2.2. Gel production

About 10 kg of sea bass mince were obtained in a Baader 694 deboning machine (Baader, Lübeck, Germany) fitted with a drum with 3 mm ∅ holes. Hake, about 10 kg of frozen fish, were thawed overnight in a refrigerator. Afterwards, skin and bones were manually removed. Resulting fish flesh was minced once in a model 84,145 meat grinder (Hobart, Troy, OH, USA), equipped with 2 cm blades and a screen with 6 mm ∅ holes. The quantities of mince, chilled water, salt (2.5%, w/w), MTGase, SDS, DTT and NEM for 1.3–1.4 kg batches were weighed (Table 1) to ensure a similar protein:water proportion. A portion of chilled water was added to SDS/DTT/NEM and mixed with the reagent for 1 min. The sea bass or hake minces were mixed with the other ingredients/additives for 2 min at 1420 rpm and 2 min at 2800 rpm in a model UM12 homogeniser (Stephan & Söhne, Hameln, Germany). Mixing was performed under vacuum and at less than 7 °C. Procedure followed Cardoso et al. (2010).

Table 1
Sample coding and main ingredients (%, w/w) of the various minced fish products.

BATCH	Sea bass mince	Water	Salt	MTGase	SDS	DTT	NEM	Total
$SB0_{CTL}$	79.1	18.4	2.5	0.0	0.0	0.0	0.0	100.0
$SB0_{SDS}$	75.8	20.7	2.5	0.0	1.0	0.0	0.0	100.0
$SB0_{DTT}$	78.4	18.9	2.5	0.0	0.0	0.2	0.0	100.0
$SB0_{NEM}$	78.4	18.9	2.5	0.0	0.0	0.0	0.2	100.0
SBa_{CTL}	77.5	19.5	2.5	0.5	0.0	0.0	0.0	100.0
SBa_{SDS}	74.2	21.8	2.5	0.5	1.0	0.0	0.0	100.0
SBa_{DTT}	76.7	20.1	2.5	0.5	0.0	0.2	0.0	100.0
SBa_{NEM}	76.7	20.1	2.5	0.5	0.0	0.0	0.2	100.0
BATCH	Hake mince	Water	Salt	MTGase	SDS	DTT	NEM	Total
$H0_{CTL}$	96.8	0.7	2.5	0.0	0.0	0.0	0.0	100.0
$H0_{SDS}$	92.9	3.6	2.5	0.0	1.0	0.0	0.0	100.0
$H0_{DTT}$	96.1	1.2	2.5	0.0	0.0	0.2	0.0	100.0
$H0_{NEM}$	96.1	1.2	2.5	0.0	0.0	0.0	0.2	100.0
Ha_{CTL}	94.9	2.1	2.5	0.5	0.0	0.0	0.0	100.0
Ha_{SDS}	90.9	5.1	2.5	0.5	1.0	0.0	0.0	100.0
Ha_{DTT}	94.1	2.7	2.5	0.5	0.0	0.2	0.0	100.0
Ha_{NEM}	94.1	2.7	2.5	0.5	0.0	0.0	0.2	100.0

2.3. Proximate composition

Moisture and ash were determined by standard AOAC procedures (AOAC, 1984), while protein was determined in a model FP-528 LECO protein/nitrogen analyser (LECO, St. Joseph, USA) and fat determined by Bligh and Dyer's method (Bligh & Dyer, 1959).

2.4. Mechanical properties

Folding test, gel strength, elasticity and texture profile analysis (TPA), namely hardness, cohesiveness, gumminess, springiness, and chewiness, were done as in previous work (Cardoso, Mendes, Pedro, & Nunes, 2008). Gel strength, elasticity, and TPA were evaluated using an Instron model 4301 texturometer (Instron Corp., Canton, USA).

Force and distance at rupture were also done as in previous work (Cardoso et al., 2009).

2.5. Water holding capacity (WHC)

WHC was measured by a modification of a published method (Sánchez-González et al., 2008), as described in a previous work (Cardoso et al., 2010).

2.6. Protein solubility

To follow protein–protein chemical interactions, protein solubility in four different extracting solutions was determined (Table 2). Soluble protein was separated as described in a previous work (Cardoso et al., 2010). Protein quantitation in the supernatants was done through absorbance measurement at 280 nm (Piñeiro et al., 1999). Results are the average of three determinations, calculated as percent protein solubilised with respect to total protein in the sample and divided by the protein solubility in SDS + DTT + U, to give a relative protein solubility. For each sample, comparison to the solubility in SDS + DTT + U (100%) enabled assessment of the relative importance of the chemical interactions targeted by the missing component.

2.7. Electrophoresis

Changes in individual proteins were followed by SDS-PAGE in 15% Excel-Gel™ (Amersham, Uppsala, Sweden) according to previous work (Cardoso et al., 2009). For optical density measurements, silver stained gels were analysed in a model GS-800 densitometer (Bio-Rad, Hercules, USA) with software Quantity One® (Bio-Rad).

2.8. Dynamic rheological measurement (DRM)

Two sets of batters (sea bass and hake) were prepared, using the same ingredients' and reagents' proportions as the gel products (Table 1). Afterwards, they were heated within a serrated plate geometry of a model RS 75 controlled-stress rheometer (Haake, Karlsruhe, Germany) and small amplitude oscillatory shear measurements were performed. The gap between the two plates