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Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing

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ABSTRACT

The paper examines the effect of High Pressure Processing (HPP) (300 MPa), the incorporation of microbial transglutaminase (MTGase) and the addition of different additives such as lysine and cystine, as potential enhancers of low-salt (0.3%) surimi gel. Effects on myosin as the molecule responsible for gelation was monitored by Fourier transform infrared spectroscopy, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and dynamic rheometry measurements. The effects on physicochemical properties of surimi gels were determined by Folding and Puncture tests and water holding capacity.

Results indicated an increase in β-sheet when HPP was applied or additives added (cystine and lysine), especially when samples are treated with MTGase. Protein aggregation due to HPP and the additives resulted in lower myosin heavy chain (MHC) band density in the SDS–PAGE. Rheometry measurements indicated that MTGase activity was prompted by the incorporation of cystine and lysine in the absence of HPP. Also, HPP assisted gelation, resulting in improved mechanical properties of the gels. Samples containing additives, with or without HPP, exhibited the highest Folding test scores, indicating greater network flexibility. Lastly, water binding capacity was also enhanced by both additives and HPP.

Industrial relevance: The industrial relevance of the present work is focused on the appropriated gelation of myofibrillar proteins which is an essential step in the elaboration of surimi-based products. Sodium chloride has an important role in that fact inducing protein unfolding and solubilization. The reduction in NaCl content, following the NAOS strategy, required the application of different technologies to facilitate surimi adequate gelation. High-pressure processing has been commonly used as an innovative technology to prolong shelf life but it can be successfully used to induce proteins gelation. Due to that ability, the use of high pressure on surimi-based products result an interesting tool to facilitate surimi gelation. The use of Microbial transglutaminase (MTGase) alone or in combination with some aminoacids such as lysine and cystine can significantly improve surimi gelation added in a very small proportion.

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1. Introduction

There is currently a generalized concern to achieve healthy products in the light of international recommendations for the reduction of NaCl intake (NAOS Strategy, 2005; EFSA, 2005). Salt reduction in the surimi industry poses a major challenge since salt solubilizes myofibrillar proteins, a necessary prior step for protein gelation (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). When NaCl content is reduced, protein gelation needs to be assisted as proteins are not properly unfolded by salt solubilization, which reduces gel-forming ability (Lanier et al., 2014). The literature reports studies of different ways to overcome this requirement such as the addition of additives, and/or various technological treatments. One of the most commonly used has been microbial transglutaminase (MTGase) (Seki, Nozawa, & Ni, 1998), but some others such as sodium pyrophosphate (Matsukawa, Hirata, Kimura, & Arai, 1995, Chang & Regenstein, 1997), ascorbic acid (Nishimura et al., 1992; Chen, Chow, & Ochiai, 1999), cystine (Chen et al., 1999), and lysine (Dickinson, 1997; Liu, Kanoh, & Niwa, 1995, Cando, Herranz, Borderías, & Moreno, 2016a) have also been assayed.

Various studies have indicated that MTGase is very useful for making surimi gels, especially if protein functionality is reduced (Moreno, Carballo, & Borderias, 2009a; Moreno, Cardoso, Solas, & Borderías, 2009b; Cardoso, Mendes, Saraiva, Vaz-Pires, & Nunes, 2010), given its ability to form bonds between the γ-carboxyamide group of a peptide-bound glutaminyl residue and a variety of primary amines resulting in ε-(γ-glutamyl) lysine isopeptide bonds that can lead to intermolecular covalent cross-linking of the peptide chains (Motoki & Seguro, 1998). Moreover, recent research has reported that in surimi, a very low concentration (0.1%) of cystine and lysine can assist the gelation (Cando et al., 2016a).

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On the other hand, high pressure processing (HPP) can also enhance protein gelation even with a poorly-solubilized actomyosin complex. High pressure processed gels have also been found to exhibit greater elasticity and more stable protein networks due to the increase of physical interactions such as hydrogen and hydrophobic bonds and dispersive interactions prompting protein gelation (Sun & Holley, 2010, Cando, Moreno, Tovar, Herranz, & Borderias, 2014). Recent research on low-salt (0.3%) surimi gels indicates that the application of 300 MPa induces myofibrillar protein unfolding and can promote functional and physicochemical properties similar to those of gels made with regular NaCl content (Cando, Herranz, Borderías, & Moreno, 2015; Moreno et al., 2015). All this suggests that there are different types of additives that can enhance bond formation and protein networks by taking advantage of increased unfolding of protein molecules caused by HPP. In this connection the combination of MTGase and HPP treatment have been previously studied in heat-induced Alaska Pollock and flying fish surimi by different authors (Zhu, Lanier, Farkas, & Li, 2014, Herranz, Tovar, Borderias, & Moreno, 2013). These researchers indicated that in both high pressure processing enhanced the gel strength and deformability in cooked surimi gels. However, the effect of low salt content over myofibrillar proteins solubilization is a limiting factor that has not been studied yet.

The object of this work is to examine the modifications induced in myofibrillar proteins when microbial transglutaminase and different additives, such as lysine and cystine, are used to enhance protein gelation in combination with high pressure processing in order to achieve a low-salt surimi gel. According to the objective, the novelty of this work is based on the approach of study focussed on the modifications over myofibrillar proteins structure and the way that influenced the subsequent gelation with low salt content.

2. Material and methods

2.1. Raw materials

Grade KA Alaska Pollock surimi (*Theragra chalcogramma*), supplied by *Angulas Aguinaga* (Guipuzcoa, Spain) in 20-Kg frozen blocks was used to elaborate the gels. Sodium chloride (Panreac, Quimica, S.A.; Barcelona, Spain) was added to solubilize the surimi protein.

The ingredients tested as gelation enhancers were: microbial transglutaminase ACTIVA GS (99% maltodextrine and 1% enzyme with an activity of approx. 100 U/g of powder; Ajinomoto Co.; North America, Inc., USA.), cystine (Merck KGaA, Darmstadt, Germany) and lysine (Panreac, Quimica, S.A.; Barcelona, Spain).

2.2. Sample preparation

Alaska Pollock surimi was homogenized under vacuum and refrigeration (<12 °C) using a Stephan homogenizer at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with a fixed NaCl content (0.3%). Different surimi doughs were prepared with addition of 0.5% MTGase, 0.1% cystine (Cys), and 0.1% L-lysine (Lys) as shown in Table 1. The concentrations of these ingredients were chosen on the basis of previous research Cando et al., 2016a; Cando, Moreno, Borderías, & Skara, 2016b). Ingredients were added directly in powder form, and transglutaminase was kept on ice to prevent inactivation. In all cases gel moisture was adjusted to 76%. Each surimi dough was stuffed into a 35 mm Krehalon casing (Amcor group Flexibles Hispania S.L., Barcelona, Spain).

Three different lots of samples were prepared for each additive -MTGase (TG), MTGase and cystine (TG-Cys) and MTG and lysine (TG-Lys) and each group was divided into two lots, which were then separated and subjected to heat treatment (Q) or to setting (S). The Q and S lots were further subdivided and either pressurized (HPP) or not. The HPP lot was pressurized at 300 MPa/10 °C/10 min and then left to set at 5 °C/24 h. The pressure level was chosen again on the basis of previous work by Cando et al. (2014, 2015)). In fact there is evidence that the pressure level used is suitable for maintaining MTGase enzymatic activity (Lauber, Noack, Klostermeyer, & Henle, 2001). Also, Moreno et al. (2009a) reported absence of any residual transglutaminase activity in restructured fish products after 6 h at 5 °C.

For sample treatment two more batches were prepared. The first (Lot S) consisted of suwari gels, which were pressurized (HP-S) or unpressurized (S), followed by setting at 5 °C/24 h (Table 1). The second batch consisted of cooked samples (90 °C/30 min). These were samples cooked after setting at 5 °C/24 h (Lot Q), and samples which were processed by HPP followed by setting (5 °C/24 h) and then cooked (Lot HP-Q).

2.3. Proximate analysis

The ash, fat, crude protein and moisture content of Alaska Pollock surimi was determined (AOAC, 2000) in quadruplicate. Crude protein content was measured by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA).

2.4. Fourier transform infrared spectroscopy

Changes occurred over protein secondary structure of suwari gels with or without HPP processing (S and HP-S) were examined by

Table 1
Samples coding: composition and treatments.

	Code	Formulation			Treatment	
		NaCl (%)	MTGase (%)	Additive (0.1%)	Pressure	Thermal treatment
Suwari	C-S	0.3	–	–	0	5 °C/24 h
	C-HP-S		–	–	300	
	TG-S		0.5	–	0	
	TG-HP-S		0.5	–	300	
	TG-Lys-S		0.5	Lysine	0	
	TG-Lys-HP-S		0.5	Lysine	300	
	TG-Cys-S		0.5	Cystine	0	
	TG-Cys-HP-S		0.5	Cystine	300	
Definitive/kamaboko	C-Q		–	–	0	90 °C/30 min + 5 °C/24 h
	C-HP-Q		–	–	300	
	TG-Q		0.5	–	0	
	TG-HP-Q		0.5	–	300	
	TG-Lys-Q		0.5	Lysine	0	
	TG-Lys-HP-Q		0.5	Lysine	300	
	TG-Cys-Q		0.5	Cystine	0	
	TG-Cys-HP-Q		0.5	Cystine	300	

Samples coding: C—control; S—suwari; HP—high pressure; Q—heating process; TG—transglutaminase; Lys—lysine; Cys—cystine.

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