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Influence of amino acid addition during the storage life of high pressure processed low salt surimi gels

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ABSTRACT

Protein gelation is the main step in the preparation of surimi-based products. Salt addition is essential in thermal gelation in order to dissolve myofibrillar proteins before heating. The manufacture of reduced-NaCl surimi-based products poses a technological challenge to the surimi gelation in that the proteins are not previously unfolded. In this study reduced-NaCl surimi gels (0.3 g/100 g NaCl) were made by adding cystine (0.1 g/100 g) or lysine (0.1 g/100 g), with or without high pressure assistance (300 MPa) to determine the influence of those additives on surimi gel and on stability over up to 28 days of chilled storage. Results indicated that the physicochemical properties achieved in the reduced-NaCl surimi gels were similar to those of the gels with regular NaCl content (3 g/100 g). Gel properties remained stable throughout chilled storage, indicating a successful gelation process and a well stabilized protein network. Although gels were microbiologically safe ($<10^6$ CFU/g) during chilled storage, sensory analysis detected off-flavour after day 14, especially in cystine samples, rendering them unacceptable. In conclusion these gels exhibit good physicochemical and sensory properties as well as microbiological and sensory stability up to day 14 of chilled storage.

1. Introduction

Texture is the most important characteristic in the preparation of surimi-based products. The chief factor in achieving the desired texture is the gel-forming ability of fish myofibrillar proteins (Sun & Holley, 2011). To achieve proper gelation, 2–3 g/100 g sodium chloride is required to facilitate protein solubilization as a first step in the gelation process (Kim & Park, 2008). In order to manufacture healthier products in line with the NAOS strategy (Ballesteros Arribas, Dal-Re Saavedra, Pérez-Farinós, & Villar Villalba, 2007), seafood products need to contain as little added NaCl as possible. Thus, the search for different technologies and ingredients to produce reduced-sodium gels with good sensory and mechanical quality is currently a challenge. There are different strategies; one possible alternative is to substitute Na^+ with other cations such as K^+, Mg^{2+} or Ca^{2+} (Tahergorabi & Jaczynski, 2012), but undesirable flavours have been found to develop in some cases (Desmond, 2006). The addition of small amounts of different ingredients such as the amino acids cystine or lysine, has been reported to improve texture (Cando, Herranz, Borderias, & Moreno, 2016b). Each of these acts in a different way. On the one hand, Cystine is a weak oxidant that maximizes the formation of disulphide bonds (Chen, Chow, & Ochiai, 1999), and on the other hand lysine can contribute to the formation of covalent crosslinks between the ε-amino group and the γ-carboxyamide group of glutaminyl residues of adjacent proteins when acting as a substrate of transglutaminase (Dickinson, 1997; Ting, Ishizaki, & Tanaka, 1999). In this context, a previous study also reported that similar physicochemical properties to those of gels with regular NaCl content could be achieved in low-NaCl (0.3 g/100 g) surimi gels if cystine and/or lysine is added (Cando et al., 2016b).

High pressure processing (HPP) causes protein unfolding at around 100–150 MPa and has been successfully used in the preparation of surimi gels. This protein unfolding is very important to assure optimum gelation (Macfarlane & McKenzie, 1976). Moreover, the application of HPP induces conformational changes in myofibrillar proteins which may result in increased bond formation, making for a better protein network and thus improving water binding capacity and mechanical properties (Farkas & Mohácsi-Farkas, 1996). However, pressure above 500 MPa causes the opposite effect, i.e. bond disruption, which hinders gel formation resulting in a poorer gel (Cando, Moreno, Tovar, Herranz, &

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Borderias, 2014). Many different authors have reported that the optimum pressure to improve fish protein gelation is around 300 MPa (Angsupanich, Edde, & Ledward, 1999; Cando, Herranz, Borderías, & Moreno, 2015; Gilleland, Lanier, & Hamann, 1997). In this connection, it has been reported that the combination of cystine and lysine allows the formation of suitable surimi gels with physicochemical properties comparable to surimi gels with regular NaCl content (Cando, Moreno, Borderías, & Skåra, 2016c).

On the other hand the addition of these amino acids could facilitate microorganism growth during chilled storage (Atlas, 2010), and also irregular flavour. Therefore, in this kind of surimi gels it is important to consider the influence of amino acids during chilled storage since there have been many reports of changes in physicochemical properties during chilled storage (Cardoso, Mendes, Pedro, Vaz-Pires, & Nunes, 2010; Rahmanifarah, Shabanpour, & Shabani, 2015) (Pérez-Mateos, Boyd, & Lanier, 2004; Sell, Beamer, Jaczynski, & Matak, 2015). These changes occurring during chilled storage are also related to HPP, which normally induces greater hardness and chewiness on the gels (Kunnath, Panda, Jaganath, & Gudipati, 2015). There is no information in the scientific literature about the evolution during chilled storage of surimi gels made with cystine and lysine in combination with high pressure.

The aim of this work was to study the evolution in chilled storage of physicochemical and sensory properties of low-sodium surimi gels with added lysine and cystine which were processed under high pressure, bearing in mind the ability of those amino acids to generate a reducing medium that could facilitate microorganism growth.

2. Materials and methods

2.1. Raw material

Alaska pollock surimi (*Theragra chalcogramma*) grade KA was supplied by Angulas Aguinaga, S.A. (Guipuzcoa, Spain) in frozen blocks of 20 kg.

The gels were prepared with sodium chloride (Merck KGaA), L-lysine (CAS: 56-89-3, Sigma-Aldrich) and L-cystine (CAS: 56-87-1, Sigma-Aldrich) as additives.

2.2. Proximate analysis

Ash, fat and moisture content of Alaska pollock were determined following the methodology described by AOAC (2005). Crude protein was measured using a LECO FP-2000 nitrogen determinator (Leco Corporation, St. Joseph, MI, USA).

Sodium content was determined by atomic absorption spectroscopy using an Atomic Absorption spectrometer with continuous high resolution (HSC AAS technology) -model ContrAA 700- Analytik Jena AG, Jena, Germany equipped with a short arc xenon lamp (GLE, Berlin, Germany) and an air-acetylene flame. A calibration curve at different concentrations was prepared from an individual trade pattern of Na, concentration 1 g/L (Panreac Chemistry, S.A., Barcelona, Spain).

2.3. Sample preparation

Alaska Pollock surimi was chopped and homogenized in a Stephan homogenizer at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with two different NaCl concentrations to make regular-NaCl control gels (3 g/100 g) and reduced-NaCl gels (0.3 g/100 g). The process was performed in vacuum conditions and the temperature was controlled so as not to exceed 12 °C throughout the process. Compositions of the samples are reported in Table 1. The dough for each sample was stuffed into 35 mm Krehalon casings (Amcor group Flexibles Hispania S.L., Barcelona, Spain) and pressurized samples were processed at 300 MPa (Stansted Fluid Power CTD, FPG 7100:-2C. Stansted, UK) for 10 min at 10 °C. Both pressurized and non-pressurized samples were stored at 5 °C for 24 h then heated (90 °C/30 min) and stored at 4 °C. Physicochemical and sensory analyses were performed once a week for four consecutive weeks (day 1, 7, 14 and 28).

Table 1
Samples treatment and composition.

Sample	NaCl (g/100 g)	Additive (0.1 g/100 g)	High pressure (MPa)
Control	3	–	–
L	0.3	lysine	–
L-HP	0.3	lysine	300
C	0.3	cystine	–
C-HP	0.3	cystine	300

2.4. Microbiological analysis

To determine the microbiota growing during storage of surimi gels, 10 g of sample was weighed and transferred to sterile bags (Sterilin, Stone, Staffordshire, UK), combined with 90 ml of buffered 0.1 g/100 g peptone water (Cultimed, Madrid, Spain) and shaken vigorously for 1 min in a Stomacher blender (model Colworth 400, Seward, London, UK). Appropriate dilutions were prepared for the following microorganism determinations: i) total bacterial counts (TBC) on spread plates of Iron Agar (Microkit, Madrid, Spain) 1 g/100 g NaCl, incubated at 15 °C for 5 days; (ii) H_2S-producing organisms, as black colonies, on spread plates of Iron Agar incubated at 15 °C for 3 days; (iii) luminescent bacteria on spread plates of Iron Agar 1 g/100 g NaCl incubated at 15 °C for 5 days; (iv) total aerobic mesophiles on pour plates of Plate Count Agar, PCA (Cultimed) incubated at 30 °C for 72 h; (v) H_2S-producing clostridia on double-layered plates of Tryptose Sulphite Cycloserine Agar (TSCA, BioMèrieux, Marcy-l'Étoile, France) incubated at 37 °C for 24–48 h; (vi) Enterobacteriaceae on double-layered plates of Violet Red Bile Glucose Agar (VRBG, Cultimed) incubated at 30 °C for 48 h; and (vii) lactic acid bacteria on double-layered plates of Man Ragosa and Sharp agar (MRSA, Merk, Kenilworth, N.J., U.S.A) incubated at 30 °C for 72 h. All analyses were performed in duplicate at 1, 7, 14 and 28 days. The day of surimi gel preparation was taken as day 1. Microbiological counts are expressed as the log of colony-forming units per gram (log CFU/g) of sample.

2.5. Colour. lightness (L*)

The colour parameters L*, a*, and b* of the surface of surimi gels were determined using a portable colorimeter (Minolta, CR-400 Konica-Minolta, Japan) (D65/2°), which was standardized using

Table 2
Sodium content of surimi gels.

Sample	Na^+ (g/100 g)	Reduction of Na^+ (%) *
Control	1.31 ± 0.01	–
C	0.25 ± 0.001	81
C-HP	0.26 ± 0.01	80
L	0.25 ± 0.003	81
L-HP	0.25 ± 0.005	81

Data are given as mean ± SD (n = 3). *Percentage of reduction of sodium in the product regarding a product with regular content (control). Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HHP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.