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The effect of initial wash at acidic and alkaline pHs on the properties of protein concentrate (kamaboko) products from sardine (*Sardina pilchardus*) samples

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Abstract

Sardine samples were initially washed at acidic (2.50, 4.00 and 5.50) and alkaline (8.50, 10.00 and 11.50) pHs, followed by two washing cycles at pH 7.2 ± 0.15. Final recoveries for total solids were 77.5%, 71.5% and 63.1% at 4.00, 2.50 and 5.50 pHs and 48.3%, 43.3% and 29.3% at 8.50, 10.0 and 11.5 pHs, respectively. Consequently, similar were the results for protein recoveries (higher at acidic conditions) and lipid elimination (higher at alkaline conditions), indicating higher solubility of molecules in alkaline solutions. Lightness and whiteness index improved due to washing cycles, the thermal process further increased them and this was more evident in the samples washed at acidic conditions ($p < 0.05$). Kamaboko gels washed at acidic conditions showed higher values in firmness and more cohesive and elastic texture ($p < 0.05$). Evaluation analysis using sum scores revealed that initial wash at pH 5.50 is the most suitable treatment for kamaboko gels from sardine.
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Keywords: Kamaboko; pH; Washing efficiency; Colour properties; Gel properties

1. Introduction

The use of alternative fish species in order to obtain surimi of good gel-forming ability is one of the aims of the fishing industry. Due to its high potential for capture and its low price, there is a sustained interest at present in small pelagic species (Alvarez, Couso, Solas, & Tejada, 1992). However, the use of pelagic species with a high lipid content, like sardine, presents particular problems, mainly due to the high lipid content, which depends on the season, showing a maximum of 220 g kg^{-1} fish around September and a minimum of 10 g kg^{-1} during February (Mendes, Gómez-Guillén, & Montero, 1997; Esquivel et al., 1997).

Other problems faced with producing surimi from small pelagic species, such as sardine, is the dark muscle content, poor stability and, in some cases, the presence of histidine, which rapidly changes after death into histamine, a possible cause of allergy (Hall & Ahmad, 1997; Suzuki, 1981). Moreover, dark muscles, both superficial and deep-seated, contain more hemoglobin and myoglobin, which play an essential role in the whiteness, one of the factors determining the quality of surimi (Chaijan, Benjakul, Visessanguan, & Faustman, 2004a, 2004b).

Ochiai, Ochiai, Hashimoto, and Watabe (2001) reported that in order to prepare high-quality surimi and process it into kamaboko of higher gel strength and better whiteness, it is necessary to remove dark muscle as much as possible. However, attempts to remove dark muscle with a meat separator at higher levels in processing resulted in lower yield of surimi and higher costs of the products. Thus, an

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intensive washing protocol is required in order to produce high quality surimi, which ideally should have good gel-forming ability, elastic texture, good taste and white appearance (Pacheco-Aguilar, Ramírez-Suárez, & Mazorra-Manzano, 2001).

The objective of this study was to apply six different washing treatments at acidic (2.50, 4.00 and 5.50) and alakline (8.50, 10.00 and 11.50) pH areas for protein concentrate (kamaboko) production from sardine (*Sardina pilchardus*) and to evaluate their effect on washing efficiency, colour and gels properties of the final product.

2. Materials and methods

2.1. Materials

Fresh sardines (*S. pilchardus*) were purchased from the local fish-market of Thessaloniki (permanent supplier of the department) during the period of November 2004 to February 2005. Sardine samples were covered with crushed ice, in order to avoid temperature rise during transportation, and directly brought to the laboratory for further processing. Whole sardines (size: 11.7–16.1 cm; weight: 13.6–33.4 g) were processed into skinless fillets manually and minced using a Clatronic FW 2684 mincing machine with a 3 mm plate.

2.2. Kamaboko production

2.2.1. Washing conditions

Equal portions of sardine mince (~500 g) were weighed in a 2 L beaker and washed in a ratio of 3:1, washing medium:sardine mince, respectively, for 10 min under continuous agitation. The average temperature during washing was 11.8 ± 1.4 °C. Three washing cycles were applied as follows:

First washing cycle: In order to ascertain the effect of pH on the quality of kamaboko, six (6) different pHs were studied (2.50, 4.00, 5.50, 8.50, 10.00 and 11.50). The acidic pHs (2.50, 4.00 and 5.50) were obtained using 0.1 M $NaH_2PO_4 \cdot H_2O$ solution, which was further enforced (for low pHs 2.50 and 4.00) with gradually added 85% H_3PO_4. The alkaline pHs (8.50, 10.00 and 11.50) were achieved using 0.1 M $Na_2HPO_4 \cdot 2H_2O$, which was also further enforced (for high pHs 10.00 and 11.50) with gradually added 30% NaOH solution. All samples were washed at the above pHs for 10 min.

Second washing cycle: All above pHs were readjusted in the range of 7.00–7.50 with phosphate solution (0.1 M $Na_2HPO_4 \cdot 2H_2O$ solution was adjusted in the range of 7.00–7.50 with gradually added 85% H_3PO_4). All samples were washed at the above pH for 10 min.

Third washing cycle: Final wash of sardine mince was conducted with 0.3% NaCl solution in order to facilitate dewatering. All samples were washed at the above solution for 10 min. The moisture content of the washed sardine mince samples was adjusted in the range of 78–80% using a cheesecloth.

2.2.2. Gel preparation

The washed sardine mince samples were (a) mixed for 1 min with salt equivalent to 2.5% of the mince's weight, (b) stuffed into metal rings of uniform dimensions (9.7 mm height, 25 mm diameter), (c) vacuum-sealed in moisture/vapour-proof film bags, (d) preheated at 40 °C for 40 min, (e) heated at 90 °C for 30 min and (f) held in refrigeration overnight prior to texture evaluation.

2.2.3. pH measurement

The pH of sardine flesh was measured with a Hanna Instruments HI 8424 microcomputer pH-meter. Samples were prepared according to Cortés-Ruiz, Pacheco-Aguilar, García-Sánchez, and Lugo-Sánchez (2001) by blending 2 g of sardine mince with 18 mL of distilled water.

2.2.4. Washing efficiency

Washing efficiency was estimated as reported by Pacheco-Aguilar et al. (2001) as the percent recovery of total solids and proteins and the percent removal of lipids and ash:

- % recovered total solids or proteins = (amount of solids or proteins recovered after washing)/(amount of solids or proteins of unwashed mince)%;
- % removed lipids or ash = 100 − [(amount of lipids or ash recovered after washing)/(amount of lipids or ash of unwashed mince)%].

2.2.5. Proximate analysis

Moisture content was determined by the CEC (Commission of European Communities) recommended method ISOR 1442 (CEC, 1979). The lipid content was determined by the Bligth and Dyer (1959) method as modified by Hanson and Olley (1963). Total protein (crude protein, $N \times 6.25$) content of sample was determined using the Kjeldahl method according to standard AOAC methods (1990). The ash content was determined by mineralization at 550 °C according to standard AOAC methods (1990).

2.2.6. Assay of protease activity

Protease activity was measured in surimi wash water, which was collected after the first, second and third washing cycle. The reaction mixture contained 2.5 mL of 0.5% casein in 0.1 M phosphate buffer pH 7.00 and 0.3 mL of the liquid waste. The mixture was incubated in a water bath at 40 °C for 30 min. Then, 2.8 mL of 5% (w/v) trichloroacetic acid (TCA) were added to stop the reaction and to precipitate protein. The mixture was allowed to stand for 1 h at room temperature. The precipitate was removed by filtration through Whatman No. 1 filter paper. The absorbance of the supernatant was measured using a Spectronic 601 (Bausch & Lomb) spectrophotometer at 280 nm in a 1 cm silica cell. A blank was run by adding the enzyme

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