Original Research Article

Evaluation, Effect of Whitening Agents on Physico-chemical and Functional Properties of Sardine Surimi (*Sardina pilchardus*)

.

ABSTRACT

The effects of hydrogen peroxide (H_2O_2), sodium bicarbonate ($NaHCO_3$) and calcium carbonate ($CaCO_3$) treatments on the colour and textural properties of sardine surimi (*Sardina pilchardus*) were studied. Principal Component Analysis (PCA) was applied in order to investigate their effects and to determine the optimum whitening agents used. Addition of $CaCO_3$ and H_2O_2 significantly improved whiteness of surimi in comparison to $NaHCO_3$ treatments ($p < 0.05$). Some textural damage and a reduction in WHC values were observed for surimi treated withH_2O_2 and $NaHCO_3$ ($p < 0.05$). PCA biplot showed that 1.5 % $CaCO_3$ tended to result in improved whiteness, WHC and textural properties since 1.5 % $CaCO_3$ sample sits closer to these functional properties vector lines than the other treatments. Whereas, 2.5 % H_2O_2 had positively affected only the whiteness parameter. Results indicated that treating mince with the appropriate type and concentration of whitening agent can improve the functional properties of surimi, particularly from fish species with darker meat such as sardine.

Aims: In order to improve whiteness and functional properties of sardine surimi (*Sardina pilchardus*), the effects of hydrogen peroxide (H_2O_2), sodium bicarbonate ($NaHCO_3$) and calcium carbonate ($CaCO_3$) treatments were studied. Principal Component Analysis (PCA) was applied in order to investigate their effects and to determine the optimum whitening agents used.
Study design: Experimental Research Design
Place and Duration of Study: Research Unit “Biopreservation and Valorisation of Agro-FoodProducts”of the Higher Graduate School of Food Industry of Tunisia. The study was conducted in 3 months.
Methodology: Sardine surimi samples were prepared with different treatments at different concentrations: calcium carbonate ($CaCO_3$), hydrogen peroxide (H_2O_2) and sodium bicarbonate ($NaHCO_3$). Proximate composition, total pigment, whiteness, water holding capacity and textural properties were investigated. Optimal levels of each whitening agent were determined using PCA.
Results: Addition of $CaCO_3$ and H_2O_2 significantly improved whiteness of surimi in comparison to $NaHCO_3$ treatments ($p < 0.05$). Some textural damage and a reduction in WHC values were observed for surimi treated withH_2O_2 and $NaHCO_3$ ($p < 0.05$). PCA biplot showed that 1.5 % $CaCO_3$ tended to result in improved whiteness, WHC and textural properties since 1.5 % $CaCO_3$ sample sits closer to these functional properties vector lines than the other treatments. Whereas, 2.5 % H_2O_2 had positively affected only the whiteness parameter.
Conclusion: Results indicated that treating mince with the appropriate type and concentration of whitening agent can improve the functional properties of surimi, particularly from fish species with darker meat such as sardine.

Keywords: Sardine surimi, whitening agent, colour, textural properties, Principal Component Analysis.

1. INTRODUCTION

Surimi is a stabilised minced fish flesh, washed with water and blended with cryoprotectants [1]. As colour and texture are functional properties responsible for the final consumer's acceptance of surimi-based products, white-fleshed species are commonly used as raw material for surimi production. However, limited access to some of the white-fish resources due to their overexploitation [2] and the abundance of dark muscle fish species which make up 40–50% of the total fish catch in the world [3] could have a great interest in using the large quantities of these low value fatty pelagic fish for human food, particularly for surimi production.

Sardine, which is one of the most common fishing species in Tunisia (21,086 T in 2012) [4] is still rather used fresh, salted or canned. In spite of its abundance, low price and good nutritional value, sardine is still underexploited. Therefore, surimi processing is an effective way to take advantage of underutilized fish species by making a more sustainable and profitable use of resources [5], especially when surimi and based-products do not exist yet, and no information regarding production of Tunisian surimi has been reported. However, the use of pelagic species presents particular problems, mainly the dark muscle content that is rich in hemoglobin and myoglobin and which play an essential role in the whiteness [6]. Therefore, the use of this small pelagic fish for surimi production is one of major challenges in transforming underutilized fish protein resources into human foods.

Washing minced fish is the initial step in obtaining a white, odorless and bland surimi as it removes compounds such as sarcoplasmic proteins, inorganic salts, low-molecular weight substances, lipids, and blood components [7, 8]. Ochiai et al. [9] suggested that to prepare high-quality surimi and process it into kamaboko of higher gel strength and better whiteness, it was necessary to remove dark muscle as much as possible. However, these attempts resulted in lower yield of surimi and higher costs of the products [6].

Hence, the use of whitening agents is the potential alternative for colour improvement of products prepared from dark fleshed-species. However, the addition of whitening agents to the fish mince may alter the texture of the surimi product [10].

Chen et al. [11] reported that colour of dark-fleshed fish surimi could be improved by leaching the mince with hydroperoxide or sodium bicarbonate solution. Calcium carbonate also has been used to whiten fish mince and to make products chalky and opaque white [1, 2]. Thus, in order to produce high quality surimi (good taste, white appearance, good gel-forming ability and elastic texture), an intensive washing protocol is required.

Therefore, the objectives of this study were to investigate the effect of calcium carbonate and washing treatments (hydrogen peroxide and sodium bicarbonate) at different concentrations on the colour and textural properties of surimi gel made from sardine (*Sardina pilchardus*) and to determine the optimal levels of each whitening agent using PCA.

2. MATERIAL AND METHODS

2.1 Material

Sardine (*Sardina pilchardus*) with an average length of 13-15 cm was caught off the Tunisian coast. It was kept in ice and transported to the laboratory in less than 12 hours after catching where it was hand beheaded, eviscerated, filleted, washed and minced.

All general chemicals used were of analytical grade purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Distilled water was used for the preparation of all solutions.

Surimi preparation

2.2 Methods

2.2.1 Surimi preparation

In order to improve the colour of the dark minced flesh, surimi samples were prepared with different treatments as follows:

2.2.1.1 Conventional washing process: Surimi used as control was prepared by conventional method as described by Rawdkuen et al. [12]. The mince was successively washed two times with cold distilled water (4 °C), then with NaCl solution (0.5 % w/v) with 1:3 (w/w) ratio of mince/water and mince/solution. Each mixture was continuously and gently stirred for 10 min and dewatered using centrifugation with a speed of 700 × g for 15 min at 4 °C (Model CE 21K, Grandiumpiant, Belluno, Italy). Finally, a cryoprotectant mixture was added to the washed mince (4 % sorbitol and 4 % sucrose w/w). Surimi was packed into polyethylene bags (100 g) and stored at -18 °C.

2.2.1.2 Hydrogen peroxide treatment: Surimi was prepared by conventional method as described by Rawdkuen et al. [12] with some modifications. The mince was successively washed (leaching process) with cold distilled water, hydrogen peroxide solution at different concentrations (1; 1.5; 2 and 2.5 % v/v) referred to as the bleaching solution and NaCl solution (0.5 % w/v) at 4 °C with 1:3 (w/w) ratio of mince/water and mince/solution. Each mixture was continuously and gently stirred for 10 min and dewatered using centrifugation with a speed of 700 × g for 15 min at 4 °C (Model CE 21K, Grandiumpiant, Belluno, Italy). Finally, the washed mince was mixed with cryoprotectant (4 % sorbitol and 4 % sucrose w/w). Surimi was packed into polyethylene bags (100 g) and stored at -18 °C.

2.2.1.3 Sodium bicarbonate treatment: Surimi was prepared by conventional method as described by Rawdkuen et al. [12] with slight modifications, as described previously, except that the second washing cycle was conducted with sodium bicarbonate at different concentrations (0.1; 0.3; 0.5 and 0.7 % w/v) [13].

2.2.1.4 Calcium carbonate treatment: Surimi was prepared by conventional method as described by Rawdkuen et al. [12] with some modifications, as described previously, except that in the final step of surimi preparation, the washed mince was mixed with cryoprotectant (4 % sorbitol and 4 % sucrose w/w) and calcium carbonate at different concentrations (0.5; 0.75; 1 and 1.5 % w/w) [2].

2.2.2 Surimi gel preparation

To prepare the gel, frozen surimi samples were partially thawed at room temperature. The surimi was then cut into small pieces with an approximate thickness of 1 cm. The moisture was adjusted to 80 % and 2.5 % salt was added. The surimi sol was stuffed into glass tubes (2.5 cm diameter) and both ends were sealed tightly. Kamaboko gel was prepared by setting the sol at 40 °C for 30 min followed by heating at 90 °C for 20 min in a water-bath. The gel was then cooled in iced water and stored at 4 °C for 24 h before analysis.

2.2.3 Proximate composition

Total protein content was determined in quadruplicate as % N × 6.25 using a LECO CHNS-932 nitrogen micro analyzer (Leco Corporation, St. Joseph, MI, USA) (Etheridge, Pesti and Foster, 1998). Total lipid content was determined by using Soxhlet extraction according to the method of AOAC [14]. Moisture content was determined according to the AOAC method [15] by drying the samples in an oven at 105 °C. Ash content was determined by heating the samples in a furnace muffle at 525 °C according to the AOAC method [15].

2.2.4 Texture analysis

Texture analysis of surimi gels was carried out using a texture analyser (Model TA-XT2 Stable Micro Systems, Haslemere, UK). Prior to testing, gels were equilibrated at room temperature (25–27 °C) and cut into three cylindrical shapes (25 mm diameter × 25 mm length). The samples were compressed at 30 % of initial height using a spherical probe (25 mm diameter), at a speed of 60 $mm.min^{-1}$. Breaking force and gel strength were evaluated.

2.2.5 Whiteness measurement

Whiteness was measured using a colorimeter (Chroma-mètre Minolta CR 400). L* (Lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) values were made on five replicate and whiteness was calculated using the following equation [16]:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (1)$$

2.2.6 Total pigment

The total pigment content was determined according to the method of Rawdkuen et al. [12]. Surimi (1 g) was mixed with 9 ml of acid acetone (90 % acetone, 8 % deionized water and 2 % HCl). The mixture was macerated with a glass rod and allowed to stand for 1 h at room temperature. The extract was filtered with a Whatman No. 1 filter paper and the absorbance was read at 640 nm against an acid acetone blank. The total pigments were calculated as hematin using the following formula:

$$\text{Total pigment (ppm)} = A_{640} \times 680 \quad (2)$$

The Total pigment content was expressed as mg/100 g sample.

2.2.7 Water-holding capacity (WHC)

Water-Holding Capacity was evaluated using a centrifuge method according to Sang-Keun et al. [17]. Samples (0.3 to 1.3 g) were equilibrated at room temperature and placed on nylon plain membrane (5.0 mm pores, Micronsep, USA) maintained in the middle position of a centrifuge tube. Water loss was determined by weighing before and after centrifugation at 1,000 × g for 15 min at 5 ºC. WHC was expressed as the percentage of the initial water remaining in the sample after centrifugation. Each value is the mean (standard deviation) of at least four determinations.

2.2.8 Statistical analysis

Statistical analyses were performed using the IBM Statistical Package for Social Sciences software (SPSS version 20.0.0 for windows, Chicago Inc., IL, USA). Comparison of means was carried out by the SNK (Student-Newman-Keuls) test with one-way analysis of variance (ANOVA). Significance of differences was defined as $p < 0.05$. The ANOVA procedure was further enhanced by the appliance of Principal Component Analysis (PCA) (XLSTAT version 2020.5) in order to investigate the effect of all treatments on the profile of the final products and to determine the optimum treatment(s).

3. RESULTS AND DISCUSSION

3.1 Effects of Hydrogen peroxide treatment

Table 1 shows the proximate compositions of surimi treated with conventional washing process (control) and surimi processed with H_2O_2 treatment at different concentrations (1-2.5 % v/v). The use of hydrogen peroxide as bleaching solution had only affected the protein content of surimi which decreased slightly but significantly when compared with the control ($p < 0.05$). This reduction means that other proteins may be eliminated. Most of the proteins eliminated during surimi processing are sarcoplasmic proteins, which are water soluble and considered to be undesirable components; other eliminated proteins include the heme pigments and blood [19]. Moreover, the decrease of protein content during washing treatment of surimi could be due to the oxidation of proteins caused by the peroxide decomposition radicals (such as hydroxyl radicals), resulting in the generation of carbonyl derivatives [20].

Analysis of colour parameters showed that H_2O_2 treatment had a beneficial effect on the colour of the sardine surimi at the concentration of 2.5%, when compared with the control, resulting in higher *L** values as shown in table 3 ($p < 0.05$). However, a highly significant reduction of the red pigment (*a** values) and also a reduction of the yellow pigment towards the grey area (*b** values) were obtained ($p < 0.05$) (**Table 1**). H_2O_2 can oxidize chromophores, leading to the decrease in redness and yellowness [21]. These results are, partially in accordance with those found by Himonides et al. [22]. Brown et al. [23] indicated that the highest 'L-value' (lightness) of the dark muscle of Alaska pollock fillets was produced by bleaching solution consisting of 2 % H_2O_2 with 1 % STP at pH 10.5. According to

Thanonkaew et al. [24], soaking the cuttlefish in 3 % NaCl and 0.5 % H_2O_2 for 15 min could improve the colour by increasing 'L-value' and decreasing the 'a-value'.

Table 1. The effect of hydrogen peroxide treatment on the physico-chemical properties of surimi from sardine (*Sardina pilchardus*)

		Concentrations tested				
		0 %	**1.00 %**	**1.50 %**	**2 %**	**2.50 %**
Proximate composition (%)	**Water**	75.5 ± 0.5[a]	75.31 ± 0.94[a]	74.52 ± 0.51[a]	75.41 ± 0.9[a]	74 ± 0.81[a]
	Protein	17.02 ± 0.2[c]	16.76 ± 0.04[b]	16.35 ± 0.04[a]	16.21± 0.07[a]	16.05 ± 0.05[a]
	Fat	3.16 ± 0.13[a]	3.14 ± 0.05[a]	3.14 ± 0.07[a]	3.16 ± 0.06[a]	3.74 ± 0.27[b]
	Ash	3.3 ± 0.61[c]	3 ± 0.05[a]	3.06 ± 0.05[a]	3.19 ± 0.04[b]	3.19 ± 0.04[b]
Colour parameters	**L***	49.26 ± 1.57[a]	47.27 ± 1.42[a]	48.34 ± 0.41[a]	48.32 ± 0.48[a]	53.44 ± 0.51[c]
	a*	1.38 ± 0.08[a]	0.31 ± 0.04[c]	0.06 ± 0.06[a]	0.19 ± 0.08[b]	1.05 ± 0.02[d]
	b*	7.04 ± 0.18[a]	2.35 ± 0.38[b]	1.98 ± 0.08[a]	1.97 ± 0.39[a]	4.37 ± 0.32[c]
Water holding capacity (%)		41.05 ± 0.58[a]	39.82 ± 0.22[a]	39.99 ± 0.18[b]	39.79 ± 0.27[c]	39.65 ± 0.78[d]
Total pigment content (mg/100 g)		402.56 ± 1.36[a]	127.61 ± 1.04[d]	114.92 ± 0.68[c]	94.06 ± 1.41[b]	68.68 ± 2.45[a]

**Mean ± SD of triplicate analyses. Values in same ligne with different letters are significantly different ($p < 0.05$).*

Whiteness is one of the prime requisites in deciding the quality and price of surimi. The results showed that whiteness of surimi treated decreased up to concentration of 2 % H_2O_2 (**Figure 1**). However, a distinct improvement in the colour was observed when washing with 2.5% H_2O_2 resulted in a higher whiteness of the surimi obtained. The increase of whiteness when washing with 2.5 % H_2O_2 indicated that H_2O_2 could improve colour of surimi to some extent. James and Mccrudden [25] investigated the effect of H_2O_2 on cod fillets and they state that the most effective concentration of H_2O_2 for whitening was 0.85 %. Young et al. [26] associated whitening of the fish samples, treated with various buffer solutions at different pH levels (2 to 8–9 and 10.5) with mainly 0.75 % H_2O_2, with an increase in pigment solubility, particularly at alkaline pH.

These findings are in accordance with the total pigment results showing a significant decrease ($p < 0.05$), in comparison with the control (402.56 ± 1.36 mg/100 g), when the concentration of H_2O_2 increased up to 2.5 % (68.68 ± 2.45 mg/100 g) (**Table 1**). The significant decrease was explained by Ninan et al. [20] as follows. Hydroperoxyl anion and hydroxyl radical derived from the decomposition of H_2O_2 are able to destroy the chemical bonds of chromophores leading to substance that either does not contain a chromophore, or contains a chromophore that does not absorb visible light.

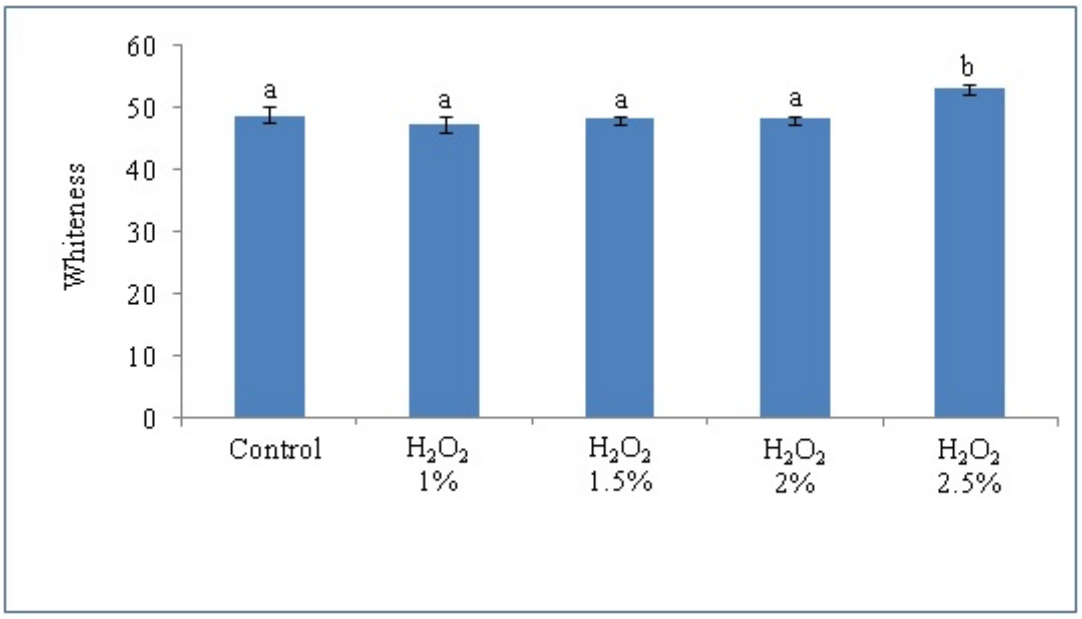
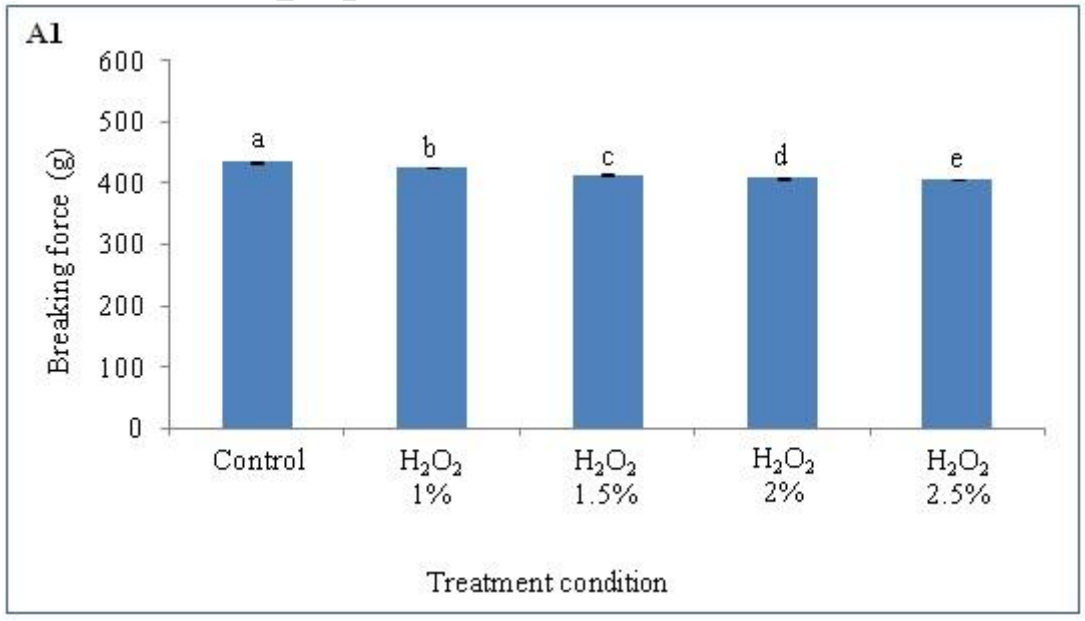


Fig. 1.Whiteness of sardine surimi (*Sardinapilchardus*) under different concentrations of Hydrogen peroxide (H_2O_2) treatment

Textural properties are other important parameters along with whiteness for deciding the quality of surimi. Surimi prepared by washing with H_2O_2 solution at different concentrations (1; 1.5; 2 and 2.5 % v/v), showed some textural damage. A marked decrease in breaking force and gel strength in comparison with the control ($p < 0.05$) were recorded (**Figure 2. A1 and A2**). When the concentration of H_2O_2 increased up to 2.5 %, the decrease in both breaking force and gel strength was noticeable ($p < 0.05$).

In the present investigation, the decrease of breaking force and gel strength might be due to the excessive denaturation of proteins induced by oxidizing agents, leading to affect the protein functionality and to a poorer gel-forming ability [22].



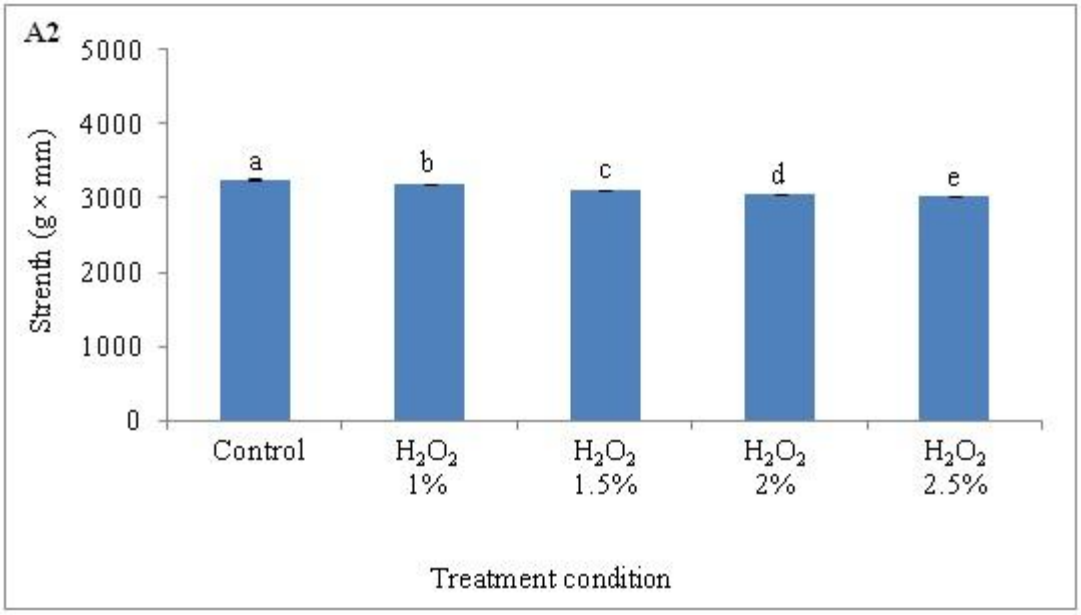


Fig. 2. Breaking force (A1) and strength (A2) of surimi gel produced from sardine (*Sardina pilchardus*) under different concentrations of Hydrogen peroxide (H_2O_2) treatment

Washing with H_2O_2 solution at all concentrations gave a surimi with reduced WHC than did water ($p < 0.05$). Values of WHC tended to decrease ranging from 41.05 ± 0.58 % to 39.65 ± 0.78 % for surimi produced with conventional and hydrogen peroxide washing (2.5 % v/v), respectively (**Table 1**).

3.2 Effects of Sodium bicarbonate treatment

0.1-0.7 % sodium bicarbonate (w/v) has been used in preparing surimi from sardine meat fish, during the washing of mince.

The effect of sodium bicarbonate washing treatment on the proximate composition of sardine surimi is shown in table 2. It can be observed that this treatment led in products with higher moisture content and lower lipid, protein and ash contents ($p < 0.05$).

Fat is considered to be an undesirable component in surimi. In this investigation, the decrease of fat content when using the sodium bicarbonate treatment is in agreement with results found by Chen et al. [11]. In their study, they reported that lipid elimination efficiency of horse mackerel (*Trachurus japonicus*) mince washed with alkaline solution was greater than that washed with cold water. Rawdkuen et al. [12] suggested that larger lipid reduction for the alkaline process (sodium bicarbonate treatment) was obtained because, at high pH, the solubilised proteins are separated from the storage lipids and the membrane phospholipids on the basis of density and solubility differences during centrifugation. Therefore, a portion of the membrane phospholipids sediment in the bottom layer of the centrifuge tube, and also neutral lipids are significantly separated on to the top.

Moreover, Chen et al. [11] found that the total protein recovery of mince washed with alkaline solution was lower than that washed with cold water. According to Kaewjumpol et al. [28], sodium bicarbonate had been found to increase pH and shift the isoelectric point, which results in transverse swelling of the myofibrils and promotes myosin extraction (i.e., salt soluble protein extraction).

The increase of moisture content in mince washed with sodium bicarbonate was also found in serveral studies ($p < 0.05$) [29, 30, 31]. This result could be due to the water absorption phenomenon of myofibrillar protein [31].

Table 2. The effect of sodium bicarbonate treatment on the physico-chemical properties of surimi from sardine (*Sardina pilchardus*)

		Concentrations tested				
		0 %	**0.10 %**	**0.30 %**	**0.50 %**	**0.70 %**
Proximate composition (%)	**Water**	75.5 ± 0.5[a]	85 ± 0.87[b]	86.33 ± 0.29[b]	87 ± 0.5[b]	89.83 ± 0.76[c]
	Protein	17.02 ± 0.2[a]	17.08 ± 0.1[d]	16.95 ± 0.04[c]	16.89 ± 0.03[b]	16.78 ± 0.03[a]
	Fat	3.16 ± 0.13[a]	2.46 ± 0.04[b]	2.53 ± 0.27[b]	2.18 ± 0.16[a.b]	1.89 ± 0.17[a]
	Ash	3.3 ± 0.61[b]	2.94 ± 0.06[c]	2.75 ± 0.07[b]	2.63 ± 0.05[a.b]	2.54 ± 0.06[a]
Colour parameters	**L***	49.26 ± 1.57[a]	48.83 ± 2.06[a]	48.78 ± 2.28[a]	48.61 ± 0.46[a]	48.54 ± 1.01[a]
	a*	1.38 ± 0.08[a]	0.7 ± 0.43[a]	0.99 ± 0.23[a]	0.93 ± 0.4[a]	0.61 ± 0.29[a]
	b*	7.04 ± 0.18[a]	2.03 ± 0.46[a]	3.2 ± 0.39[a]	3.72 ± 0.60[a]	2.24 ± 1.09[a]
Water holding capacity (%)		41.05 ± 0.58[a]	29.87 ± 0.55[b]	29.82 ± 0.20[b]	29.74 ± 0.16[b]	27.6 ± 0.53[a]
Total pigment content (mg/100 g)		402.56 ± 1.36[a]	398.02 ± 3.86[d]	399.65 ± 2.75[c]	395.52 ± 1.36[b]	394.84 ± 1.36[a]

**Mean ± SD of triplicate analyses. Values in same ligne with different letters are significantly different ($p < 0.05$).*

The effect of water (tap water) and $NaHCO_3$ solution (0.1–0.7 % w/v) on the colour of sardine surimi was evaluated (**Figure 3**). Usually, '*L** values' were used to indicate differences in lightness and even whiteness, however, a difference of 0.72 units in *L** values (**Table 2**) between control and sample treated with 0.7 % $NaCO_3$ does not convey the difference in visual whiteness between these samples.

As consequence, whiteness values of surimi was not significantly ($p > 0.05$) affected by ingredient. Whiteness values ranged from 48.46 ± 0.14 (0.1 %) to 48.78 ± 0.22 (0.7 %) for the treated samples compared with 48.75 ± 0.38 for the control. Since whiteness was not ameliorated, we can consider that sodium bicarbonate treatment was not useful. These results were similar to those found by Hunt and Park [32] when comparing the effect of bicarbonate sodium on the gelling properties of Alaska pollock surimi. Choi and Park [33] reported that the lower whiteness of gels treated with alkalin process was due to the remaining hemoproteins in the recovered protein. According to Chaijan et al. [34], tap water removed less myoglobin than the salt containing solutions (NaCl, $NaHCO_3$, and sodium phosphate buffer). Salt compounds in washing solutions may weaken and break the bonds between muscle and myoglobin, thereby releasing more myoglobin, which is then solubilized in water and removed through washing.

These results are justified by the slight reduction of total pigment content ($p < 0.05$) noted in samples treated with sodium bicarbonate (**table 2**). The decrease was greater when using 0.7 % $NaHCO_3$.

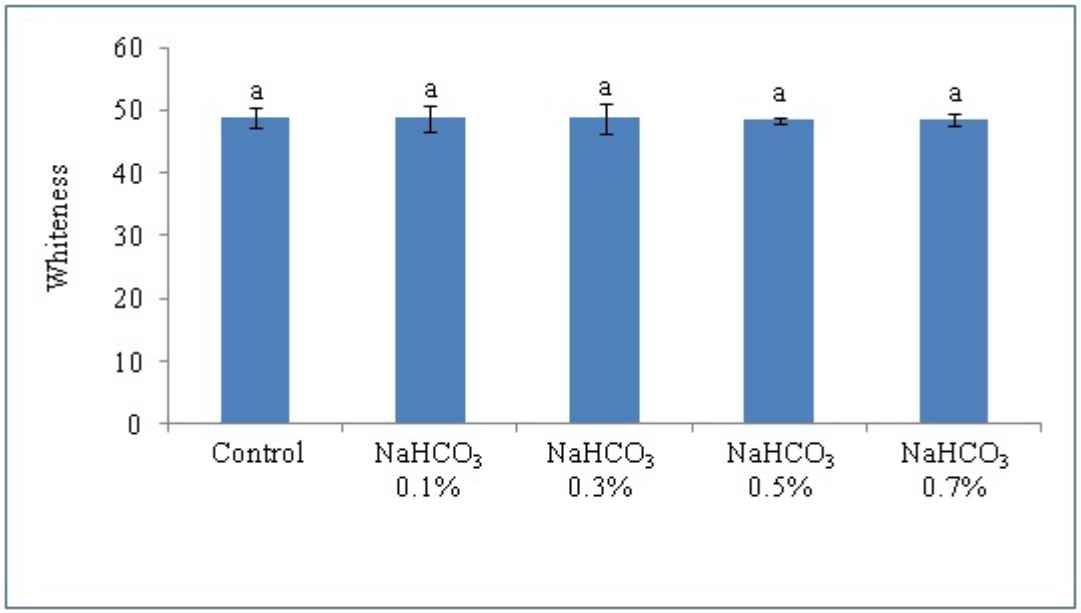
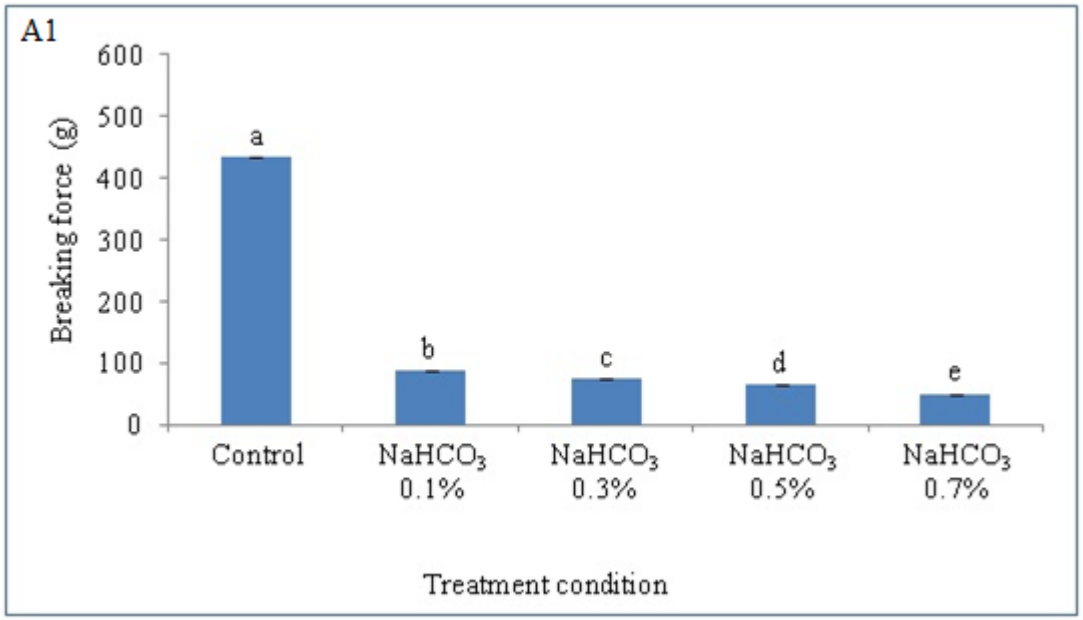


Fig.3. Whiteness of sardine surimi (*Sardina pilchardus*) under different concentrations of Sodium bicarbonate ($NaHCO_3$) treatment

Breaking force and gel strength data of sardine surimi are shown in figures 4.A1 and A2. Generally, the use of this ingredient resulted in reduced breaking force and gel strength values when compared with those of surimi made with conventional washing method ($p < 0.05$). Increasing concentration of $NaHCO_3$ used resulted in a decrease in breaking force (from 88.36 ± 0.33 g for samples treated with 0.1 % $NaHCO_3$ to 50,53 ± 0.12 g for samples treated with 0.7 % $NaHCO_3$) and gel strength values (from 662.75 ± 0.57 g × mm for samples treated with 0.1 % $NaHCO_3$ to 379 ± 0.36 g × mm for samples treated with 0.7 % $NaHCO_3$) ($p < 0.05$).



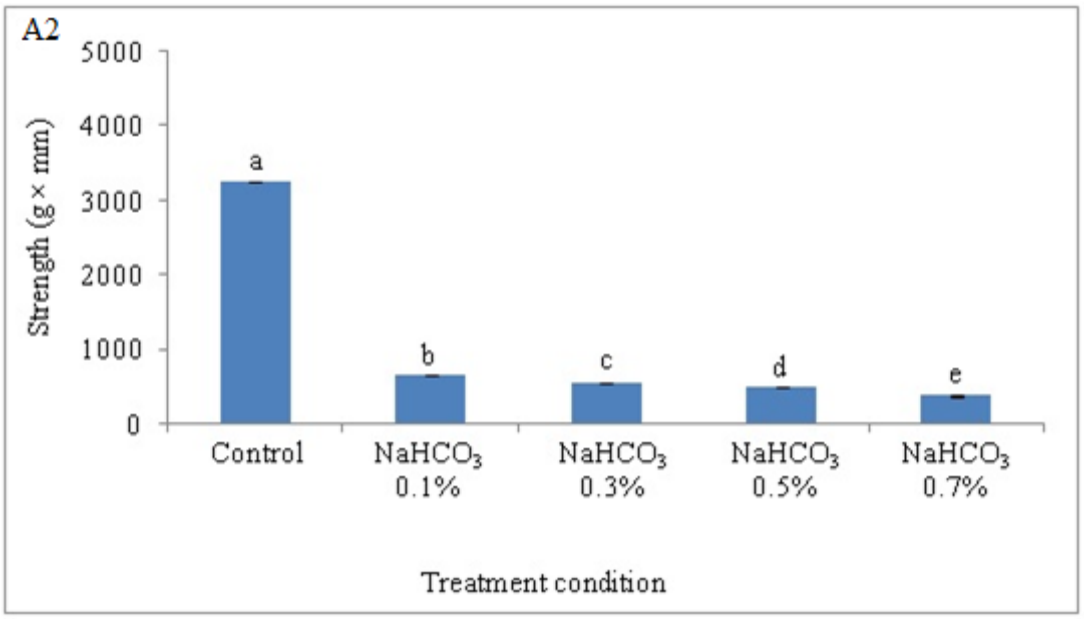


Fig. 4. Breaking force (A1) and strength (A2) of surimi gel produced from sardine (*Sardina pilchardus*) under different concentrations of Sodium bicarbonate ($NaHCO_3$) treatment

The reduced breaking force and gel strength values observed when using $NaHCO_3$ treatment can be due to excess moisture absorption during washing cycle. According to Karayannakidis et al. [6], the observed differences in the textural characteristics of the produced fish gels were mainly attributed to differences in final moisture content as well as in the chemical composition of the sardine mince, since various amounts of proteins, lipids and ash were recovered by applying the alkaline and conventional washing treatments. Moreover, Rawdkuen et al. [12] reported that some sarcoplasmic proteins that remain in pH-shifting processes may be bound to the myofibrils during the heat treatment, thus decreasing the strength of the gel. However, in other studies, sodium bicarbonate found to improve gel texture of Alaska Pollock and Pacific whiting surimi due to increased myosin heavy chain cross-linking caused by increasing pH [32, 35].

WHC is one of the most important functional properties of protein gels associated with the stability of surimi gel-based restructured seafood. The results of the water holding capacity (WHC) depicted in the table 2 showed substantially different behaviors among the surimi gels with different treatments. For samples prepared with sodium bicarbonate treatment, WHC decreased as the concentration of $NaHCO_3$ increased ($p < 0.05$). The continuously decrease of WHC values with increasing $NaHCO_3$ concentrations was possibly due to the poor gel network of surimi treated which could not imbibe water and led to high water releases. Chaijan et al. [36] reported that the gels of sardine and mackerel muscle prepared by the alkaline process showed higher expressible moisture than those from the conventional washing method.

3.3 Effects of Calcium carbonate treatment

Proximate composition of control sample without addition of calcium and samples with calcium has been analyzed and given in table 3. The one-way ANOVA showed that ingredient did not significantly affect proximate composition in comparison with control ($p > 0.05$).

Table 3. The effect of calcium carbonate treatment on the physico-chemical properties of surimi from sardine (*Sardinapilchardus*)

		Concentrations tested				
		0 %	**0.50 %**	**0.75 %**	**1 %**	**1.50 %**
Proximate composition (%)	**Water**	75.5 ± 0.5^{a}	73.65 ± 0.76^{a}	74.37 ± 0.8^{a}	74.58 ± 0.62^{a}	75.26 ± 0.66^{a}
	Protein	17.02 ± 0.2^{a}	17.05 ± 0.08^{a}	17 ± 0.11^{a}	16.95 ± 0.09^{a}	17.05 ± 0.04^{a}
	Fat	3.16 ± 0.13^{a}	3.5 ± 0.5^{a}	3.1 ± 0.07^{a}	3.59 ± 0.46^{a}	3.15 ± 0.06^{a}
	Ash	3.3 ± 0.61^{b}	3.34 ± 0.15^{b}	2.91 ± 0.12^{a}	3.06 ± 0.06^{a}	3.05 ± 0.05^{a}
Colour parameters	**L***	49.26 ± 1.57^{a}	55.78 ± 0.7^{b}	56.25 ± 1.18^{b}	57.09 ± 1.12$^{b.c}$	58 ± 0.31^{c}
	a*	1.38 ± 0.08^{a}	1.56 ± 0.11^{a}	2.17 ± 0.05^{b}	2 ± 0.35^{b}	2.15 ± 0.37^{b}
	b*	7.04 ± 0.18^{a}	7.47 ± 0.76$^{a.b}$	8.3 ± 0.31^{b}	6.54 ± 0.96^{a}	8.38 ± 0.4^{b}
Water holding capacity (%)		41.05 ± 0.58^{a}	48.35 ± 0.54^{b}	48.41 ± 0.45^{b}	49.26 ± 0.26^{b}	55.71 ± 0.31^{c}
Total pigment content (mg/100 g)		402.56 ± 1.36^{a}	400.97 ± 2.75^{a}	405.05 ± 3.49^{a}	399.84 ± 1.36^{a}	401.65 ± 2.39^{a}

**Mean ± SD of triplicate analyses. Values in same ligne with different letters are significantly different ($p < 0.05$).*

In order to compare individual differences in lighteness (*L** value), redness (*a** value) and yellowness (*b** value) between standard and whitened samples, a statistical comparison of these parameters was carried out. Our results indicated that adding calcium carbonate in the range of 0.5 %-1.5 % had influenced significantly *L**, *a** and *b** values (**Table 3**).

Generally, washing steps lead to ameliorate the colour of the fish flesh. Further improvements in the degree of lightness and whiteness index were achieved during addition of calcium carbonate ($p < 0.05$) (**Figure 5**). The whiteness values for surimi samples ranged from 55.12 ± 0.13 to 57.7 ± 0.21 when 0.5 to 1.5 % calcium carbonate were added.

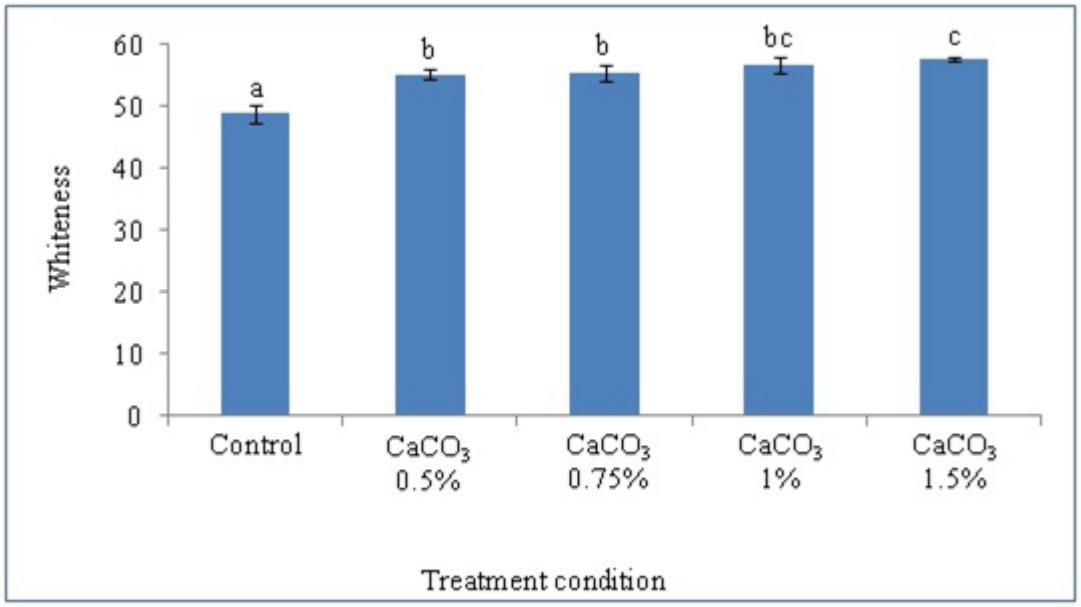
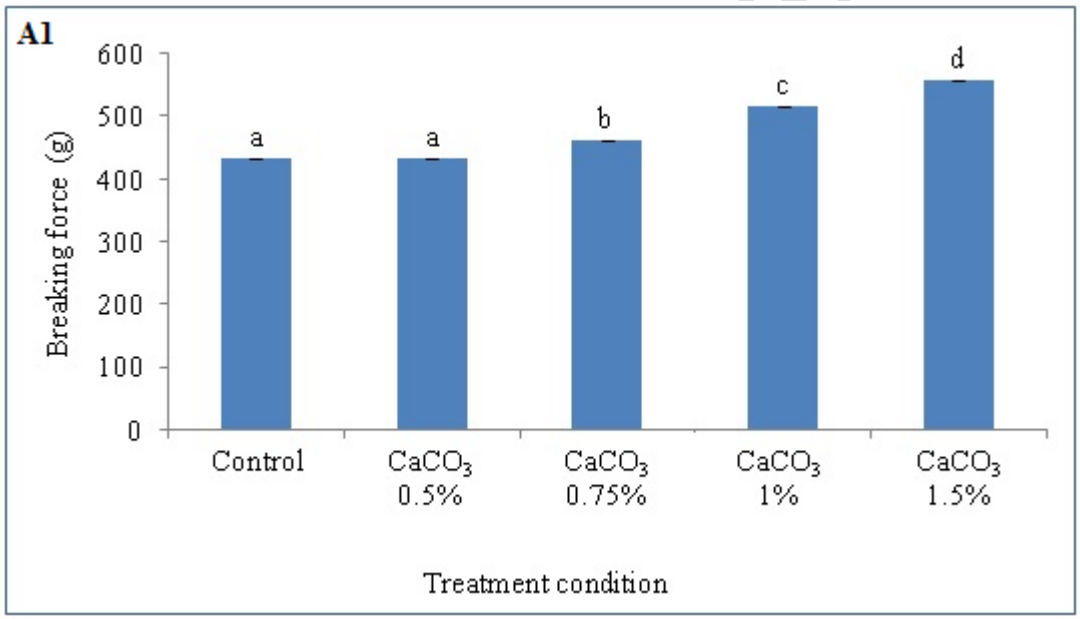


Fig.5. Whiteness of sardine surimi (*Sardinapilchardus*) under different concentrations ofCalcium carbonate ($CaCO_3$) treatment

These results are partially in accordance with those found by Hsu and Chiang [10]. In their study, they reported that addition of calcium carbonate to golden threadfin bream surimi, in the range of 0–2 %, showed no significant influence on the *b** values, but 3 units increase in the *L** values. According to Lee et al. [37], products appear whiter because, at neutral pH, particles of calcium carbonate would not dissolve and are believed to bring about a light scattering effect. Moreover, in surimi processing, washing minced meat with fresh cold water removes most of the sarcoplasmic proteins constituting myoglobin, haemoglobin and other unwanted substances and further whiteness of surimi can be increased [38].

However, no significant differences in the total pigments of standard and treated mince, at any concentrations, were observed ($p > 0.05$) (**table 3**).

In order to determinate the quality and the efficiency of production methods, gel properties were followed. Breaking force and gel strength results of sardine surimi gel were compared at different calcium carbonate concentrations (**Figure 6.A1 and A2**). Without calcium compounds, control sample ought to possess a breaking force and gel strength of 433.36 ± 0.24 g and 3249.09 ± 0.11 g × mm, respectively. Generally, addition of calcium carbonate resulted in highly values ($p < 0.05$) with maximal results (556.6 ± 0.09 g for breaking force and 4174.5 ± 0.16 g × mm) were attained by adding 1.5 % calcium carbonate.



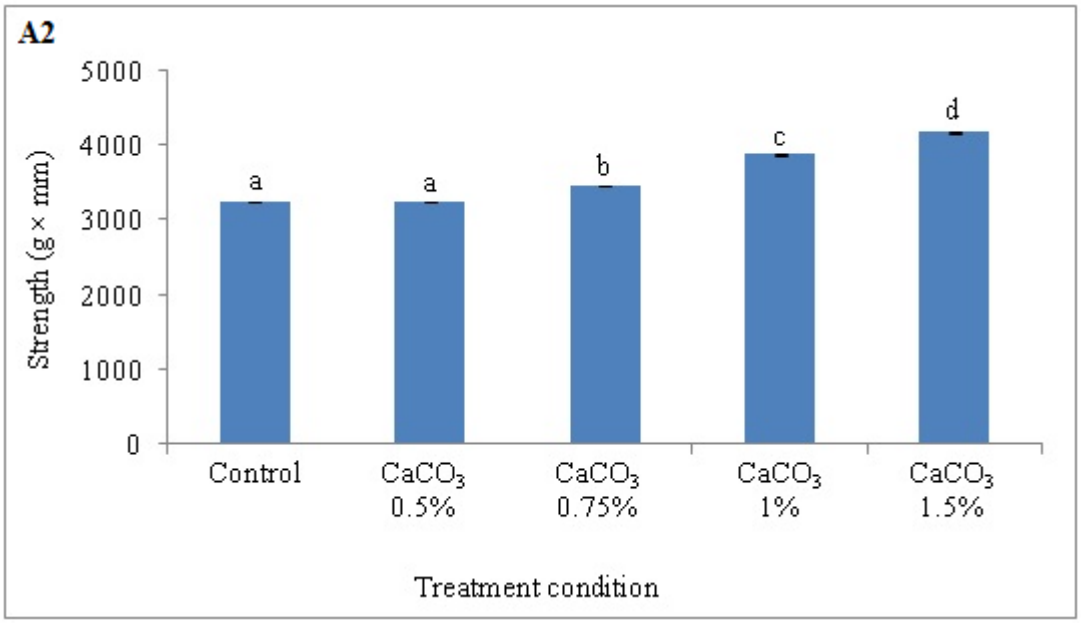


Fig. 6. Breaking force (A1) and strength (A2) of surimi gel produced from sardine (*Sardina pilchardus*) under different concentrations of Calcium carbonate ($CaCO_3$) treatment

Improvement of surimi gel properties with increasing of $CaCO_3$ concentrations was also demonstrated by Benjakul et al. [2] who reported that breaking force and deformation of the mixed SSA grade surimi gel increased with increasing concentration of calcium carbonate added ($p < 0.05$). This result suggested that addition of calcium ion has an enhanced role for endogenous transglutaminase activity in myosin heavy chain cross-linking during gelation as reported by Lee and Park [38] and Benjakul et al. [2]. Lee and Park [39] found that addition of calcium compounds led to reduction of myosin heavy chain and formation of high molecular weight components (>205 kDa). The appearance of high molecular weight components by cross-linking of myosin heavy chain were accompanied by increased gel strength. In their study, Wan et al. [40] reported that the breaking strength was Ca^{2+}-dependent and increased to a maximum value at 2 mM Ca^{2+}.

The WHC of untreated and treated sardine surimi with calcium carbonate at different concentrations is depicted in table 3. A distinct improvement in the WHC was observed when calcium carbonate was added ($p < 0.05$), especially for the sample added with 1.5 % $CaCO_3$. This finding indicated more bound or entrapment water in matrix of proteins. Use of calcium might have improved and strengthened gel network and ability to hold water as exhibited by improved breaking force and gel strength in samples.

3.4 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical procedure, using orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. By plotting the PCs, we can view the interrelationships between different variables, detect and interpret sample patterns, groupings, similarities, or differences [41]. PCA was applied to physico-chemical data in order to evaluate the differences and similarities among the treatments and to highlight the typical production(s) method(s) point of view whiteness and textural properties.

The first 2 PCA biplots (F1 and F2) accounted for 86.15 % (59.54 % and 26.61 %) of the variability in the data (**Figure 7**).

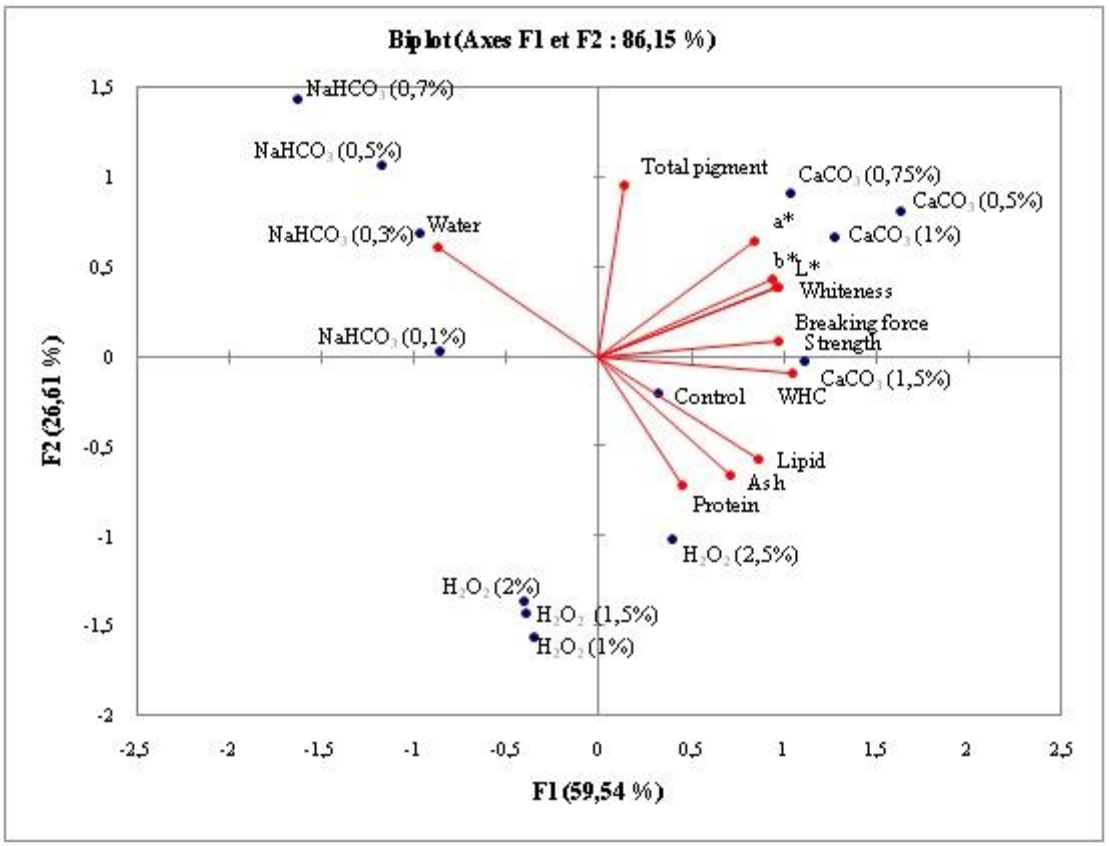


Fig. 7. Principal component analysis biplot (PCA) of the physico-chemical data of different samples of sardine surimi under various treament conditions

Vector lines with the same direction and close to each other show positive correlation between physico-chemical parameters, those with inverse direction (180°) show negative correlation, whereas if they are separated by 90°, they are independent [18]. Samples located close to a vector line of a physico-chemical parameter show strong correlation (**Figure 7**).

The figure showed that WHC, whiteness, *L** value, breaking force and strength are located close to one another indicating that they are positively correlated with each other. Moisture, which is located in the opposite quadrant, showed no correlation with the other physico-chemical parameters (**Figure 7**).

Control, H_2O_2 (2.5 %) and all $CaCO_3$ treatments are located in the right quadrant, which indicates a greater tendency for WHC, whiteness, *L*a* b** values, breaking force, strength and protein, ash, lipid and total pigments contents. However, $CaCO_3$ (1.5 %)treatment sits closer to the functional properties (Whiteness, WHC, breaking force and gel strength) vector lines than the other $CaCO_3$ treatments. The rest of H_2O_2 and all the $NaHCO_3$ treatments lie in the left quadrant.

All the $NaHCO_3$ treatments sit in the upper-left quadrant which indicate a strong correlation with moisture content (Figure 7).

From this analysis, we noted that different production methods led to significant changes in the quality of surimi samples.

These findings showed that optimal treatments are $CaCO_3$ (1.5 %) and H_2O_2 (2.5 %). Addition of 1.5 % $CaCO_3$ improved whiteness and textural properties, however the use of 2.5 % H_2O_2 during washing step showed some textural damage but resulted in a higher whiteness.

From the safety point of view, hydrogen peroxide has a short half-life in the environment and lack of any residues of toxicological concern. Thus, the residue of this compound in washed mince would be negligible owing to his instability. Additionally, the washing media was removed from the mince, leading to the lower amount of H_2O_2 remaining [27]. On the other hand, it was demonstrated that residual H_2O_2 trapped in the minced flesh is converted via catalase, which exists in the flesh cells, into oxygen and water which are non-toxic products. So H_2O_2 can be characterised as a processing aid rather than a food additive [22].
According to the EFSA Panel on Food Additives and Nutrient Sources added to Food [42], in the EU, calcium carbonate is authorised as a food additive. It was previously evaluated by the Joint FAO/ WHO Expert Committee on Food Additives (JECFA) in 1965, when the committee established an Acceptable Daily Intake (ADI) not limited. Calcium carbonate is also included in the list of substances that may be added for specific nutritional purposes in foods for particular nutritional uses and in Directive 2002/46/EC relating to food supplements. The Panel notes that the estimated exposures to calcium from all sources, including the use of calcium carbonate as a food additive, taken together with intakes of calcium from food fortification and from supplements are below the UL of 2500 mg/day for calcium from all sources established by the Scientific Committee for Food (SCF) in 2003. The Panel concludes that trace levels of adventitious nanoscale material within macroscale calcium carbonate are not of toxicological concern.

4. CONCLUSION

Whitening agents used in this study directly affected the physico-chemical properties of surimi made from sardine (*Sardina pilchardus*).Addition of various concentrations of calcium carbonate during surimi preparation lead to enhance whiteness. Textural properties also indicated increased values of breaking force and strength indicating gel strengthening along with increased whiteness. Bleaching with 2.5 % H_2O_2 significantly improved the *L** value, whiteness and total pigments content of surimi ($p < 0.05$).However,some textural damage and a reduction in WHC values were observed for surimi treated with$NaCO_3$ ($p < 0.05$). Proper selection of the type and concentration is very important when whitening agent isused to modify the colour and textural properties of sardine surimi. Thus, PCA bipolt was used and showed that, generally, 1.5 % $CaCO_3$ and 2.5 % H_2O_2 were the most appropriate whitening agents in term of gel property and colour improvement.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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