# Characterization of Sardine Minced Flesh (*Sardinella aurita*) Washed with Different Solutions

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**ABSTRACT.** Minced sardine was washed with sodium bicarbonate (0.5%) and water to evaluate their effectiveness in removing components such as protein, fat, ash and non-protein nitrogen. Mince washed with $NaHCO_3$ showed greater reductions in protein and nitrogen compounds and had a lighter color, while water washed mince showed highest reductions in ash and lipid components. Moisture and pH increased with the number of washing exchanges. Centrifugation after each washing cycle did significantly reduce the components compared to a one step centrifugation dewatering process. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: <getinfo@haworthpressinc.com> Website: <http://www.HaworthPress.com>]*

**KEYWORDS.** Mince, washing, sardine

## *INTRODUCTION*

Surimi, a washed fish mince, has great potential as a functional protein and could be a substitute for traditional animal and vegetable proteins (Maza, 1995). Tropical countries could utilize sardine and mackerel to develop sea-

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food products, but, due to high fat and myoglobin content, it is necessary to develop methods to remove undesirable components (Putro, 1989). Roussel and Cheftel (1988) reported that products made from sardine mince are characterized by a high fat content, dark colored meat and strong odors, which decrease its frozen storage life. Washing minced fish is the initial step in obtaining a white, odorless and bland surimi as it removes substances that promote protein denaturation during frozen storage and enhances the functional properties of proteins (Lee, 1984). Various methods have been developed to effectively process fish into surimi, including washing meat with $NaHCO_3$ solution to remove fat and dark muscle (Putro, 1989; Hennigar et al., 1988; Min et al., 1987; Nishioka, 1993).

The objective of this study is to determine chemical and physical characterization of minced sardine flesh washed with different solutions. Special emphasis is being given to the quantity of protein, fat, ash and non-protein nitrogen removed from minced sardine flesh after treatment with 0.5% sodium bicarbonate solution.

## *MATERIALS AND METHODS*

### *Sample Preparation*

Sardines (*Sardinella aurita*) used in this investigation were obtained form Central Fisheries Market and taken to the Food Science and Technology Institute where they were stored overnight in ice. The fish were headed and gutted, boned, and washed to remove remaining visceral tissue. Fillets were minced using a Yanagiya N-16 (S) fish separator machine, equipped with a 0.4 mm drum. Then mince was divided into nine batches (150 g each). Six mince batches were separately washed three times with 0.5% $NaHCO_3$ using a 1:5 solution:flesh ratio. Three of the batches were centrifuged after each washing step, and the remainder were centrifuged after the three washing steps. Three separate batches were utilized for a water-wash process. Wash water was cooled with ice to 4-8°C and added to the mince. This was gently stirred for 5 min and then allowed to stand for an additional 5 min, until meat had settled and water decanted. Final dewatering was carried out by using a Model Sorlvat Centrifuge operating at 2000 rpm × 15 min at 5°C. Washing efficiency was measured by comparing moisture, protein, ash, solid and fat contents remaining after each washing cycle.

### *Chemical Analyses*

Proximate composition of each treatment was determined in triplicate, according to AOAC procedures (AOAC, 1980). The pH was determined

using a digital pH meter (model 8417 HANNA Instruments). Trimethylamine (TMA) of the separated flesh and minced meat was determined by picric acid procedure (AOAC, 1980). Phosphorous and calcium analysis content was performed by standard methods (AOAC, 1980). Luminance (L* value), redness (a* value) and yellowness (b* value) were measured with a Hunter Color Difference Meter, based on a white standard tile (L* = 92.8; a* = −1.1; and b* = 0.4).

Volatile nitrogen was measured by distillation with MgO as catalyst (Standby, 1976). Non protein nitrogen (NPN) was obtained using trichloroacetic acid protein precipitation by method of Murray and Gibson (1972), and determined by micro Kjeldahl methods. Measurement of oxidative rancidity was estimated using 2-thiobarbituric acid (TBA) reagent with EDTA and PG 0.5% (Rhee, 1978). Extractable total protein was determined using method described by Arai (1974). Sarcoplasmic protein was determined using method described by Hasshimoto and Watanabe (1979). Wastewater from each washing cycle was analyzed by micro Kjeldahl method (AOAC, 1980).

## *RESULTS AND DISCUSSION*

Physical and chemical characteristics of sardine washed mince are shown in Table 1. Washing increased the moisture content and was the highest in mince washed with sodium bicarbonate (81.79%). This could be due to the water absorption phenomenon of myofibrillar protein described by Adu et al. (1983). Susuki (1987) reported that several washings increased hydrophilic properties of muscle, original muscle swelling, and increased difficulty of

TABLE 1. Proximate Composition of Sardine (*Sardinella aurita*) Washed Minced with Different Solutions*

| | UNWASHED | WASHED WITH 0.5% $NaHCO_3$ | WASHED WITH WATER |
|---|---|---|---|
| MOISTURE (%) | 74.32 ± 0.31 | 81.79 ± 0.16 | 74.41 ± 0.32 |
| ASH (%) | 1.27 ± 0.021<br>(4.06) ± 0.021 | 0.74 ± 0.012<br>(5.08) ± 0.012 | 0.44 ± 0.037<br>(2.62) ± 0.037 |
| PROTEIN (%) | 14.67 ± 0.43<br>(55.01) ± 0.43 | 10.01 ± 0.014<br>(58.68) ± 0.014 | 12.72 ± 0.17<br>(53.33) ± 0.17 |
| LIPID** (%) | 7.65 ± 0.16 | 4.38 ± 0.0082 | 3.48 ± 0.19 |
| pH | 6.2 | 8.6 | 6.89 |

* Data presented in table are means ± standard deviations of 3 measurements.
Dry weight values in parentheses.

water removal. Roussel and Cheftel (1988) reported a moisture increase from 74% to 81% in mince washed with sodium bicarbonate 0.5%. Babbitt (1986) reported a moisture increase of 7.6% for washed mince of Alaska pollock.

Ash content of mince decreased with washing exchanges, with the lowest in mince washed with water (4.06% to 2.62%). The ash content in mince washed with sodium bicarbonate was 5.08%. This could be due to sodium incorporation into mince by the sodium bicarbonate washing solution. Adu et al. (1983) reported a reduction from 4.43% to 1.30% (80%) of ash in water washed mince. Pacheco-Aguilar et al. (1989) reported an ash reduction of approximately 73% in water washed mince in Pacific whiting.

On a dry-weight basis, protein content of washed sardine mince increased compared to unwashed mince, while washing mince with sodium bicarbonate increased protein concentration from 55.01% to 58.68%. Roussel and Cheftel (1988) reported that washing produced a water-soluble protein reduction and an apparent increase in myofibrillar protein concentration. Adu et al. (1983) found a significant protein loss when wet mince was analyzed, and stated that this resulted from increased water retention and loss of other compounds during washing procedure.

Fat content decreased with different treatments showing the greatest reduction in washed mince with water (7.65 to 3.48), which represents a reduction of 57% on a dry weight basis. Adu et al. (1983) reported that repeated washing helped to remove fat content (65%). Roussell and Cheftel (1988) stated that final fat content was proportional to initial fat content. They found 80-85% removal of fat when mincemeat was washed with alkali solution. The sardine mince washed with sodium bicarbonate and water showed a pH increase from 6.2 to 8.6 and 6.9, respectively, with the highest for $NaHCO_3$ washed mince. This pH increase is due to the alkali solution utilized for washing, which is incorporated to mince.

L*, a* and b* color parameters of sardine mince are shown in Table 2. L* value of washed sardine increased markedly with 0.5% $NaHCO_3$ solution from 30.9 for unwashed mince to 49.5, while in all treatments the a* value

TABLE 2. L*, a* and b* Color Parameters of Washed Sardine (*Sardinella aurita*) Minced with Different Solutions

| | UNWASHED | WASHED WITH 0.5% $NaHCO_3$ | WASHED WITH WATER |
|---|---|---|---|
| a* | 6.8 | 0.6 | 4.2 |
| b* | 17.5 | 15.2 | 21.1 |
| L* | 30.9 | 49.5 | 29.0 |

Data presented in table are means of 5 measurements.

decreased, with lowest shown for $NaHCO_3$ washed sardine mince (0.6). The b* value showed an increase in water washed mince. Roussell and Cheftel (1988) reported a L* value around 60 on washed sardine mince with alkali solution, while the redness value a* decreased. They reported that progressive removal of pigments such as hemoglobin, myoglobin and possibly skin pigments, led to a grayish color of the mince.

The dewatering effects on mince washed with 0.5% $NaHCO_3$ solution are shown in Table 3. No significant differences were observed among the treatments, although mince centrifuged after each washing step showed higher values for proximate composition. Pacheco-Aguilar et al. (1989) reported that dewatering between multiple water exchanges was more efficient to remove lipid than a single exchange.

Since sardine mince washed with 0.5% sodium bicarbonate showed the highest reduction of soluble protein, a specified analysis of nitrogen compound removal was done. Physical and chemical evaluations of washed mince are summarized in Table 4. Moisture increased with washing exchanges, and after the third exchange, the original value increased 6.33%. Lee (1986) reported that excessive agitation or a long washing time often resulted in hydration of the muscle tissue and difficulty in water removal from washed meat during the dewatering operation. Therefore, residence time should be kept to a minimum, just long enough for adequate extraction. Although some solids are removed during washing, final weight of washed mince can increase significantly (Adu et al., 1983).

Ash content decreased with washing, the highest reduction being after the first washing, from 6.78 to 4.97, representing a 34% reduction based on unwashed mince. Pacheco-Aguilar et al. (1989) reported a reduction of 79.84% of ash, and stated that the amount of removed ash after one washing

TABLE 3. Effects of Water Removal on Sardine (*Sardinella aurita*) Minced with $NaHCO_3$ (0.5%)*

| | 0.5% $NaHCO_3$ (1) | 0.5% $NaHCO_3$ (2) |
|---|---|---|
| MOISTURE (%) | 81.90 ± 0.30 | 81.91 ± 0.19 |
| SOLID (%) | 18.10 ± 0.30 | 18.08 ± 0.19 |
| ASH (%) | 0.75 ± 0.01 | 0.71 ± 0.014 |
| PROTEIN (%) | 10.19 ± 1.18 | 10.74 ± 0.51 |
| LIPID (%) | 4.48 ± 0.35 | 4.54 ± 0.17 |

(1): Centrifuged after three washing exchanges with a water removal between them.
(2): Centrifuged after three washing exchanges without water removal between them.
* Data presented in table are means ± standard deviations of 3 measurements.

TABLE 4. Physical and Chemical Characteristic of Washed Sardine (*Sardinella aurita*) Minced with $NaHCO_3$ (0.5%) Solution

| | UNWASHED MINCE | FIRST WASH | SECOND WASH | THIRD WASH |
|---|---|---|---|---|
| MOISTURE (%) | 79.74 ± 0.22 | 82.73 ± 0.45 | 84.47 ± 0.10 | 86.07 ± 0.17 |
| ASH (%) | 1.38 ± 0.03 (6.78) ± 0.03 | 0.83 ± 0.04 (4.97) ± 0.04 | 0.68 ± 0.038 (4.34) ± 0.038 | 0.061 ± 0.08 (4.44) ± 0.08 |
| PHOSPHOROUS (P mg %) | 0.47 ± 0.021 | 0.29 ± 0.012 (38.3)[a] | 0.093 ± 0.001 (80.42)[a] | 0.063 ± 0.005 (87.45)[a] |
| CALCIUM (g %) | 1.56 ± 0.038 | 1.11 ± 0.06 (28.47)[a] | 0.97 ± 0.01 (35.76)[a] | 0.87 ± 0.03 (42.38)[a] |
| PROTEIN (%) | 13.95 ± 0.34 | 12.55 ± 0.078 | 10.34 ± 0.26 | 9.45 ± 0.39 |
| PROTEIN (% dry weight) | 69.32 ± 1.69 | 74.25 ± 0.45 | 66.58 ± 1.69 | 68.47 ± 2.83 |
| SOLID (%) | 20.25 ± 0.22 | 17.0 ± 0.45 (15.66)[a] | 15.53 ± 0.1 (22.8)[a] | 13.93 ± 0.17 (31.36)[a] |
| LIPID (%) | 4.58 ± 0.18 | 3.45 ± 0.07 | 3.28 ± 0.08 | 3.14 ± 0.11 |
| TBA (μg/g) | 9.38 ± 0.44 | 5.37 ± 0.02 (40.88)[a] | 4.08 ± 0.05 (54.36)[a] | 3.97 ± 0.02 (56.13)[a] |
| pH | 6.75 | 7.96 | 8.24 | 8.26 |
| TMA (mg N %) | 3.07 ± 0.12 | 0.88 ± 0.22 (71.6)[a] | 0.32 ± 0.016 (89.68)[a] | 0.28 ± 0.02 (90.97)[a] |
| N-TVN (mg-N %) | 18.73 ± 1.75 | 10.23 ± 1.27 | 6.86 ± 0.25 | 6.29 ± 0.27 |
| **NPV (g N/g) | 35.0 | 14.0 (60)[a] | 13.8 (60.57)[a] | 8.7 (75.14)[a] |
| **P. SARCOPLASMIC | 28.46 | 7.8 (72.59)[a] | 7.8 (72.59)[a] | 6.2 (78.21)[a] |

[a]: reduction %
Dry weight in parentheses.
** NPN: non protein nitrogen; sarcoplasmic protein.
Data presented in table are means ± standard deviations of 3 measurements.

exchange depended upon the amount of water in contact with a unit weight of mince fish flesh, but not on a directly proportional basis. Adu et al. (1983) stated that greatest reduction in ash (80%) was found in washed mince 4:1 water:flesh ratio. Mestiri et al. (1992) reported a 39.2% reduction on washed mince 1:5 water:flesh ratio. On wet-weight basis, protein content of sardine mince decreased with washing steps from 13.95% to 9.45%; however, protein content on a dry-weight basis increased from 69.32% to 68.47%.

After the first washing procedure, 68.79% of solids were recovered, similar to the yield obtained by Pacheco-Aguilar et al. (1989). Adu et al. (1983) reported 63% for solid and 77% for protein recoveries for rockfish, using two exchanges at a 4:1 water:flesh ratio. Babbitt (1986) reported 28% loss of solids and 75% total protein recovery for Alaskan pollock. Lee (1986) reported that the amount of protein extracted increased markedly with a washing time extended up to 9-12 minutes. Ortiz (1990) stated that higher protein reduction on cachama (*Colossoma macroporum*) and sardine mince occurred after the first washing step and decreased with additional washing steps with a 1:3 mince:water ratio and one minute agitation. Lipid was reduced from 4.58% to 3.14%, which represents a reduction of 32% based on a dry weight basis, the greatest reduction occurring in the first washing exchange mince. Roussell and Cheftel (1988) reported 80% lipid reduction on washed sardine mince with 0.5% $NaHCO_3$ solution in a 10 minute exchange, and stated that removal of lipid component depended on initial lipid content in mincemeat.

The first washing step removed 71.6% of TMA, and losses increased with subsequent washing exchanges. Pacheco-Aguilar et al. (1989) stated that dewatering between washing steps was more efficient to remove TMAO. Adu et al. (1983) reported that mince washing greatly reduced the amount of TMAO in washed flesh. They also stated that although TMAO remaining in dried washed flesh was completely degraded to TMA and DMA during storage at $-30°C$, the amount of TMA and DMA formed was quite low as compared with dried fillet and dried unwashed mince flesh. The greatest reduction in TBA was observed in the first washing (57.25%) (Table 4). Gutierrez (1990) reported that washed mince from cachama (*Colossoma macroporum*) stored at $-20°C$ developed less oxidative value than unwashed mince. Tseo et al. (1983) stated that leaching did not prevent the increase in oxidative rancidity or thiobarbituric (TBA) values associated with refrigerated deboned flesh; however, TBA values were significantly lower than in unwashed flesh. They indicated that washing improved product quality in frozen fish stored for an extended period of time.

Phosphorus and calcium components decreased with the number of washing exchanges from 0.47 mg% for phosphorus and 1.56 g% for calcium, to 0.063 mg% and 0.87 g%, respectively, after the third washing step. Based on unwashed mince values, it represents a reduction of 87% for phosphorus and 56% for calcium. Adu et al. (1983) reported a 68% phosphorus reduction in different fish species washed with water and two washing exchanges, using a 1:4 mince:water ratio. Mestiri et al. (1992) reported a mineral reduction of 39.2% during the water wash process of fish mince.

$NaHCO_3$ washed mince produced a progressive increase in pH, being higher after the third washing exchange, varying from 6.75 to 8.26. Shimizu et al. (1992) stated that pH of washing water affected water retention during the washing process, as well as water holding capacity and gel forming ability. They found that Pacific mackerel mince leached by alkali solution decreased the denaturation rate as the muscle pH increased, and recommended use of an alkaline leaching solution to maintain pH around 6.5-7.0.

One of the objectives of washing mince was the reduction of nitrogenous compounds such as sarcoplasmic proteins. The non-protein nitrogen losses increased notably with washing exchanges, and ranged from 60% in the first washing step to 75.14% after the third exchange, whereas sarcoplasmic protein reduction increased from 72.59% to 78.21% after the third washing step. Roussell and Cheftel (1988) reported a 50% reduction in total nitrogen in sardine mince with $NaHCO_3$ after the third washing. Nunes et al. (1992) reported a 35% reduction of sarcoplasmic proteins during the first washing exchange, and stated that this reduction was less in following washing steps. They found that flavor compounds, pigments and other substances were removed with the washing exchange. Adu et al. (1983) reported a 77% protein reduction during the washing process.

The effects of washing on nitrogenous compound reduction on wastewater was analyzed and shown in Table 5. Nitrogenous components, such as sarcoplasmic protein and non-protein nitrogen (NPN), were removed in the two first washing steps, with the highest nitrogen content (15.9 g N/g) found in the first waste water and decreasing afterward. Salas (1994) reported that 81.2% of total nitrogen and 88.2% of protein nitrogen were extracted after the first and second washing exchanges. Giaimo (1982) studied the effect of washing exchanges over different species, and stated that non-protein nitrogen was eliminated through the third washing, then variation was less significant.

TABLE 5. Nitrogen Content Waste Water from Washed Sardine (*Sardinella aurita*) Minced with $NaHCO_3$ (0.5%) Solution*

| | FIRST WASHED | SECOND WASHED | THIRD WASHED |
|---|---|---|---|
| NON-PROTEIN NITROGEN (g N/g) | 2.2 ± 0.22 | 1.8 ± 0.16 | 0.76 ± 0.022 |
| PROTEIN NITROGEN (g N/g) | 13.76 ± 0.21 | 4.29 ± 0.16 | 2.63 ± 0.13 |
| TOTAL NITROGEN (g N/g) | 15.9 ± 0.082 | 5.88 ± 0.10 | 3.36 ± 0.07 |

* Data presented in table are means ± standard deviations of 3 measurements.

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