# INFLUENCE OF WASHING AND FROZEN STORAGE ON THE MYOFIBRILLAR PROTEIN FRACTION IN SARDINE MINCE FLESH.

## Influencia del lavado y almacenamiento congelado en la fracción de las proteínas miofibrilares de la pulpa de sardina.

***Marinela Barrero*** [1]***, Ynes Castillo y Makie Kodaira*** [2]

[1,2] *Instituto de Ciencia y Tecnología de Alimentos. Facultad de Ciencias. Universidad Central de Venezuela. Caracas 1042-A. Venezuela. E-mail: mbarrero@ciens.ucv.ve*

**ABSTRACT**

Frozen storage of fish species, such as sardine, result in detrimental changes in functional properties that determine storage life. Sardine meat is characterized by high fat content, dark meat, and sarcoplasmic proteins that inhibit gel formation. Washing mince flesh with solutions such as sodium bicarbonate is very effective for removing undesirable components. The objective of this research was to study the effects of frozen storage at -30°C in the myofibrillar protein fraction of sardine mince flesh washed with 0.5% sodium bicarbonate solution. Samples of sardine-minced flesh were washed three times with a 0.5% of sodium bicarbonate solution and centrifuged at 3000 rpm for 15 minutes. These samples were divided in lots of 100 g. packed in plastic bags and stored at -30°C, and analyzed every 30 days for 150 days. The myofibrillar proteins were extracted using a phosphate buffer (tris HCl, KCl, EDTA, pH 7.6), and evaluated by SDS-PAGE. The bands were analyzed and digitalized with a Gel Doc 2000 and Quality One 4.1.1 by Bio-Rad. The main bands of myofibrillar protein were identified by comparison of these against a prestained molecular weight standard. After 60 days there was deterioration of the myofibrillar protein fraction with apparent molecular weight between 220 and 65KD, and the formation of molecular aggregates at high molecular weight occurred. After 120 days due to myofibrillar protein deterioration, protein and peptides with low molecular weight were formed and increasing throughout frozen storage. Understanding the mechanism involved in the deterioration of the mince flesh during frozen storage we would enable to help the establishment of quality parameters and ability to predict storage life for that product.

**Key words:** Sardine, frozen storage, myofibrillar protein, sodium bicarbonate, washing.

**RESUMEN**

El almacenamiento congelado de especies pesqueras, como la sardina, resulta en cambios significativos en sus propiedades funcionales las cuales determinan su tiempo de vida en almacenamiento. La pulpa de sardina se caracteriza por un alto contenido de grasa, músculo oscuro, y proteínas sarcoplasmáticas que inhiben la formación de geles a base de esta pulpa. Aplicando tratamiento de lavado a la pulpa de sardina se remueven compuestos indeseables para la preparación de productos a base de esta pulpa y a la vez aumentando su tiempo de vida en anaquel. El objetivo del presente estudio fue evaluar el efecto del almacenamiento en congelación a –30°C sobre la fracción de las proteínas miofibrilares de la pulpa de sardina tratada con soluciones al 0,5% de bicarbonato de sodio. Lotes de pulpa de sardina se le aplicó tratamiento de lavado con una solución de bicarbonato de sodio al 0,5% y luego centrifugadas a 300 rpm por 15 min. para la eliminación del agua remanente. Lotes de 100 gr. fueron empacados en bolsas de polipropileno y almacenadas a –30°C y analizadas cada 30 días durante150 días. Las proteínas nmiofibrilares fueron extraídas con buffer fosfato (tris HCl, KCl, EDTA, pH 7,6), y evaluadas por la técnica de electroforesis, SDS-PAGE. Las bandas de las diferentes proteínas y sus productos de degradación fueron analizadas y digitalizadas utilizando un Gel Doc 2000 y un programa Quality One 4.1.1 de Bio-Rad. Las principales bandas y sus productos de degradación fueron identificados por comparación de estos contra un estándar de peso molecular. A los 60 días se observó el comienzo del deterioro de las proteínas miofibrilares con pesos moleculares aparentes entre 220 y 65KD, y la formación de agregados moleculares de alto peso molecular. A los 120 días este deterioro se hace más pronunciado apareciendo gran cantidad de bandas de bajo peso molecular, péptidos, los cuales incrementan a medida que transcurre el tiempo de almacenamiento congela-

Recibido: 14 / 11 / 2005. Aceptado: 23 / 01 / 2007.

do. Sin embargo, estos cambios son menos severos que los observados en la pulpa de sardina sin tratamiento (control). La evaluación de los cambios que envuelven el deterioro de la pulpa de sardina en congelación podría ayudar a establecer parámetros de calidad y permitir predecir el tiempo de vida útil de los productos a base de estas pulpas.

**Palabras clave:** Sardina, almacenamiento congelado, proteínas miofibrilares, tratamiento con bicarbonato de sodio.

## INTRODUCTION

The sardine (*Sardinella aurita*) is a very important low cost fish resource in Venezuela. The annual catch is about 110,000 metric tons [14] with large amount of this catch used for food and canned products and a large amount of this catch is used for animal food and canned products. The consumption of fresh sardine or its frozen sardine products is not well accepted by the consumer because of the high fat content, large percentage of dark muscle, and high concentration of sarcoplasmic proteins. One alternative to increase sardine consumption could be the production of sardine mince flesh. Sardine mince flesh production is a relatively simple process in which muscle is separated from bones yielding a dark flesh meat. The process of producing mince flesh combines muscle components such as lipids, sarcoplasmic proteins, and digestive enzymes, inorganic salts, and low molecular weight organic substances that induce myofibrillar protein denaturation. Myofibrillar proteins are the most important muscle component since they are responsible for the texture attributes and functional properties of muscle in foods [4, 5, 15].

Washing treatment on fish mince flesh helps remove those components that produce denaturation of myofibrillar protein and help to increase gel formation and myofibrillar protein concentration for further mince flesh based product production. The importance of washing treatment is to removes pro-oxidants and components susceptible to lipid oxidation. Several studies have been conducted using washing treatment solutions such as sodium chloride, sodium bicarbonate; sodium phosphate and water to enhance the quality of fish mince flesh [4, 5, 10, 12, 16, 19, 20]. These studies stated that treatment of washing on the fish mince flesh significantly reduces soluble proteins, pro-oxidative enzymes, lipids, and increased gel-forming ability and improved color properties of the final product.

Frozen storage of fish mince flesh has been largely used for preservation of food by decreasing microbial. Conversely, during frozen storage fish mince flesh become unstable and undergoes a number of alterations that determine the end of its storage life. Frozen storage induces protein aggregation, causing hardening of the muscle. Myofibrillar proteins undergo denaturation and aggregation when the water and associated solutes in the tissue are lost due to dehydration by freezing. This process produces an undesirable texture for the products elaborated from this raw material. Hydrophobic interactions have been identified as a cause of lower extractability and reduction of the functionality of the myofibrillar protein. Similarly, during frozen storage formaldehyde increases its interaction with myofibrillar proteins accelerating their denaturation and aggregation [1, 3, 7, 8, 9, 13, 17]. Moreover, several researchers have concluded that washing, fish mince, will decrease the stability of its products when frozen due to the removal of oxidative compounds and the increased polarity of the residual lipids [10, 12, 20].

For a better understanding of the effects of storage and the subsequent deterioration of the mince flesh during frozen storage, and to help establish quality parameters that would be used to predict storage life for products made with sardine mince flesh the present study evaluated the effects of frozen storage at -30°C for 150 days on the myofibrillar protein fraction of sardine flesh washed with 0.5% sodium bicarbonate solution.

## MATERIALS AND METHODS

### Material

Sardines were caught from Sucre State, Venezuela, and transported in insulated boxes with ice to the Food Science Technology Institute in Caracas. After reaching the laboratory, the fish were deheaded, gutted and treated with 0.5% sodium bicarbonate ($NaHCO_3$) solution (1:5 mince flesh:water) (FIG. 1) following the procedure stated by Barrero and Bello [5]. Mince flesh from sardines treated with $NaHCO_3$ 0.5% solution and a control (sardines mince flesh that were not washed) were divided into 100 g lots, packed, frozen at –30°C and stored at -30°C for 150 days until analysis.

**Total protein extractable:** Total protein content was determined by micro-Kjeldhal method A.O.A.C. [2]. Total extractable protein in saline solution was determined according to the method of Arai [1], with the following modifications: 10g of mince flesh was homogenized with buffer saline (0.45M KCl, 3.38 mM $K_2PO_4$ and 15.5 $Na_2HPO_4$; I= 0.5, pH 7.5). After 24 hr the supernatant was collected and protein content was determined by micro-Kjeldhal method A.O.A.C. [2].

**SDS-PAGE:** Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Hashimoto *et al*. [11]. Protein extraction was performed following the procedure stated by Ashie *et al*. [3]; 5 g of mince flesh was homogenized with buffer (tris HCl, KCl, EDTA, pH 7.6). Extracted protein was adjusted to 80 ug/ul following method stated by Lowry et al. [16] and subjected to electrophoresis in 12% polyacrylamide. Protein molecular weight standard markers ranging from 14.300 to 200.000 DA were purchased from Gibco BRL MA7405. After electrophoresis, the gels were stained with Coomassie Blue R-250 for 20 minutes and distained with 10% Acetic acid, 10% methanol and 80% distilled

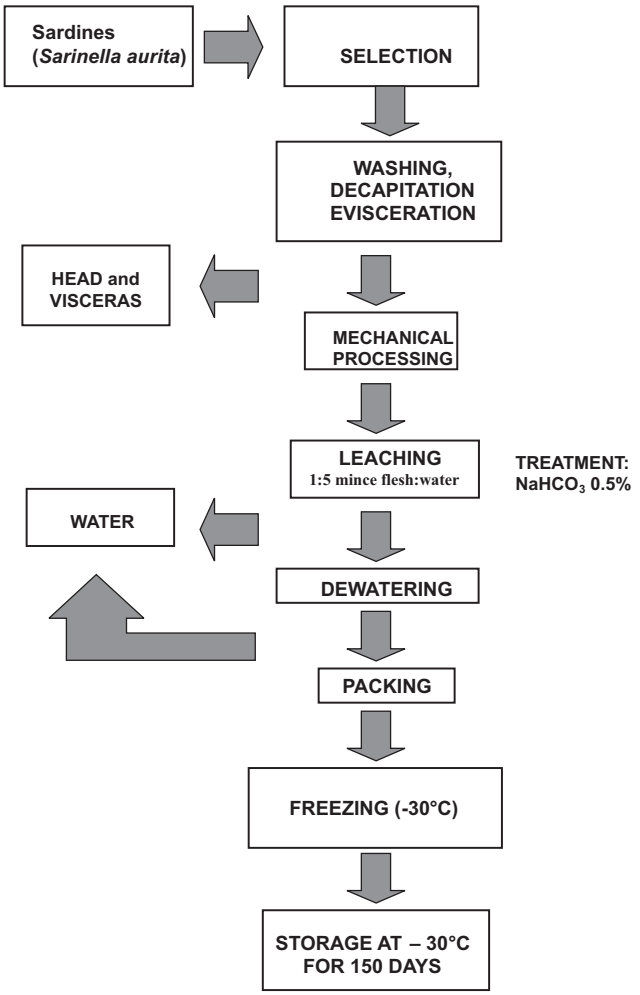


**FIGURE 1. SARDINE MINCE FLESH MANUFACTURING STEPS/** DIAGRAMA DE FLUJO PARA LA ELABORACION DE PULPA DE SARDINA.

water solution for 24 hr. The bands of proteins were digitized and their optic density obtained using Gel Doc 2000 Bio-Rad and analyzed by Quantity One 4.1.1 Bio-Rad software.

**Statistic analysis:** All data were analyzed using Staf Grafic 6.0 (Manugistics, Inc., Rockville MD, USA). The total extractable protein was evaluated using ANOVA al a significant level of 95%. The dependent variable was the concentration for each days evaluated.

## RESULTS AND DISCUSSION

### Total extractable protein

The initial amount of protein extractable in saline solution was significantly ($P < 0.05$) higher for sardine mince flesh control (7.79%) compared to sardine mince flesh treated with 0.5% $NaHCO_3$ solution (6.83%) (TABLE I). Total extractable protein decreased from 6.22% to 3.67% for the control after 150 days of frozen storage, representing 41% of the total extractable protein. The most drastic change from 7.91% to 3.67% (53%) was between 120 and 150 days of storage at –30°C. Conversely, sardine mince flesh treated with 0.5% $NaHCO_3$ solution decreased drastically from 6.83% to 2.81% representing 58% of the total extractable protein after 30 days of storage at -30°C; thereafter the protein extractable decreased 46% after 150 days of the frozen storage. Lack of protein solubility during frozen storage was due to interactions responsible for aggregation of the myofibrillar proteins Benjakul *et al*., [6] stated that these interactions included disulfide bridges as well as formaldehyde formation. They evaluated physicochemical changes of some tropical fish muscle proteins during frozen storage and found that formaldehyde is an effective cross-linker that induces aggregation of protein thereby decreasing protein solubilization. They also noted a decrease in saline protein solubilization due to the exposure of reactive sulfydryl groups that induce oxidation or disulfide exchange. Moreover, formaldehyde is responsible for oxidation of sulfydryl groups inducing protein aggregation. Similarly, Careche *et al.* [8] stated that the myosin heavy chain is the most involved protein in aggregate formation. They evaluated the influence of frozen storage temperature to the type of aggregation of miofibrillar proteins in cod (*Gadus morhua)* fillets concluding myosin was more involved than actin in the aggregates at –30°C.

The difference in protein extractability between sardine mince flesh treated with 0.5% $NaHCO_3$ solution and the control could be due the lack of protection effect of the myofibrillar protein by components such as lipids that are removed during washing treatment resulting in protein aggregation at the beginning of frozen storage. Montero et al. and Tejada *et al*. [18, 19] stated the protective effect during frozen storage is due to the lipids contents. They reported that the washing process and cryoprotectants could modify the organization of the myofibrillar protein favoring aggregation during frozen storage.

*TABLE I*

**TOTAL EXTRACTABLE PROTEIN (g/100g ± SE) FOR SARDINE (*Sardinella aurita*) TREATED WITH 0.5% $NaHCO_3$ SOLUTION AND CONTROL, STORED AT -30°C FOR 150 DAYS/** PROTEINA TOTAL EXTRAIBLE (g/100g ± SE) PARA SARDINA (*Sardinella aurita*) ACONDICIONADA CON UNA SOLUCIÓN DE $NaHCO_3$ AL 0,5% Y ALMACENADA A -30°C POR 150 DÍAS

| Storage time (days) | Treatment | |
|---|---|---|
| | Control | $NaHCO_3$ 0.5% |
| 0 | $7.79^a \pm 1.13$ | $6.83^b \pm 1.95$ |
| 30 | $6.22^a \pm 0.81$ | $2.81^b \pm 0.89$ |
| 60 | $6.94^a \pm 0.60$ | $3.19^b \pm 1.09$ |
| 90 | $7.91^a \pm 1.25$ | $4.34^b \pm 1.07$ |
| 120 | $4.53^a \pm 0.25$ | $2.16^b \pm 0.64$ |
| 150 | $3.67^a \pm 0.08$ | $1.52^b \pm 0.20$ |

Result from 4 replications. ANOVA statistical analysis. Assay performed in three replications. a,b- means not followed by the same letter within row differ ($P<0.05$).

**SDS-PAGE**

The electrophoresis pattern of myofibrilar protein for sardine mince flesh control and mince flesh treated with 0.5% $NaHCO_3$ solution during frozen storage at –30°C varied between treatments (FIGS. 2 and 3, TABLES II and III). The most important myofibrillar protein bands, 200 KDa, 41 KDa, 35 KDa and 31 KDa, were correlated to each standard molecular weight marker. Optic density (OD) of myofibrillar proteins increased intensity of the band and band number during frozen storage. As frozen storage advanced, the protein extracted with saline solution increased for low molecular weight products (LMWP) 30 days and after 60 days after initiation of frozen storage for the control and sardine mince flesh treated with 0.5% $NaHCO_3$ solution respectively. This could be attributed to the higher proteolytic enzyme activity, high lipid content being oxidized, and trimethylamine (TMA) content that produced LMWP in the control samples. Low molecular weight proteins were responsible for the high protein extractable values obtained for the control during frozen storage. Conversely, sardine mince flesh treated with 0.5% $NaHCO_3$ solution had lower of LMWP at the beginning of frozen storage due to the washing treatment, which eliminated low molecular weight proteins, sarcoplasmic proteins and low molecular weight compounds that can affect degradation during frozen storage. However, sardine mince flesh treated with 0.5% $NaHCO_3$ solution contained a higher proportion of high molecular weight products (HMWP) throughout frozen storage. These high proportions of HMWP could be due to the production of proteins aggregations which increased the intensity of the bands between 200 to 45 KDa regions. Since myosin and HMWP are responsible for hydrophobicity, extractable protein from sardine mince flesh treated with 0.5% $NaHCO_3$ solution decreased during frozen storage. The decrease in protein extraction during frozen storage has been reported elsewhere. Tejada et al. [19] concluded that myofibrillar proteins decreased significantly as frozen storage advanced due to the gradual change of salt-extracted proteins in the protein composition. They also stated that during frozen storage there was an increase in high molecular weight band, which did not enter the gel. Futher, Montero et al. [17] evaluated chemical and functional properties of sardine (*Sardina pilchardus W.*) dark and light muscle proteins during frozen storage and the effect of washing on mince quality. They stated that the decrease in soluble protein in the treated mince was due in part to the production of high molecular weight polymers through the increase of disulfide bonds. Also, the loss of Ca-ATPase activity due to oxidation of SH-groups on the actomyosin indicated aggregation or denaturation and this loss of activity increased considerably in the first month of storage. After 60 days of frozen storage in 0.5% $NaHCO_3$ solution, the LMWP increased in number of eletrophoretic bands until the end of storage.

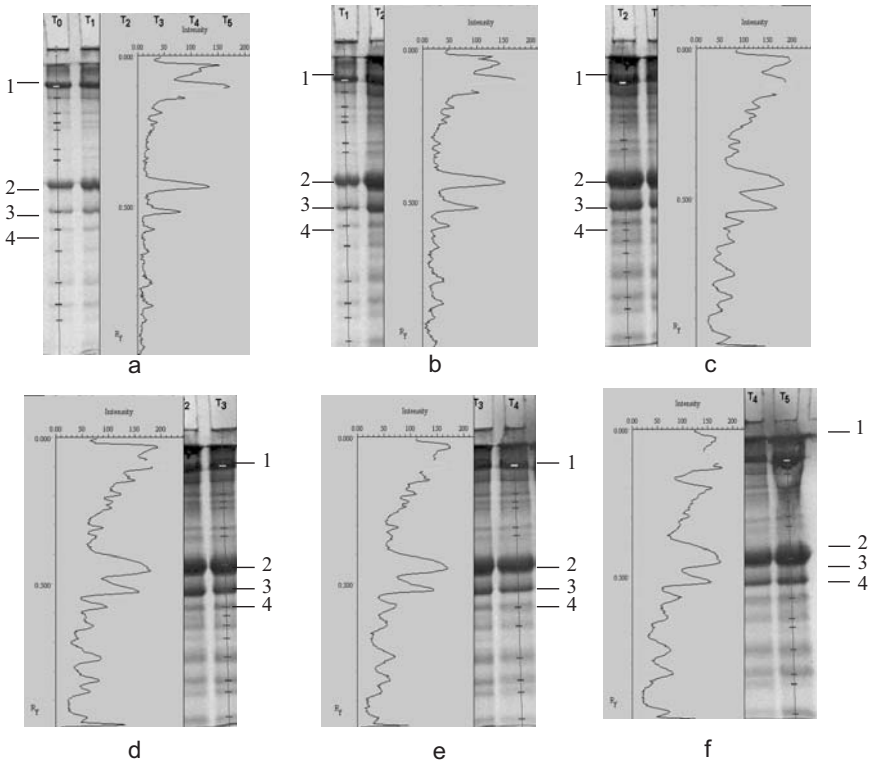


**FIGURE 2. SDS-PAGE AND OPTIC DENSITY OF PROTEIN EXTRACTED FROM SARDINE MINCE FLESH *(Sardinella aurita)* STORED AT – 30°C. A- 0 B- 30 C- 60 D-A- 90 E- 120 F- 150 DAYS. 1- 200 KDA, 2- 41 KDA, 3- 35 KDA, 4-31 KDA./**
**SDS-PAGE Y DENSIDAD OPTICA DE PROTEINAS EXTRAIDAS DE LA PULPA DE SARDINA (*Sardinella aurita*) ALMACENADA A – 30ºC. A- 0 B- 30 C- 60 D-A- 90 E- 120 F- 150 DIAS. 1- 200 KDA, 2- 41 KDA, 3- 35 KDA, 4-31 KDA.**

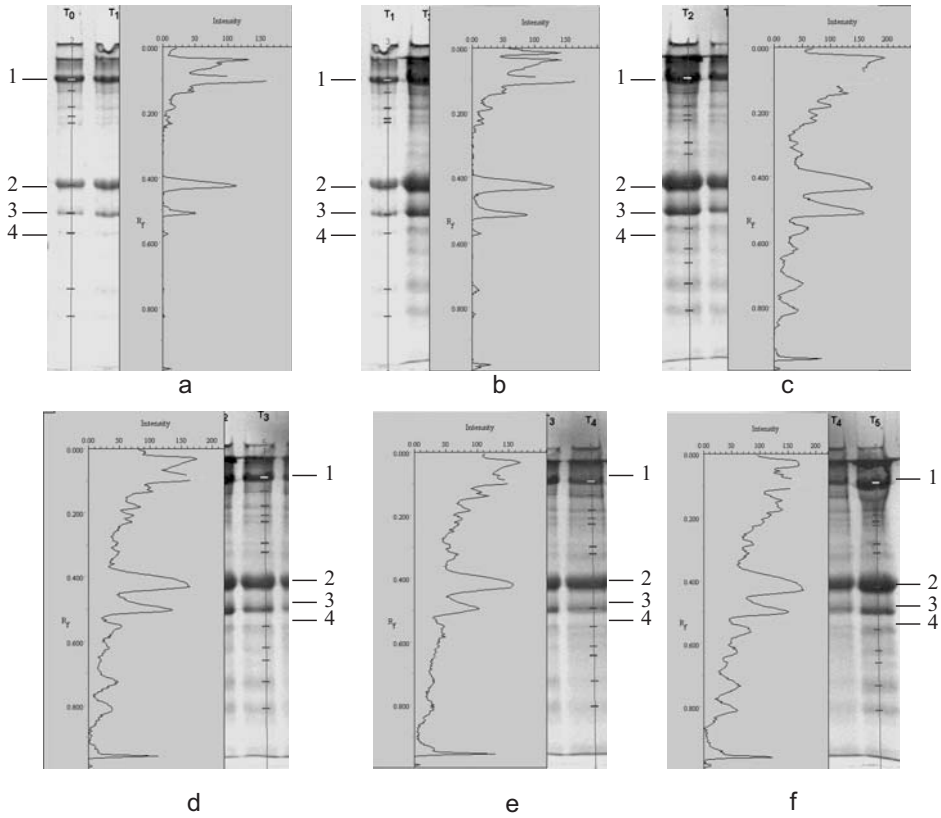


**FIGURE 3. SDS-PAGE AND OPTIC DENSITY OF PROTEIN EXTRACTED FROM SARDINE MINCE FLESH (*Sardinella aurita*) TREATED WITH 0.5% $NAHCO_3$ AND STORED AT - 30°C. A- 0 B- 30 C- 60 D- 90 E- 120 F- 150 DAYS. 1- 200 KDA, 2- 41 KDA, 3- 35 KDA, 4-31 KDA/** SDS-PAGE Y DENSIDAD OPTICA DE LAS PROTEINAS EXTRAIDAS DE LA PULPA DE SARDINA (*Sardinella aurita*) ACONDICIONADA CON $NaHCO_3$ 0.5% Y ALMACENADA A - 30°C. A- 0 B- 30 C- 60 D- 90 E- 120 F- 150 DAYS. 1- 200 KDA, 2- 41 KDA, 3- 35 KDA, 4-31 KDA.

*TABLE II*

**PROTEIN MOLECULAR WEIGHT (KDA) OBTAINED BY SDS-PAGE AND ANALYZED BY OPTIC DENSITY OF SARDINE MINCE FLESH (CONTROL) DURING FROZEN CONDITIONS AT - 30°C. /** PESO MOLECULAR DE LAS PROTEINAS OBTENIDAS POR SDS-PAGE Y ANALIZADAS POR DENSIDAD OPTICA DE LA PULPA DE SARDINA (CONTROL) DURANTE EL ALMACENAMIENTO CONGELADO A - 30°C

| Line # | Standard | 0 days | 30 days | 60 days | 90 days | 120 days | 150 days |
|---|---|---|---|---|---|---|---|
| 1 | 220.950 | 166.729 | 166.032 | 152.806 | 171.829 | 171.480 | 0.000 |
| 2 | 96.740 | 128.481 | 127.745 | 117.477 | 124.082 | 123.709 | 0.000 |
| 3 | 71.770 | 93.622 | 94.344 | 91.787 | 0.000 | 123.709 | 89.725 |
| 4 | 45.470 | 81.660 | 82.223 | 80.970 | 0.000 | 92.261 | 83.147 |
| 5 | 28.680 | 73.932 | 76.281 | 74.167 | 75.586 | 82.331 | 74.172 |
| 6 | 19.740 | 59.257 | 59.998 | 60.376 | 59.489 | 75.353 | 61.933 |
| 7 | 14.530 | 53.182 | 54.798 | 0.000 | 54.787 | 59.865 | 55.969 |
| 8 | | 41.491 | 41.287 | 41.650 | 41.680 | 55.120 | 41.238 |
| 9 | | 35.505 | 35.496 | 34.606 | 34.985 | 41.813 | 35.373 |
| 10 | | 31.766 | 31.737 | 31.633 | 0.000 | 35.478 | 31.934 |
| 11 | | 0.000 | 27.916 | 0.000 | 0.000 | 31.678 | 27.873 |
| 12 | | 21.909 | 21.695 | 0.000 | 28.109 | 27.991 | 0.000 |
| 13 | | 18.777 | 18.577 | 0.000 | 0.000 | 22.117 | 0.000 |
| 14 | | 0.000 | 16.919 | 22.444 | 22.226 | 18.779 | 18.434 |
| 15 | | | 14.558 | 18.718 | 18.637 | 17.265 | 17.278 |
| 16 | | | | 17.327 | 17.252 | 14.486 | 14.153 |
| | | | | 14.393 | 14.653 | | |

*TABLE III*

**PROTEIN MOLECULAR WEIGHT (KDA) OBTAINED BY SDS-PAGE AND ANALYZED BY OPTIC DENSITY OF SARDINE MINCE FLESH (CONTROL) DURING FROZEN CONDITIONS AT - 30ºC/ PESO MOLECULAR DE LAS PROTEINAS (KDA) OBTENIDAS POR SDS-PAGE Y ANALIZADAS POR DENSIDAD OPTICA DE LA PULPA DE SARDINA (CONTROL) DURANTE EL ALMACENAMIENTO CONGELADO A - 30ºC**

| Line # | Standard | 0 days | 30 days | 60 days | 90 days | 120 days | 150 days |
|---|---|---|---|---|---|---|---|
| 1 | 220.950 | 189.347 | 189.767 | 198.198 | 207.003 | 202.960 | 202.960 |
| 2 | 96.740 | 145.767 | 143.058 | 139.982 | 149.413 | 146.633 | 131.575 |
| 3 | 71.770 | 100.630 | 98.866 | 103.258 | 105.527 | 101.445 | 108.259 |
| 4 | 45.470 | 88.031 | 86.245 | 89.268 | 90.299 | 89.485 | 95.848 |
| 5 | 28.680 | 81.213 | 82.373 | 84.287 | 83.325 | 83.544 | 88.466 |
| 6 | 19.740 | 43.896 | 43.733 | 65.509 | 65.509 | 64.500 | 83.544 |
| 7 | 14.530 | 36.344 | 35.995 | 58.712 | 59.794 | 59.971 | 87.459 |
| 8 | | 31.701 | 31.613 | 44.018 | 44.305 | 44.715 | 67.504 |
| 9 | | 22.857 | 22.708 | 36.703 | 37.182 | 37.789 | 61.631 |
| 10 | | 19.631 | 19.479 | 32.656 | 33.082 | 33.201 | 44.426 |
| 11 | | | | 28.384 | 28.532 | 28.795 | 37.303 |
| 12 | | | | 26.259 | 26.533 | 27.321 | 32.774 |
| 13 | | | | 23.426 | 23.548 | 23.637 | 28.329 |
| 14 | | | | 20.154 | 20.259 | 20.450 | 26.486 |
| 15 | | | | | | | 23.273 |
| 16 | | | | | | | 20.239 |

## CONCLUSION

A remarkable difference between the control and washing treatment with 0.5% $NaHCO_3$ solution during frozen storage is that the washing treatment with 0.5% $NaHCO_3$ solution decreased protein denaturation (decrease solubilization of protein) keeping protein on stable conditions for further utilization. Washing treatment with 0.5% $NaHCO_3$ resulted enhance sardine mince flesh and could be recommended to decreased protein denaturation, increase storage life of further products made from sardine mince flesh. Further investigations are needed using other washing treatments such as sodium chloride, water or their combination, all of what could increase storage life of sardine mince flesh.

## ACKNOWLEDGMENTS

This study was supported by Consejo de Desarrollo Científico y Humanístico, Universidad Central de Venezuela, project PI 03-32-3843-2000.

## BIBLIOGRAPHIC REFERENCES

[1] ARAI, K. “Evaluation of fish quality from muscle protein studies”. In: **Sakana no hinshitss (Quality of fish) by Koseishe.** Ed Japanese Society of Scientifuc Fosheries. Tokio 55-80pp. 1974.

[2] ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS A.O.A.C. Official methods of analysis”. Washington, DC. 868-937pp. 1984.

[3] ASHIE, IN; SIMPSON, BR; ROMASWAMY, HS. Changes in texture and microstructure of pressure-treated fish muscle tissue during chilled storage. **J. Muscle Food.** 8:13-32. 1997.

[4] BARRERO, M.; BELLO, R. Caracterization of sardine minced flesh (*Sardinella aurita*) washed with different solutions. **J. of Agric. of Food Prod. Technol.** 9(3): 105-112. 2000a.

[5] BARRERO, M.; BELLO, R. Cambios en la composición de los ácidos grasos de la pulpa de sardina (*Sardinella aurita*) lavada con una solución de bicarbonato de sodio al 0,5 %. **Rev. Científ. FCV-LUZ.** X(2): 136-143. 2000b.

[6] BENJAKUL, S.; VISESSANGUAN, CHT.; TANAKA, M. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. **Food Res. Intern.** 36:787-795. 2003.

[7] CASTRILLÓN, A.M; NAVARRO, P.; PONTES-ALVÁREZ, E. Changes in chemical and nutricional quality of fried sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheting. **J. of Sci. Food Agric.** 75:125-132. 1997.

[8] CARECHE, M.; DEL MAZO, M.L.; TORREJÓN, P.; TEJADA, M. Importance of frozen storage temperature in the type of aggregation of myofibrilar proteins in cod (*Gadus morhua*) fillets. **J. of Agric. Food Chem.** 46: 153-1546. 1998.

[9] CARECHE, M.; HERRERO, A.M.; RODRÍGUEZ-CASADO, M.L.; DEL MANZO, M.L.; CARMONA, P. Structural changes of hake (*Merluccius merluccius L*) fillets: Effects of freezing and frozen storage. **J. Agric. Food Chem.** 47: 952-959. 1999.

[10] CHEN, H.; CHIU, E.; HUANG, J. Color gel-forming properties of horse mackerel (*Trachurus japonicus*) as related to washing conditions. **J. of Food Sci.** 62(5):985-991. 1997.

[11] HASHIMOTO, K.; WATANABE, S.; KONO, M.; SHIRO, K. Muscle protein composition of sardine and mackerel. **Bull of the J. Soc. of Sci. Fish.** 45(11): 1435-1441. 1979.

[12] HUIDOBRO, A.; TEJADA, M. Solubilization of protein aggregates formed during frozen storage of hake, sardine and mixed minces. **27th WEFTA Meeting**, Madrid, Spain, October 19-22. 2pp. 1997.

[13] HUIDOBRO, A.; TEJADA, M. Foaming capacity of fish minces during frozen storage. **J. Food. Agric.** 60: 263-270. 1998.

[14] INSTITUTO NACIONAL DE INVESTIGACIONES AGRÍCOLAS (INIA). Ministerio de Agricultura y Tierra. Condiciones ambientales en zona nororiental del país consideradas causas de escasez de sardinas. Caracas, Venezuela. Boletín de Noticias Pesqueras. 2pp. 2006.

[15] LIN, T.M.; PARK, J.W.; MORRISSEY, M.T. Recovered protein and reconditioned water from surimi processing waste. **J. of Food Sci.** 60:4-9. 1995.

[16] LOWRY, O.H.; ROSEBROUGH, N.J.; FARR, A.L.; RANDALL, R.J. Protein Measurement with the Folin Phenol Reagent. **J. of Biol. Chem.** 193:265-275. 1951.

[17] MARTÍ DE C., M.A; GÓMEZ-GUILLEN, M.C.; MONTERO, P. Influence of frozen storage on textural properties of sardine (*Sardina pilchardus*) mince gels. **Food Chem**. 60(1): 85-93. 1997.

[18] MONTERO, P.; PARDO, M.V.; GÓMEZ-GUILLÉN, M.C.; BORDERIAS, J. Chemical and functional properties of sardine (*Sardina pilchardus W*) dark and light muscle proteins during frozen storage. Effect of washing on mince quality. **Food Sci. Tech. Intern.** 5(2): 139-147. 1999.

[19] TEJADA, M.; CARECHE, M.; TORREJÓN, P.; DEL MAZO, M.; SOLAS, M.T.; GARCÍA, M.L.; BARBA, C. Protein extracts and aggregates forming in minced cod (*Gadus morhua*) during frozen storage. **J. Agric. and Food Chem.** 44: 3308-3314. 1996.

[20] UNDELAND, I.; EKSTRAND, B.; LINGNERT, H. Lipid oxidation in minced Herring (*Clupea harangus*) during frozen storage. Effect of washing and precooking **J. Agric. Food Chem.** 46: 2319-2328. 1998.