CHEMICAL, MICROBIOLOGICAL AND SENSORY EVALUATION OF A DRIED SALTED PRODUCT PRODUCED FROM SARDINES *(Sardinops caerula)* AND CEREALS[1]

Josefina C. Morales de León[2], *Michael Morrisey*[3] *and Ma. Elena Vallin*[2]

Instituto Nacional de la Nutrición, Salvador Zubirán (INNSZ), México, D.F., México

SUMMARY

The chemical, microbiological and sensory stability of a dried salted product prepared from sardines and cereals was evaluated during a 12-week storage period. The product is named NUTRIPEZ and was produced in 20 kg-lots, both with and whithout smoke treatment. The product was stored under both tropical and room conditions in Mexico City, and packaged in two types of flexible packaging. Rancidity analyses were done by the peroxide value, TBA test and sensory evaluation. The results demostrated that both the smoking of the product and the cellopolyal packaging, offered the best protection against oxidation.

INTRODUCTION

Fishery development programs have dramatically increased in the less developed countries over the last decade, and offer a great potential for many of them to improve the nutritional level of lower income families. In Mexico, this has not occurred due in part to traditionalism in the Mexican diet—which in the past has not included fishery products—, to the poor quality of the

Manuscrito modificado recibido: 17-12-90.

1 Este proyecto fue financiado parcialmente por el Consejo Nacional de Ciencia y Tecnología de los Alimentos (CONACYT).

2 Instituto Nacional de la Nutrición Salvador Zubirán (INNSZ), Departamento de Ciencia y Tecnología de los Alimentos, Vasco de Quiroga 15, Col. Tlalpan CP 14000, México, D.F.

3 Present address: University of Rhode Island, Department of Science and Nutrition, Kingston, RI 02881, USA.

fishery products now offered in the market, and to the apparent high cost of fresh fish because of an inefficient distribution system (1). For these reasons, in Mexico fishery products are not as well accepted as in many other countries. Furthermore, there is a general lack of information concerning the nutritional aspects of many of the fish species captured in Mexican waters.

Fresh fish represents the highest demand for any fishery product, but there remain the problems of proper handling and preservation of these and other products that require refrigeration, in a country where many of the lower income level families cannot afford electricity. Moreover, the most common edible species are beyond the purchasing power of these families as well. Because of this fact, development of new products that have extended shelf-life, would be more appropiate in a program directed to expand the utilization of fish in the lower income brackets.

These products therefore would be prepared applying traditional methods such as salting, smoking and drying, and would use underutilized fish species, that would allow the development of inexpensive products that could be stored for relatively long periods of time.

The National Nutrition Institute, precisely, has been conducting research in this area for several years, with some promising results. We have developed a product called NUTRIPEZ[4] which is cheap to produce, has a good nutritional composition, and an adequate consumer acceptance (1). The main ingredient, sardine, is the lowest cost per kg fish caught in Mexican waters, but it is a fat fish, susceptible to spoilage and oxidation. The purpose of this experiment, therefore, was to evaluate the chemical, microbiological and sensory characteristics of NUTRIPEZ under different storage and packaging conditions. It is hoped that the information derived from these experiments may allow the establishment of adequate mechanisms for its commercialization, in areas of the country where high-protein foods are needed.

MATERIAL AND METHODS

The raw materials, eviscerated Monterrey sardine *(Sardinops caerula)*, defatted soybean flour, cornmeal, salt and a mixture of spices (onion, pepper, chile, garlic), were obtained from different markets in Mexico City. Formulation and production of the product was also done in Mexico City, which is distant from the sardine unloading ports.

Sardines are a highly perishable product, and the only means of transferring them to the pilot plant was by freezing the raw material before transportation.

Proximate analysis (protein, fat, crude fiber, water and ashes) was carried out by the AOAC methods (2) for all of the raw materials used in the experiment. Microbial analysis count (coliforms, molds and yeasts) was performed by the procedures described for food analysis by Fernández (3). Randicity of the final product was determined by the peroxide value method, modified by Pearson (4), and by Bligh and Dyer (5); and the 2-thiobarbituric acid (TBA) test was carried out according to the distillation method of

4 Registered name.

Tarladgis (6) and Robles (7). Water activity was determined utilizing a digital instrument NOVASINA AG (CH-8050 Zurich Switzerland Model DAL-20), and a sensory analysis was accomplished by a preference test which evaluated the taste on a seven-point hedonic scale (8).

For the above-mentioned test, 10 untrained judges were selected from the laboratory personnel to evaluate each of the samples. The product NUTRIPEZ was evaluated by preparing a taco filling that would not mask the rancidity of the product (9).

Two 20 kg samples, each of both smoked an unsmoked NUTRIPEZ were prepared according to the procedures shown in Figure 1. The two products were then stored during a 12-week period at 23°C and 50% relative humidity (simulating the storage conditions in a lukewarm zone) and 35°C and 80% relative humidity (simulating storage conditions in a tropical zone). Both the smoked and unsmoked samples were each divided, for storage, into three batches. One of them served as control and was stored without any packaging whereas the other two were packaged either in a cellopolyal[5] or a low-density polyethylene flexible package. Samples were taken at 7-day intervals, and water activity, peroxide value, and TBA tests were run on each sample.

Microbiological analysis was done at 0, 6 and 12 weeks, while proximate analysis was performed at 0 and 12 weeks. The results were statistically analyzed by analysis of variance and the "t" Student's test to determine if significant differences existed between the samples.

RESULTS AND DISCUSSION

Results of proximate analysis of the raw materials (Table 1), used for producing NUTRIPEZ, indicate that the main protein sources were the defatted soy flour (51.2% protein, dry basis) and the Monterrey sardine (58.85% dry basis).

Fat concentration was 34.4%, dry basis, and water activity of the sardine was found to be 0.969.

With respect to the microbiological analysis (Table 2), the corn flour had and aerobic mesophilic count of 15,100 CFU/gram and the mold count was 270/g, which are within the limits set by the Official Mexican Code (Norma Oficial Mexicana, 1980), for corn products (10).

The defatted soy flour results were 4,800 CFU/g for the aerobic mesophilics, and 110 CFU/g for the mold count. There are not official standards for this product, but they were within the limits set by the supplier. The Monterrey sardine had an aerobic mesophilic count (150,000 CFU/g) considerably below the limits established by the Official Mexican Code (Norma Oficial Mexicana) (11) for fresh fish with 1×10^6 CFU/gram. In all of the raw materials, the yeast count was found to be negative.

Chemical analysis on the Monterrey sardine showed a peroxide value of 65.29 meq/kg; and a TBA test value of 121.50 mg/kg. These results were in the range of results described by the Canadian Department of Fisheries (12) and Sinnhuber and Yu (13), for fatty fish, especially if they had been previously kept in frozen storage. Khayat and Schwall (14), showed that the oxidation process continues throughout cold storage. This finding has important ramifications for our study, due to the dificulty encountered in receiving fresh

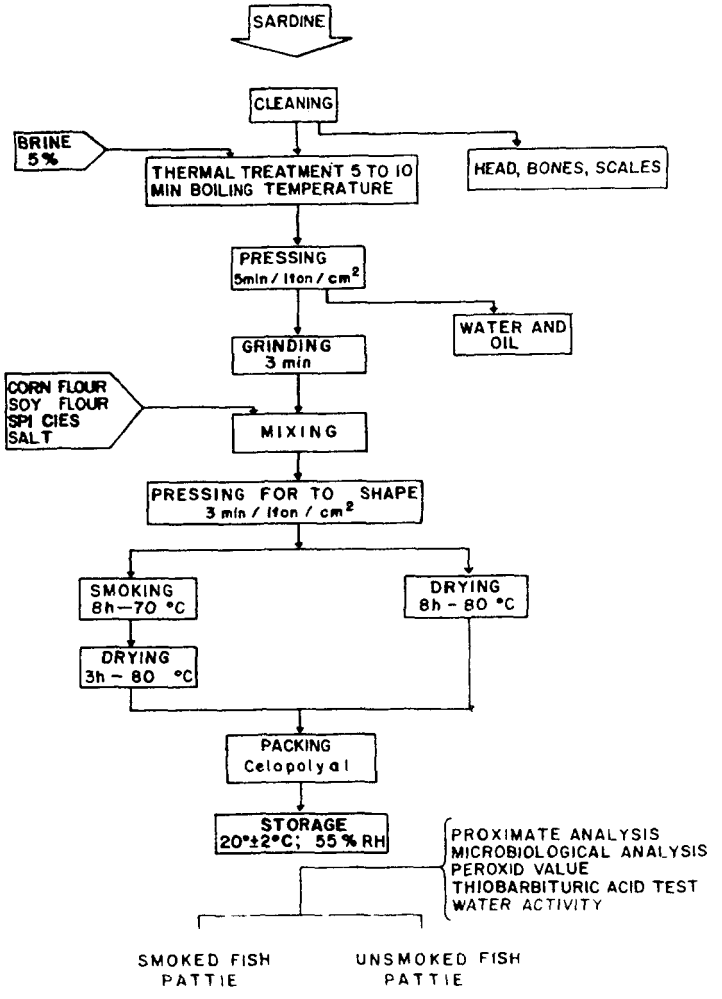


FIGURE 1

Procedure for the preparation of the sardine fish patty (NUTRIPEZ), smoked and unsmoked

TABLE 1

PROXIMATE ANALYSIS OF RAW MATERIAL
g/100g

Determination	Corn flour	Defatted soy flour	Sardine	Spices
Humidity	11.7	8.0	68.7	8.6
Ashes	1.3	6.4	1.3	6.2
Protein*	9.4	47.3	18.4	—
Fat	3.9	1.6	10.7	7.3
Crude fiber	3.2	3.7	—	33.5
Carbohydrates**	70.5	33.0	0.9	44.4

* Nitrogen X 6.25.
** By difference.

TABLE 2

MICROBIAL ANALYSIS OF RAW MATERIALS

Raw material	Total count (CFU/g)	Mold CFU/g	Yeast MPN/g	Coliforms	
				Total	MPN/g
Corn flour	15,100	270	0	7.3	-3.0
Deffated soy flour	4,800	110	0	-1.0	-3.0
Sardine	150,000	50	0	Negative	Negative
Spices	4'600,000	2,100	0	460.0	11.0

unfrozen fish for the production of NUTRIPEZ.

The proximate analysis results for the two different preparations of NUTRIPEZ are detailed in Table 3, where minimum differences in composition between smoked and unsmoked patties can be observed.

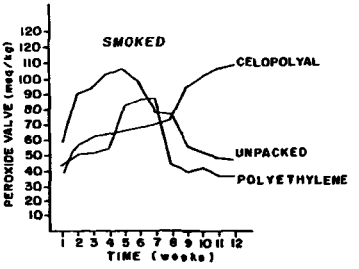
The results of the peroxide (PV) test for both smoked and unsmoked NUTRIPEZ stored at 23°C/50% RH, are presented in Figure 2. The smoked product without protective packaging reached a maximum peroxide value in the fifth week of storage (105.03 meq/kg) followed by the product wrapped in polyethylene, which peaked in the seventh week (85.35 meq/kg), and that wrapped in cellopolyal, which reached a maximum value in the twelfth week (105.03 meq/kg). The unsmoked product (Figure 2), showed a similar behavior as the smoked sample without packaging, and had a value of 110.02 meq/kg at the fifth week, while the packaged material had a lower value of 103.0 meq/kg for the same period of time These results demonstrated some potential antioxidant properties of the smoked product, as well as the fact of lending a *sui generis* taste to the product itself. The recorded decrease in the

TABLE 3

PROXIMAL ANALYSIS OF SMOKED (S) AND UNSMOKED (US) SARDINE PATTIES[1]
g/100g

Determination	S		US	
	(db)[2]	(hb)[3]	(db)[2]	(hb)[3]
Humidity	—	2.8	—	2.4
Ashes	12.9	12.8	11.9	11.6
Protein[4]	39.9	38.7	38.9	37.8
Fat	10.8	10.5	10.5	10.3
Crude fiber	3.4	3.3	2.8	2.7
Carbohydrates[5]	33.0	31.9	35.9	35.2

1 One day after they were prepared.
2 Dry basis (db).
3 Humidity basis (hb).
4 Nitrogen X 6.25.
5 Carbohydrates by difference.



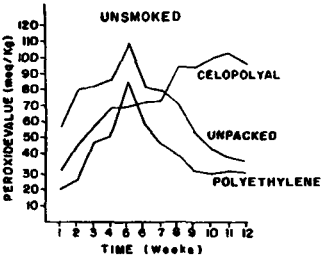


FIGURE 2

Peroxide value of NUTRIPEZ during storage at 23°C/50% RH

PV in the unpackaged and polyethylene packaged products was probably due to the breakdown of the unstable peroxides into secondary products that are characteristic of rancid products.

At tropical conditions (35°C/80% RH), the degree of rancidity in all products was accelerated, as Figure 3 depicts. All values were higher at each time period tested, than those tested at temperate environmental conditions. Under both conditions, the cellopolyal gave the best protection against oxidation.

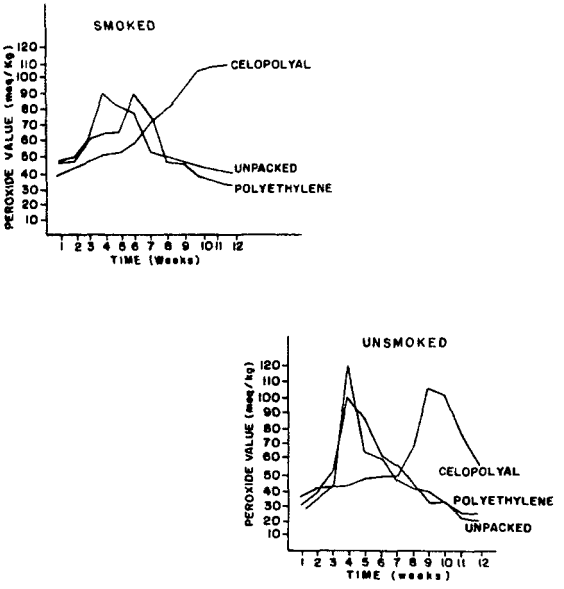


FIGURE 3

Peroxide value of NUTRIPEZ during storage at 35°C/80% RH

Results from the TBA analysis are given in Figure 4. The data reveal that at 23°C/50 RH, there is some protection from oxidation in the smoked product. As demonstrated for the peroxide test, the celopolyal packaging also gave a TBA protective effect for both samples. Similar results are shown in Figure 5, at 35°C/80% RH.

NUTRIPEZ is a dried-salted cake (124 g each) with a significant lipid content. The lipids source is sardine itself; therefore, the product contains highly polyunsaturated lipids, very susceptible to oxidation. In fresh or "wet" fish products, the limits given for detectable rancidity or acceptance of a product is a TBA number of 10-20 for fatty fish. All of our samples registered higher values. There is justifiable concern for the safety of foods with oxidized lipids and high TBA numbers.

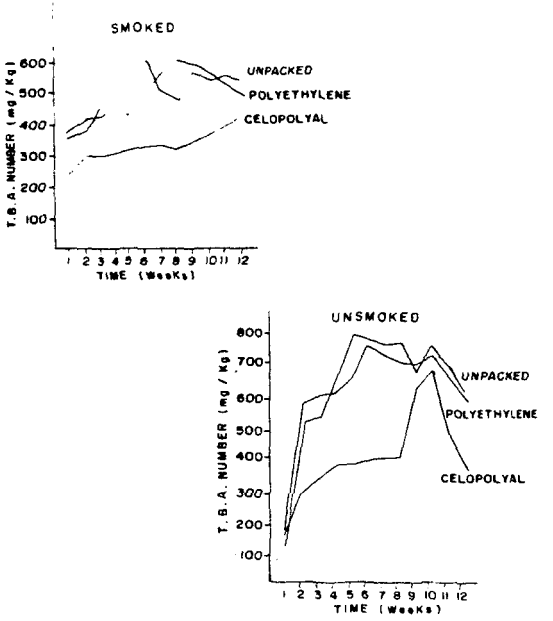


FIGURE 4

TBA number of NUTRIPEZ during storage at 23°C/50% RH

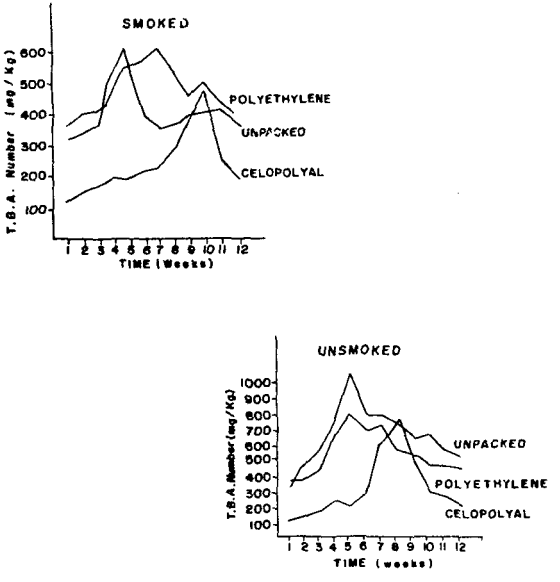


FIGURE 5

TBA number of NUTRIPEZ during storage at 35°C/80% RH

It has been reported that malonaldehyde (one of the oxidation products that is measured by the TBA test) and its acetal, tetraethoxy propane, is toxic to rats at high levels (15).

Carcinogenicity of these compounds is in dispute, due to the contamination of test samples with other compounds. Nonetheless, these considerations should be carefully weighed before any program for the production of dried fatty fish products on a commercial scale is initiated. It was of interest for the authors to determine if this rancidity rating in the dried product would carry through to the final prepared product, and be rejected by consumers. For this purpose, a taste panel was therefore run on all products, with the results shown in Table 4. The products with the highest acceptance rating were both the smoked and plain product packaged in cellopolyal, with an acceptance rating comprised between 2.9 and 4.1 (good to acceptable).

TABLE 4

SENSORIAL EVALUATION[1] OF THE SMOKED (S) AND UNSMOKED (US) SARDINE PATTIES

Sample	Grades					
	Time (Weeks)					
	Zero (0)		Six		Twelve	
	S	US	S	US	S	US
	23°C/50% RH					
Unpacked	3.5	4.1	3.9	4.1	3.9	4.7
Polyethylene	3.5	4.1	4.3	4.2	3.9	4.2
Cellopolyal	3.5	4.1	3.8	3.7	3.6	4.1
	35°C/80% RH					
Unpacked	3.5	4.1	3.7	4.7	4.3	4.9
Polyethylene	3.5	4.1	3.3	4.2	3.8	4.2
Cellopolyal	3.5	4.1	4.3	4.1	3.7	2.9

1 Seven points hedonic scale. One is excellent, and 7 is the worst.

Determination of water activity during storage of the patties under the different conditions, gave the results presented in Tables 5 and 6. As can be appreciated, in general the behaviour of the values given for water activity were similar under both storage conditions. An increase on the water activity was detected for the patties without any packaging and for the patties wrapped with polyethylene in comparison with the patties wrapped with cellopolyal, wherein the values of water activity were significantly reduced.

In all cases, as the microbiological tests demonstrated, the water activity was significantly low to stop the development of microorganisms.

TABLE 5

WATER ACTIVITY OF THE SMOKED (S) AND UNSMOKED SARDINE (US) PATTIES DURING THE STORAGE AT 23°C/50% RH

Time (Weeks)	Samples					
	Unpacked		Polyethylene		Cellopolyal	
	S	US	S	US	S	US
1	0.154	0.148	0.152	0.143	0.155	0.146
2	0.159	0.623	0.155	0.170	0.229	0.186
3	0.555	0.644	0.524	0.512	0.229	0.124
4	0.586	0.675	0.598	0.571	0.172	0.122
5	0.640	0.787	0.480	0.607	0.294	0.127
6	0.633	0.657	0.649	0.508	0.228	0.124
7	0.564	0.632	0.593	0.484	0.222	0.076
8	0.677	0.656	0.518	0.477	0.130	0.092
9	0.581	0.582	0.463	0.462	0.067	0.082
10	0.525	0.563	0.469	0.468	0.081	0.064
11	0.515	0.543	0.450	0.466	0.055	0.049
12	0.515	0.523	0.444	0.462	0.053	0.048

TABLE 6

WATER ACTIVITY OF THE SMOKED (S) AND UNSMOKED SARDINE (US) PATTIES DURING THE STORAGE AT 35°C/80% RH

Time (Weeks)	Samples					
	Unpacked		Polyethylene		Cellopolyal	
	S	US	S	US	S	US
1	0.159	0.145	0.156	0.147	0.158	0.149
2	0.661	0.549	0.275	0.213	0.210	0.150
3	0.710	0.690	0.536	0.562	0.342	0.373
4	0.898	0.725	0.721	0.605	0.251	0.166
5	0.700	0.720	0.584	0.707	0.314	0.162
6	0.601	0.661	0.570	0.608	0.266	0.152
7	0.584	0.621	0.530	0.597	0.248	0.146
8	0.649	0.703	0.623	0.539	0.088	0.123
9	0.582	0.634	0.556	0.496	0.087	0.110
10	0.572	0.582	0.544	0.558	0.082	0.059
11	0.565	0.581	0.537	0.537	0.076	0.045
12	0.540	0.578	0.519	0.531	0.071	0.025

TABLE 7

MICROBIAL ANALYSIS OF SMOKED (S) AND UNSMOKED SARDINE (US) PATTIES DURING STORAGE

Sample	Time (Weeks)					
	Zero (0)		Six		Twelve	
	S	US	S	US	S	US
	(Total count 9CFU/g)					
	23°C / 50% RH					
Unpacked	44,000	55,000	60,000	100,000	26,000	32,000
Polyethylene	44,000	55,000	26,000	24,000	6,200	6,800
Cellopolyal	44,000	55,000	10,000	10,000	9,100	4,300
	35°C / 80% RH					
Unpacked	44,000	55,000	69,000	110,000	24,000	42,000
Polyethylene	44,000	55,000	28,000	67,000	19,000	40,000
Cellopolyal	44,000	55,000	15,000	24,000	12,000	16,000

TABLE 8

PROXIMATE ANALYSIS OF SMOKED (S) AND UNSMOKED SARDINE (US) PATTIES AT THE END OF THE STORAGE PERIOD[1]

Determination	Unpacked		Polyethylene		Cellopolyal	
	S	US	S	US	S	US
	At 23°C / 50% RH					
Humidity	12.5	12.9	5.9	7.6	2.3	2.4
Ashes	11.5	10.4	12.3	11.0	12.8	11.6
Protein[2]	34.9	34.0	37.5	35.9	38.9	38.0
Lipids	9.4	9.2	10.2	9.7	10.6	10.3
Crude fiber	2.9	2.4	3.2	2.6	3.3	2.7
Carbohydrates[3]	28.8	31.1	30.9	33.2	32.1	35.0
	At 35°C / 80% RH					
Humidity	10.6	11.0	9.6	10.0	2.3	2.0
Ashes	11.7	10.6	11.9	10.7	13.0	13.0
Protein[2]	35.7	34.6	36.0	35.0	38.0	37.0
Lipids	9.7	9.3	9.8	9.5	10.0	10.0
Crude fiber	3.0	2.5	3.0	2.5	3.0	3.0
Carbohydrates[3]	29.3	32.0	29.7	32.3	33.7	35.0

1 Twelve weeks.

2 Nitrogen x 6.25.

3 By difference.

In the microbiological analysis, both the smoked and the unsmoked product at both climatic storage conditions given in Table 7, showed that the product that had been packaged in cellopolyal, exhibited the lowest number of aerobic mesophilic bacteria. The smoked NUTRIPEZ systematically exhibited fewer bacterial counts than the unsmoked product, inferring a bactericidal effect of the smoking procedure, as reported by Borgstrom (16) and Morales (9).

In all cases, the presence of coliform bacteria, molds or yeasts was not detectable. In this sense, the product maintained hygienic qualities throughout its storage.

The proximate composition of the NUTRIPEZ product in both presentations, smoked and unsmoked, and under both climatic conditions, was determined at the end of the experiment. The results, as shown in Table 8, demostrate that the product did not undergo significant changes during the storage tests, with the exception of water content.

Only the product packaged in cellopolyal maintained a water content below 3%, which is another positive effect of this type of packaging.

CONCLUSION

The packaging material cellopolyal both slowed down the rancidity due to oxidation and helped maintain the low water content of the semidried product during storage under different temperature and humidity conditions. On the other hand, stability of NUTRIPEZ both smoked and unsmoked, during three months of storage, was acceptable from a microbiogical point of view. The rancidity of the product increased at different rates in all batches during storage; however, the product that was wrapped in cellopolyal was the most acceptable as judged by a sensory panel after three months of storage.

RESUMEN

EVALUACION DE LA ESTABILIDAD QUIMICA, MICROBIANA Y SENSORIAL DE UN PRODUCTO SECO SALADO DE SARDINA (*Sardinops caerula*), ADICIONADO DE CEREALES

En el Instituto Nacional de la Nutrición Salvador Zubirán se ha venido desarrollando una línea de investigación para la conservación de pescado por métodos tradicionales de secado y ahumado, habiendo obtenido un producto registrado como NUTRIPEZ que se elabora con sardina, adicionada de cereales, soya y condimentos.

En el presente trabajo se planteó como objetivo evaluar la estabilidad química, microbiana y sensorial del producto NUTRIPEZ, durante su almacenamiento. Se utilizaron dos lotes de 20 kg cada uno, de NUTRIPEZ ahumado y sin ahumar. Los dos productos se almacenaron durante tres meses a 23°C/50% HR y a 35°C/80% HR, empacados en dos materiales flexibles: celopolial y polietileno de baja densidad, versus un lote testigo sin empaque.

Los resultados revelaron que el empaque de celopolial brinda una mayor protección contra la rancidez y el desarrollo microbiano. La aceptación entre las dos presentaciones de NUTRIPEZ no acusó diferencias significativas.

BIBLIOGRAPHY

1. Bourges, R.H., L.J. Morales & H. Sierra. Development of a compressed product made from sardine. **Arch. Latinoamer. Nutr.,** 35 (3): 438-446, 1985.
2. Association of Official Analytical Chemists. **Official Methods of Analysis of the AOAC.** 12th ed. Washington, D.C., The Association, 1975, p. 22, 130, 15, 135 and 137.
3. Fernández, E. & M. Costarrica. **Técnicas Microbiológicas en Técnicas para el Muestreo y Análisis Microbiológico de los Alimentos.** Dirección General de Salud Pública, S.S.A.,.México, 1975.
4. Pearson, D., **Laboratory Techniques in Food Analysis**. London, Butterworth & Co. Publishers Ltd., 1973.
5. Bligh, G.E. & J.W. Dyer. A rapid method of total lipid extraction and purification. **Can. J. Biochem. Physiol., 37** (8): 911-917, 1959.
6. Tarladgis, G.B., M.B. Watts & T.M. Younathan. A distillation method for the quantitative determination of malonaldehyde in rancid foods. **J. Am. Oil Chem. Soc., 37** (1): 44-48, 1960.
7. Robles, C.M., E. Cervantes & J.P. Ke. **Recommended Method for Testing the Objective Rancidity Development in Fish-Based TBA Formation**. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1089. Department of Fisheries and Oceans. Fisheries Development Branch, Halifax, Nova Scotia, Canada, 1982.
8. Larmond, W. **Laboratory Methods for Sensory Evaluation of Food.** Canada Department of Agriculture Publication 1639, 1977.
9. Morales de León, J., H. Bourges, F.M. de León & M.Y. López. Estudios de aceptación de tortas secas de sardina (*Sardinops caerula)*, realizado en una comunidad rural. **Tecnología de Alimentos, México, 18** (5): 27-31, 1983.
10. Norma Mexicana, Harina de Maíz Nixtamalizado, F. 46 México, 1980.
11. Proyecto de Normas Microbiológicas y Químicas para el Control Sanitario de Agua, Bebidas y Alimentos. México, Dirección General de Investigación en Salud Pública, 1974.
12. Canadian Department of Fisheries and Oceans. **Operational Ground Fish and Laboratory Methods Guide.** Halifax, Nova Scotia, Canada, Fisheries Development Branch, 1984.
13. Sinnhuber, O.R. & T.C. Yu. 2-Thiobarbituric acid method for the measurement of randicity in fishery products. The quantitative determination of malonaldehyde. **Food Technol., 12** (1): 9-11, 1985.
14. Khayat, A. & D. Schwall. Lipid oxidation in seafood. **Food Technol., 37** (7): 130-140, 1983.
15. Pearson, A.M., J.I. Gray, A.M. Walzak & N.A. Horenstein. Safety implications of oxidized lipids in muscle foods. **Food Technol., 37** (71): 212, 1983.
16. Borgstrom, G. **Fish as Food**. Vol. 1 and III, New York, N.Y., Academic Press, 1962.