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Vitamin Content of Swordfish (*Xhiphias gladius*) Affected by Salting and Frying

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***Abstract*—**The swordfish (*Xiphias gladius*) is a large oceanic fish of high commercial value, which is widely distributed in waters of the world's oceans. They are considered to be an important source of high quality proteins, vitamins and essential fatty acids, although only half of the population follows the recommendation of nutritionists to consume fish at least twice a week. Swordfish is consumed worldwide because of its low fat content and high protein content. It is generally sold as fresh, frozen, and as pieces or slices. The aim of this study was to evaluate the effect of salting and frying on the composition of the water-soluble vitamins (B_2, B_3, B_9 and B_{12}) and fat-soluble vitamins (A, D, and E) of swordfish. Three loins of swordfish from Pacific Ocean were analyzed. All the fishes had a weight between 50 and 70 kg and were transported to the laboratory frozen (-18 ºC). Before the processing, they were defrosted at 4 ºC. Each loin was sliced and salted in brine. After cleaning the slices, they were divided into portions (10×2 cm) and fried in olive oil. The identification and quantification of vitamins were carried out by high-performance liquid chromatography (HPLC), using methanol and 0.010% trifluoroacetic acid as mobile phases at a flow-rate of 0.7 mL min^{-1}. The UV-Vis detector was used for the detection of the water- and fat-soluble vitamins (A and D), as well as the fluorescence detector for the detection of the vitamin E. During salting, water and fat-soluble vitamin contents remained constant, observing an evident decrease in the values of vitamin B_2. The diffusion of salt into the interior of the pieces and the loss of constitution water that occur during this stage would be related to this significant decrease. In general, after frying water-soluble and fat-soluble vitamins showed a great thermolability with high percentages of retention with values among 50–100%. Vitamin B_3 is the one that exhibited higher percentages of retention with values close to 100%. However, vitamin B_9 presented the highest losses with a percentage of retention of less than 20%.

***Keywords*—**Frying, HPLC, salting, swordfish, vitamins.

I. Introduction

SWORDFISH *(Xiphias gladius*) belongs to Xiphiidae family, a pelagic and migratory fish with a worldwide distribution. Swordfish presents a high protein value as well as important ω-3 fatty acids and fat and water soluble vitamins [1], which make it very attractive to the seafood industry. In 2016, Spain captured about 80% of swordfish in Europe and represents a main position worldwide [2]. This fact contributed to the development of a consolidated transformer sector of fishery products that integrates different subsectors. Among them, the most important subsector is the canning industry [3]. However, the elaboration of canned products entails the need to know the chemical composition and microbiological parameters of the raw material and the biochemical and microbiological changes that occurred during the process. The canning process includes salting, frying and sterilization treatments, which can deteriorate of important nutritional components present in the raw food.

Vitamins are essential organic compounds, which in very small amounts in the diet are essential for the normal course of physiological functions, including growth, reproduction and conservation of health and life [4], [5]. Fat-soluble vitamins can be stored in appreciable amounts by the animal organism, whereas water-soluble vitamins are not stored but are rapidly excreted, with the exception of vitamin B_{12} [4]. Animal origin foods tend to contain a greater proportion of fat-soluble vitamins than water-soluble ones, which is reversed in vegetables [6]. The main problem of vitamins stems from its instability in food, together with the losses caused by the conditions of processing and cooking. It may be affected by specific parameters as temperature, oxygen, light, humidity, pH and duration of exposure [5]. The objective of this study was to know the content of water and fat-soluble vitamins and its stability after salting and frying in swordfish from the Pacific Ocean.

II. Material and Methods

A. Sample Preparation

Three swordfish with a weight between 50 and 70 kg were caught at the Pacific Ocean by Organización de Palangreros Guardeses (ORPAGU), from A Guarda, Pontevedra (NW Spain). Its loins were transported frozen (-18 °C) to the laboratory and were allowed to defrost at 4 °C. Each loin was sliced and submerged in brine (8%) for 12 h, with the fish to brine ratio at 1:2. After removal from the brine, slices were cleaned and divided into portions (10×2 cm) to finally be fried. The frying treatment was carried out in olive oil at 120 °C for about 7 minutes. Samples were taken from raw fish, after the end of the salting, and after frying, in each of the batches. The losses in the different stages were calculated for the three swordfish loins.

B. Chemical and Reagents

Vitamin standards were purchased from Sigma Aldrich (Madrid, Spain). HPLC-grade solvents ―acetonitrile, methanol, and water― were from Panreac (Barcelona, Spain). Analytical grade solvents ―potassium hydroxide (KOH), trifluoroacetic acid (TFA), hexane, and ethanol― were from

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Sigma Aldrich (Madrid, Spain).

C. Extraction of Water-Soluble Vitamins

The extraction was conducted following the procedure described by [7] with some modifications. Homogenized sample was weighted (1 g) and dissolved in 4 mL of a methanol: 0.010% TFA pH 3.9 solution (50:50). The mixture was stirred for 15 min and centrifuged (14,000 *x g*, 15 min, 4 °C). The supernatant was collected and dried on a rotary evaporator under vacuum (90 min, 70 °C). The resulting residue was dissolved in 1.5 mL of methanol, filtered through a 0.45 µm Teflon membrane filter and taken to a vial for further analysis. All samples were extracted and injected into the chromatograph at least in duplicate.

D. Extraction of Fat-Soluble Vitamins

This method was similar to the one proposed by [8]. Homogenized sample was weighted (2.5 g) and mixed with 4 mL of 50% KOH and 6 mL of ethanol. After 22 h dark storage, n-hexane (10 mL) and distilled water (5 mL) were added to the sample, shaken and centrifuged (5 min, 3500 *x g*), in order to extract the unsaponifiable matter. The organic phase (6 mL) was evaporated to dryness in a rotatory vacuum evaporator (15 min, 30 °C). The residue was dissolved in 1.5 mL of methanol and filtered through a 0.45 µm Teflon membrane filter before injection into the column. All samples were extracted and injected into the chromatograph at least in duplicate.

E. Chromatographic Apparatus

Water and fat-soluble vitamins were determined with a liquid chromatograph equipped with vacuum degasser (SCM 1000), quaternary pump (P4000), autosampler (AS3000) with column oven and tray temperature control, UV-Vis photodiode array detector (UV6000LP), and fluorescence detector (FL3000) controlled with Chrom-Quest 4.1.

F. Chromatographic Conditions for Sample Analysis

Reversed-phase chromatographic column Ultrasphere ODS C_{18} (250 mm x 4.6 mm, 5 µm particle size, Beckman, Fullerton, EE.UU.) was used. The flow rate was 0.7 mL min^{-1}. Auto sampler injection was 30 µL. Mobile phase composition was 0.010% TFA at pH 3.9 and methanol. The gradient elution started at 95:5 and was constant in the first 4 min, then decreased to 2:98 during the next 10 min, was kept constant for the next 17 min and finally linearly increased up to 95:5 in the last 5 min. The column effluent was monitored with UV-vis photodiode array detector at 265, 280, and 325 nm, and with fluorescence detector at 294 and 330 nm for emission and excitation, respectively. The column remained thermostated at 35±1 °C during the analysis.

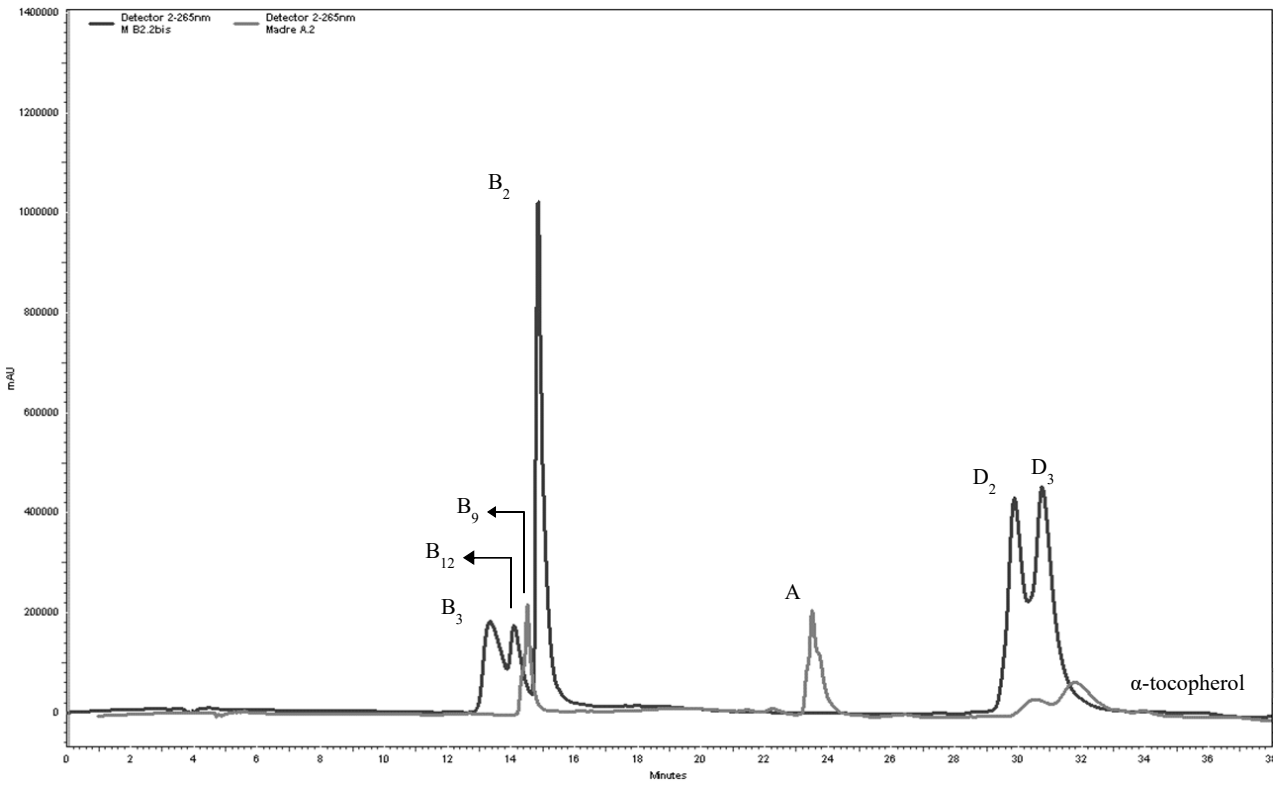

Fig. 1 Representative chromatograms of the vitamin stock solutions at 265 nm

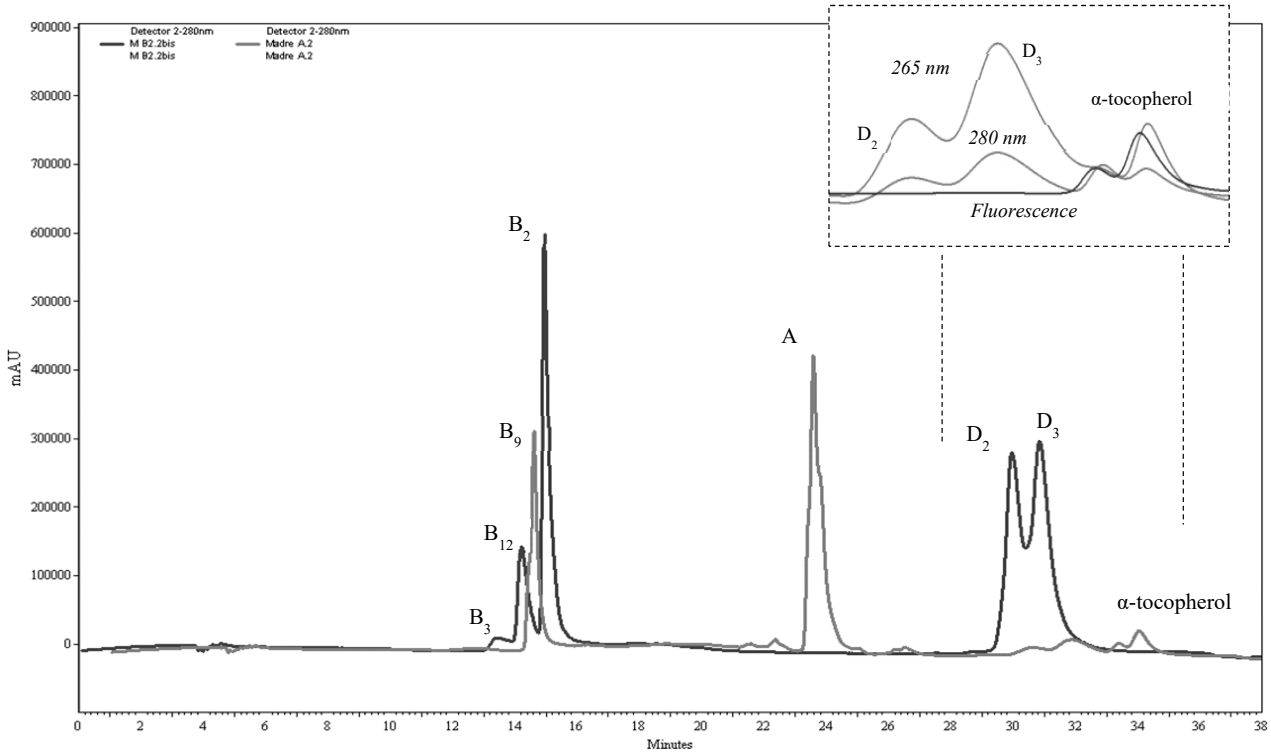

Fig. 2 Representative chromatograms of the vitamin stock solutions at 280 nm, and fluorescence detector for α-tocopherol (small box)

G. Standards

Vitamins stock solutions were prepared in methanol: 0.010% TFA (50:50) in appropriate proportions (Fig. 1). Vitamins B_2 (riboflavin), B_3 (niacin), B_9 (folic acid), B_{12} (cobalamin), A (retinol), D_2 (ergocalciferol), D_3 (cholecalciferol), and α-tocopherol were quantified (hereafter referred as B_2, B_3, B_9, B_{12}, A, D, α-tocopherol, respectively). Standard curves, as well as the detection and quantification limits, were determined following the procedure described in [9]. At the same time, 10 blanks were extracted, being treated under the same conditions as the samples. Recovery tests were performed by spiking the samples with vitamins ten times with 20, 30, and 40% of each of the vitamins (data not shown). Repeatability and reproducibility tests were performed by extracting a sample five times on three different days. The coefficients of variation were less than 2%. Samples from the tests (preserved from light) were also injected after 4, 8 and 12 h after extraction, observing coefficients of variation below 2%.

H. Statistical Analysis

The statistical analysis was based on the one-way analysis of variance (ANOVA) with a confidence interval of 95% ($P < 0.05$) using the Least Significant Difference (LSD) test. All statistical tests were performed with the Statistical 8.0 program for Windows (Tulsa, OK, USA). Mean values and standard deviations were used.

III. Results and Discussion

A. Water-Soluble Vitamins Changes after Salting and Frying

According to the sensitivity of the wavelengths and/or fluorescence detection (data not shown) different selections were made. Vitamins B_2 and B_3 were quantified by 265 nm, and vitamins B_9 and B_{12} by 280 nm.

In the raw swordfish, the content of *vitamin B_2* (Table I) was within the range of values determined in swordfish by other authors [4], [10]. Moreover, it was in line with those quantified for other fish species (Table II). The content significantly decreased after being subjected to the salting and remained constant after frying (Table I). This fact is relating with the leaching of this compound [4]. Thus, during salting a loss of constitution water was occurred, hence a loss of this water-soluble vitamin. This vitamin is very resistant to heat and oxidation [16], but very sensitive to light, especially at high temperatures [17]-[19]. Therefore, a great stability can be observed by this vitamin after frying, as the content of vitamin B_2 did not decrease compared to the fish after salting (Table I).

TABLE I
Water-soluble Vitamins Content in Swordfish

Vitamin	Raw	Salted	Salted+Fried
B_2 (mg/100 g)	0.023 ± 0.002^a	0.004 ± 0.004^b	0.006 ± 0.006^b
B_3 (mg/100 g)	6.16 ± 1.07^a	6.36 ± 0.79^a	8.19 ± 1.24^a
B_9 (μg/100 g)	0.78 ± 0.03^a	0.79 ± 0.13^a	0.19 ± 0.05^b
B_{12} (μg/100 g)	3.22 ± 1.09^{ab}	2.48 ± 0.44^a	3.70 ± 0.45^b

[a-b] Values with different superscripts were significantly different ($P<0.05$).

Vitamin B_3 contents (Table I) were within the wide range of values determined by other authors in other species of raw or cooked fish (Table II). In raw fish, the contents are slightly higher than those determined by [13] in swordfish and minor to those contributed by [10] and [4] in this variety of fish (Table II). The values obtained remain constant during the salting and frying stage (Table I). This fact may be due to the great stability owned by this vitamin [20].

Vitamin B_9 contents (Table I) were lower than those found in the literature in different fresh or processed fish by other authors (Table II). Although the values remained constant during the salting of the pieces, vitamin B_9 exhibited a large decrease after frying, around 80% (Table I). This variation can be due to a significant denaturation after submitting the fish portions to the high temperatures for a prolonged time during frying [21].

The values of *vitamin B_{12}* content determined (Table I) were within the range of values determined by [10], [4], and [13] in swordfish (Table II). These values decreased slightly (not significantly) during the salting of the pieces, and they increased during frying. This fact may be explained by an increase in the concentration of the content in this vitamin due to the lightly loss of constitution water. Nevertheless, the loss of this vitamin was not high indicating its good stability [22].

TABLE II
WATER-SOLUBLE VITAMINS CONTENT PER 100 g OF FISH IN DIFFERENT SPECIES FROM OTHER STUDIES

Species	B_2 (mg)	B_3 (mg)	B_9 (µg)	B_{12} (µg)	Reference
Catfish	0.03	1.13	--	--	[11]
Catfish, fried	0.01	0.04	--	--	[11]
Catfish, baked	0.02	0.73	--	--	[11]
Catfish, grilled	0.07	2.03	--	--	[11]
Catfish, microwaved	0.02	0.05	--	--	[11]
Catfish	0.12	2.4	82.5	2.4	[4]
Porbeagle-mackerel shark	0.18	8.0	--	--	[4]
Shark	0.062	2.938	3	1.49	[12]
Salmon	0.15	10.4	26	5	[10]
Atlantic salmon	0.380	7.860	25	3.18	[12]
Salmon	0.041	--	10	--	[13]
Salmon, boiled	0.081	3.0	8.4	--	[13]
Salmon, grilled	0.12	4.4	10	--	[13]
Sturgeon	--	5.62	--	1.27	[14]
Swordfish	0.05	9	15	5	[10]
Swordfish	0.50	8.2	--	5.7	[4]
Swordfish	0.043	4.1	14	1.4	[13]
Swordfish	0.053	7.760	2	1.70	[12]
Swordfish, cooked	0.063	9.254	2	1.62	[12]
Albacore	--	--	--	6.05	[15]
Albacore	0.2	17.8	15	5	[10]
Skipjack tuna	0.04	7.2	--	4.2	[4]
Bluefin tuna	0.12	8.5	--	3.4	[4]
Tuna	0.2	17.8	15	5	[10]
Tuna	0.251	8.654	2	9.43	[12]
Tuna	0.048	10	8.3	2.4	[13]
Tuna, canned in vegetable oil	0.022	9.8	14	--	[13]

--: no data.

The true retention (%TR) for the water-soluble vitamins after frying was obtained adopting the approach suggested by [23] for small size food ítems, following (1):

$$\%TR = \frac{\text{nutrient content per g cooked food} \times \text{g food after cooking}}{\text{nutrient content per g raw food} \times \text{g food before cooking}} \times 100 \quad (1)$$

It must be taken into account that (1) contemplates the product losses produced during the different processes (defrosting, salting, slicing, cleaning, and frying).

According to the %TR calculated by (1), it can be observed that the vitamin B_2 presented the lowest retention after salting (Table III). On the other hand, after frying vitamin B_9 was strongly affected, since only 12.7% was retained in the swordfish (Table III). It can be appreciated that after the two processes studied (salting and frying), vitamins B_3 and B_{12} presented good retention percentages, exceeding a 60%TR (Table III).

TABLE III
WATER-SOLUBLE VITAMINS CONTENT IN PERCENTAGE OF TRUE RETENTION (%TR) OF THE WATER-SOLUBLE VITAMINS AFTER SALTING AND FRYING

Vitamin	%TR after salting	%TR after salting+ frying
B_2	16.9	13.63
B_3	99.5	69.6
B_9	98.6	12.7
B_{12}	75.0	60.1

B. Fat-Soluble Vitamin Changes after Salting and Frying

According to the sensitivity of the wavelengths and/or fluorescence detection, vitamin D was quantified by using 265 nm, vitamin A by 280 nm, and α-tocopherol by the fluorescence detector.

The *vitamin A* content in raw fish was 228.95±13.71 µg/100 g (Table IV). The values obtained were lower than those determined by [10] in swordfish and clearly superior to those quantified by [4] and [13], both in raw and cooked swordfish (Table V). Moreover, there is a great variation between the data found in the scientific literature for swordfish (36–500 µg/100 g) and other species (Table V). Although the vitamin A content decreased not significantly after salting, the decrease was significant after frying, in line with the data obtained by other authors for cooked fish in similar studies (Table V). This may be due to the fact that its resistance to heat treatments is rapidly lost when the treatment is in the presence of oxygen [5].

TABLE IV
FAT-SOLUBLE VITAMIN CONTENT IN SWORDFISH

Vitamin	Raw	Salted	Salted+Fried
A (µg/100 g)	228.95±13.71^{a}	181.38±52.41^{ab}	162.60±24.82^{b}
D (µg/100 g)	33.50±4.44^{a}	31.47±4.82^{a}	25.71±4.64^{a}
α-tocopherol (mg/100 g)	1.20±0.19^{a}	1.19±0.28^{a}	1.22±0.29^{a}

$^{a-b}$ Values with different superscripts were significantly different (P<0.05).

The *vitamin D* contents obtained in all samples of swordfish (Table IV) were higher than those presented by other authors (Table V). The vitamin D content remained stable after salting and frying, due to the high thermostability characteristic of the

fat-soluble vitamins [26]. However, a decrease (not significant) can be observed after frying, which may be due to the exposure of oxygen at elevated temperatures [27], [5]. Vitamin D_2 was also counted to detect the possible contribution of this vitamin by the oil. No significant increases were observed in this vitamin (Table V).

In raw swordfish, *α-tocopherol* content (1.20 ± 0.19 mg/100 g) was within the range of values determined in fresh swordfish by other authors, as well as in similar varieties of fish (Table V). The content of this vitamin in foods treated in vegetable oil increases or remains stable because vegetable oil is a good source of this vitamin [28], [29]. Therefore, no changes were found in α-tocopherol content after salting or frying (Table IV) and these values were lower than those shown by other authors (Table V). This may be due to the instability in presence of oxygen, light and peroxides (which occurs as a result of the auto-oxidation of unsaturated fat) of this vitamin [30].

TABLE V
FAT-SOLUBLE VITAMINS CONTENT PER 100 g OF FISH IN DIFFERENT SPECIES FROM OTHER STUDIES

Species	*A*(μg)	*D* (μg)	*α-tocopherol* (mg)	Reference
Salmon	40	--	--	[12]
Salmon	--	--	54.40	[24]
Salmon, cooked	--	--	48.95	[24]
Salmon	13	8	2	[10]
Salmon	--	11.26-33.23	--	[25]
Salmon, baked	--	14.26-26.08	--	[25]
Salmon	33	11	--	[13]
Salmon, grilled	70	9.2	4.3	[13]
Salmon, boiled	65	11	5.3	[13]
Swordfish	500	7.2	1	[10]
Swordfish	--	9.75	--	[25]
Swordfish	475.5	--	0.44	[4]
Swordfish	<0.64	(1.3)	0.088	[13]
Swordfish	36	13.9	2.02	[12]
Swordfish, cooked	43	16.6	2.41	[12]
Albacore	4	20	0.9	[10]
Tuna	60	25	1	[10]
Tuna	655	5.7	1	[12]
Tuna	--	1.50	--	[25]
Tuna, baked	--	8.15	--	[25]
Bluefin tuna	288.9	--	1.20	[4]
Skipjack tuna	15.6	--	3.00	[4]
Tuna	11	4.2	0.64	[13]
Tuna, canned in vegetable oil	23	<0.70	1.9	[13]

--: no data; < Values below the detection limit; ( ) values between the detection limit and the quantification limit.

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The fat-soluble vitamins true retention (%TR) calculated by (1) contemplates product losses during the different processes (Table VI).

In general, fat-soluble vitamins studied were not highly affected by the salting process, since high %TR was showed. On the other hand, the %TR in vitamin A and D decreased to more than a half after frying. However, in the case of α-tocopherol the decrease was smaller probably influenced by the possible contribution of this vitamin from the olive oil used in the frying process.

TABLE VI
FAT-SOLUBLE VITAMIN CONTENT IN PERCENTAGE OF TRUE RETENTION (%TR) OF THE WATER-SOLUBLE VITAMINS AFTER SALTING AND FRYING

Vitamin	%TR after salting	%TR after salting+frying
A	77.1	37.2
D_3	91.4	40.2
α-tocopherol	96.5	53.2

IV. CONCLUSION

From this study it can be concluded that the salting process in the swordfish loin portions studied did not affect the contents of water- and fat-soluble vitamins, with the exception of the B_2 vitamins that it is lost in a large proportion. The subsequent frying process was supported to a greater extent by vitamins B_3 and B_{12} and B_2 (since it did not lose more than it had lost in the salty). Vitamin B_9 was strongly affected by the frying treatment. However, fat-soluble vitamins seem to tolerate better this treatment better, despite being affected by exposure to oxygen during the frying treatment.

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REFERENCES

[1] N. Cobas, S. Martínez, and I. Franco, *Pez Espada: Pesca, Composición Química y Valor Nutricional*. Vigo, Spain: Universidade de Vigo. Servizo de Publicacións; 2016.

[2] N. Abid and M. Idrissi, "Descripción del pez espada (SWO)," in *Manual de ICCAT. Comisión Internacional para la Conservación del Atún Atlántico,* Publicaciones ICCAT, 2006, ch. 2.1.9.

[3] MERCASA, *Productos de la pesca y la acuicultura*. (online). 2016. (Accessed 24/01/2019). Available from: http://www.mercasa-ediciones.es/alimentacion_2016/pdfs/Sectores/Productos_pesca_y_acuicultura_2016.pdf.

[4] S.P. Lall and M.P. Parazo, "Vitaminas del pescado y del marisco," in *El Pescado y los Productos Derivados de la Pesca*, A. Ruiter, and B.P. Sanz, Ed. Acribia, Zaragoza, 1999, ch. 6.

[5] E. Lešková, *et al.* "Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models," *J. Food Comp. Anal.,* vol. 19, no. 4, pp. 252–276, Sept. 2013.

[6] S. Dergal, "Vitaminas y nutrientes inorgánicos," in *Química de los Alimentos*, 4rd ed. S. Dergal, Ed. México: Pearson Educación, 2006, pp. 363–400.

[7] B. Klejdus, *et al.* "Simultaneous determination of water- and fat-soluble vitamins in pharmaceutical preparations by high-performance liquid chromatography coupled with diode array detection," *Anal. Chim. Acta*, vol. 520, no. 1, pp. 57–67, Aug. 2004.

[8] T. Saldanha, A.C.H.S. Frankland, M.E. Nogueira, and N. Bragagnolo, "HPLC Separation and determination of 12 cholesterol oxidation products in fish: Comparative Study of RI, UV, and APCI-MS Detectors," *J. Agric. Food Chem.*, vol. 54, no. 12, pp. 4107–4113, Jun. 2016.

[9] J.C. Miller, and J.N. Miller, *Statistic of Analytical Chemistry*. Englewood Cliffs, N.J.: Prentice-Hall, 1999.

[10] O. Moreiras, A. Carbajal, L. Cabrera, and C. Cuadrado, "*Tablas de*

Composición de Alimentos". Madrid: Pirámide, 2013.

[11] B. Ersoy, and A. Özeren, A, "The effect of cooking methods on mineral and vitamin contents of African catfish," *Food Chem.,* vol. 115, no. 2, pp. 419–422, July 2009.

[12] USDA, Nutrient Database for Standard Reference, Laboratory, Agricultural Research Service. (online). 2014. (Accessed 10/01/2019). Available from: http://www.nal.usda.gov/fnic/foodcomp/search/.

[13] M.G. Dias, M.V. Sánchez, H. Bártolo, and L. Oliveira, "Vitamin content of fish and fish products consumed in Portugal", *EJEAFChe.,* vol. 2, no. 4, pp. 510–513, Sept. 2003.

[14] A. Badiani, *et al.*, "True retention of nutrients upon household cooking of farmed portion-size European sea bass (*Dicentrarchus labrax* L.*),"* *LWT-Food Sci. Technol.*, vol. 50, no.1, pp. 72–77, Jan. 2013.

[15] D.A. Dobreva, *et al.,* "Black sea fish and shelfish as essential source of vitamin B12," *Int. J. Sci. Rep.*, vol. 4, no. 8, pp. 199–203, Aug. 2018.

[16] F. Hoffmann-La Roche, *Vitamin Compendium: the properties of the vitamins and their importance in human and animal nutrition.* Basle, Switzerland: Roche, 1976.

[17] A.S. Al-Khalifa, and A.A. Dawood, "Effects of cooking methods on thiamin and riboflavin contents of chicken meat," *Food Chem.*, vol. 48, no. 1, 1993, 69–74.

[18] E. Mašková, J. Rysová, V. Fiedlerová, and M. Holasová, "Vitamin and mineral retention in meat in various cooking methods," *Czech J. Food Sci*., vol. 12, no. 5, pp. 407–416, 1994.

[19] A. Lassen, M. Kall, K. Hansen, and L. Ovesen, "A comparison of the retention of vitamins B_1, B_2 and B_6 and cooking yield in pork loin with conventional and enhanced meal-service systems," *Eur. Food Res. Technol.*, vol. 215, no. 3, pp. 194–199, Sept. 2002.

[20] G.F.M. Ball, *Water-soluble Vitamin Assay in Human Nutrition.* London, NY: Chapman & Hall, 1994.

[21] R.R. Eitenmiller, and W.O. Laden, *Vitamin Analysis for the Health and Food Science*. Boca Raton, Florida: CRC Press, 1999.

[22] F. Watanabe, "Vitamin B_{12} Sources and Bioavailability," *Exp. Biol. Med.*, vol. 232, no. 10, pp. 1266–1274, Nov. 2007.

[23] E.W. Murphy, P.E. Criner, and B.C. Gray, "Comparison of methods for determining retentions of nutrients in cooked foods," *J. Agric. Food Chem.,* vol. 23, no. 6, pp. 1153–1157, Nov. 1975.

[24] A. Polat, *et al.*, "Tocopherol content of commercial fish species as affected by microwave cooking," *J. Food Biochem.*, vol. 37, pp. 381–387, Sept. 2011.

[25] W.C. Byrdwell, *et al.*, "Vitamin D levels in fish and shellfish determined by liquid chromatography with ultraviolet detection and mass spectrometry," *J. Food Comp. Anal.,* vol. 30, no. 2, pp. 109–119, Jun. 2013.

[26] D. Kilcast, "Effect of irradiation on vitamins," *Food Chem.*, vol. 49, no. 2, pp. 157–164, 1994.

[27] D. Lund, "Effects of heat processing on nutrients," in *Nutritional Evaluation of Food Processing,* E. Karmas, and R.S. Harries, Ed. Dordrecht: Springer, 1988, pp. 319–354.

[28] B. Holland, *et al.*, "Fish and Fish Products" in *The Composition of Foods*, 5th ed. McCance and Widdowson's, Cambridge: Royal Society of Chemestry, 1993.

[29] A.H. Simonne, and R.R. Eitenmiller, "Retention of vitamin E and added retinyl palmitate in selected vegetable oils during deep-fat frying and fried breaded products," *J. Agric. Food Chem.*, vol. 46, no. 12, pp. 5273–5277, 1998.

[30] M.A. Murcia, M. Martínez-Tomé, I. Del Cerro, F. Sotillo, and A. Ramírez, "Proximate composition and vitamin E levels in egg yolk: losses by cooking in a microwave oven," *J. Sci. Food Agric.*, vol. 79, no. 12, pp. 1550–1556, Aug. 1999.