

Fat-Soluble Vitamins Contents in Atlantic Mackerel from the North East of Tunisia

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Abstract – The main purpose of this study was carried out in order to estimate fat soluble vitamins contents in Atlantic Mackerel fillets from the northeastern coast of Tunisia. Retinol, α -tocophérol and cholecalciferol were separated and analyzed using HPLC system. The experimental procedure used for extraction consisted on saponification, normal phase separation with UV and fluorescence detection for tocopherol and retinol. While cholecalciferol determination was carried out using reverse phase HPLC. Results showed that fillets of *Scomber scombrus* are a substantial natural source of A, D and E vitamins (6.54; 8.31; 445 μ g/100g).

Keywords — *Scomber Scombrus*, Fat Soluble Vitamins, HPLC Determination.

I. INTRODUCTION

Sea food products are generally high in polyunsaturated fatty acids (PUFAs) mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have benefic effects on human health such as the prevention of some cardiovascular diseases [1], hypertension, autoimmune disorder and cancer. They are also considered as a high source of vitamins and minerals [2]. Known as fat soluble vitamins, retinol, α -tocophérol and cholecalciferol contents differ largely between fish species.

Vitamin A activity is related to the presence of retinol and carotenoids. Fish species are able to transform easily carotenoids into vitamin A [3]. The most common pigment in fishes is asthaxanthine, which is an important carotenoid in salmon, shellfish and shrimps [4]. Fish species convert asthaxanthine, canthaxanthine and isozéaxanthine into β -carotene and then in retinol [5] via retinal, in the intestine [6]. Retinol is found in the liver of marine and freshwater fish species, zeaxanthin and lutein were found as the main carotenoids in the eggs [7]. In wild rainbow trout astaxanthin is the major carotenoid pigment responsible for its flesh pink color, while both astaxanthin and canthaxanthin are used for pigmenting farmed trout [8]. Carotenoids have an important function as pigments related to fish's communication; in fact fishes are able to change their color through both hormonal and neuronal control of chromatophores, where carotenoids represent the major constituents [9].

Vitamin A has an antioxidant activity [10]; it promotes vision, bone and muscle growth and has an effect on many body functions such as gene transcription, reproduction, maintenance of healthy epithelial tissue [11] and embryonic development and hematopoiesis. Also, it has an immune function and then protects the skin and the body

against infections. Hypovitaminosis and hypervitaminosis A can lead to abortion and embryonic malformation [12].

Vitamin E exists in different forms, (α , β , γ , δ) tocopherols and their corresponding tocotrienols, each form has its own biological activity. Characterized by their antioxidant activity, tocopherols react with peroxide radicals, and inactivate them [13]. Alpha-tocopherol is the main and the most active form of vitamin E [14]. Considered as a natural antioxidant [15] it prevents the rancidity of oils and inhibits the process of per-oxidation of polyunsaturated fatty acids and cell membranes compounds [16]. Vitamin E cannot be synthesized by humans; food intake makes the highest contribution.

In marine organism α -tocopherol is the principal vitamin E with low concentration [17]; which is influenced by a large number of factors as: storage, seasonal variations, characteristic of fish species and their diet composition [18].

Having some beneficial properties, it decreases the risks of cardiovascular diseases, cancer, and prevents the sexual impotence [19]. In addition, vitamin E supplements play a role against diabetes and cystic fibrosis [20]; also, it is used in the chemical industry as additive for food and cosmetic products [21].

Vitamin D is referred to two fat soluble prohormones, vitamin D₂ (ergocalciferol, derived from plants) and vitamin D₃ (cholecalciferol, produced by animals); ingested vitamin D₂ and endogenously produced D₃ are converted to the biological active form of vitamin D, in human body [22]. Recent studies indicate that vitamin D₃ is more active than vitamin D₂ [23].

Vitamin D is responsible for a wide range of functions, one of the well established is related to calcium absorption and homeostasis with the role in bone mineralization particularly in relation to rickets and osteomalacia [24], in tandem with calcium, vitamin D may be involved in the prevention of osteoporosis, fracture incidence and falls, particularly for old people [25],[26].

Vitamin D deficiency induces many diseases including psoriasis, multiple sclerosis, inflammatory bowel diseases, diabetes, hypertension, cardiovascular diseases, and various cancers [27],[28]. An increased vitamin D status is linked to significant morbidity and mortality risk reduction [29].

Foods that make the highest contribution to dietary intakes of vitamin D is related to habitual dietary patterns and vary from country to another, however fish and fats, shellfish, fish liver oils, oily fish, egg yolk and mushrooms are considered as the richest sources of vitamin D [30].

In sea food product, the only vitamin D determined is vitamin D₃. Fish accumulate only vitamin D₃ or convert vitamin D₂ into D₃ [31] which concentration can vary significantly both between and within species and according to whether they are wild or farmed. In fish tissues vitamin D originates from their feeding habits chain with phyto- and zooplankton, containing both of vitamin D₂ and D₃ [32].

The aim of this study is to investigate oil soluble vitamins in Atlantic mackerel fillets from the northeastern coast of Tunisia.

II. MATERIAL AND METHODS

A. Biological material

In order to quantify fat soluble vitamins composition in *Scomber scombrus* fillets, 10 to 20 selected fresh fishes caught during June 2011 from the northern east coast of Tunisia Bizerta region, the fishing harbor of (Zarzouna). Specimens were randomly selected with an average length and weight of 24.6cm and 81.17g. When received in the laboratory, samples were immediately washed several times and then eviscerated, filleted and mixed to provide the raw materials for recovery of lipid for oil-soluble vitamins analysis. All the analysis were carried out in triplicates.

B. Chemicals and Standards

Solvent used in the extraction process and chromatography analysis were for HPLC grade. The extractor solvents (hexane; ascorbic acid; 1, 4-dioxane; petroleum ether; ethanol; potassium hydroxide; acetonitrile; 2-propanol) were purchased from Merck (Darmstadt, Germany). Internal standards (DL- α -tocopherol and tocopherol-isomers (β , γ , and δ)) were purchased from Merck (Darmstadt, Germany), vitamin D₂ and D₃ from Sigma Aldrich (Steinheim, Germany) and (all-trans-retinol) was purchased from Fluka (Buchs, Switzerland).

For α -tocopherol, all-trans-retinol and vitamin D₂ and D₃ standard solutions were prepared in ethanol at the desired concentration then they were diluted in ethanol or n-hexane as appropriate. To avoid oxidation, these solutions were prepared in dark in the same day of the analysis and concentrations were confirmed spectrophotometrically, using known absorption coefficients of each vitamin. Quantification of each vitamin was done with the internal standard method and peak areas were identified by comparisons of retention times with those of standards used for vitamins determination.

C. Analysis method

1. Saponification and extraction

Vitamins analysis was based on saponification and extraction of the unsaponifiable matter. Vitamin A, D and E contents determination was made according to the European standard: EN 12 823-1(2000); EN 12 821 (2000) and EN 12 822 (2000) respectively. In this trend, 10 to 30 g of homogenized biomass samples were saponified into 250 ml balloon by adding extraction solution (100 ml of ethanol, 50 ml of 50% KOH and 1g of ascorbic acid). The

solution was boiled in water bath (80°C) and the whole flask content was transferred in separatory funnel (500 ml) and 100 ml of distilled water and 150 ml of petroleum where added. The solution was shaken to separate the layers. The organic layer was evaporated by rotary evaporator and then filtrated.

The entire saponification procedure was practiced under nitrogen atmosphere in dark with avoidance as much possible direct light and with ascorbic acid addition as an antioxidant. Aliquot of 20 μ l was injected in the HPLC system. Vitamin D saponification and extraction was performed exactly as described for retinol and α -tocopherol.

For both vitamins A and E analysis, peak was identified by comparing with those of authentic standards and their contents were calculated on a weight basis. Peak identification and purity was operated with normal-phase chromatography system with UV (all-trans-retinol) and fluorescence detections (tocopherols), for vitamin D compounds analysis, reverse-phase chromatography with UV detection was used after the semi-preparative clean-up procedure.

2. Semipreparative HPLC

Purification and quantification of vitamins were carried out using normal phase HPLC, which consists on varian vista 5500 liquid chromatography System. Vitamin A injection volume was 20 μ L; eluent A (980ml hexane, 20 ml isopropanol); flow rate, 1 mL/min; fluorescence detector, excitation at 325 nm, emission at 480 nm; column (Maxsil 5 Silica 250 \times 4.00 mm).

For vitamin E we used the same chromatographic conditions but eluent A was composed of (970 ml hexane, 30 ml 1,4 dioxane); flow rate, 1 mL/min; with fluorescence detector, and excitation is at 293 nm, and the emission is at 326 nm; column (Maxsil 5 Silica 250 \times 4.00 mm); whereas technique applied for vitamin D is both of reversed-phase and normal-phase systems, in fact clean-up semipreparative HPLC was performed first by a normal-phase analytical column with a mobile phase containing (propanol /n-hexane). Vitamin D and provitamin D fractions were collected separately and then evaporated and easily redissolved in the eluent for the analytical reversed-phase chromatography.

3. Analytical HPLC

The analytical HPLC system consisted on a Varian vista liquid chromatography System with UV detector set at 265 nm and a column (5mm, 25 cm \times 4.6 mm) with guard column (C18, Waters). Temperature of the column oven was 25°C. Mobile phase contained 93% of methanol and 7% of water. Flow rate of the mobile phase was 1 ml/min, and the injection volume was about 20 μ l. Vitamin D₃ quantification was made using vitamin D₂ as an internal standard. Quantification was based on peak areas [33]. Standard curves were obtained daily by standard injections. The variation of detector response and the retention times were obtained by standard injection.

III. RESULTS

The analytical chromatograms of the studied vitamins are presented in (Figure 1). Using an internal standard for quantification of vitamin A, peak response was linear and the injection concentration contains retinol from 0.125 to 2.5 μ g ($R^2 = 0.999$).

For vitamin D determination, peak area response was linear and tested range was from 0.001 to 0.2 μ g ($R^2 = 0.998$). As observed for vitamin A and D, the detector response of α -tocopherol was linear ($R^2 = 0.9999$).

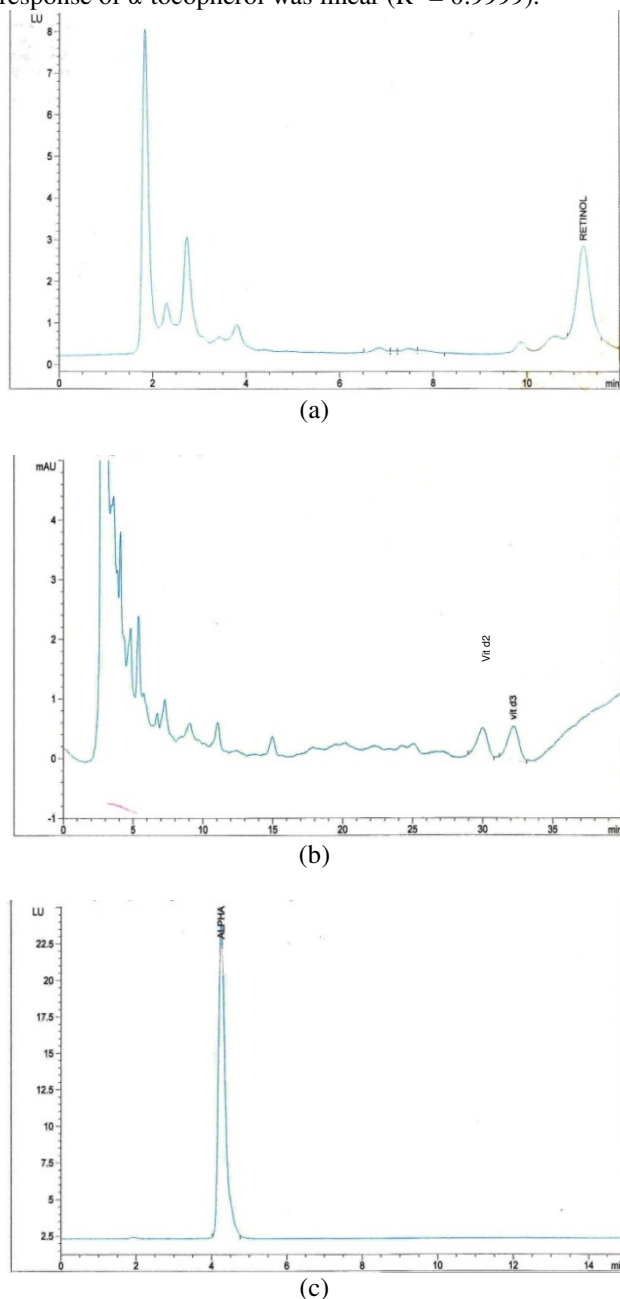


Fig.1. Analytical chromatograms of retinol (a), vitamin D (b), and α -tocopherols (c) in Atlantic Mackerel fillets.

As indicated in (Table 1) Oil soluble vitamin contents in studied samples were high in vitamin E comparatively with retinol and vitamin D contents.

Table 1: Values of Oil soluble vitamins in Mackerel fillets: Means (\pm) Standard deviation

	Vitamin A	Vitamin D	Vitamin E
Content μ g/100g	6.54 \pm (0.03)	8,31 \pm (0.15)	445 \pm (0.04)

IV. DISCUSSION

In our study, the obtained results of the analyzed vitamins were lower than those of other analysis made on Atlantic mackerel [34] where retinol, vitamin D and α -tocopherol levels were about (430; 14.7 and 3300 μ g/100g). In mackerel liver, vitamin D ranges from 2.5 to 28 μ g/100g [35], in fact our finding is included in this range (8.31 μ g/100g). According to the UK Food Standard Agency [36] vitamin D content of raw Atlantic Mackerel is about 8.8 μ g/100g, this value is in accordance with our finding, but comparatively with US National Nutrient Database [37] our value is lower than 16.1 μ g/100g. Other study on Atlantic Mackerel [38] show that vitamin D content was about (41.6 ng/g) this value is significantly lower than our finding.

When compared with other fish species as fin-fish (Pacific cod), it was found that vitamin A content was about 8 μ g/100g [39]; this value is close to our finding. In Horse Mackerel, vitamins A and E were about 141 μ g/100g and 0.773 mg/100g [40], these values are higher than results obtained in this data. Study on Baltic herring fillets show that vitamin D and all-trans-retinol contents were about 8 and 7 μ g/100g respectively, whereas α -tocopherol content was about 6220 μ g/100g [41], values relative to our study were close to these results for retinol and cholecalciferol, whereas for vitamin E concentration our result are significantly lower. However, in mackerel muscles it was reported that vitamin E values ranging from (16 to 18 μ g/g) [42]. In Baltic herring vitamin D was ranged from (5.7 to 15.4 μ g/100g) [43] results of our analysis are included in this range.

Farmed fishes may have additional α -tocopherol in their diet [44]; and when they are fed diets with high vitamin E level it will be accumulated in all tissues, and a large portion is redistributed to ovary [45].

The diversity of values noted in the literature and the differences with our results may be related to the influence of different factors such as the parts of the analyzed tissues, the different times and localities, geographic availability [46], the fishing seasons, the environmental factors, the biological cycle, physiological state and maturity, the feeding habit, the size and the sex and the part of fish used for analysis [32]. These factors are known to affect variability in nutrient composition, particularly for vitamins [47]. Indeed, in our study analyzed specimens were caught in June, 2011. This period is related to the post spawning time in which lipid value decreases significantly. By another hand, fishes were very small (24.6 cm) and the analyzed mussels were section from the middle of the body and there was a mixture of dark and white tissue. Sampling period corresponded to high temperature, affecting its fatty acids composition and probably vitamin E requirement [48]; likewise toxic

inorganic and organic contaminants [10] and concentration of some antioxidants [49].

V. CONCLUSION

We conclude that Atlantic mackerel muscle from the north east of Tunisia, represents a real substantial and natural source of oil soluble vitamins. Method used for extraction and separation with HPLC system induce a selective and sensitive detection of each vitamin studied in fish samples.

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