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Original Research Article

Black Sea fish and shellfish as essential source of vitamin B_{12}

Diana A. Dobreva[1]*, Albena V. Merdzhanova[1], Mona D. Stancheva[1], Doychin I. Terziyski[2], Veselina Z. Panayotova[1]

[1]Department of Chemistry, Medical University of Varna, Varna, Bulgaria
[2]Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria

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***Correspondence:**
Dr. Diana A. Dobreva,
E-mail: didobreva@gmail.com

ABSTRACT

Background: Vitamins are very important for the human body because they are necessary for many biological absorption processes of other nutrients, for cells and tissues growth and repair. Because of this, there are many recommendations on daily vitamins intake, approved by the different country and world food organizations. Usually under the term "vitamin B_{12}" is understood only cyanocobalamin, but actually this name is general and covers all potentially biologically active "cobalamins"- a group of cobalt-containing compounds. Animal foods, as different meat, milk, eggs, fish and shellfish are considered as the main dietary sources of this vitamin. They contain different forms (methylcobalamin and deoxyadenosylcobalamin) of vitamin B_{12} in different amounts. There is limited information in the scientific literature about the vitamin B_{12} content in black sea fish and shellfish. The aims of the present work were to determine and compare vitamin B_{12} contents as well as relative daily intake of vitamins in different fish and shellfish species from Black Sea waters.
Methods: Vitamin B_{12} was analysed spectrophotometrically. The method for quantitative analysis includes extraction from the edible tissue and enzymatic hydrolysis to release the cobalt ions.
Results: The quantities of vitamin B_{12} found in the edible tissue of the analysed samples ranged from 0.63 μg.100 g^{-1} ww to 21.5 μg.100 g^{-1} ww.
Conclusions: The observed results confirm that all fish and molluscs samples deliver significant quantities of the water-soluble vitamin B_{12}.

Keywords: Cyanocobalamin, UV-Vis, Spectrometry, Cobalt-complexes, Mussel

INTRODUCTION

Vitamin B_{12} is essential for the human body - the synthesis of red blood cells, DNA, some neurotransmitters and metabolites. It is responsible for maintaining the activity of the immune system, supports a number of processes in the central and peripheral nervous system and plays a key role in the implementation of key processes and protects against many diseases.

All water-soluble vitamins (B-group and C), with the exception of vitamin B_{12}, were found in organisms of plant origin. Sources of biologically active forms of cobalamins were only organisms of animal origin and some bacteria. Vegetable foods were considered not to contain biologically active forms of vitamin B_{12}.[1] Compared to those of other vitamins, the vitamin B_{12} molecule is the largest (molecular mass of 1355.4 g.mol^{-1}) and the most complex. The term "vitamin B_{12}" is a generic name that includes all potentially biologically active forms – "cobalamines" (a group of cobalt-containing compounds).[2] As the main sources of vitamin B_{12} were considered meat, milk, eggs, and fish and crustaceans.[3,4] They mainly contain two forms of vitamin methylcobalamine and 59-deoxyadenosylcobalamine, which function as coenzymes in methionine biosynthesis, as well as in the metabolism of fatty acids in mammalian cells.[2,5] The absorption and bioavailability of vitamin B_{12} in the body strongly depends on the gastrointestinal absorption of the different individuals.[1] On consuming or supplementing with excessive doses, the amount that exceeds absorption body's ability was release through the urine.[6] Most often, vitamin B_{12} deficiency in the human body was usually caused by malabsorption, combined with inadequate dietary intake.[7-9]

Studies conducted among a vegan group indicate that they need additional vitamin B_{12} intake because they do not consume foods of animal origin.[8] Vegetarians do not suffer from a severe lack because they regularly consume dairy products and eggs, which contains some of the daily vitamin B_{12} needed.

The need from vitamin B_{12} quantitation is based on the requirement of clinical analysis (control of blood serum and plasma levels), food analysis (amount in different food matrices), and pharmaceutical substance analysis. Vitamin B_{12} can be quantitated by various methods such as microbiological, colorimetric, spectrophotometric, atomic absorption, atomic emission and liquid chromatographic.[10] The choice of a suitable method for quantitative analysis depends on different factors: sample type, purpose of analysis, pre-treatment procedure, time of analysis, type and quantities of reagents, sensitivity of the method, and the price. The most commonly used method for quantification of vitamin B_{12} in complex matrices like blood and serum, characterized by high resolution and sensitivity, is high performance liquid chromatography (HPLC).[11,12] However, in routine food analysis, the spectrophotometric method also provides the necessary sensitivity. It is characterized by speed, high sensitivity and accuracy, and relatively low cost. The analysis is to determine the content of cobalt ions in the aqueous sample extract by means of a colorimetric reaction.[13] The quantitative analysis of the vitamin B_{12}, in the selected fish and shellfish species, was performed by a spectrophotometric method.

There is limited information in the scientific literature about the cyanocoblamin's content in Black Sea fish and shellfish. Because of this, the aims of the present work were to determine and compare vitamin B_{12} contents as well as relative daily intake of vitamins in different fish and shellfish species from Black Sea waters.

METHODS

Reagents and instrumentation

All used reagents were with analytical grade purity, purchased from Sigma Aldrich – cobalt (II) chloride ($CoCl_2$), acetate buffer (0.1 M, pH 4.0), sodium cyanide (NaCN), α-amylase, pepsin, pyrogallol red, nitric acid (conc. HNO_3), sulfuric acid (conc. H_2SO_4), water and ethanol. For the purposes of the experiment, from the listed reagents were prepared the following solutions: 1 $mg.ml^{-1}$ cobalt (II) solution in 0.09 M nitric acid, 0.02% pyrogallol red, 1% NaCN.[12,14]

The spectrophotometric analysis of vitamin B_{12} was performed on a Hach Lange DR3900 spectrophotometer. It is a single-ray instrument operating in the visible area of electromagnetic spectrum. The quantitative determination of the Co (II) content in the aqueous extracts of the analyzed samples was carried out by measuring their absorption. For this purpose, the spectrophotometer was calibrated by measuring the intensity of Co (II) and pyrogallol red complex in solutions with increasing concentration. Upon construction of the calibration curve, five different standard solutions of $CoCl_2$ in aqueous nitric acid were used in a concentration range of 0.025-0.4 μg/ml.

The colored complex of each of the five solutions was synthesized directly in the glass cuvette of the apparatus. Their absorption was measured at λ_{max} =475 nm. In the cuvette were mixed 1 ml of the corresponding cobalt solution and 0.5 ml of a 0.02% solution of pyrogalol red. The absorption was read as measured against a blank sample (distilled water with 0.5 ml pyrogallol red solution). The described procedure was used to measure the absorptions of the 5 standard solutions of Co (II). With the obtained data was constructed the calibration curve (Figure 1). The shown linearity of the method was 0.9846.

Samples

All samples - ten Black Sea fish (garfish, sprat, grey mullet, horse mackerel, bonito, bluefish, goby, turbot, shad and red mullet) and shellfish (black mussel and rapana) were used as samples for the present study (table 1). The fish and mollusk species were purchased from Varna local fish markets. All samples were immediately frozen and stored at -20^{o}C in a home fridge. Biometric characteristics as mean weight (g) and mean length (cm) were determined and presented in Table 1.

Samples preparation

Before quantitative spectrometric analysis of vitamin B_{12}, it is necessary to extract from samples. The enzymatic hydrolysis is a traditionally used method for complex matrices. Heudi et al used the enzymes α-amylase and pepsin, 1% NaCN, at pH ~ 4 acetic acid buffer.[12] Under these conditions the samples were hydrolyzed for 3 hours at 37^{o}C. Thereafter, the enzymes were deactivated by prolonged (35 min) heating at 100 - 120° C.

The samples were cooled to room temperature in an ice water bath, filtered through a medium-speed "white blend" filter. The filtrates were used in the subsequent steps of the experiment. The solutions, containing the sample extract of vitamin B_{12}, were subjected to acid decomposition of the cobalamine molecule for the separation of cobalt ion.[15] A mixture of conc. HNO_3:

conc. H_2SO_4 =10:1 was added to a portion of the collected filtrate and the resulting solution was evaporated to near dryness. The residue was neutralized and transferred to a measuring flask and distilled water was added to the mark. The solution was used to perform a spectrophotometric quantitative analysis of the cobalt ions content. Four parallel samples were processed for each fish species, mussel and rapana (two with and another two without a standard additive). The evaluation of the extraction procedure was carried out using the method of standard addition.

Table 1: Biometrical and biological characteristics of analyzed fish and shellfish.

No	Species	n	Food/habitat	Length, cm mean±SD	Weight, g mean±SD
1.	Sprat *(Spratus sprattus sullinig)*	21	Zooplankton/pelagic	11.5±0.2	10.5±0.3
2.	Red mullet (*Mullus barbatus*)	18	Zooplankton and crustaceans/demersal	14.5±1.5	39.3±3.0
3.	Horse mackerel (*Trachurus Mediterraneus)*	18	Carnivore/pelagic	14.9±1.5	22.9±2.3
4.	Goby *(Neogobius melanostomus)*	16	Carnivore/demersal	16.7±1.1	69.4±2.4
5.	Shad *(Alosa pontica)*	7	Carnivore/ pelagic	26.8±2.1	325.0±5.0
6.	Bluefish (*Pomatomus saltatrix*)	12	Carnivore/pelagic	18.5±1.1	60.0±3.0
7.	Grey mullet *(Mugil cephalus)*	7	Herbivore/ pelagic	32.0±2.5	290±4.5
8.	Garfish (*Belone belone*)	7	Carnivore/pelagic	35.0±1.2	52.0±3.5
9.	Bonito *(Sarda sarda)*	5	Carnivore/pelagic	40.0±1.5	420.0±5.5
10.	Turbot *(Pseta maxima)*	3	Carnivore/demersal	45.0±2.0	1400.0±10.0
11.	Black mussel (*Mytilus galloprovincialis*)	50	Herbivore/demersal	5.5±0.5	12.0±0.5
12.	Rapana (*Rapana venosa*)	15	Carnivore/demersal	10.0±0.5	155.0±5.0

n=number of specimens.

Spectrophotometric analysis

Quantitative determination of cobalt ions in samples was performed analogously to standards. The analyzed solution was prepared directly in the spectrophotometer's cuvette. Combine 1 ml of sample and 0,5 ml of 0,02% pirogalol red solution. The absorption of resulting complex was measured against a blank sample (distilled water with 0.5 ml pyrogalol red). The amount of cobalt ions was determined by the built-in standard curve.

RESULTS

Table 2: Vitamin B_{12} content in black sea fish and shellfish.

No	Species	Vitamin B_{12}, μg.100g^{-1}ww
1	Sprat	0.75 ± 0.07
2	Horse mackerel	5.75 ± 0.48
3	Goby	21.50 ± 4.60
4	Grey mullet	5.70 ± 0.80
5	Shad	18.88 ± 3.70
6	Bluefish	13.20 ± 3.10
7	Bonito	6.05 ± 1.40
8	Turbot	19.50 ± 4.30
9	Garfish	0.63 ± 0.08
10	Red mullet	17.40 ± 2.20
11	Black mussel	22.35 ± 3.81
12	Rapana	19.05 ± 2.60

Quantitative determination of the content of Co (II) in ten fish species and Black Sea mussel and rapana was carried out. The data obtained from the analysis were recalculated and they are shown in Table 2 as micrograms vitamin B_{12} per 100 grams of raw edible tissue (μg.100g^{-1}ww) of the test specimens (mean±standard deviation).

DISCUSSION

The shown data variety was in the range of 0.63 μg.100 g^{-1}ww to 22.35 μg.100 g^{-1}ww. Half of the fish analyzed species showed a content of this vitamin below 10 μg100 g^{-1} ww, the lowest values were calculated of 0.75 μg.100 g^{-1} ww in sprat and 0.63 μg.100 g^{-1} ww in garfish. With exception of shad (18.88 μg.100 g^{-1} ww), all other fish with high levels of vitamin B_{12} were demersal species - 21.5 μg.100 g^{-1}ww for goby, 19.50 μg.100 g^{-1} ww for turbot, 17.40 μg.100 g^{-1}ww for red mullet and rapana (19.05 μg.100 g^{-1} ww) and mussel (22.35 μg.100 g^{-1} ww).

There are only few studies in the scientific literature presenting results for cyanocobalamin content in raw edible fish or shellfish tissues.[11,16] Ahmadnia and collective investigated vitamin B_{12} content in grey mullet fish and the kilka (similar to sprat fish). The found amounts were significantly higher than our data - 13 μg.100 g^{-1} ww for grey mullet and 5.7 μg.100 g^{-1} ww for kilka.

Lebiedzinska and colleagues studied the vitamin B_{12} content in salmon's edible tissue. The presented result (2.8 μg.100 g^{-1} ww) was similar to our data, but falls within the low amount value.[11]

Some databases also present results for the cyanocobalamine content in fish and shellfish - whole food catalog and USDA food composition database. The first of them showns data for bluefish (5.39 μg.100 g^{-1} ww), shad (0.15 μg.100 g^{-1} ww) and turbot (2.20 μg.100 g^{-1} ww).[17] The results were several times higher than ours.

The USDA food composition database shows quantities of vitamin B_{12} for eel, mackerel and turbot – 3.00 μg. 100 g^{-1} ww, 13.67 μg.100 g^{-1} ww and 2.20 μg.100 g^{-1} ww, respectively.[18] The presented values for eel (like garfish) and mackerel were close to those obtained in our study, while the content of the shad was lower. The data for vitamin B_{12} content in rapana are very scarce in the scientific literature. The Japanese database presents a result for the amount of vitamin B_{12} in rapana tissue which is close to that in our research.

Results for cyanocobalamin in mussel were published by Ramasamy Santhanam in the book Nutritional Marine Life, as well as in the American database The Self Nutrition Data. Their results are close to those found in our study - 12 μg. 100 g^{-1} ww and 17 μg. 100 g^{-1} ww, respectively.[19]

The estimation of the edible tissue of the analyzed Black Sea fish and shellfish as a source of vitamin B_{12} was based on the comparison of the specified amount of vitamins in the recommended daily intake tables for Bulgaria.[20] The comparison was made for the age group up to 60 years.

The results shown in Figure 1 represent a percentage of the recommended daily intake (RDI) for vitamin B_{12} (for 100 grams of edible tissue).

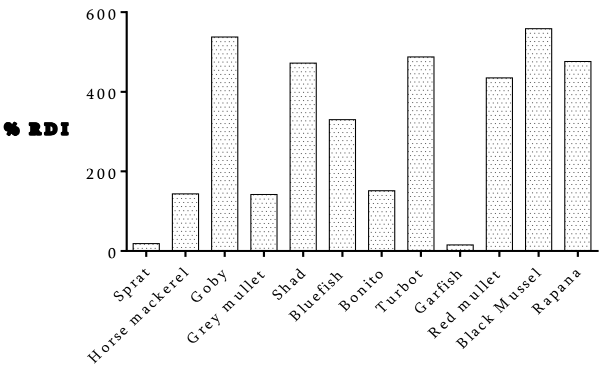

Figure 1: Percentage of the RDI* of vitamin B_{12} in Black Sea fish and shellfish.

*Relative daily intake (RDI).

Almost all of analyzed fish and shellfish, excluding sprat and garfish, provide significant amounts of vitamin B_{12} above the RDI. One hundred grams of edible tissue of gray mullet, bonito and horse mackerel contain almost doubled amounts of RDI. In red mullet, turbot, shad, goby, black mussel and rapana, the quantities were almost seven times higher than the required daily intake.

CONCLUSION

The vitamin B_{12} content was studied in twelve Black Sea samples of animal origin - ten fish and two molluscs. With the exception of two fish species (sprat and garfish), all others provide significant amounts of the analyte. With a significantly higher content of cyanocobalamin were distinguished all demersal fish, rapana and mussel. Recalculated with RDI, they provide over 500% of the required daily amount of vitamin B_{12}. Bluefish, gray mullet, bonito and horse mackerel contain much less cyanocobalamin, but they also provide significantly more than 100% RDI.

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