

РЕЗЮМЕТА НА НАУЧНИТЕ ТРУДОВЕ

на

гл. ас. Диана Атанасова Добрева, дх

за участие в конкурс

за заемане на академична длъжност ДОЦЕНТ

в област на висшето образование

4. Природни науки, математика и информатика, професионално направление

4.2. Химически науки, по научна специалност „Химия“

обявен за нуждите на Катедра Химия в ДВ бр.36/27.04.2018г.

Резюметата на научните трудове са представени както следва:

- I.** Автореферат на дисертационен труд
- II.** Равностойни на монографичния труд пълнотекстови публикации
- III.** Публикации в български и чуждестранни научни издания
- IV.** Глава от книга
- V.** Учебно помагало
- VI.** Избрани резюмета от научни форуми

I. Автореферат на дисертационен труд	
1	АВТОРЕФЕРАТ на ДТ на гл. ас. Д. Добрева на тема „ <u>Мастноразтворими витамини в черноморски и сладководни риби</u> “, за присъждане на образователна и научна степен „ДОКТОР“ по научна специалност „Биоорганична химия, химия на природните и физиологично активни вещества“, Варна, 2015

МЕДИЦИНСКИ УНИВЕРСИТЕТ „ПРОФ. Д-Р ПАРАСКЕВ СТОЯНОВ“ – ВАРНА

**ФАКУЛТЕТ ПО ФАРМАЦИЯ
КАТЕДРА ХИМИЯ**

ИАНА АТАНАСОВА ДОБРЕВА

**МАСТНОРАЗТВОРИМИ ВИТАМИНИ
В ЧЕРНОМОРСКИ И СЛАДКОВОДНИ РИБИ**

АВТОРЕФЕРАТ

**НА ДИСЕРТАЦИЯ ЗА ПРИСЪЖДАНЕ НА ОБРАЗОВАТЕЛНАТА
И НАУЧНА СТЕПЕН „ДОКТОР“**

**по научна специалност „Биоорганична химия, химия на природните и
физиологично активни вещества“**

Научни ръководители: проф. Мона Станчева, дхн
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ВАРНА, 2015

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Дисертационният труд се състои от 179 страници, включващи 46 фигури, 46 таблици. Цитирани са 210 литературни източника, 197 от които на латиница и 13 на кирилица.

Дисертационният труд е обсъден на заседание на разширен катедрен съвет на Катедрата по Химия при Медицински университет–Варна и насочен за защита пред научно жури.

Дисертантът е главен асистент в Катедрата по Химия, при Медицински университет – Варна.

Експерименталната работа по дисертационния труд е извършена в научната лаборатория на Катедрата по Химия.

Защитата на дисертационния труд ще се състои на г. от ч. в.....аудитория на Медицински Университет "Проф. Д-р Параскев Стоянов" - Варна.

ВЪВЕДЕНИЕ

Витамините обхващат група от разнообразни по химичен състав и структура органични съединения, необходими за растежа и развитието на организма и поддържане на неговото здраве. Те не могат да бъдат синтезирани в човешкия организъм, което определя необходимостта от вноса им с храната. Изключение прави само витамин D, който се синтезира в организма, но в количества, които са недостатъчни за задоволяване на физиологичните потребности.

Витамините се разделят на две главни групи според разтворимостта си - водоразтворими и мастноразтворими. Към мастноразтворимите витамини се отнасят четири групи витамини - А, D, Е и К, които съдържат в структурите си полиизопренови вериги. Мастноразтворимите витамини имат главно структурна и регулаторна функция. Витамин А играе важна роля в процесите фоторецепция и репродукция. Витамин D регулира калциевата хомеостаза – повишава резорбцията и отлагането на калций в костите, стимулира растежа, предпазва от костни фрактури. Витамин Е се вгражда в липидния бислой на мембранните структури и предпазва ПНМК от окисление и автоокисление, повлиява генната експресия, подобрява съдовата дилатация.

В последните години се доказва редица специфични функции на мастноразтворимите витамини, отвъд добре известните. Така, витамин D е важен регулатор на имунитета, има антиканцерогенно действие. Витамин К регулира костното изграждане и резорбция, има антиатерогенен ефект.

Световната здравна организация проучва рисковете от развитие на дефицит на тези витамини. Тя препоръчва ежедневен прием на витамини А, D₃ и Е в достатъчни количества. Това количество се дефинира като „препоръчителен дневен прием“ от съответния витамин. Министерството на здравеопазването в България е изготвило и публикувало своите препоръки в „Наредба № 23 от 19 юли 2005г. за физиологичните норми за хранене на населението“.

Храните от растителен и животински произход са основен източник на мастноразтворими витамини. Рибните тъкани се считат за особено богати на тези нутриенти. Количествата на мастноразтворимите витамини в тях значително варират в зависимост от водния басейн, вида, различните представители от даден рибен вид, както и сезона на улов. Оценено е, че ядливите рибни части доставят значими количества от препоръчителния дневен прием на мастноразтворими витамини за човека. Изключение прави витамин К, който се открива в много ниски концентрации, само в някои рибни видове.

В България проучванията върху съдържанието на мастноразтворими витамини в черноморски риби са много малко. Не се откриват систематични изследвания за рибни видове от българската акватория на Черно море и сладководните водоеми във вътрешността на страната. Липсват данни за промените, настъпващи с тези витамини в рибната тъкан, при съхранението и термичната обработка на рибите. Това определя и целта на настоящата работа.

ЦЕЛ И ЗАДАЧИ НА ДИСЕРТАЦИОННИЯ ТРУД

ЦЕЛ: Да се проучи съдържанието на all-trans-ретинол, холекалциферол и α -токоферол в традиционно консумирани в България черноморски и сладководни видове риба и да се извърши оценка на съдържанието на тези витамини по отношение на препоръчителния им дневен прием.

За постигането на тази цел ще бъдат разработени следните задачи:

1. Оптимизиране и валидиране на течно-хроматографски метод за определяне съдържанието на all-trans-ретинол, холекалциферол и α -токоферол в ядивна рибна тъкан.
2. Определяне съдържанието на витамини А, D₃ и Е в ядивната тъкан на традиционно консумирани рибни видове чрез оптимизирания течно-хроматографски метод;
3. Оценка на ефекта на съхранение на рибна тъкан при ниски температури и термична обработка, върху съдържанието на изследваните мастноразтворими витамини;
4. Оценка на качеството на рибите като естествен източник на витамин А, витамин D₃ и витамин Е.

II. Равностойни на монографичния труд пълнотекстови публикации

2.	M. Stancheva, A. Merdzhanova, D. Dobрева , L. Makedonski Fatty Acids Composition and Vitamin A, D and E Content of Sprat (<i>Sprattus Sprattus</i>) and Goby (<i>Neogobius Rattan</i>) from Bulgarian Black Sea <i>Ovidius University Annals of Chemistry</i> , 21, 1, 23-28, 2010. ISSN: 1223-7221
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Fatty acid composition and fat-soluble vitamins content of sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea

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Abstract Sprat and goby are commercially important Bulgarian Black Sea fish species. The fatty acid (FA) composition was analyzed by Gas Chromatography with MS detector. Lipid extraction was done according to the Bligh and Dyer method. The monounsaturated FA accounted were 26.93 % for sprat and 30.38 % for goby and palmitoleic (C 16:1) and oleic (C 18:1) acids were dominants in this group. In comparison with other groups, the polyunsaturated FA showed the high level in goby – 37.60% including eicosapentaenoic (C 20:5 n3, EPA), docosahexaenoic (C 22:6 n3, DHA) acids, and lower level on sprat – 34.33%. The level of n 3 polyunsaturated fatty acid was higher than the total n 6 polyunsaturated fatty acid in the all analyzed Black Sea fish species. HPLC method was used for determination of Vitamin A (all-trans-retinol), Vitamin D₃ (cholecalciferol) and Vitamin E (α-Tocopherol) content. The results from fat-soluble vitamins show the differences between sprat and goby. The present studies suggest that both fish species are good sources of n 3 fatty acids and vitamins A, D₃ and E.

Keywords: Black Sea fish, fatty acids, PUFA, Vitamin A, Vitamin D₃, Vitamin E

1. Introduction

Fish is considered as a valuable source of essential fatty acids, vitamins and low levels on saturated fatty acids and cholesterol. The significance of long chain polyunsaturated fatty acids such as n-3 PUFA has gained attention because of their prevention of human cardiovascular diseases. The vitamins are organic compound that are necessary in very small amounts in the diet and fish is one of the main source of vitamins. Vitamins forms are heterogeneous group of substances and are vital nutrients and the absence of vitamins causes serious physiological problems. They regulate metabolic processes, control cellular functions and prevent different diseases.

Black Sea appears to be one of the important fish basins influencing greatly the economy of all countries around the basin. Bulgarian's fishery catch are mainly based on small pelagic fishes namely sprat (*Sprattus sprattus*), horse mackerel (*Trachurus trachurus*) and others. The fatty acids and vitamins data for different marine fish species especially originating from Canada, Norway, Japan are

available in literature .However information about the fatty acids and vitamin contents of Bulgarian Black Sea fish species is lacking. One report was encountered in the literature, in witch was mentioned FA and vitamin E content in sprat and mackerel [16].

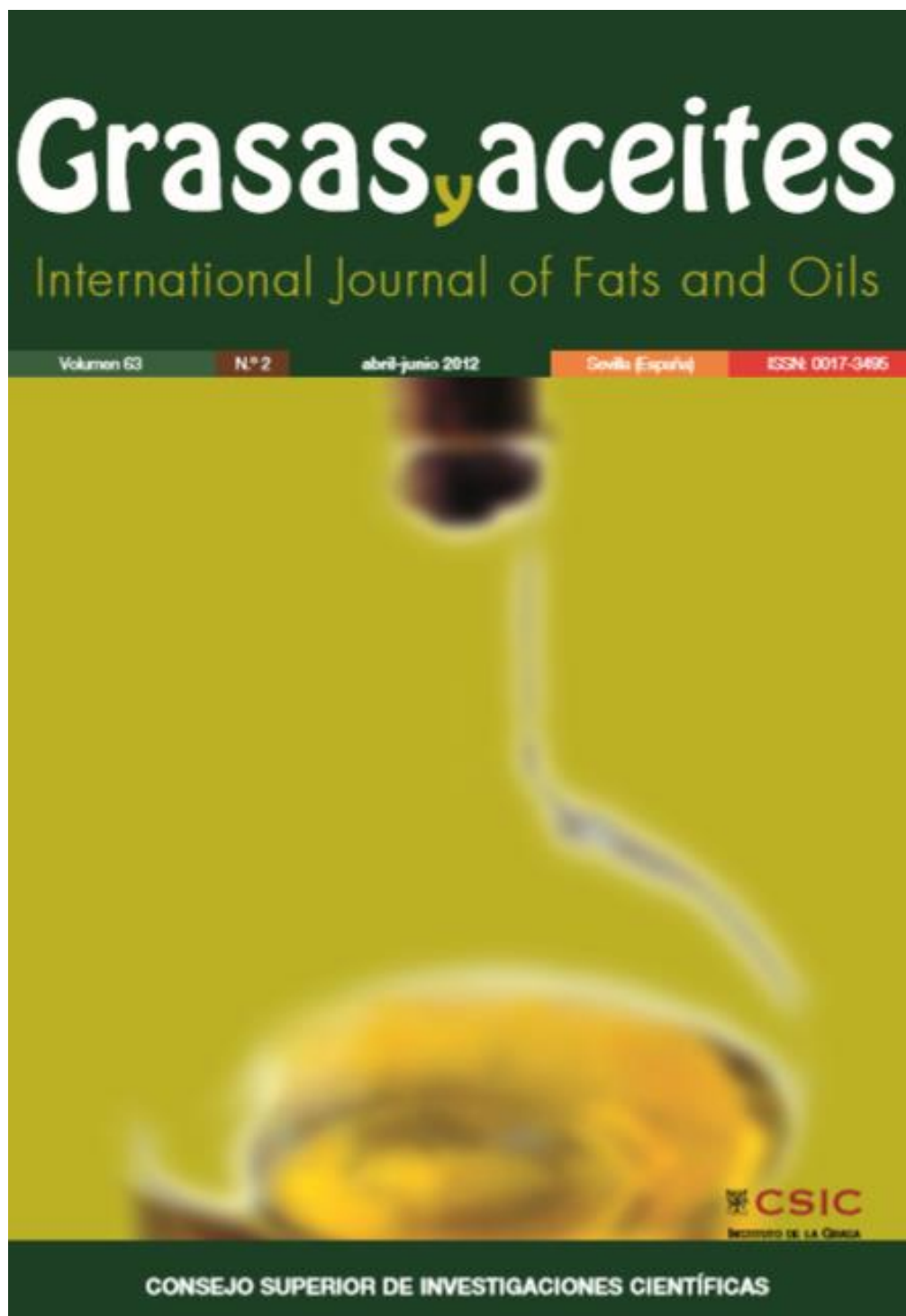
The objective of our study was to collect information on fatty acid composition fat-soluble vitamin content of two of commercially important Bulgarian fish species. Black sea sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) were selected. Their total lipids, fatty acid composition and vitamin A, D₃ and E contents were determined.

2. Experimental

2.1. Sampling of fish species

Samples of the commercially important Bulgarian fish species sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Kavarna (North Bulgarian Black seacoast) were purchased from Varna local fishmarket during non-spawning season (november 2008). Twenty-five specimens with

3.	M. Stancheva, B. Galunska, D. A. Dobрева , A. Merdzhanova Fat Soluble Vitamin And Fatty Acid Contents In Bulgarian Black Sea Fish Species <i>Grasas y Aceites</i> , 63, 2, 152-157, 2012. ISSN: 0017-3495; eISSN: 1988-4214
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GRASAS Y ACEITES, 63 (2),
 ABRIL-JUNIO, 152-157, 2012,
 ISSN: 0017-3495
 DOI: 10.3989/gya.069611

Retinol, alpha-tocopherol and fatty acid content in Bulgarian Black Sea fish species

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RESUMEN

Retinol, alfa-tocoferol y contenido en ácidos grasos de especies de peces búlgaros del Mar Negro.

El objeto de la investigación presentada es definir y comparar los lípidos totales, el perfil de ácidos grasos y el contenido de retinol y alfa-tocoferol en el tejido comestible de cuatro especies de peces con importancia comercial del Mar Negro búlgaro —espadín (*Sprattus Sprattus*), gobio de boca negra (*Neogobius Melanostomus*), chicharro (*Trachurus Trachurus*) y sábalo del Mar Negro (*Caspialosa Pontica*). Dos vitaminas liposolubles son analizadas simultáneamente mediante cromatografía líquida de alta eficacia (HPLC). El contenido mayor de retinol se encuentra en el espadín ($142.3 \pm 4.4 \mu\text{g}/100\text{g}$), y de alfa-tocoferol en el chicharro ($1112.7 \pm 39.2 \mu\text{g}/100\text{g}$). El contenido de ácidos grasos ha sido analizado mediante cromatografía gaseosa/espectrometría de masas (GC/MS). El contenido de ácidos grasos (AG) omega-3 (n3) es considerablemente más alto que el contenido de ácidos grasos (AG) omega-6 en todas las especies analizadas. La proporción n6/n3 está en el intervalo recomendado (0.2-1.5) para el espadín, el gobio de boca negra y el sábalo del Mar Negro. Los niveles relativamente altos de retinol, alfa-tocoferol, relaciones de ácidos grasos, n6/n3 AG y PUFA/SFA muestran que todas estas especies de peces poseen buenas propiedades nutricionales.

PALABRAS CLAVE: *Alosa pontica* – *Mar Negro* – *Neogobius*

levels of retinol and alpha-tocopherol, FA composition, n3/n6 FA and PUFA/SFA ratios indicate that these fish species have good nutritional quality.

KEY-WORDS: *Alosa pontica* – *Black sea* – *Neogobius rattan* – *Sprattus sprattus* – *Trachurus medditeraneus ponticus*.

1. INTRODUCTION

Fish is an important component of a healthy diet, providing a number of substantial nutrients that are essential for achieving a balanced nutrition for children, adults and the elderly. Fish tissue is a good source of fats, proteins, vitamins and minerals. Lipids of marine fish species are rich sources of fat soluble vitamins and both saturated and unsaturated fatty acids (Tocher, 2003). The fat soluble vitamins are essential nutrients related to a diversity of biologically important processes in the human body. Retinol takes place in photo reception, regulates gene expression and cell proliferation, bone growth and reproduction. The biologically active isomer of vitamin E - alpha-tocopherol acts as an antioxidant protecting membrane structures and lipo proteins from oxidation (Anderson J. and Youna L., 2008).

4.	<p>M. Stancheva, D.A. Dobрева Bulgarian Marine and Freshwater Fishes as a Source of Fat-Soluble Vitamins for a Healthy Human Diet <i>Foods</i>, 2, 332-337, 2013. ISSN 2304-8158</p>
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Article

Bulgarian Marine and Freshwater Fishes as a Source of Fat-Soluble Vitamins for a Healthy Human Diet

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Abstract: The aim of the present study evaluates the fat-soluble vitamins all-*trans* retinol (vitamin A), cholecalciferol (vitamin D₃) and α-tocopherol (vitamin E) content in the fresh edible tissue of Bulgarian fish species: marine—grey mullet (*Mugil cephalus*) and bonito (*Sarda sarda*), and freshwater—rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*). The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction with *n*-hexane. All-*trans* retinol, cholecalciferol and α-tocopherol were analyzed simultaneously using RP-HPLC\UV\FL system with analytical column C18 ODS2 Hypersil™. The fat soluble vitamins content (μg per 100 g wet weight) in the fresh edible fish tissue of analyzed fishes are in the ranges: vitamin A from 2.7 ± 0.4 to 37.5 ± 3.4 μg/100 g ww; vitamin D₃ from 1.1 ± 0.1 to 11.4 ± 0.6 μg/100 g ww; vitamin E from 121.4 ± 9.6 to 1274.2 ± 44.1 μg/100 g ww. Three fat-soluble vitamins occur in higher amounts in rainbow trout and grey mullet species. According to recommended daily intake (RDI), they are a good source of cholecalciferol.

Keywords: *Oncorhynchus mykiss*; *Cyprinus carpio*; *Mugil cephalus*; *Sarda sarda*; fat soluble vitamins; HPLC/UV/FL

1. Introduction

Fish is an essential source of both macronutrients—proteins and fats, and micronutrients—vitamins and minerals. Therefore, fish consumption is an important component of a balanced human diet [1].

5.	M. Stancheva, A. Merdzhanova, D.A. Dobрева , L. Makedonski Common carp (<i>Cyprinus caprio</i>) and European catfish (<i>Sillurus glanis</i>) from the Danube River as a source of fat soluble vitamins and fatty acids <i>Czech Journal of Food Science</i> , 32, 1, 16-24, 2014. ISSN 1805-9317
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Common Carp (*Cyprinus caprio*) and European Catfish (*Sillurus glanis*) from the Danube River as Sources of Fat Soluble Vitamins and Fatty Acids

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Abstract

STANCHEVA M., MERDZHANOVA A., DOBREVA D.A., MAKEDONSKI L. (2014): Common carp (*Cyprinus caprio*) and European catfish (*Sillurus glanis*) from the Danube River as sources of fat soluble vitamins and fatty acids. *Czech J. Food Sci.*, 32: 16–24.

The total content of fat soluble vitamins and their percentages in the recommended daily intake for humans per 100 g portion, fatty acids composition, the atherogenic (IA) and thrombogenicity (IT) indices in two freshwater fish species – Common carp (*Cyprinus caprio*) and European catfish (*Sillurus glanis*) were investigated. Retinol contents in fresh edible tissues of the Common carp and European catfish were found to be 30.8 ± 3.4 mg/100 g wet weight (ww) for the Common carp 30.8 ± 3.4 µg/100 g ww and 1.9 ± 0.1 µg/100 g ww for the European catfish, cholecalciferol contents 14.8 ± 1.0 and 3.1 ± 0.1 µg/100 g ww, and α -tocopherol contents 2764.5 ± 44.0 and 2182.5 ± 31.5 µg/100 g ww, respectively. The sum of monounsaturated FA accounted for 50.02% (catfish) and 23.15% (carp). Polyunsaturated FA (PUFA) showed a higher level in the carp (36.75%) and a lower one in the catfish (21.64%). Both fishes are good sources of cholecalciferol in terms of the recommended daily intake of vitamins established in Bulgaria. Three fat soluble vitamins, n-3 PUFAs content, and IA value were higher in carp. IT values were similar for both species.

Keywords: RDI; atherogenicity index (IA); thrombogenicity index (IT); HPLC; GC-MS; human health

Fish are characterised by significant contents of various components beneficial for human health. They are one of the most important dietary sources of fat soluble vitamins and polyunsaturated fatty acids (PUFA), their contents, however, strongly depend on the fish species, gender, maturation, environmental factors, and feeding type (TOCHER 2003; STEVENS 2006).

Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet. They control a variety of biologically important processes in the human body. All-trans retinol participates in photoreception, regulates the gene expression, bone growth, teeth development, reproduction, etc. Cholecalciferol promotes and enhances the absorption and metabolism of calcium and phosphorus. α -Tocopherol

is an important antioxidant as it protects the membrane structures, essential fatty acids, and vitamins A from oxidation (RIBAROVA 2007; ANDERSON 2008).

Various epidemiological studies have demonstrated the key role of fish consumption in the prevention of coronary heart diseases (KRIS-ETHERTON *et al.* 2003). The nutritional benefits of fish consumption are mainly attributed to the effects of ω -3 polyunsaturated fatty acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action. Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFAs (LIX *et al.* 2006). PUFAs can affect platelet function by interacting with membrane proteins and serving as

Supported by the National Science Fund, Ministry of Education and Science of Bulgaria, Project No. DVU 440/2008.

6.	<p>M. Stancheva, A. Merdzhanova, B. Galunska, D. A. Dobрева The Effect Of Steaming Process On Fat Soluble Vitamins' Content And Fatty Acid Profile In Bluefish And Rainbow Trout Fillets <i>Animal Review</i>, 1, 1, 1-10, 2014. Online ISSN: 2409-6490 Print ISSN: <u>2412-3382</u></p>
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THE EFFECT OF STEAMING PROCESS ON FAT SOLUBLE VITAMINS' CONTENT AND FATTY ACID PROFILE IN BLUEFISH AND RAINBOW TROUT FILLETS

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ABSTRACT

Fatty acid composition and all-trans-retinol, alpha-tocopherol, and cholecalciferol content was determined and compared in raw and steamed Bluefish and Rainbow trout. Total lipids were extracted by Bligh and Dyer method followed by GC-MS. All-trans-retinol, cholecalciferol and alpha-tocopherol were analyzed simultaneously using HPLC. In comparison with raw fish fillets, analyzed fat soluble vitamins content in steamed fish fillets for the Trout and Bluefish decreased significantly to about 54.2% and 49.8% for retinol and 32.6% and 43.5% for alpha-tocopherol, respectively. After steaming, the cholecalciferol amounts in processed fillets decreased significantly only in Rainbow trout (23.5%), whereas in Bluefish the losses were non-significant. After cooking, the polyunsaturated fatty acid content changed significantly in the Rainbow trout (45.8%), whereas the variations in the Bluefish were minor. The major PUFA in all samples were linoleic acid (LA) and docosahexaenoic acid (DHA). PUFA/SFA ratios were between 1.01 and 1.68 for both species. Steaming increases PUFA/SFA ratio by 8.33% in Rainbow trout, but does not affect this ratio in Bluefish.

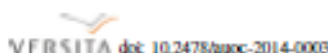
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Keywords: Black sea, Fish nutrition, Human health, *Oncorhynchus mykiss*, *Pomatomus saltatrix*, Thermal processing.

1. INTRODUCTION

The Rainbow trout and Bluefish are commercially important fish species in Bulgaria. The Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely farmed fishes in our country. It is

7.	A. Merdzanova, D. A. Dobрева , M. Stancheva, L. Makedonski Fat Soluble Vitamins Content And Fatty Acid Composition Of Wild Black Sea Mussel, Rapana And Shrimp <i>Ovidius University Annals of Chemistry</i> , 25, 1, 15-23, 2014. ISSN: 1223-7221
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Fat soluble vitamins and fatty acid composition of wild Black sea mussel, rapana and shrimp

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Abstract Many studies suggest that marine molluscs are one of the most important dietary sources of fat soluble vitamins (E, D₃ and A) and essential fatty acids (FA). The most commercially important species from the Bulgarian Black Sea are the Black mussel, rapana and shrimp. There is scarce information in the scientific literature about fat soluble vitamins and FA composition of these Black Sea molluscs. The aims of the present study are to determine and compare fat soluble vitamins content as well as relative daily intake, FA composition and atherogenic index (IA), thrombogenicity index (TI) and flesh-lipid quality index (FLQ) in wild Black Sea mussel (*Mytilus galloprovincialis*), rapana (*Rapana venosa*) and shrimp (*Crangon crangon*). Fat soluble vitamins were analysed simultaneously using RP-HPLC system. The FA profile was analysed by GC-MS. All of the analysed samples presented significant amounts of vitamin E, followed by vitamin A and D₃. Black Sea molluscs are excellent sources of fat soluble vitamins, especially for vitamin D₃ - one survey provides more than 100% of the RDI established in Bulgaria. The FA composition of total lipids showed significant differences and the present study revealed that SFA content was significantly higher than MUFA ($p < 0.001$) and PUFA ($p < 0.001$) (SFA > PUFA > MUFA) in shrimp and mussel whereas rapana showed opposite trends (PUFA > SFA > MUFA). The omega3/omega6 and PUFA/SFA ratios of the analysed species were greater than the FAO/WHO recommendations.

Keywords: *Mytilus sp.*, *Rapana sp.*, *Crangon sp.*, fat soluble vitamins, fatty acids, Bulgarian Black Sea coast

1. Introduction

Mytilus galloprovincialis (Lamarck, 1819) is a marine mollusk with the highest ecological and economic importance in the Black Sea ecosystem including in Bulgaria marine area, known as mass species and marine bio-resource potentially exploitable for human consumption [1]. Among the Black Sea mussels the Black mussel (*M. galloprovincialis*) is the most widespread. It can be found all over the Black Sea – in depth to 65 meters and in the Bay covering areas about 15–20 meters. *M. galloprovincialis* is a filter feeding animal, which depends on phytoplankton, organic detritus, bacteria and probably dissolved organic matter in the water as sources of food [2].

Rapana venosa is a marine snail with fast growth rate and tolerance to low salinity; high and low temperatures, water pollution and oxygen deficiency.

This species have a documented impact on both natural and cultivated populations of mussels and other molluscs, and significant negative changes in the ecosystem. Rapa whelk (*R. venosa*) is a very voracious predator; introduced into the Black Sea in the early 1940s, it is blamed for the decline in the native, edible bivalve fauna. Since the 1980s, rapana has become a valuable commercial resource: its meat is exported to Japan for food and recently it has also been included in the diet of those native to the Black Sea area. According to some recent reports, annual Rapa whelk catches from Turkey and Bulgaria exceed 13,000 t year⁻¹ [3].

Crangon crangon (Linnaeus, 1758) is a marine coastal decapod species with a wide distribution range along the European coast including Black sea. Brown shrimp (*C. crangon*) inhabits mainly soft bottom (sandy, sandy-mud and muddy substrata), estuarine and marine shallow areas and may occur at

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8.	<p>M. Stancheva, A. Merdzhanova, D. A. Dobрева Fat soluble vitamins, cholesterol and fatty acid composition of wild and farmed black mussel (<i>Mytilus galloprovincialis</i>) consumed in Bulgaria <i>Journal of Aquatic Food Product Technology</i>, 26, 2, 181-191, 2016 ISSN: <u>1049-8850</u></p>
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Journal of Aquatic Food Product Technology



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Fat Soluble Vitamins, Cholesterol, and Fatty Acid Composition of Wild and Farmed Black Mussel (*Mytilus galloprovincialis*) Consumed in Bulgaria

Mona Stancheva, Albena Merdzhanova & Diana A. Dobрева

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Date: 03 May 2017, At: 12:05

Fat Soluble Vitamins, Cholesterol, and Fatty Acid Composition of Wild and Farmed Black Mussel (*Mytilus galloprovincialis*) Consumed in Bulgaria

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ABSTRACT

The most commercially important mollusk species from the Bulgarian Black Sea is the black mussel (*Mytilus galloprovincialis*). There is limited information about fat soluble vitamins, cholesterol, and fatty acid content of the Bulgarian Black Sea mussel. The aims of the present study are to determine and compare the fat soluble vitamin contents as well as relative daily intake of vitamins, cholesterol, fatty acid content, and lipid quality indices (atherogenic, thrombogenic) in the wild and farmed black mussels. Fat soluble vitamins and cholesterol were analyzed simultaneously using reverse phase high performance liquid chromatography. The fatty acid composition was analyzed by gas chromatography-mass spectrometry. In both mussels, fat soluble vitamins A and E were in high amounts, but they were a better source of vitamin D₃. Cholesterol contents were 67.54 ± 0.50 mg/100 g ww (wild) and 49.88 ± 0.30 mg/100 g ww (farmed). The fatty acid distributions of wild and farmed mussels are: saturated > polyunsaturated > monounsaturated fatty acid. The n3/n6 and polyunsaturated/saturated fatty acid ratios were greater than that recommended by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). A 100-g edible portion of both mussels contained from 0.252 g (wild) to 0.425 g (farmed) of eicosapentaenoic acid (EPA, C20:5n-3) + docosahexaenoic acid (DHA, 22:6n-3).

KEYWORDS

Black mussel; *Mytilus galloprovincialis*; fat soluble vitamins; fatty acids; cholesterol

Introduction

Mytilus galloprovincialis (Lamarck, 1819) is the marine mollusk with the highest ecological and economic importance in the Black Sea ecosystem, including the Bulgarian marine area. Bivalve molluscs, particularly marine mussels such as *Mytilus* spp., have been used as indicator organisms in environmental monitoring programs due to their wide distribution, sedentary lifestyle, and tolerance to a large range of environmental conditions (Petrova and Stoykov, 2011a).

The black mussel (*Mytilus galloprovincialis*) is the most widespread among all Black Sea mussels and can be found everywhere in the Black Sea—at a depth of up to 65 m and in the Bay covering places about 15–20 m (Petrova and Stoykov, 2011b). On the other hand, the culture of marine organisms in the Black Sea region is a relatively recent development and not widespread yet. However, prospects for mussel culture in the Black Sea are quite high due to the favorable salinity, temperature, topography, food availability, reproductive potential, and socioeconomic conditions in this area. *M. galloprovincialis* is a filter feeding animal that depends upon phytoplankton, organic detritus, bacteria, and probably dissolved organic matter in the water as sources of food. Mussel (*Mytilus* spp.) farming is the most efficient way to convert the organic matter produced by marine organisms of the first link of the food chain (as phytoplankton) into nutritious human food.

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9.	<p>A. Merdzhanova, D. A. Dobрева, L. Makedonski Comparison of polyunsaturated fatty acid and fat-soluble vitamins content of cooked Shad (<i>Alosa immaculata</i>) <i>International Journal of Agricultural Technology</i>, 12, 6, 1043-1056, 2016 ISSN: 2630-0192</p>
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Journal of Agricultural Technology 2016 Vol 12(6): 1043-1056
 Available online <http://www.ijat-aatsea.com>
 ISSN 1686-9141

Comparison of polyunsaturated fatty acid and fat-soluble vitamins content of cooked Shad (*Alosa immaculata*)

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Merdzhanova, A., Dobрева, D.A., Makedonski, L. (2016) Comparison of polyunsaturated fatty acid and fat-soluble vitamins content of cooked Shad (*Alosa immaculata*). *Journal of Agricultural Technology* 12(6): 1043-1056.

This study presents the evaluation of the effect of cooking (steaming and grilling) on lipids, fatty acids profile, nutritional quality indices (NQI) and fat soluble vitamins content of Black Sea shad (*Alosa immaculata*). The Bligh and Dyer's method was used for total lipid content determination. The fatty acid methyl esters were analysed by GC/MS and fat soluble vitamins - by RP-HPLC. The two cooking methods increased the levels of the saturated fatty acids (SFA), whereas grilling process decreased monounsaturated fatty acids (MUFA) quantity in shad tissue. Omega-3 polyunsaturated FAs (n-3 PUFA) levels significantly decreased after both thermal processes. Steaming doesn't affect the omega-6 (n-6) PUFA - their amounts remain almost unchanged. The vitamin A value decreases significantly after steaming, whereas vitamin D₃ and E remain almost unchanged. Among the three fat soluble vitamins, the grilling process affects significantly mainly vitamin A and E. In conclusion - both cooking methods are suitable for preserving the lipid nutrition quality of shad edible tissue.

Keywords: *Alosa immaculata*, Fatty acids, Fat soluble vitamins, Grilling, Steaming

Introduction

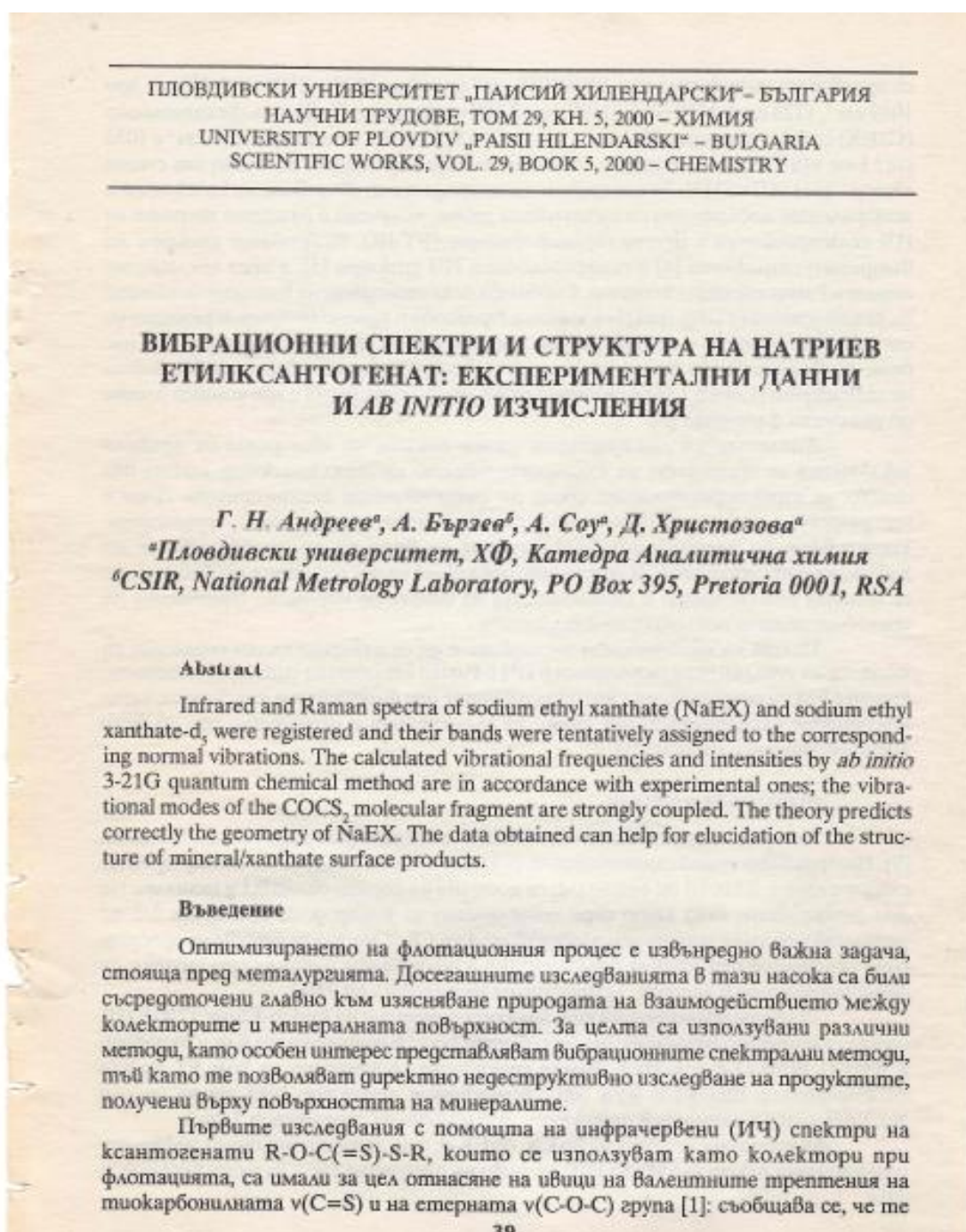
Various epidemiological studies have demonstrated the key role of fish consumption in prevention of coronary heart diseases (Kris-Etherton et al., 2003; Ruxton, 2011). The nutritional benefits of fish consumption are mainly attributed to the effects of omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action. Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFAs (Lee et al., 2006). The second essential nutrients of fish tissue are the fat soluble vitamins which control a diversity of biologically important processes in human body. It is well known that the marine fish fatty acid (FA) composition is characterized by high levels of n-3 PUFA (Eicosapentaenoic acid, EPA, C20:5 n-3; Docosahexaenoic acid, DHA, C22:6 n-3) and vitamin D₃ content.

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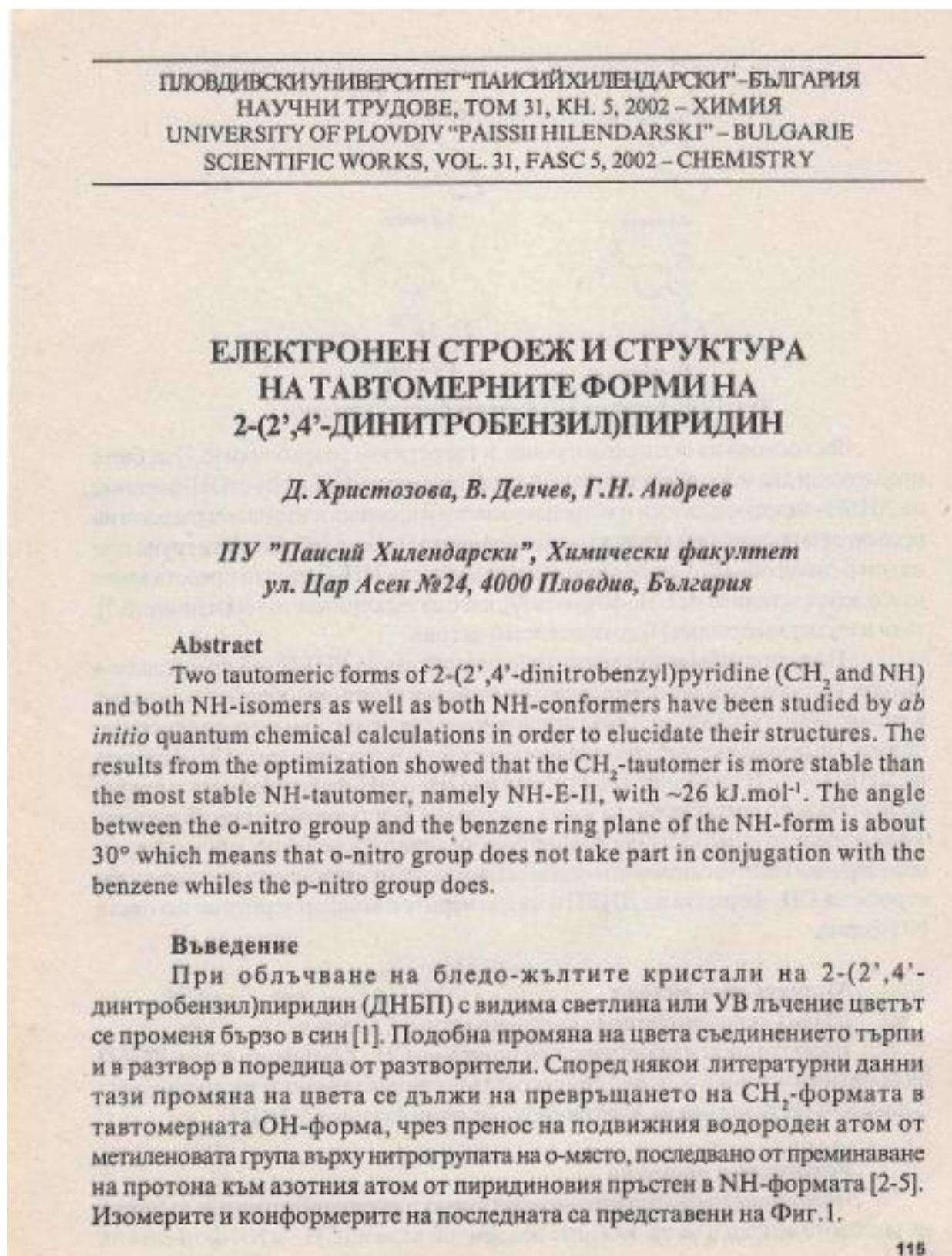
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III. Публикации в български и чуждестранни научни издания

10.	Г. Н. Андреев, А. Бързев, А. Соу, Д. Христовова (Добрева) Вибрационни спектри и структура на натриев етилксантогенат: експериментални данни и <i>ab initio</i> изчисления ПУ „П. Хилендарски” – <i>Научни трудове – Химия</i> , 29, 5, 2000. ISSN: 0861-3184
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11.	<p>Д. Христозова (Добрева), В. Делчев, Г. Н. Андреев Електронен строеж и структура на тавтомерните форми на 2-(2',4'-динитробензил) пиридин ПУ „П. Хилендарски” – <i>Научни трудове – Химия</i>, 31, 5, 2002. ISSN: 0861-3184</p>
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12.	G. N. Andreev, B. Shrader, D. A. Hristozova (Dobрева) , V. B. Delchev, J. S. Petrov, P. Rademacher Vibrational spectra and structure of the photochromic 2-(2',4'-dinitrobenzyl)pyridine <i>Journal of Molecular Structure</i> , 645, 77-78, 2003. ISSN: 0022-2860
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Journal of Molecular Structure 645 (2003) 77–87

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Vibrational spectra and structure of the photochromic 2-(2',4'-dinitrobenzyl)pyridine

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Received 16 August 2002; accepted 11 October 2002

Abstract

The molecular structure of the pale yellow crystals of 2-(2',4'-dinitrobenzyl)pyridine (CH₂ form) and its photo induced 'enamine' NH tautomer (dark blue crystals) have been studied by means of vibrational spectra and ab initio calculations. The Raman spectrum of the photo-sensitive CH₂ form was registered by NIR FT-Raman spectroscopy by means of the Nd:YAG laser as an excitation source. Ab initio calculations have been performed for the CH₂ and NH tautomers at the Hartree–Fock level using a 6-21G** basis set. The theoretical geometrical parameters for the isolated 2-(2',4'-dinitrobenzyl)pyridine molecule (CH₂ form) are close to the literature X-ray diffraction data. According to the theory the dihedral angle between the benzene and pyridine ring planes in the NH photo induced tautomer is about 46°, the *ortho*-nitro group is twisted about 25° towards the benzene ring plane, whereas the *para*-nitro group is coplanar to the benzene ring. The assignment of the fundamental vibration frequencies of both 2-(2',4'-dinitrobenzyl)pyridine tautomers CH₂ and NH have been performed on the basis of Raman and infrared spectra and ab initio force field calculations. The computed frequencies are in coincidence with the registered ones; the mean deviations are between 23.7 and 28.5 cm⁻¹.

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Keywords: Vibrational spectra; 2-(2',4'-Dinitrobenzyl)pyridine; Photochemistry; Ab initio calculations; Band assignment

1. Introduction

When irradiated with visible or ultraviolet light the pale yellow 2-(2',4'-dinitrobenzyl)pyridine crystal [1] (or in solution in different solvents [2]) undergoes

a color change to deep blue which decays slowly to its original color in the dark. This reversible photo-induced reaction was described first by Tschitochiba bin [1] who proposed a mechanism involving a proton transfer from the methylene group to the pyridine nitrogen atom. Later an alternative mechanism was proposed [2] assuming a proton transfer to the *ortho*-nitro group forming an aci-nitro structure. Crystallographic and optical spectroscopic investigation of

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E-mail address: andreev@argon.acad.bg (G.N. Andreev).

13.

V. Delchev, **D. Hristozova (Dobrev)**, A. Terziyski, J. Petrov
 Thermodynamic analysis of the stable isomers of Adamantylidene-[1-(2,5-dimethyl-3-furyl)ethylidene]succinic anhydride: a DFT level study
Bulgarian Chemical Communications, 37, 4, 344–349, 2005. ISSN: 0324-1130

Bulgarian Chemical Communications, Volume 37, Number 4 (pp. 344–349) 2005

Thermodynamic analysis of the stable isomers of Adamantylidene-[1-(2,5-dimethyl-3-furyl)ethylidene]succinic anhydride: a DFT level study

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¹ Department of Chemistry, University of Plovdiv, 4000 Plovdiv, Bulgaria

² Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Dedicated to the memory of Prof. Bejdar Jordanov

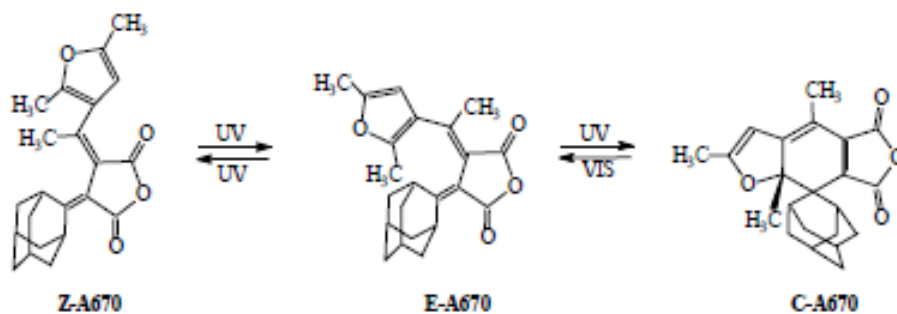
Received September 12, 2005

The photoisomers of Adamantylidene-[1-(2,5-dimethyl-3-furyl)ethylidene]succinic anhydride were studied at the B3LYP/6-31G level of theory. It was found out that the most stable isomer is E and the most unstable one – C. Electronic and thermodynamic analyses of the mutual interconversions of the isomers revealed that the conversion of isomer E (colourless) and Z (coloured) into C (coloured) is forbidden. However, this process can occur under special conditions (irradiation, solvents etc.) as it is shifted toward the more stable forms – the E and Z ones.

Key words: Photochromism, Furfurylfulgides, Thermodynamics, DFT calculations.

INTRODUCTION

The fulgides are derivatives of bis-alkylidenesuccinic anhydride, which have pronounced photochromic properties. These compounds are known since the beginning of the twentieth century, when Stobbe synthesized a series of their phenyl derivatives [1,2]. When irradiated with UV/VIS light the fulgides undergo a nearly quantitative conversion into their coloured forms [3]. For example, Adamantylidene-[1-(2,5-dimethyl-3-furyl)ethylidene]succinic anhydride (called A670) undergoes photochemical isomerization according to Scheme 1:



The UV/VIS irradiation can provoke a photocyclization of A670. The compound has shown a marked increase in the quantum efficiency (30%) by photoisomerization [3, 4]. The fulgides are interesting because they are potential materials for reversible optical information storage medium since they undergo colour changes upon irradiation. The irradiated products can easily revert back to the original structures and colours either thermally or photochemically [5]. These properties make the fulgides suitable also for preparing holograms [6], photochromic lenses, integrated optics, sunlight attenuation, sensor protection [5] and optical switches [7].

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 E-mail: vdelchev@pu.acad.bg

14.	<p>М. Станчева, Д. Добрева, А. Мерджанова, Б. Галунска Определяне на витамин А и витамин Е чрез високо-ефективна течна хроматография в риба кая (<i>Neogobius fluviatilis</i>) от българското черноморско крайбрежие ПУ „П. Хилендарски“ – <i>Научни трудове - Химия</i>, А, 36, 5, 45-50, 2008. ISSN: 0861-3184</p>
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ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ“ – БЪЛГАРИЯ
 НАУЧНИ ТРУДОВЕ, ТОМ 36, КН. 5, 2008 – ХИМИЯ, СБ. А
 PLOVDIV UNIVERSITY „PAISII HILENDARSKI“ – BULGARIA
 SCIENTIFIC PAPERS, VOL. 36, BOOK 5, 2008 – CHEMISTRY

**ОПРЕДЕЛЯНЕ НА ВИТАМИН А И ВИТАМИН Е
 ЧРЕЗ ВИСОКО-ЕФЕКТИВНА ТЕЧНА ХРОМАТОГРАФИЯ
 В РИБА КАЯ (*Neogobius fluviatilis*)
 ОТ БЪЛГАРСКОТО ЧЕРНОМОРСКО КРАЙБРЕЖИЕ**

*Станчева М., Добрева Д. А., Мерджанова А., Галунска Б.
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ABSTRACT

This study presents simultaneous determination of Vitamin A (all-trans retinol) and Vitamin E (α-tocopherol) in tissue samples from Bulgarian Black Sea Coast fish species by high-performance liquid chromatography method.

The method was applied to samples of Black Sea goby fish (*Neogobius fluviatilis*) and includes two stages: extraction of tocopherol and retinol from the fish tissue and subsequent quantitative HPLC determination. Quantitative determination of the fat soluble vitamins in the hexane extracts has been done by HPLC with UV-detection on RP-column Nucleosil (25 cm x 0,46 cm). The elution of tocopherol and retinol from the chromatographic column was done by mobile phase composed of 100% methanol at flow rate 0.9 ml/min. Tocopherol was detected at wavelength 295 nm and retinol at 325 nm.

Mean concentration in fresh material were 47.93 µg/100g for Vitamin A and 0.5 mg/100 g for Vitamin E. Our results are in good agreement with the data from the literature for other fish species.

Keywords: retinol, α-tocopherol, blacksea fish species, HPLC

ВЪВЕДЕНИЕ

Рибата е ценен хранителен продукт – източник на белтъци, калций и фосфор. В нейните липиди се съдържат и голямо количество есенциални полиненаситени висши мастни киселини (ω-3, ω-6 ПНВМК). Други ценни компоненти в рибната тъкан са също важните за човешкия организъм мастно- и водо-разтворими витамини А, Е, Д, В₁, В₂, В₁₂, ниацин.

Витамин А (all-trans-ретинол) изпълнява важни функции в организма. Той е необходим за поддържане на нормалното зрение, тъй като е важна съставка на

15.	<p>M. Stancheva, D. Dobрева, A. Merdzhanova, B. Galunska Vitamin Content and Fatty Acids Composition of Rainbow trout (<i>Oncorhynchus mykiss</i>) PU „Paisii Hilendarski“, <i>Scientific Papers - Chemistry, A</i>, 37, 5, 117-124, 2010. ISSN: 0204-5346</p>
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ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ“ – БЪЛГАРИЯ
НАУЧНИ ТРУДОВЕ, ТОМ 37, КН. 5, 2010 – ХИМИЯ, СБ. А
PLOVDIV UNIVERSITY „PAISII HILENDARSKI“ – BULGARIA
SCIENTIFIC PAPERS, VOL. 37, BOOK 5, 2010 – CHEMISTRY

VITAMIN CONTENT AND FATTY ACIDS COMPOSITION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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ABSTRACT

The aim of present study is to evaluate the composition and the content of fatty acids (FA) and fat soluble vitamins (A, E, D₃) in the edible tissue of farmed rainbow trout from the region of Central Bulgaria.

All-trans-retinol (vit. A), cholecalciferol (vit. D₃) and α-tocopherol (vit. E) were analyzed simultaneously using HPLC system with UV (vitamin A and D₃) and fluorescence detection (vitamin E). The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter. Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters were performed using gas chromatography system with MS detection.

It was found that the lipid fraction contains substantial amounts of palmitic, palmitoleic, stearic, linolenic, arachidonic and docosahexaenoic fatty acids and fat-soluble vitamins. The retinol content in the fresh edible tissue of rainbow trout (*Oncorhynchus mykiss*) was 22.3±2.0 μg/100g; cholecalciferol – 6.0±0.29 μg/100g and α-tocopherol – 809.1± 56.0 μg/100g.

Linoleic acid (15.81%), docosahexaenoic acid (9.40%) and arachidonic acid (4.21%) were the most dominant polyunsaturated fatty acids, about 33% of total FA content. Palmitic acid (12.93%), tetracosanoic acid (3.76%) and oleic acid (3.57%) were found to be the dominant of the saturated and unsaturated FA in rainbow trout fillets.

Keywords: fat-soluble vitamins, PUFA, HPLC, GS/MS, trout

INTRODUCTION

Fish tissue is a good source of fats, proteins, vitamins and minerals and important component of balanced diet. Omega-3 and omega-6 fatty acids (FA), as well as fat-soluble vitamins are essential compounds of fish lipids and exclusively provided by the diet.

Being component of membrane lipids, the essential FAs maintain the integrity, flexibility and permeability of membranes, provide substrate for the biosynthesis of biologically active eicosanoids. It has been shown that omega-3 FAs exert antihypertensive, antiarrhythmic, antidepressive, and immunomodulatory effect. Acting as an-

16.	<p>D.A. Dobрева, B. Galunska, M. Stancheva Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue Varna Medical University, <i>Scripta Scientifica Medica</i>, 43, 1, 276-279, 2011. ISSN 1314-6408</p>
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Scripta Scientifica Medica, 2011; vol. 43 (1), pp 35-38

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LIQUID CHROMATOGRAPHY METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF FAT SOLUBLE VITAMINS IN FISH TISSUE

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Reviewed by: assoc. prof. L. Macedonski

ABSTRACT

The aim of the present study was to develop simple and accurate small-scale method for simultaneous determination of retinol, α -tocopherol, ergocalciferol, and cholecalciferol in edible fish tissue. High performance liquid chromatography was the method of choice since it provides rapid, sensitive and accurate detection of all four fat-soluble vitamins and requires small amounts of sample. The sample preparation procedure was improved using single reaction tube for both hydrolysis and extraction of the analytes. The overall recovery exceeded 76% for retinol and ergocalciferol, 93% for alpha-tocopherol, and 83% for cholecalciferol. The method precision (relative standard deviation) was below 10% for all analytes. The method was verified on real fish tissue samples and the results for the tested fat-soluble vitamin contents were in a good agreement with the data given by other authors.

Key words: fat soluble vitamins, HPLC, fish tissue

INTRODUCTION

Lipids of marine fish species are rich source of fat soluble vitamins, related to a diversity of biologically important processes in human body. Vitamin A (retinol), takes place in photoreception, regulates gene expression and cell division, bone growth, reproduction. The biologically active isomer of vitamin E - alpha-tocopherol acts as antioxidant protecting membrane structures and lipoproteins from oxidation. Vitamin D₃ (cholecalciferol) and its plant isomer vitamin D₂ (ergocalciferol) are of vital importance for regulation of calcium and phosphate homeostasis. Fat-soluble vitamins have been determined so far, in many different samples, by a variety of techniques. Among them the most widely applied are the chromatographic techniques mainly HPLC, which provides rapid, sensitive and accurate methods for vitamin determination and has the advantages of solvent economy and easy coupling with other techniques. It also requires small amounts of sample. However, most of the already reported methods measure indi-

the determination of fat-soluble vitamins include UV, diode array (11,15) fluorimetric (14,17), and electrochemical (12), as well as MS detection (3,6). Concerning sample preparation, it is recommended to use short time and gentle extraction methods, sometimes in a darkened place, since these vitamins are unstable during common procedures.

The aim of the present work was to develop a simple, fast and accurate method for simultaneous determination of four fat-soluble vitamins: retinol (A), cholecalciferol (D₃), ergocalciferol (D₂), and alpha-tocopherol (E) in edible fish tissue.

MATERIAL AND METHODS

Instrumentation and chemicals: A high-performance liquid chromatograph (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors were used. All solvents were of HPLC grade. Methanol and water was obtained by Sigma-AldrichTM, USA. Substances of vitamins A, D₃, D₂ and alpha-tocopherol were all of analytical grade and were supplied by Supelco (Sigma-AldrichTM, USA).

17.	<p>D. A. Dobрева, M. Stancheva, A. Merdzhanova, L. Makedonski Fatty Acid profile and Vitamin A and E content in Horse mackerel (<i>Trachurus mediterraneus</i>) <i>Asian Chemistry Letters</i>, 15, 1 and 2, 91-100, 2011. ISSN: 0971-9822</p>
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**Fatty Acid profile and Vitamin A and E content in
Horse mackerel (*Trachurus mediterraneus*)**

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The aim of this study was to measure and evaluate of the total lipid, fatty acid profil and Vitamin A and Vitamin E content of Black Sea horse mackerel or scad (*Trachurus mediterraneus pontica*) catch from Bulgarian Black Sea and the same fish species from Greek coast of Mediterranean Sea (*Trachurus mediterraneus mediterraneus*). The results from analysis showed that the sample of Black Sea scad (Spring 08) contain 5.01 g total lipid per 100 g raw weight and 2.40 g total lipid per 100 g raw weight (Autumn 08) while Greek scad present 7.90 g total lipid per 100 g raw weight (Spring 09). The fatty acid composition was analysed by Gas Chromatography with MS detector. The level of total ω 3 polyunsaturated fatty acid was higher than the total ω 6 polyunsaturated fatty acid in the all analyzed Black Sea fish species. The vitamins content was determinate by HPLC with UV detector. The results from measure show the differences for Vitamin A and E contents between Black Sea horse mackerel and Mediterranean horse mackerel.

1. Introduction

On the recommendations of the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) it is advisable to consume annually at least 15-20 kg fish per capita [1]. In Bulgaria, the Black Sea is the main resource for fishing. It is a unique semi-closed basin with slow water circulation, relatively low salinity (e.g. compared with the Mediterranean), high eutrophication, inhabited by about 140 fish species only 15 of which are commercially important. Mediterranean horse mackerel (*Trachurus mediterraneus*) ranks second in the annual fish catch. According to a research on that catch (biometric and biological features – Georgiev, Kolarov, 62; Cantis, Jonescu, 79) it is concluded that in the Black Sea (the Marmara Sea as well) exists a subspecies named *Trachurus mediterraneus ponticus*, Aleev 59 which is smaller than the Mediterranean horse mackerel (*Trachurus mediterraneus mediterraneus*) and inhabits the western and north-western parts of the Black Sea. The two populations differ in lipid

18.	<p>M. Stancheva, D. Dobрева, A. Merdzhanova Fatty acids composition and fat soluble vitamins content of bighead carp (Aristichthys Nobilis) PU „Paisii Hilendarski“, <i>Scientific Papers – Chemistry</i>, 38, 5, 221-232, 2011. ISSN: 0204-5346</p>
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ПЛОВДИВСКИ УНИВЕРСИТЕТ „ПАИСИЙ ХИЛЕНДАРСКИ“ – БЪЛГАРИЯ
НАУЧНИ ТРУДОВЕ, ТОМ 38, КН. 5, 2011 – ХИМИЯ
UNIVERSITY OF PLOVDIV „PAISII HILENDARSKI“ – BULGARIA
SCIENTIFIC PAPERS, VOL. 38, BOOK 5, 2011 – CHEMISTRY

FATTY ACIDS COMPOSITION AND FAT SOLUBLE VITAMINS CONTENT OF BIGHEAD CARP (*ARISTICHTHYS NOBILIS*)

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ABSTRACT

In the present study fatty acid composition and fat soluble vitamins content were analyzed in two season's samples (spring and autumn) freshwater bighead carp (*Aristichthys nobilis*).

Analysis of fatty acid methyl esters was performed by gas chromatography system with MS detection. Vitamins A, D₃ and E were analyzed simultaneously using RP-HPLC system. The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter.

The fatty acid and vitamins contents of the investigated fish species showed significant seasonal changes. The spring bighead carp characterized with saturated fatty acid (SFA) (37.5%) and mono unsaturated fatty acids (MUFA) (22.1%), and poly unsaturated fatty acids (PUFA) (40.4%), including essential omega 3 fatty acids (23.0%). The autumn samples showed higher SFA (40.5%) and MUFA (34.8%), and lower PUFA (24.6%), due to reduced omega 3 fatty acids (9.7%).

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19.	M. Stancheva, D. A. Dobрева , B. Galunska Retinol, cholecalciferol and alpha-tocopherol contents of Bulgarian Black Sea fish species <i>Ovidius University Annals of Chemistry</i> , 23, 1, 31-34, 2012. ISSN: 2286-038X
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Retinol, cholecalciferol and alpha-tocopherol contents of Bulgarian Black Sea fish species

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Abstract The aim of the present study is to determine and to compare the content of retinol, cholecalciferol and alpha-tocopherol in edible tissue of two Black sea fishes - Garfish (*Belone belone*) and Turbot (*Psetta maxima*). All-trans-retinol (vitamin A), cholecalciferol (vitamin D₃) and alpha-tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column. The mobile phase was composed of 97:3 = MeOH:H₂O. Retinol and cholecalciferol were monitored by UV detection at $\lambda_{max} = 325$ nm and $\lambda_{max} = 265$ nm, respectively. Alpha-tocopherol was detected by fluorescence at $\lambda_{ex}=288$ nm and $\lambda_{em}=332$ nm. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Quantities of all-trans-retinol and cholecalciferol were higher in garfish tissues while alpha-tocopherol content in turbot showed seven times higher values.

Keywords: retinol, cholecalciferol, alpha-tocopherol, turbot, garfish, HPLC

1. Introduction

Fish is considered as a valuable source of essential nutrients – macronutrients as proteins and fats, and micronutrients as vitamins and minerals, and is an important component of balanced human diet. Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet.

Fat soluble vitamins control a variety of biologically important processes in human body. All-trans retinol (vitamin A), takes place in photoreception and regulates gene expression and cell division, bone growth, teeth development, reproduction etc. Cholecalciferol (Vitamin D₃) promotes and enhances the absorption and the metabolism of calcium and phosphorus. Alpha-tocopherol is vitamin E isomer with the highest biological activity. Its main role is as antioxidant, protecting membrane structures, essential fatty acids, and vitamins A and C against oxidation [1].

Fish production in Bulgaria comes mainly from commercial fishing. The catches in the Black Sea account about 77.3 % of total fish production for the

country. Aquaculture production (including fresh water fish farming and marine farming of fish and mussels) accounts approximately 13.8 % of the total fish production [2].

As a delicious Black sea fishes Turbot (*Psetta maxima*) and Garfish (*Belone belone*) are consumed in significant amounts in Bulgaria.

Garfish is characterized with long thin body and long needle like mouths. Adult species can reach up to 1 m in length and are mostly silver in color with a blue to green back side and green skeleton. They are typical pelagic fishes. The garfishes are omnivorous – they prefer to feed on zooplankton and small fishes [3].

Some of the Black Sea fish species have migratory character (like garfish), others are non-migratory and they are subject of perennial fishing. One of this species is the Black sea turbot. *Psetta maxima* is one of the flatfish species. They are characterized with white meat and usually low lipid levels. The turbot live near the marine floor – in softer bottoms of mud and sandy mud. They fed with crustaceans, squid, and with a variety of small

20.	A. Merdzhanova, M. Stancheva, D.A. Dobрева , L. Makedonski Fatty acid and fat soluble vitamins composition of raw and cooked Black Sea horse mackerel <i>Ovidius University Annals of Chemistry</i> , 24, 1, 27-34, 2013. ISSN: 2286-038X
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Fatty acid and fat soluble vitamins composition of raw and cooked Black Sea horse mackerel

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Abstract. The fat soluble vitamins, as well as n3 and n6 fatty acids (FA) are essential compounds of fish lipids and exclusively provided by the diet. Fish is sometimes eaten raw, but it is usually thermal processed before consumption. Temperature processing of fish tissue enhances its taste, inactivates pathogenic microorganisms and increases its shelf life. The fat soluble vitamins (vitamins A, D₃ and E) and fatty acids are considered to be susceptible to oxidation during heating (cooking) process. The aim of the present study was to evaluate the effect of steaming (10 min at 90°C) and frying (5 min on the each side with sunflower oil) on fat soluble vitamins and fatty acids composition in Horse mackerel (*Trachurus mediterraneus*) fish fillets. Vitamins A, D₃ and E were analyzed simultaneously using RP-HPLC. The fatty acid composition was analyzed by GC-MS. The amounts of vitamin A (retinol) in cooked fish fillets (for both heat treatments) decreased significantly, compared to their content in the raw samples. In contrast vitamin D₃ (cholecalciferol) content affects only by steaming, while changes on vitamin E (alpha-tocopherol) was observed solely after frying process. The highest content of monounsaturated fatty acids (MUFA) were observed after steaming, whereas fried samples presented higher values of polyunsaturated fatty acids (PUFA) due to significant increase in linoleic acid (C18:2n6). During steaming did not reduce significant n3 and n6 PUFA levels, while frying caused a large reduction of n3 PUFAs. The ratio of n3/n6 was markedly lower in fried samples than in raw and steamed mackerel. In conclusion the Black Sea Horse Mackerel is a good source of vitamin D₃, vitamin E and n3 PUFAs. After steaming and frying process there were minimum losses in the contents of cholecalciferol and alpha-tocopherol, while retinol was reduced nearly a half. The process of frying affects most significantly three fatty acids groups, whereas after steaming was observed little influence on fatty acids profile.

Keywords: *Trachurus mediterraneus*, steaming, frying, vitamins, fatty acids, human health

1. Introduction

The Black Sea appears to be one of the important fish basins influencing greatly the economy of all Black Sea countries. The small pelagic species Horse mackerel (*Trachurus mediterraneus*, Aleev 59), which inhabits the western and north-western parts of the Black Sea [1], is of key importance for Bulgarian fisheries for economic and social reasons (number of fishermen involved) and as livelihood support for population. This carnivore species represents about 50% of the Bulgarian summer pelagic catches, and a considerable percentage of the Black Sea total catches and plays an important role to provide essential nutrients for the population.

Marine fish, especially carnivores, are characterized by low levels of omega-6 (n6) fatty acids (linoleic acid LA, C18:2 n6) and high levels of omega-3 (n3) PUFAs (eicosapentaenoic acid, EPA C20:5n3; docosahexaenoic acid, DHA C22:6n3) in particular, which are essential for the human health [2]. Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet. Vitamins A and E act as natural antioxidants in the living organisms. All-trans-retinol is very important for the visual system in humans; alpha-tocopherol (alpha-TP) is significant for the normal reproduction and muscle development and cholecalciferol promotes and enhances the absorption and metabolism of calcium and phosphorus. It is well-

21.	<p>D.A. Dobрева, A. Merdzhanova, M. Stancheva Effect of frozen storage on fat soluble vitamins content in fish fillets Varna Medical University, <i>Scripta Scientifica Medica</i>, 45, 3, 23-26, 2013. ISSN 1314-6408</p>
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ORIGINAL ARTICLES

EFFECT OF FROZEN STORAGE ON FAT SOLUBLE VITAMINS CONTENT IN FISH FILLETS

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ABSTRACT

Fat-soluble vitamins content (all-trans-retinol, alpha-tocopherol and cholecalciferol) in edible tissue of Bluefish (*Pomatomus saltatrix*), a typical Black sea pelagic fish, and in Rainbow trout (*Oncorhynchus mykiss*), a typical farmed freshwater fish, were determined and compared on raw state and after frozen storage.

The sample preparation procedure includes saponification and consequent extraction of fat-soluble vitamins with n-hexane. The extract was dried under nitrogen flow and redissolved in methanol. HPLC analysis of methanolic samples was performed on ODS2 Hypersil (250x4,6, 5µm) column with a mobile phase of methanol:water = 97:3. The quantification of fat-soluble vitamins was by the method of standard addition. Retinol and cholecalciferol were monitored by UV detection and alpha-tocopherol was detected by fluorescence.

The retinol and cholecalciferol contents in fresh edible tissue of Black sea Bluefish (38.5±2.4 µg.100g⁻¹ww and 11.2±1.2 µg.100g⁻¹ww, respectively) were close to values in the freshwater fish Rainbow trout (58.9±2.6 µg.100g⁻¹ww and 14.9±1.1 µg.100g⁻¹ww, respectively). Alpha-tocopherol content was several fold higher in Rainbow trout (1648.9±68.8 µg.100g⁻¹ww) than in Black sea Bluefish (427.1±37.1 µg.100g⁻¹ww).

Long period of storage affected mostly retinol and alpha-tocopherol contents in two fish species. While cholecalciferol content remained almost unchanged.

Key words: *Retinol, Alpha-Tocopherol, Cholecalciferol, Frozen storage, Fish fillet*

INTRODUCTION

Fish tissue fats are rich source of fat soluble vitamins and both saturated and unsaturated fatty acids. Fat soluble vitamins are essential nutrients controlling a diversity of biologically important processes in human body. All-trans-retinol (vitamin A) is a fat-soluble unsaturated isoprenoid necessary for growth, differentiation and maintenance of epithelial tissues,

and also for reproduction. It takes place in photoreception and regulates gene expression and cell division, bone growth, teeth development etc (10). Cholecalciferol (vitamin D₃) plays crucial role for the development, growth, and maintenance of a healthy skeleton from birth until death. Its major function is to maintain calcium homeostasis (6). Alpha-tocopherol (vitamin E) is an important antioxidant which protects against lipid peroxidation (which could contribute to cell membrane weakness), and essential fatty acids and vitamins A from oxidation (2).

Moreover the fat soluble vitamins are considered to be especially susceptible to oxidation. Freezing is one of the easiest and most preferred method of preserving foods. Frozen foods retain their original flavor, color and more of their nutrients. But their final quality is highly dependent on primary condi-

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22.	<p>V. Panayotova, M. Stancheva, D. Dobрева Alpha-tocopherol and ergocalciferol contents of some macroalgae from Bulgarian Black Sea coast <i>Analele Universitatii "Ovidius" Constanta - Seria Chimie</i>, 24, 1, 13-16, 2013. ISSN: 2286-038X</p>
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Alpha-tocopherol and ergocalciferol contents of some macroalgae from Bulgarian Black Sea coast

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Abstract The aim of the present study was to determine and compare α -tocopherol and ergocalciferol content in four macroalgae from Bulgarian Black sea coast. *Ulva rigida*, *Cladophora vagabunda*, *Cystoseira barbata* and *Cystoseira crinita* were used for evaluation of corresponding fat soluble vitamins content. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Ergocalciferol (vitamin D₂) and α -tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL system (Thermo Scientific Spectra SYSTEM) equipped with RP analytical column. The mobile phase was composed of 97:3 = MeOH:H₂O. Ergocalciferol was monitored by UV detection at λ_{max} = 265nm, while α -tocopherol was detected by fluorescence at λ_{ex} =288nm and λ_{em} =332nm. Alpha-tocopherol content in algal tissues ranged from 1.68±0.38mg/100g d.w. in *Cladophora vagabunda* to 29.13±1.08mg/100g d.w. in *Cystoseira barbata*. Ergocalciferol was detected only in *Ulva rigida* samples.

Keywords: macroalgae, ergocalciferol, α -tocopherol, HPLC

1. Introduction

Seaweeds belong to a group of plants known as algae. They are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health. Algae have been used since ancient times as food, fodder, fertilizer and as source of medicine. Nowadays seaweeds represent an inexhaustible source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins [1].

Seaweeds are a good source of some water- (B1, B2, B12, C) and fat-soluble (β -carotene with vitamin A activity, vitamin E) vitamins. Vitamin E is the most abundant fat-soluble vitamin of non-saponifiable lipids in many algae. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing blood pressure (vitamin C), prevention of cardiovascular

diseases (β -carotene), or reducing the risk of cancer (vitamins E and C, carotenoids) [2]. Vitamin E is one of the most important fat-soluble vitamins with a strong antioxidant activity. Its special function is lipid protection from peroxidation. It exists in eight forms: α , β , γ , δ -tocopherols and α , β , γ , δ -tocotrienols. The α -forms showed the highest antioxidant effect [3]. Further, the connection of vitamin E and decrease of blood pressure was reported [4].

Algal products have been used in food, cosmetic and pharmaceutical industries. An expanding market for these products is a fact and is facing a new challenge of growing algae on a large scale without harming any further the marine environment. Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. Brown seaweeds (*Cystoseira crinita* and *Cystoseira barbata*) and green seaweeds (*Ulva rigida* and *Cladophora vagabunda*) are widespread along the coastal area. Seaweeds are still under-utilized in Bulgaria because the knowledge about their chemical composition is still limited. It was reported that Black Sea *Ulva* spp. and *Cystoseira* spp. extracts have antioxidative and antibacterial activities [5,6,7].

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| 23. | D. Dobрева
Black Sea and Freshwater Fish as Valuable Sources of Vitamin D ₃
<i>Scripta Scientifica Pharmaceutica</i> , 1, 1, 44-47, 2014. ISSN 2367-5500 |
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ORIGINAL ARTICLES

BLACK SEA AND FRESHWATER FISH AS VALUABLE SOURCES OF VITAMIN D₃

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ABSTRACT

Fat soluble vitamins are essential nutrients in important biological processes in the human body. Vitamin D₃ (cholecalciferol) promotes and enhances the absorption and metabolism of calcium and phosphorus in our body. Nowadays there is a plethora of evidence suggesting that, in addition to its important role in maintaining bone health, vitamin D is involved in the amelioration of cell ageing and prevention of cardiovascular diseases, diabetes, immune dysfunctions and some cancers. This article presents information about vitamin D₃ contents in a broad range of fish species available on Bulgarian local fish markets. The aim is to increase consumers' awareness and encourage them to eat fish.

Keywords: *Bulgarian fish, cholecalciferol, fish tissue, health, RDI*

INTRODUCTION

Vitamin D₃ (cholecalciferol) is synthesized by living cells in the skin or ingested by consumption of high-fat animal tissues (16). The human organism produces it endogenously by means of sunlight irradiation (4,13).

The biologically active form of vitamin D₃ (as a hormone in the human body) is 1, 25-dihydroxylated form (calcitriol). The hormonal functions of vitamin D₃ include regulation of bone and muscle health (skeletal and heart), the immune system, regulation of insulin and glucose levels, calcium and phosphorus metabolism. It increases the absorption and deposition of calcium in the bones, helps convert inorganic phosphorus to organic, stimulates bone

growth, prevents muscle cramps and bone fractures (5,13,23,28).

Sufficient amounts of vitamin D₃ in the body have a proven effect against the development of various diseases and/or disorders. A number of studies have demonstrated the beneficial effect of cholecalciferol supplementation in the prevention of cardiovascular diseases, high blood pressure, diabetes type I and II, osteoporosis, multiple sclerosis (11,12,13,28). Much discussed is the role of vitamin D₃ in the prevention of cancer (4,28).

Recommendations on the average daily intake of vitamins have been approved and implemented by healthcare organizations in many countries. They aimed at specific groups of individuals - by age and gender: infants and children (divided into subgroups according to age - months and years), adolescents, adults (three subgroups - women, men and over 65 years old), pregnant women and lactating women (2, 21).

It was found that people living in warm areas with more sunlight during the year do not need additional vitamin D supplementation. According to Holik et al., 1980, the required daily intake of vitamin D₃ can be obtained by 30 min daily exposure to sunlight

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24.	<p>D. Dobрева, A. Merdzhanova, L. Makedonski, M. Stancheva Seasonal Changes In Fatty Acid Composition And Fat Soluble Vitamins Content Of Grass Carp And Common Carp <i>Agricultural Science and Technology</i>, 6, 3, 271-277, 2014. ISSN: 1313-8820</p>
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AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 6, No 3, pp 271 - 277, 2014

Seasonal changes in fatty acid composition and fat soluble vitamins content of grass carp and common carp

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Abstract. The aim of the present study is to evaluate the seasonal changes in total lipids and fatty acid (FA) composition, and fat soluble vitamins (A, D, E) content in the edible tissue of two freshwater fish species – grass carp and common carp. The FA and vitamins contents of the investigated fish species showed significant seasonal changes. All spring samples were characterized by lower saturated fatty acid (SFA) levels (from 35.9% to 36.0%) and higher polyunsaturated fatty acids (PUFA) (from 14.6% up to 30.1%), including the essential omega 3 PUFA compared to the autumn specimens. The autumn carp and grass carp showed significant decrease of the monounsaturated fatty acids (MUFA) levels. All autumn samples showed lower PUFA (from 13.3% to 30.0%) values, due to reduction of linoleic acid (C 18:2 n6) levels in carp and grass carp. PUFA/SFA and n-3/n-6 ratios decreased in all fish species in the autumn season. The fat soluble vitamins content of the analyzed species in the two seasons were in the range: from 8.56±0.68 µg/100g to 24.44±5.8 µg.100g⁻¹ wet weight (ww) for all-trans-retinol; from 5.41±0.33 µg.100g⁻¹ ww to 30.87±5.1 µg.100g⁻¹ ww cholecalciferol and from 1051.8±38.470 µg.100g⁻¹ ww to 3133.1±57.8 µg.100g⁻¹ ww α-tocopherol, respectively. All analyzed cyprinid species in both seasons are good sources of fat soluble vitamins and PUFAs, which makes them a desirable part of the human diet.

Keywords: common carp, grass carp, fatty acids, fat soluble vitamins, seasonal changes

Introduction

Fishes are regarded as important natural food sources of various beneficial components such as omega-3 (n-3) fatty acid (FA) and fat soluble vitamins, which are necessary for a healthy diet. The nutritional benefits of fish consumption are mainly attributed to the effects of n-3 polyunsaturated fatty acids (PUFA) (Kris-Etherton et al., 2003). Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of these PUFAs (Lee et al., 2006). PUFA can affect platelet function by interacting with membrane proteins, but their effect depends on the FA chain length and the degree of saturation. Individual saturated fatty acids (SFA) such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) increase LDL cholesterol and platelet aggregation

(*Cyprinus carpio*) and the grass carp (*Ctenopharyngodon idella*) are highly bred members of this family in freshwater farms and dam lakes in our country (NAFA, 2007). The common carp is omnivore fish, which is tolerant to large variations of quality of ambient conditions. This species is not susceptible to disease and is tolerant to handling. Grass carp use the natural food in fish ponds very well and their percentage ranges from 20–30% compared to carp. This herbivore fish exploit the ecological potential of fish ponds very well and make production more economical (Čirković et al., 2012). A few studies have examined the factors influencing FA and fat soluble vitamins contents in cultured carp species (Circovic et al., 2012; Ljuboevic et al., 2013). The chemical composition of cyprinids is quite well known mainly due to the importance of this species in some Central and Eastern European countries. Despite the

25.	<p>A. Merdzhanova, D.A. Dobрева, M. Stancheva Quality Evaluation of Dietary Lipid of Channel Catfish (<i>Ictalurus Punctatus</i>) from Bulgaria <i>Bulgarian Journal of Agricultural Science</i>, 21, 1, 202-207, 2015. ISSN 2534-983X</p>
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Bulgarian Journal of Agricultural Science, 21 (Supplement 1) 2015, 000–000
Agricultural Academy

QUALITY EVALUATION OF DIETARY LIPID OF CHANNEL CATFISH (ICTALURUS PUNCTATUS) FROM BULGARIA

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Abstract

MERDZHANOVA, A., D. DOBREVA and M. STANCHEVA, 2015. Quality evaluation of dietary lipid of channel catfish (*Ictalurus punctatus*) from Bulgaria. *Bulg. J. Agric. Sci.*, Supplement 1, 21:

Fish lipids are important components of diet due to their significance as energy, essential fatty acids and fat soluble vitamins sources. No data is available on fatty acid (FA) composition and fat soluble vitamins content of freshwater channel catfish from Bulgarian fish market. The objectives of the present work were to investigate the total lipid content, FA profile, lipid quality indices (atherogenic, thrombogenic), fat soluble vitamins (A, E and D₃) as well as relative daily intake of vitamins of Channel catfish (*Ictalurus punctatus*). The potential nutritional and medicinal value of FA composition and vitamins content to consumers were evaluated. The FA composition was analyzed by GC–MS. Fat soluble vitamins were analyzed simultaneously using RP–HPLC. The FA distribution of catfish is: SFA>MUFA>PUFA. The n3/n6 and PUFA/SFA ratios were greater than the recommended by FAO/WHO. A portion of 100 g contained 0.245 g of EPA+DHA n-3 PUFA. Catfish tissue presented significant amounts of vitamin E (1374.5±158.1 µg.100⁻¹ ww), followed by vitamin A (36.2±0.7 µg.100⁻¹ ww) and D₃ (17.7±0.7 µg.100⁻¹ ww). This species is excellent source of fat soluble vitamins, especially for vitamin D₃ – one survey provides more than 300% of the RDI established in Bulgaria. This study provides specific nutritional information with respect to the consumption of channel catfish for nutrient balance as foodstuff. Since fish tissue is a valuable source of essential nutrients, a detailed analysis for evaluation the nutrient composition and content on fish lipids is needed.

Key words: channel catfish, fatty acids, lipid quality indices, vitamin E, vitamin A, vitamin D₃

Abbreviations: FA – fatty acid; GC–MS – gas chromatography with mass detector; RP–HPLC – reverse phase – high pressure liquid chromatography; SFA – Saturated fatty acids; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; n-3 PUFA – omega-3 polyunsaturated fatty acid; n-6 PUFA – omega-6 polyunsaturated fatty acid; EPA – eicosapentaenoic fatty acid, n-3 PUFA; DHA – docosahexaenoic fatty acid, n-3 PUFA; RDI – recommended daily intake

Introduction

Fishes are regarded as important natural food sources of various beneficial components such as omega-3 (n-3) FA and fat soluble vitamins, which are necessary for a healthy diet. The nutritional benefits of fish consumption are mainly attributed to the effects of n-3 PUFAs (Kris-Etherton et al., 2003). Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects

of these PUFAs (Lee et al., 2006). PUFA can affect platelet function by interacting with membrane proteins, but their effect depends on the FA chain length and the degree of saturation. Individual saturated fatty acids (SFA) such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) increase LDL cholesterol and platelet aggregation (Kris-Etherton et al., 2003; Lee et al., 2006). Several studies have shown that freshwater fishes have a high capacity for transformation of C18 essential fatty acids (EFA) as 18:3 n-3 (alpha-linoleic

26.	<p>D. A. Dobрева, A. Merdzhanova, M. Stancheva The impact of Different Cooking Methods on Fat Soluble Vitamins' Content and Fatty Acid Composition of the Black Sea Shad (<i>Alosa immaculata</i>) <i>Scientific Works of University of Food Technologies</i> – LXII, 2015, 310-314. ISSN 2535-1311</p>
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НАУЧНИ ТРУДОВЕ НА
УНИВЕРСИТЕТ ПО ХРАНИТЕЛНИ
ТЕХНОЛОГИИ - ПЛОВДИВ
ТОМ LXII
2015 г.



SCIENTIFIC WORKS OF
UNIVERSITY OF FOOD
TECHNOLOGIES
VOLUME LXII
2015

Влияние на различни готварски методи върху съдържанието на
мастноразтворими витамини и мастни киселини в Черноморски Карагъоз
(*Alosa immaculata*)

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The impact of Different Cooking Methods on Fat Soluble Vitamins' Content and
Fatty Acid Composition of the Black Sea Shad (*Alosa immaculata*)

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Abstract

The aim of the present study was to evaluate the effect of steaming (10 min at 90°C) and grilling (15 min, 220°C) on lipid, fat soluble vitamins' contents, and the fatty acid's group profile of Shad (*Alosa immaculata*).

The total lipid content was determined according to Bligh&Dyer method. Fat soluble vitamins were determined by RP-HPLC. Fatty acid methyl esters were analysed by GC/MS.

The amount of vitamin A in steamed fillets decreases significantly compared to raw samples, whereas vitamin D₃ and E remain almost unchanged. Among the three fat soluble vitamins, the grilling process affects significantly only vitamin A and E. The steaming tends to increase the saturated fatty acid (FA) levels, whereas grilling increases monounsaturated fatty acids. The steaming, results in significant decrease in omega-3 FA levels. After grilling the omega-6 amounts remain almost unchanged, whereas the omega-3 polyunsaturated FA were significantly decreased.

After steaming and grilling the shad showed insignificant losses of vitamin D and E contents, while retinol was reduced nearly in half. The FA composition of shad tissue did not reveal great losses in omega-3 FA after heat treatments. We can conclude that both cooking methods are suitable for preserving the biological active components in shad.

Keywords: *Alosa immaculata*, grilling, n-3, n-6, steaming, fatty acids, fat soluble vitamins

Introduction

The oily fish are a rich source of biologically active substances, such as fat soluble vitamins (A, D₃ and E) and both saturated and unsaturated fatty acids [13]. The vitamins are essential nutrients which control a diversity of biologically important processes in human body. Fish tissue lipids have been intensively studied due to their cardio-protective effects. Two long chain omega-3 polyunsaturated fatty acids (n3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are identified as the most likely active constituents [2].

The consumption of raw fish fillets is very limited in Bulgaria and the Western society.

Therefore information about fat soluble vitamins' content and FAs in raw fish may have limited application for human health. Moreover these nutrients are considered to be especially susceptible to oxidation during cooking processes such as steaming, boiling, grilling, baking and frying. Temperature processing of fish fillets inactivates pathogenic microorganisms and enhances its taste, but influences the amounts of fat soluble vitamins and essential fatty acids.

The black Sea Shad (*Alosa immaculata*) is an anadromous species which inhabits the Black Sea while for spawning migrates to the Danube River [14]. It is an economically important species - object of commercial fishing.

The aim of the present study was to evaluate the effect of the steaming and grilling processes on total

27.	A. Merdzhanova, D. A. Dobрева , S. Georgieva Nutritional evaluation of aquaculture mussels (<i>M. galloprovincialis</i>) from the Black Sea, Bulgaria <i>Ovidius University Annals of Chemistry</i> , 27, 1, 1-7, 2016. ISSN: 2286-038X
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Nutritional evaluation of aquaculture mussels (*M. galloprovincialis*) from the Black Sea, Bulgaria

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Abstract. In recent years black mussels are one of the most commercially important species from the Bulgarian Black Sea. The marine mollusks are valuable healthy food, low in calories and fats and high in proteins. They are a major dietary source of fat soluble pigments - astaxanthin, carotenoids and polyunsaturated fatty acids (PUFA). To our knowledge the information about the nutritional quality of mussels from the Bulgarian Black Sea waters, based on chemical composition, fat soluble pigments, cholesterol and PUFA content is very limited. The aim of the present study is to determine and compare protein, lipid, carbohydrate and energy values, fat soluble pigments, cholesterol and fatty acid composition in farmed mussels (*Mytilus galloprovincialis*) from the Bulgarian northern and southern parts of the Black Sea coast. The mussel samples were analyzed for lipids (Bligh & Dyer method), crude proteins (Kjeldahl method), carbohydrates and moistures according to the AOAC (1990) methods. Fatty acids were analyzed by the GC-MS system. Fat soluble pigments and cholesterol were analyzed simultaneously by the RP-HPLC system. Lipid and protein content were found to be higher in mussels from the northern region. In accordance with the Commission Regulation (EC) No. 116/2010 all analyzed mussel samples can be classified as high in protein and low in fats and carbohydrates. The amount of cholesterol, contained in all mussel populations is significantly low, while the omega-3 (n-3) is significantly higher than the omega-6 PUFA. A portion of 100 g edible tissue provides 0.500 g more of the required amount of eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) n-3 PUFA according to EFSA (2012). It can be concluded that the studied mussel aquaculture in the Black Sea is beneficial food for the human health and it is advisable to be part of a proper or a preventive diet of Bulgarian consumers.

Keywords: black mussels, astaxanthin, n-3 PUFA, energy value.

1. Introduction

Bivalve mollusks like *Mytilus galloprovincialis*, (Lamarck, 1819) constitute a highly nutritive seafood of increasing demand on international markets.

Moreover, the mussels contain higher omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs) with several potential cardio protective effects and antithrombotic action. Carotenoids possess high antioxidant potential and some studies report their use

28.	<p>D.A. Dobрева, V.Z. Panayotova, R.S. Stancheva, M. Stancheva Simultaneous HPLC determination of fat soluble vitamins, carotenoids and cholesterol in seaweed and mussel tissue <i>Bulgarian Chemical Communications</i>, 49, G, 112-117, 2017. ISSN: 0324-1130</p>
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Bulgarian Chemical Communications, Volume 49, Special Issue G (pp.112–117) 2017

Simultaneous HPLC determination of fat soluble vitamins, carotenoids and cholesterol in seaweed and mussel tissue

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The aim of the present study was to develop simple method for simultaneous determination of fat soluble vitamins (all-trans retinol, cholecalciferol, ergocalciferol and α -tocopherol), carotenoids (β -carotene and astaxanthin) and cholesterol in seaweeds and mussel tissue. Reversed-phase high performance liquid chromatography system combine with UV and fluorescent detection was the method characterized with rapid, sensitive and accurate detection of all components. Extraction procedure requires small amounts of sample. The sample preparation included saponification and liquid-liquid extraction of the analytes. The method precision (relative standard deviation) was below 10% for all analytes. The method shows good linearity of all investigated components and analysis time – 32 min. The method was applied on real seaweed and mussel tissue samples and the results for the tested fat soluble analyte contents were in a good agreement with the data given by other authors.

Key words: *all-trans retinol, calciferol, α -tocopherol, astaxanthin, cholesterol*

INTRODUCTION

Many studies suggest that marine mollusks and edible seaweeds are valuable healthy food, low in calories and fats, and high in proteins and bioactive compounds [1, 2]. Seaweeds also have been used as permanent source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics, as fodder and fertilizer [3]. Mollusks and different seaweeds are amongst the most important dietary sources of fat soluble vitamins – A, D₃ and E. They are also rich in carotenoids, which act as antioxidants [4, 5]. Overall mollusk production in Bulgaria relies on two sources – commercial fishing (for mussel and sea snail *Rapana*) and marine aquaculture (consists of mussel only) [6].

Lipid content of different marine algae is only 1-5% of dry matter and exhibits an interesting polyunsaturated fatty acid composition (particularly ω -3 and ω -6) [7]. Seaweed and mollusks tissue is a good source of some water- (B₁, B₂, B₁₂ and C) and fat soluble (β -carotene with vitamin A activity, ergocalciferol and cholecalciferol and α -tocopherol)

vitamins, carotenoids and cholesterol content of Black mussel and different algae from Black Sea is scarce. The aim of the present work was to develop a simple and accurate method for simultaneous determination of seven fat soluble biologically active components: all-trans-retinol (vitamin A), cholecalciferol (vitamin D₃), ergocalciferol (vitamin D₂), α -tocopherol (vitamin E), β -carotene, astaxanthin and cholesterol in matrices from animal and plant origin.

EXPERIMENTAL

Instrumentation and chemicals

The chromatographic analysis was performed on HPLC system (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors. All solvents were of HPLC grade specification, obtained by Sigma-AldrichTM, USA. Substances of all-trans-retinol, ergocalciferol, cholecalciferol, α -tocopherol, β -carotene, astaxanthin, cholesterol and L-ascorbic acid were all with analytical standard specifications and were supplied by Supelco (Sigma-AldrichTM, USA).

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| 29. | D. A. Dobрева, A. Merdžhanova, L. Makedonski
Fat-soluble nutrients and fatty acids in skin and fillet of farmed rainbow trout.
<i>Bulgarian Chemical Communications</i> , 49, G, 123-129, 2017. ISSN: 0324-1130 |
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Bulgarian Chemical Communications, Volume 49, Special Issue G (pp.118–123) 2017

Fat soluble nutrients and fatty acids in skin and fillet of farmed rainbow trout

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This study compares the fat soluble components in the muscle and edible skin parts of farmed rainbow trout (*Oncorhynchus mykiss* W.) filets, sampled at two growth stages, from fish markets from Bulgaria. Insufficient information is available about the differential fat soluble pigments, cholesterol, vitamins and fatty acid compositions of rainbow trout filets when eating them with or without the skin left on. Vitamins A, D₃ and E, β -carotene and cholesterol were analyzed simultaneously using HPLC system with UV and FL detection (vitamins A and E). Total lipids were extracted according to Bligh and Dyer method. Analysis of fatty acid methyl esters (FAME) were performed by GC/MS. The average lipid content, the cholesterol and vitamin E amounts and the saturated fatty acids were significantly higher in the skin than in the muscle, whereas the proportion of vitamin A and D₃, eicosapentaenoic acid (C20:5 ω -3) and docosahexaenoic acid (C22:6 ω -3) were higher in the muscle.

Key words: *Oncorhynchus mykiss*, vitamins, carotenoids, cholesterol, PUFA

INTRODUCTION

It is well-known that fish consumption has nutritional and health benefits in humans. Rainbow trout (*Oncorhynchus mykiss* W.) is one of the most consumed fish species in Bulgaria and also of interest to aquaculture because of the rapid growth rate and excellent nutritional qualities of the meat [1]. Nutritional quality of fish depends especially on tissue lipid composition including fat soluble vitamins, fatty acids, cholesterol and β -carotene. In the scientific literature, chemical composition of fish is investigated from different points of view. Recently a special interest in fish lipid composition has risen because of its advantageous effects of human health which depend on its fatty acid (FA) and fat soluble vitamins content. Moreover, the optimal quantities of polyunsaturated FA/saturated FA, ω -3/ ω -6 FA ratios are considered as informative indices for nutritional quality. Comparative investigations on farmed rainbow trout lipids, FA, fat soluble vitamins content and cholesterol from Turkey were performed by Harlioglu A. (2012) [2]. Previous studies [3, 4] have reported data on the proximate and fatty acid composition of rainbow trout. Despite these facts, available information about the

cholesterol and fatty acid profile of rainbow trout filets and skin. This is the first study on β -carotene, cholesterol, vitamins and FA composition of farmed rainbow trout filets and skin in Bulgaria. The presented results will be useful when determining what differences might exist in nutrient ingestion, depending on whether a rainbow trout fillet is consumed with or without the skin.

EXPERIMENTAL

Sample collection

Samples of rainbow trout were purchased from Varna fish market during March 2015. Fish was raised in two fish farms (Plovdiv region, Hvoina village and Dospat Dam Lake) and fed on commercial feed mixtures. Analyzed specimens were divided in two groups (with three specimens in each group): group I (Rainbow trout I) – weighing 300 - 400 g; group II (Rainbow trout II) – weighing 700 - 900 g. Each specimen was filleted, muscle tissue was separated from the skin. Two medium samples were prepared.

Vitamins, pigments and cholesterol analysis

Saponification and extraction: Two skin and two muscle tissue samples were homogenized and used for evaluation of all-trans-retinol,

30.	V. Panayotova, A. Merzhdhanova, D.A. Dobрева , M. Zlatanov, L. Makedonski Lipids of Black Sea algae: unveiling their potential for pharmaceutical and cosmetic applications <i>Journal of IMAB</i> , 23, 4, 1747-1751, 2017. ISSN: 1312-773X
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Journal of IMAB
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Journal of IMAB - Annual Proceeding (Scientific Papers). 2017 Oct-Dec;23(4)

Original article

LIPIDS OF BLACK SEA ALGAE: UNVEILING THEIR POTENTIAL FOR PHARMACEUTICAL AND COSMETIC APPLICATIONS

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ABSTRACT

Background: Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. The red algae *Gelidium crinale* (Rhodophyta) and brown algae *Cystoseira barbata* (Phaeophytes) are among the most abundant species along the Bulgarian Black Sea shore. Yet information about their lipid composition is limited.

Purpose: Present study was conducted to investigate biologically active substances in two underexplored seaweed lipids. Total lipids, total phospholipids, fat soluble vitamins and carotenoids were analysed. In addition, the specific distribution of fatty acids group among the total lipids and total phospholipids were elucidated.

Material/Methods: The saponifiable lipid fraction was derivatized into fatty acid methyl esters (FAMES) and analysed by gas chromatography–mass spectrometry (GC-MS) to identify and quantify the fatty acids. The fat soluble non-saponifiable lipids were identified by high-pressure liquid chromatography coupled with UV/Vis and fluorescence detectors (HPLC-UV-FL).

Results: Results showed that Rhodophyta and Phaeophytes have high concentrations of polyunsaturated fatty acids (PUFA), particularly from the n-3 series, thereby being a good source of these compounds. They presented a “healthy” n-6/n-3 ratio. Both seaweed species showed considerably high amounts of α -tocopherol, β -carotene and astaxanthin.

Conclusions: The study reveals that lipids from Black Sea algae have a high potential as natural sources of biologically active ingredients. They are balanced source of fatty acids and contained beneficial antioxidants, such as α -tocopherol, β -carotene and astaxanthin.

Keywords: *Cystoseira barbata*, *Gelidium crinale*.

of bioactive natural products which may have a significant role in health promotion, mainly in diseases prevention and treatment. Moreover, seaweeds have been used since ancient times as food, sources of medicine, but despite their abundance, nowadays they are poorly exploited. This article focuses on the Black Sea red and brown algae species lipid composition. Algal total lipid content is usually low, but they contain a high proportion of polyunsaturated fatty acids (PUFA) combined with other interesting secondary metabolites as vitamins, pigments, proteins etc. PUFA are of the utmost importance for human metabolism. Besides their structural role, they possess other beneficial effects, like antioxidant activities, prevention of cardiac diseases, inhibition of tumour progression, anti-inflammatory, etc. Such properties are indicative of the potential of PUFA for nutraceutical and pharmaceutical purposes [1]. Algal lipids are a good source of vitamins, important not only due to their biochemical functions and antioxidant activity but also due to other health benefits [2]. The main source for long-chain PUFA is fish oil. Bearing in mind that fish is a declining resource and that there is an increasing commercial interest in such fatty acids (FA), an alternative source must be found [3]. Bulgarian Black Sea coast is rich in algae, regarding algal biodiversity and biomass, yet information about fat soluble vitamins and carotenoid content and FA composition of native species is still limited. Moreover, the lack of studies of the bioavailability of algal lipids currently limits their nutritional evaluation and assessments of possibility for pharmaceutical and cosmetic applications in Bulgaria. The objective of this study was to provide knowledge on the composition and reveal the potential of two seaweed species – *Gelidium crinale* (Rhodophyta) and *Cystoseira barbata* (Phaeophytes) from the Black Sea as alternative sources of the functional in-

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| 31. | A. Merdzhanova, I. Ivanov, D.A. Dobreva , L. Makedonski
Fish Lipids as a Valuable Source of Polyunsaturated Fatty Acids
<i>Acta Scientifica Naturalis</i> , 4, 70-75 2017. ISSN: 2367-5144 |
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ASN, Vol 4, No 1, Pages 70-75, 2017

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Fish Lipids as a Valuable Source of Polyunsaturated Fatty Acids

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Abstract: This article presents information about omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acid (PUFA) contents in a broad range of commercially important fish species available on Bulgarian fish markets. The aim is to raise consumers' awareness and encourage them to eat fish. Fish species from the Black Sea coast have relatively high proportion of n-3 PUFAs, of which more than 80% is by EPA (eicosapentaenoic acid, C 20:5 n-3) and DHA (docosahexaenoic acid, C 22:6 n-3). Extensive epidemiological studies show that fish consumption is inversely associated with the incidence of cardiovascular diseases (CVD), stroke and the functioning of the brain. About 0.5 g of omega-3 (EPA+DHA) a day or two servings of oily fish a week are required to reduce the risk of death from CVD. PUFA needs should be satisfied not only with food additives but with fish lipids containing food.

Key words: omega-3, omega-6, Black Sea fish, health benefits

Introduction

The optimal fatty acid (FA) composition of a diet is an important factor in disease prevention and health promotion. The FAO/WHO [7] conclude that "Adequate amounts of dietary fat are essential for health. In addition to their contribution to meeting energy needs, intake of dietary fat must be sufficient to meet the requirements for essential fatty acids". It is well known that fish lipids are one of the best sources of omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA). Thus, a more frequent consumption of fish is recommended. Fish is traditionally viewed as a key element of a healthy balanced diet. National dietary recommendations, and those relating to the Mediterranean diet, include advice on regular fish consumption. In Bulgaria, this advice is included in Food-based Dietary Guidelines for Adults in Bulgaria [8]. Fish consumption in different countries varies widely with Japan, Sweden, Norway and Denmark (55-85 g daily) having the highest, moderate in Greece, France, Great Britain (30-25 g/day) and low (below 20 g daily) in Romania, Hungary and many others. The negative trend in recent 2-3 decades in Bulgaria is towards a decrease in fish consumption from 12-14

32.	V. Panayotova, D. Dobрева , A. Merzdhanova, M. Stancheva, L. Makedonski Seasonal changes in fatty acid composition and alpha-tocopherol content in <i>Cystoseira barbata</i> <i>Proceedings of the Institute of Fishing Resources</i> , 28, 73-77, 2017. ISSN 0204-7764
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Proceedings of the Institute of Fishing Resources, Volume 28, 2017

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Seasonal changes in fatty acid composition and alpha-tocopherol content in *Cystoseira barbata*

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Abstract

Целта на настоящото проучване е да се проучат сезонните промени в съдържанието на общи липиди, мастнокиселинния състав и съдържанието на токоферол в кафявите водорасли *Cystoseira barbata*. Мастнокиселинният състав и съдържанието на витамин Е показват значителни промени в изследваните сезони. Пролетните проби се характеризират с по-високи количества наситени мастни киселини (НМК – от 62% на 70%) и мононенаситени мастни киселини (МНМК – 8% до 13%) и по-ниско съдържание на полиненаситени мастни киселини (ПНМК от 29% на 16% от общите мастни киселини). Пониските количества ПНМК се дължат главно на значителното намаляване на линоловата (C18:2n-6) и арахидоновата киселини (C20:4n-6) през пролетта. Съдържанието на токоферол намалява значително в пролетния сезон, съответно от 7.2 до 4.7 mg.g-1 липид.

Keywords: Cystoseira barbata, Black Sea, fatty acids, a-tocopherol

Introduction

Algae have been used since ancient times as food, fodder, fertilizer and as source of medicine. Their lipid fraction exhibits an interesting PUFA composition particularly omega 3 (n-3) and omega 6 (n-6) acids. These fatty acids play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. Seaweeds are a good source of vitamins, especially α -tocopherol. Algae and their extracts are of interest in cosmetics as components of sun lotions and as regenerating and anti-wrinkle products.

33.	<p>A. Merdzhanova, D. A. Dobreva, V. Panayotova Comparison of Fatty Acids, Cholesterol, Fat Soluble Vitamins and Carotenoids Content of Skin and Edible Tissue of Farmed African Catfish (<i>Clarias Gariepinus</i>, Burchell 1822) <i>Ovidius University Annals of Chemistry</i>, 29, 1, 41-47, 2018. ISSN: 2286-038X</p>
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Comparison of fatty acids, cholesterol, fat soluble vitamins and carotenoids content of skin and edible tissue of farmed African catfish (*Clarias gariepinus*, Burchell 1822)

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Abstract. African catfish (*Clarias gariepinus*) is new species for the Bulgarian market. It is a valuable source of biologically active components that play an important role in human diet, but there is lack of information for the quality of its dietary lipids. This study focuses on the assessment of skin and edible tissue lipid quality of farmed African catfish based on lipid content and detailed fatty acids, fat-soluble vitamins, cholesterol and carotenoids composition. Fatty acid composition was determined by gas chromatography with mass spectrometer (GC/MS) after lipid extraction. Vitamins A, D₃ and E, beta-carotene, astaxanthin and cholesterol were analyzed simultaneously using high performance liquid chromatography (HPLC) with ultraviolet and fluorescence detectors. Lipids, cholesterol, astaxanthin and monounsaturated fatty acids (MUFA) were significantly higher in skin, whereas vitamin A and E, polyunsaturated fatty acids (PUFA) were higher in muscle tissue. Vitamin D₃ showed comparable amounts in both tissues. Eicosapentaenoic acid (C20:5n3) and docosahexaenoic acid (C22:6n3) which are important indicators for fish lipids quality presented significantly high amounts. A portion of 100 g filet without skin contains approximately 600 mg. Results confirmed that African catfish meat – with or without the skin, can be valuable and preferable source of biologically active lipids.

Keywords: African catfish, fatty acids, vitamins, cholesterol, carotenoids

1. Introduction

According to FAO [1] and FAO-FISHSTAT [2] more than 40 % of consumed fish come from farming. Many studies are focused on the importance of fish as protein source but also discuss its importance as valuable source of essential long chain fatty acids, fat soluble vitamins and pigments [3]. In addition, the benefits of fish consumption for vulnerable groups

gariepinus, Burchell, 1822) as interesting species with high potential for fish farming [5]. Although the importance of these species, only limited investigations on nutritional composition on Silurid species as channel catfish were carried out [6].

Until now, there is no information concerning fatty acid (FA) composition, cholesterol, fat soluble vitamins and carotenoids in African catfish skin and muscle from Bulgarian market available in the

34.	A. Merdzhanova, D. A. Dobрева , V. Panayotova The comparison of proximate composition, fatty acids and fat-soluble vitamins content of the black sea sprat (<i>sprattus sprattus</i> l.) During catching seasons <i>Annals. Food Science and Technology</i> (приета, под печат) ISSN 2344-4916
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THE COMPARISON OF PROXIMATE COMPOSITION, FATTY ACIDS AND FAT-SOLUBLE VITAMINS CONTENT OF THE BLACK SEA SPRAT (*SPRATTUS SPRATTUS* L.) DURING CATCHING SEASONS

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Abstract

*The aim of the present study was to determine and compare the seasonal changes in proximate composition, fatty acid profile and fat soluble vitamins content in spring and autumn sprat (*Sprattus sprattus*) from the Bulgarian Black Sea waters. Crude protein was in the range 16.10 – 17.15%, fat content was from 4.20 to 6.65g/100g wet weight (w.w.). The fatty acid (FA) and vitamin's contents showed significant seasonal changes. The spring sprat was showed lower saturated fatty acid (SFA, 31.7%), higher mono unsaturated fatty acids (MUFA, 34.7%) and insignificantly lower polyunsaturated fatty acids (PUFA, 33.6%) compared to the autumn samples. In both seasons omega-3 (n-3) PUFA levels were higher than omega-6 (n-6) PUFA and presented over than 50% of total PUFAs. Different amounts of alpha-tocopherol were found in both seasons – 701.2 µg/100g ww (spring). The higher amounts of all-trans retinol (142.3 µg/100 g ww) and cholecalciferol (11.9 µg/100 g ww) were found in spring samples. Regardless of the observed seasonal changes in proximate and FA composition, fat soluble vitamins and n-3 PUFA contents sprat species caught from the Bulgarian part of Black Sea are excellent sources of the analysed components and can be recommended for healthy human diet.*

Key words: proximate composition, fat soluble vitamins, human health, *Sprattus sprattus*

1. INTRODUCTION

The quality of fish tissue is function of their body compositions and energy values, which vary among different seasons and species. Determination of proximate composition as protein, carbohydrates, lipids, and moisture contents is often necessary to ensure that fish tissues have a good nutrition quality and that they meet the requirements of food regulations and commercial specifications (FAO, 2010). In recent years a number of investigators have assessed proximate composition of fish species as shad, horse mackerel, garfish and goby from Southeast and Northwest part of the Black Sea (Guner et al., 1998; Boran and Karacam, 2011). However studies of seasonal changes in fat soluble vitamins contents and fatty acid profile of one of the most commercially important species as sprat from the Bulgarian part of Black Sea are lacking. During the last 20 years, the sprat species (*Sprattus sprattus* L.) has been most abundant and main subject of perennial fish caught in the Western Black Sea and for Bulgarian local fish markets. Sprat is domestic (non-migrated) species. It forms big schools and undertakes seasonal movements between foraging (inshore) and spawning (open sea) areas (NAFA, 2007; Tserkova,

35.	G. Georgieva, D. A. Dobрева , V. Panayotova, A. Merdzhanova Phenolic compounds in edible plants <i>Varna Medical Forum</i> (под печат)
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ФЕНОЛНИ СЪЕДИНЕНИЯ В ЯДЛИВИ РАСТЕНИЯ

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АБСТРАКТ

През последните години значително нараства интереса към изследването на химичния състав на ядливи растения, особено на съдържанието им на биологично активни съединения с лечебно-протективен потенциал. Такива растения традиционно се използват както в европейската, така и в азиатската култури, за подобряване на аромата и вкуса, както и за обогатяване на хранителната стойност на ястията. Ядливите растения се консумират както в сурово състояние като салати, добавки, подправки, гарнитур, така и след термична обработка.

Важна характеристика на ядливите растения е голямото разнообразие на естествени биологично активни вещества - включително флавоноиди и фенолни киселини, антоцианини и др., които се съдържат в листата или други техни части. Полифенолите са голяма група вторични растителни метаболити. Най-често срещаните фенолни съединения в растителната храна са фенолните киселини и флавоноидите.

Известно е, че приема на фенолни съединения влияе позитивно върху човешкото здраве. Тази група вещества се характеризират с доказани противовъзпалителни, противоалергични и противовирусни свойства, както и с потенциал за превенция на някои сърдечно-съдови заболявания, хипертония, диабет и др. Тези свойства се дължат на двойната роля на фенолните съединения - като антиоксиданти и като субстрати.

В България има сравнително малко и ограничена информация за състава на полифенолни киселини и флавоноиди в ядливи растения. Разширяване обхвата на подобна информация би предоставило възможност за информиран избор храна, от страна на консуматора, както и употребата им във фармацията (фитотерапия, лечебна козметика), включване в лечебни хранителни режими – като хранителни добавки и компоненти на функционални храни и др.

Ключови думи: фенолни киселини, флавоноиди, функционални храни

37.	<p>M. Stancheva, D.A. Dobрева, R. Stancheva and V. Panayotova Fat soluble vitamins, carotenoids and cholesterol content of wild and farmed black mussel (<i>Mytilus galloprovincialis</i>) Donostia–San Sebastián, Spain, <i>Aquaculture Europe</i> 2014</p>
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Aquaculture Europe 2014, Donostia–San Sebastián, Spain

**FAT SOLUBLE VITAMINS, CAROTENOIDS AND CHOLESTEROL
 CONTENT OF WILD AND FARMED BLACK MUSSEL (*Mytilus galloprovincialis*)**

M. Stancheva, D.A. Dobрева, R. Stancheva and V. Panayotova

Medical University of Varna, Department of Chemistry, Faculty of Pharmacy, 55 Marin Drinov St., 9000 Varna (Bulgaria). E-mail: didobрева@gmail.com

Introduction

Many studies suggest that marine molluscs are valuable health food, low in calories and fats, and high in proteins and bioactive compounds (Borodina and Soldatov, 2010; Desnica et al., 2011). They are one of the most important dietary sources of fat soluble vitamins A, D₃ and E. Mussels are also rich on carotenoids, which act as antioxidants (Orban et al., 2002; Lorenz, 2010). The production of mollusks in Bulgaria relies on 2 main sources – commercial fishing (mussel and sea snail *Rapana*) and marine aquaculture, which consists only of mussels (NSP, 2007). Black mussel is one of the most commercially important species from Bulgarian Black Sea. There is limited information in the scientific literature about fat soluble vitamins, carotenoids and cholesterol content of Black Sea Black mussel.

The aims of the present work are to determine and compare fat soluble vitamins, astaxanthin, beta-carotene and cholesterol in wild and farmed Black Sea Black mussel (*Mytilus galloprovincialis*).

Materials and methods

Mussel samples were purchased from different mussel farms and Varna local fish market during autumn 2013. The edible tissue of four farmed and one wild mussel, were used for evaluation the vitamins A, D₃ and E, astaxanthin, beta-carotene and cholesterol contents. An aliquot of the homogenized sample (1.000±0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 0.5M methanolic potassium hydroxide were added. Six parallel samples of edible tissue were prepared and subjected to saponification at 50°C for 30 min. The components of interest were extracted with n-hexane:dichloromethane = 2:1 solution. The extract was evaporated under nitrogen and dry residue was dissolved in methanol:dichloromethane and injected (20µl) into the liquid chromatography system.

38.	S. Vutov, A. Merdzhanova, D. A. Dobreva , M. Stancheva Fatty acid compositions and fat soluble vitamin's of marine and freshwater aquacultured fish species Rotterdam, Netherlands, <i>Aquaculture Europe</i> 2015
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Aquaculture Europe 2015, October 20-23, Rotterdam

FATTY ACID COMPOSITIONS AND FAT SOLUBLE VITAMIN'S OF MARINE AND FRESHWATER AQUACULTURE FISH SPECIES

S. Vutov, A. Merdzhanova, D. A. Dobreva, M. Stancheva
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St., 9000 Varna (Bulgaria). E-mail: didobreva@gmail.com;

Introduction

The worldwide decline of ocean fisheries stocks has provided impetus for rapid growth in fish and shellfish farming. Aquaculture has been the world's quickly developing sector of food production branch. Farming fish currently accounts for over one-quarter of all fish directly consumed by humans (FAO, 2014). Many studies suggest that fish are valuable health food, high in proteins, bioactive compounds and beneficial fats. Farming of fish helps to control both - the nutrition duality and their safety as a food (NOAA Fisheries, 2011). The some of freshwater fish species, which have been an object of breeding in Bulgaria are warm water Channel catfish (*Ictalurus punctatus*) and cold-water Rainbow trout (*Oncorhynchus mykiss*) (Hadjinkolova, 2010). The marine aquaculture consists mainly from mussels and Barramundi (*Lates calcarifer*) (feed outside the Black Sea) (NSP, 2007). The other aquacultured marine fish, available on Bulgarian markets, are Gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). These fish are some of the most commercially important species from Bulgaria. There is limited information in the scientific literature about fat soluble vitamin's and fatty acids composition in edible tissue of listed above species. The aims of the present study are to determine and to compare total lipids, fatty acids composition and fat soluble vitamin A, D₃ and E, in traditionally consumed farmed marine and freshwater fish.

Materials and methods

Fish samples were purchased from different farms and Varna local fish markets. The edible tissue of three marine and two freshwater fish, were used for evaluation the vitamins A, D₃ and E contents, total lipids and fatty acid compositions.

Fatty acids (FA) analysis: Portions of homogenate (5.000±0.001 g) were extracted in triplicate according to Bligh & Dyer (1959). Total lipid content of edible tissue was determined gravimetrically after extraction and evaporation. The portion of chloroform fraction was methylated by base-catalyzed transmethylation (2M KOH in methanol) and n-hexane (EN ISO 5509: 2000). The fatty acids methyl esters were analyzed by GC-MS.

Vitamin's analysis: An aliquot of the homogenized sample (1.000±0.005 g) was weighed and 1% of methanolic L-ascorbic acid and 1.0M methanolic KOH were added. The samples were subjected to saponification at 80°C for 20 min. The analytes were

IV. Глава от книга

36.	<p>A. Merdzhanova, D. A. Dobрева, V. Panayotova Assessment of Proximate and Bioactive Lipid Composition of Black Sea Mussels (<i>M. galloprovincialis</i>) from Bulgaria <i>Biological Resources of Water, Chapter 9</i> IntechOpen, Available on-line: April 25th 2018 DOI: 10.5772/intechopen.71909. ISBN: 978-1-78923-081-9</p>
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Assessment of Proximate and Bioactive Lipid Composition of Black Sea Mussels (*M. galloprovincialis*) from Bulgaria

Albena Merdzhanova, Diana A. Dobрева and Veselina Panayotova

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.71909>

Abstract

Farmed marine mussels from genera *Mytilus* are important for the human diet by providing high levels of proteins, omega-3 polyunsaturated fatty acids (PUFAs), fat soluble vitamins and carbohydrates. Recently, black mussels are commercially important species from the Bulgarian Black Sea. The aim of this study was to assess the seasonal changes in proximate composition and to focus on the lipid bioactive components such as fatty acids, cholesterol, fat-soluble vitamins (A, E and D₃), and carotenoids (astaxanthin, beta-carotene) in farmed mussels (*M. galloprovincialis*) from the northern part of the Bulgarian Black Sea coast. All analyzed samples presented high protein and low lipid content. The fatty acids (FA) profile was characterized by the highest amount of PUFA, as 22:6 omega-3 (n-3) dominated, regardless of the seasons. In all seasons, the content of n-3 was significantly higher than the omega-6 PUFA. The amounts of cholesterol were in the range 62.3 (summer) to 78 (autumn) mg 100⁻¹ g ww. The highest amounts of vitamin D₃ (3.1 μg 100⁻¹ g ww), vitamin E (2525 μg 100⁻¹ g ww), astaxanthin (0.470 mg 100⁻¹ g ww), and beta-carotene (0.445 mg 100⁻¹ g ww) were found in the summer season. The studied mussel aquaculture from Bulgaria presented a high beneficial potential in all seasons in terms of human health protection.

Keywords: *M. galloprovincialis*, astaxanthin, cholesterol, fat soluble vitamins, seasonal changes, omega-3PUFA

1. Introduction

V. Учебно помагало	
39.	Учебно помагало за упражнения по Аналитична химия, 2012 авторски колектив: доц. М. Станчева, гл. ас. Д. Добрева, гл. ас. Ст. Георгиева



МЕДИЦИНСКИ УНИВЕРСИТЕТ – ВАРНА

КАТЕДРА ПО ХИМИЯ

УЧЕБНО ПОМАГАЛО

ЗА УПРАЖНЕНИЯ ПО АНАЛИТИЧНА ХИМИЯ

на Магистър фармацевти



2012 г.

Учебното помагало е написано от доц. Мона Станчева, която чете лекциите по Аналитична химия на студентите от специалност „Фармация“ и от гл. ас. Д. Добрева и гл. ас. Ст. Георгиева, които водят упражненията по дисциплината.

ПРОГРАМА ЗА УПРАЖНЕНИЯ ПО АНАЛИТИЧНА ХИМИЯ ЗА СПЕЦИАЛНОСТ "МАГИСТЪР ФАРМАЦЕВТ"

I СЕМЕСТЪР

Упражнение	ТЕМА
1	Правила за работа в химическа лаборатория. Лабораторни съдове и техники в аналитичната химия. Разтвори. Концентрация на разтворите. Процентна концентрация.
2	Моларна концентрация на разтвори. Качествен анализ. Качествени реакции и системен мод на катионите от първа аналитична група.
3	Нормална концентрация на разтвори. Качествени реакции на катионите от втора и трета аналитични групи.
4	Качествени реакции на катионите от четвърта и пета аналитични групи катиони. Задачи от концентрация на разтворите.
5	Контролно върху концентрация на разтвори. Качествен анализ на някои аниони с биологично значение. Анализ на конкременти.
6	Протолитична теория. Водороден показател (рН), методи за определяне. Буферни разтвори, буферна крива, рН на буфери.
7	Контролна задача върху качествен анализ на катиони и аниони, водороден показател, методи за определяне и буферни разтвори.
8	Количествен анализ. Обеман анализ – стандартни разтвори, индикатори, изчисления. Киселинно-основен обеман анализ. Титрувални криви. Титруване на силни киселини и основи.
9	Киселинно-основен обеман анализ. Титрувални криви. Титруване на слаби протолити и полипротонни киселини.
10	Комплексометричен обеман анализ. Определяне на Са и Mg катиони в техни разтвори. Приложение на комплексометрията: определяне твърдост на вода, определяне на калциеви йони в разтвор на Са-глюконат и на магнезиеви йони в разтвор на Mg-аспартат.
11	Окислително-редукционни процеси. Окислително-редукционен обеман анализ. Перманганометрия. Определяне концентрацията на оксалова киселина и съдържание на водороден пероксид в перхидрол.
12	Контролна задача върху обеман количествен анализ.
13	Окислително-редукционен обеман анализ. Йодометрия - определяне количеството на витамин С във фармацевтични препарати. Количествено определяне на глюкоза.
14	Утачен обеман анализ. Киселинно-основно титруване в неводна среда. Титруване в среда от метанол. Определяне концентрацията на бензоена киселина.

Упражненията са по 3 учебни часа.	
II СЕМЕСТЪР	
Упражнение	ТЕМА
1	Методи за пробоподготовка. Разделяне и концентриране. Екстракционно отделяне и доказване на Pb^{2+} и Ca^{2+} .
2	Инструментални методи. Електрохимични методи за анализ. Потенциометрия. Определяне на pK_a на оцветна киселина по метода на потенциометричното титруване.
3	Потенциометрия. Построяване на титрувална крива на фосфорна киселина.
4	Оптични (спектрални) методи. Спектрофотометрия. Определяне концентрацията на салицилова киселина по метода на стандартната права.
5	Спектрофотометрично количествено определяне на витамин B_{12} .
6	Спектрофотометрично определяне на акрифлазин и метиленово синьо в общ разтвор.
7	Колоквиум върху методи за пробоподготовка, потенциометрия и спектрофотометрия.
8	Хроматография. Видове хроматография. Принцип на колонна, хартиена и тънкослойна хроматография.
9	Газова хроматография - принцип на метода. Приложение.
10	Високоэффективна течна хроматография – принцип на метода. Приложение.
11	ВЕТХ метод за количествено определяне съдържанието на кофеин в различни проби.
12	Атомно-абсорбционна спектрометрия. Принцип на метода и приложения – посещение на аналитична лаборатория.
13	Контролно върху хроматографски методи.
14	Обработка на експерименталните резултати.
15	Контрол качеството на анализа. Валидиране на метод. Принцип на добрата лабораторна практика.
Упражненията са по 2 учебни часа.	

VI. Избрани резюмета от научни форуми

1.	<p>The 1st International Vitamin Conference 19-22 May 2010, Copenhagen, Denmark Mona Stancheva, Diana Dobрева, Albena Merdzhanova, Bistra Galunska, „<i>The Effect of Storage and Processing on Fat Soluble Vitamin Content in Bulgarian Freshwater and Black Sea Fishes</i>“</p>
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Poster session 3G

Abstract no.: 96

Title:

The effect of storage and processing on Fat-soluble vitamin content in Bulgarian Freshwater and Black Sea fishes

Name:

Mona Stancheva

Organisation and address:

Medical university
 Chemistry
 55 Marin Drinov street, 9002, Varna, Bulgaria

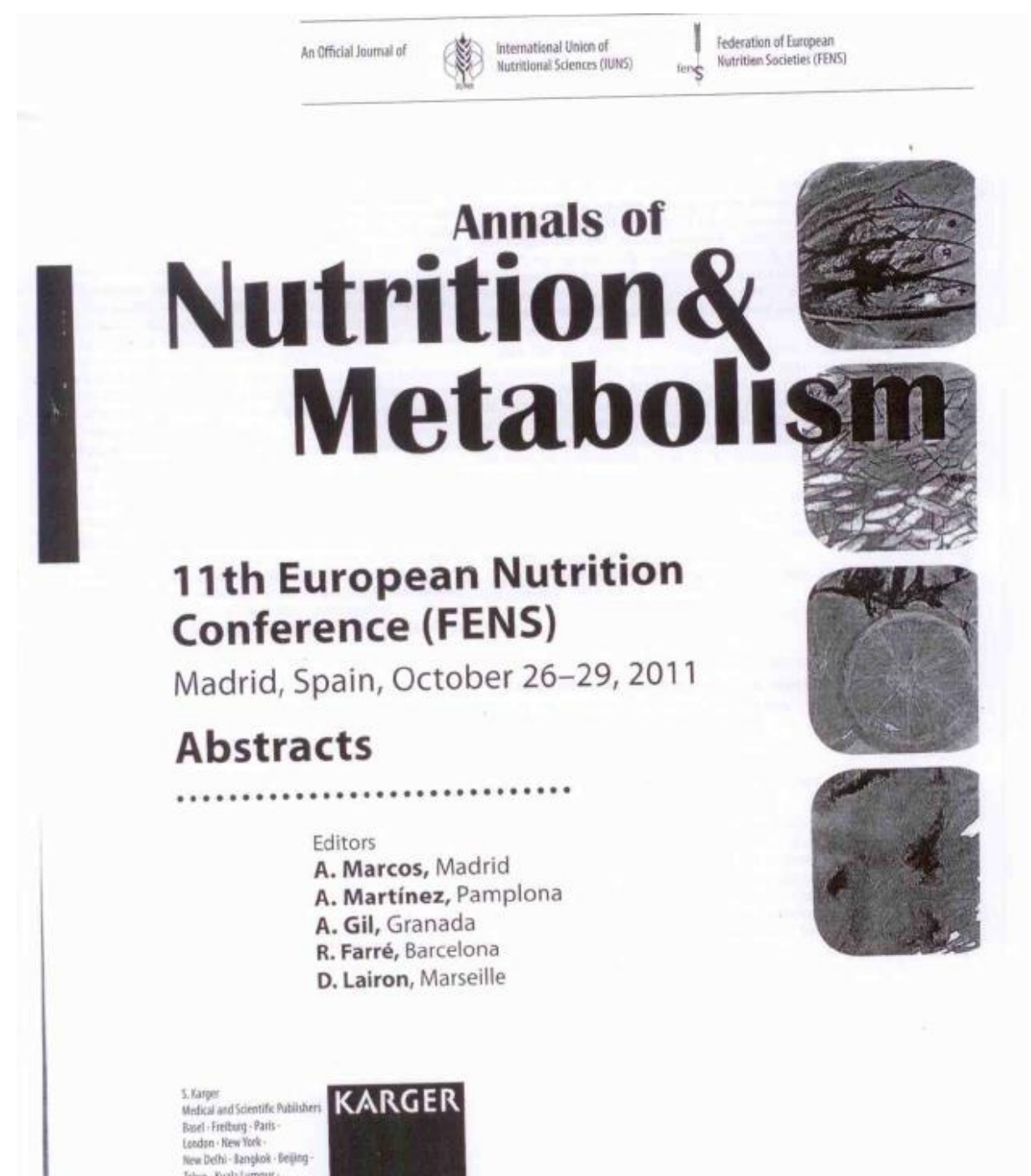
Co-authors:

Dobрева, D., Galunska B., Makedonski L.

Abstract text:

Fishes are a good source of fat soluble vitamins necessary for healthy diet. The contents of retinol, alpha-tocopherol, ergosterol, and cholecalciferol (vitamins A, E, D2 and D3) in two of most commonly eaten species of fish in Bulgaria were determined and compared. Fat-soluble vitamin contents in Bluefish (*Pomatomus saltatrix*), a typical Black sea pelagic fish, and in Brown trout (*Salmo trutta fario*), a typical Balkan freshwater fish, were analyzed in fresh, frozen and boiled edible fish tissue samples. The sample preparation procedure includes saponification and consequent extraction of fat-soluble vitamins with n-hexane. The extract was dried under nitrogen flow and redissolved in methanol. HPLC analysis of methanolic samples was performed on ODS2 Hypersil (250x4,6, 5µm) column with a mobile phase of methanol:water = 97:3. The quantification of fat-soluble vitamins was by the method of standard addition. The retinol content in the fresh edible tissue of Black sea Bluefish (26.0±2.0 µg/100g) was almost the same as in the Balkan freshwater fish Brown trout (22.3±2.0 µg/100g). Cholecalciferol (vitamin D3) content was higher in Black sea Bluefish (8.6±1.0 µg/100g) than in Brown trout (6.0±0.29 µg/100g) which was higher in alpha-tocopherol (809.1±56.0). In both fishes, the content of vitamin D2 was under the detection limit of the method. Surprisingly after steaming (15 min. at 80-85°C) there were almost no losses in the content of all fat-soluble vitamins per 100g raw tissue. Key words: fat-soluble vitamins, Bulgarian Black sea and freshwater fishes.

2.	<p>11th European Nutrition Conference FENS Madrid 2011, October 26-29 Stancheva M., Dobrev D.A., Galunska B., „<i>Vitamin A, D₃ and E Contents Of Eight Marine and Freshwater Fish Species</i>“</p>
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27/400. Innovation in Food for Optimal Nutrition
Vitamins A, D3 and E content of eight marine and freshwater fish species

M. Stancheva; D. Dobрева; B. Galunska.

Varna, Bulgaria.

Introduction: Fishes are a good source of fat soluble vitamins. They are essential nutrients controlling a diversity of biologically important processes in human body. Retinol takes place in photoreception, regulates gene expression and cell proliferation, bone growth, reproduction etc. The biologically active isomer of vitamin E - alpha-tocopherol acts as an antioxidant protecting membrane structures and lipoproteins from oxidation.

Objectives: The aims of the present study were determined and compared contents of retinol, cholecalciferol and alpha-tocopherol in eight of most commonly eaten species of fish in Bulgaria. For evaluation the fat soluble vitamins content in the edible tissue were used four marine - Atlantic bonito (*Sarda sarda*), grey mullet (*Mugil cephalus*), Turbot (*Psetta maxima*), Garfish (*Belone belone*) and four freshwater Bulgarian fish species - wild Catfish (*Silurus glanis*), Common carp (*Cyprinus carpio*), breeding (Rainbow trout (*Oncorhynchus mykiss*) and Common carp (*Cyprinus carpio*).

Method/Design: All-trans-retinol (vitamin A), cholecalciferol (vitamin D3) and alpha-tocopherol (vitamin E) were analyzed simultaneously using HPLC with UV and fluorescence detection. Retinol and cholecalciferol were monitored by UV detection. Alpha-tocopherol was detected by fluorescence detection.

The sample preparation procedure includes saponification and liquid-liquid extraction of the unsaponifiable matter with n-hexane.

Results: The fat soluble vitamins content in the fresh edible fish tissue of analyzed species are in the ranges: for all-trans-retinol from $37.5 \pm 3.5 \mu\text{g}/100\text{g}$ to $2.7 \pm 0.2 \mu\text{g}/100\text{g}$; cholecalciferol - $9.38 \pm 0.5 \mu\text{g}/100\text{g}$ - $1.1 \pm 0.1 \mu\text{g}/100\text{g}$ and alpha-tocopherol - $2836.6 \pm 56.0 \mu\text{g}/100\text{g}$ - $121.3 \pm 7.8 \mu\text{g}/100\text{g}$.

Conclusions: Results showed that most fish species were characterized by good fat soluble vitamin contents.

Key Words: Keywords: Vitamin A, Vitamin D3, Vitamin E, Hplc Uv/Fl, Fish Species 27/401. Nutrition and Healthy Lifestyle

27/404. Innovation in Food for Optimal Nutrition
Improvement of intracellular glutathione content in baker's yeast for nutraceutical application

A. Musatti¹; M. Rollin²; M. Manzoni³.

¹PhD student, University of Milan, Italy; ²Senior Researcher, University of Milan, Italy; ³Associate Professor, University of Milan, Italy.

Introduction: Glutathione (GSH) is the most abundant non-protein thiol compound widely present in living organisms where

it plays a pivotal role in bioreduction, protection against oxidative stress, and in detoxification of toxic metabolites. These characteristics make this active tripeptide of interest in the food additive industry and sports nutrition. It is synthesized intracellularly by the consecutive action of g-glutamylcysteine synthetase, feedback inhibited by GSH, and GSH synthetase.

Objectives: The research was aimed at improving intracellular GSH concentration in samples of baker's yeast (*Saccharomyces cerevisiae*), by investigating the influence of amino acids concentration on GSH yield, applying a post-fermentative procedure.

Method/Design: Trials were performed suspending cells (5% dw) in a reaction solution, containing the chosen amino acids at different concentration, glucose as energy source, and ammonium and magnesium salts. A Face Centered Central Composite Design (FCCD) was applied, with 4 variables (cysteine-A, glycine-B, serine-C, glutamic acid-D) tested at two levels (0-4 g/L) with two replicates, for a total of 30 trials. GSH was evaluated by HPLC at 24 h incubation after cell permeabilization, and results analyzed employing the Design Expert software.

Results: By applying a power transformation ($\lambda = -1.6$), the equation model was found significant ($R^2 0.9851$, predicted $R^2 0.9599$), and A, B, C, D, AC, BC, A2 and D2 were the significant model terms. Cysteine confirms to be a crucial element, to be set at least at 2 g/L. Glycine can be omitted from the biotransformation solution only if serine is present at high concentration (4 g/L). Glutamic acid has to be set at intermediate level (2 g/L) to achieve the highest GSH levels (1.7% dw).

Conclusions: Baker's yeast cells with increased intracellular GSH levels, suitable for nutraceutical applications, can be obtained in a post fermentative biotransformation process by means of combining the concentration of amino acids.

Key Words: glutathione, Design of Experiments, *Saccharomyces cerevisiae*, intracellular metabolite.

27/425. Innovation in Food for Optimal Nutrition
Aldehydes as lipid oxidation markers in wheat and buckwheat crackers

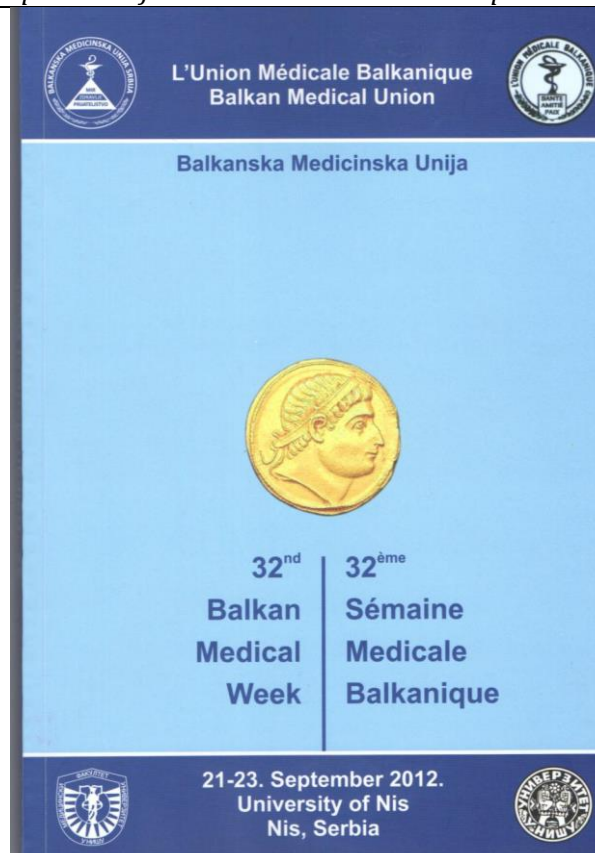
A. Mandić; I. Sedej; M. Sakac; A. Mišan.

Institute for Food Technology, Novi Sad, Serbia.

Introduction: Lipid hydroperoxides, as primary products of lipid oxidation, are unstable and rapidly decompose yielding in a range of secondary lipid oxidation products. Some of them, such as aldehydes, are highly specific to the oxidative degradation of particular polyunsaturated fatty acids. Gas chromatographic analysis of those volatile compounds, has been widely used for monitoring the food products deterioration, while only few dealing with bakery products.

Objectives: The aim of this work was to validate the proposed SHS GC FID method for determination of five aldehydes selected as markers in bakery products with high content of fats. Developed method should be appropriate to monitor the aldehydes present in freshly baked and stored crackers.

3.	32nd Balkan Medical Week 21-23 September 2012, Nis, Serbia, M. Stancheva, Merdzhanova A., Dobrev D. A. , Makedonski L., <i>Fat soluble vitamins content and fatty acids composition of Danube river's common carp and catfish</i>
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32nd Fat soluble vitamins content and fatty acids composition of Danube river's common carp and catfish

Stancheva M, Merdzhanova A, Dobrev DA

Medical University of Varna, Department of Chemistry, Faculty of Pharmacy, 9000 Varna, Bulgaria

Introduction. Many studies suggest that fish is one of the most important dietary sources of vitamin A, vitamin D₃ and vitamin E and also essential fatty acids (FA), but their contents depend on the species. There is limited information in the scientific literature about the nutritive composition of Bulgarian freshwater species.

Objectives. The aims of the present work are to determine and compare fat soluble vitamins and fatty acids contents as well as relative daily intake of vitamins, atherogenic index and thrombogenicity index in one of the most commonly eaten freshwater fish species in Bulgaria – common carp (*Cyprinus carpio*) and catfish (*Silurus glanis*).

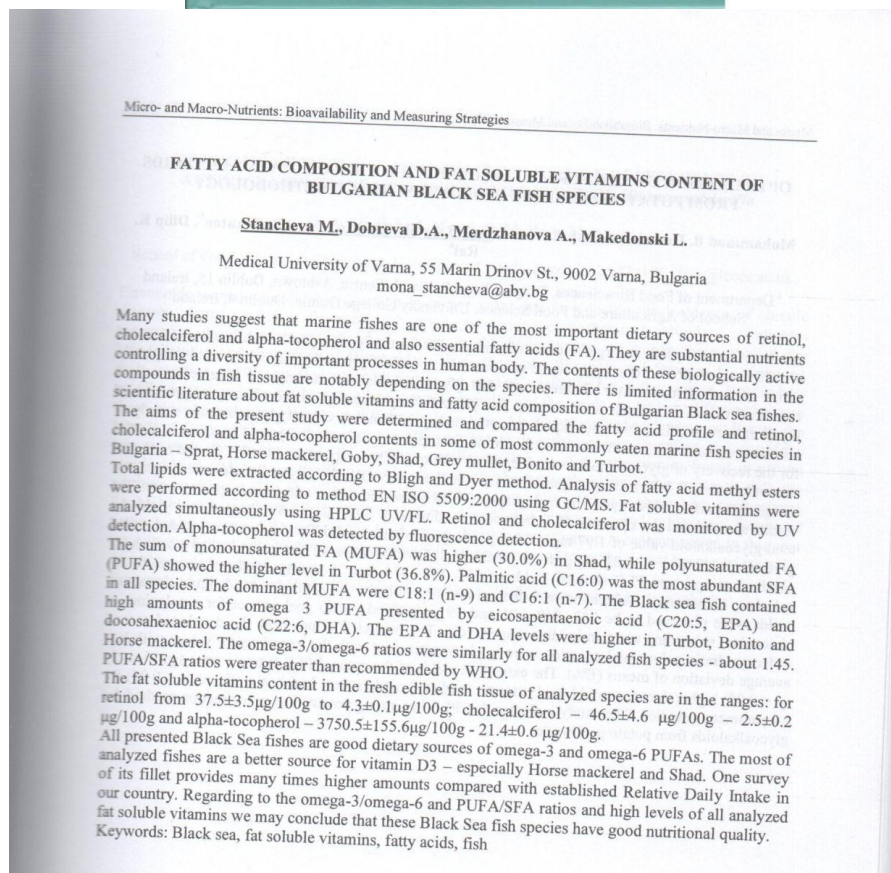
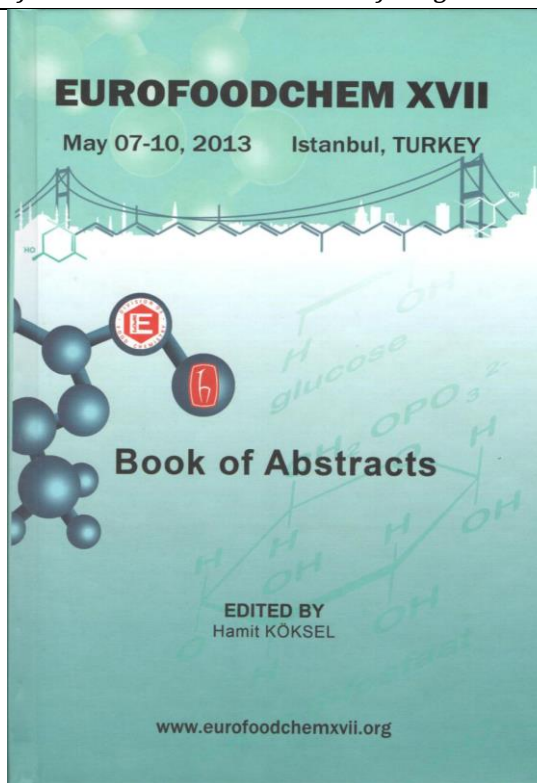
Methods. Vitamins A, D₃ and E were analyzed simultaneously using reverse-phase-high-performance liquid chromatography (RP-HPLC) system. The fatty acid (FA) composition was analyzed by gas chromatography-mass spectrometry (GC-MS).

Results. The fat soluble vitamins' content in fresh edible tissue of common carp and catfish was: vitamin A – 30.8±3.4 µg/100 g and 1.9±0.1 µg/100 g, for vitamin D₃ – 14.8±1.0 µg/100 g and 1.1±0.1 µg/100 g, and for vitamin E 2764.5±44.0 µg/100 g and 2182.5±31.5 µg/100 g, respectively. The amount of monounsaturated fatty acids (MUFA) accounted for catfish was 50.02% and for carp – 23.15%, while polyunsaturated fatty acids (PUFA) showed the higher level in carp (36.75%) and lowest for catfish (21.64%).

Conclusion. Three fat soluble vitamins are in higher amounts in common carp specimens. They are a better source especially for vitamin D₃ – one survey of its fillet provides almost three times higher amount than established Recommended Daily Intake (RDI) in our country. Catfish and carp are good dietary sources of n3 and n6 PUFAs.

Key words: *Cyprinus carpio*, fatty acids, *Silurus glanis*, vitamin A, vitamin D₃, vitamin E

4.	<p>EuroFoodChem XVII May 07-10.2013, Istanbul, Turkey Mona Stancheva, Diana A. Dobрева, Albena Merdzhanova, Lubomir Makedonski, „<i>Fatty acid composition and fat soluble vitamins content of Bulgarian Black Sea fish species</i>“</p>
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5.

21st European Congress on Obesity, ECO 2014

28-31 May 2014, Sofia, Bulgaria

Dobrev D.A., Merdzhanova A, Stancheva M, "Effect of thermal processing on fat soluble vitamin contents in black sea shad (*alosa pontica*)"

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ABSTRACTS



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Conclusion: With simple messages and an efficient methodology, FOOD contributes to health prevention at the workplace with the combination of external factors: offer and demand are reconnected.

T2:PO.046

Effect of thermal processing on fat soluble vitamin contents in Black Sea shad (*Alosa pontica*)

Diana Dobrev, Albena Merdzhanova, Mona Stancheva

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Introduction: Fishes are regarded as important natural food sources of fat soluble vitamins, which are necessary for healthy diet. Temperature processing of fish tissue enhances its taste, inactivates pathogenic microorganisms and increases its shelf life. The fat soluble vitamins are considered to be especially susceptible to oxidation during cooking process before consumption.

The aim of the present study was to evaluate the effect of steaming (10 min at 90°C) and grilling (7 min on each side) on retinol (vitamin A), cholecalciferol (vitamin D3) and alpha-tocopherol (vitamin E) contents in Shad filets.

Methods: The sample preparation procedure includes saponification and extraction of fat soluble vitamins with n-hexane. HPLC analysis was performed on RP column with a mobile phase of methanol:water = 97:3.

Results: The retinol, cholecalciferol and alpha-tocopherol content in fresh fish filets are $4.4 \pm 0.1 \mu\text{g}/100\text{g}$, $45.1 \pm 1.5 \mu\text{g}/100\text{g}$ and $1971.0 \pm 62.9 \mu\text{g}/100\text{g}$, respectively. The amount of vitamin A in steamed filets decreases significantly (~40%), when compared to its content in the raw samples. In contrast vitamin D3 and vitamin E remain almost unchanged. Among three fat soluble vitamins, the grilling process affects significantly only vitamin A and vitamin E content.

Conclusion: The sample preparation procedure includes saponification and extraction of fat soluble vitamins with n-hexane. HPLC analysis was performed on RP column with a mobile phase of methanol:water = 97:3.

T2:PO.048

Whey protein, as a weight reduction dietary supplement

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Introduction: Satiety is important in regulating food intake and has important public health significance in the control of obesity. Dairy products and/or dairy components have been shown to suppress short-term food intake, increase subjective satiety and stimulate the mechanisms known to signal satiation and satiety.

Results: Whey protein as a dairy product has potential as a functional food component to contribute to the regulation of body weight by providing satiety signals that affect both short-term and long-term food intake regulation. Whey ingestion activates many components of the food intake regulatory system. Whey protein is insulinotropic so cause satiety. Whey is much higher than other proteins in branched chain amino acids (BCAA), especially leucine, a proposed benefit to food intake regulation and the maintenance of lean body mass. Leucine enters the brain from the blood more rapidly than any other amino acid and its importance to hypothalamic regulation of food intake has been shown recently. Whey cause different nutrient related responses of anorexigenic hormones including CCK, GLP-1 (glucagon-like peptide-1), GIP (Gastric inhibitory polypeptide), PYY (peptide YY).

Conclusion: Whey protein affects satiation and satiety by the actions of: (1) whey protein fractions per se; (2) bioactive peptides; (3) amino-acids released after digestion; (4) combined action of whey protein and/or peptides and/or amino acids with other milk constituents. Based on results whey protein could be used as a weight control dietary supplement.

6.	<p>14th International Nutrition & Diagnostics Conference Prague, Czech Republic – September 2 - 5, 2014</p> <p>Dobrev D., Galunska B., Merdzhanova A., Stancheva M., „Vitamin A, D₃ and E contents and polyunsaturated fatty acid composition of turbot (<i>Psetta maxima</i>), red mullet (<i>Mullus barbatus</i>) and goby (<i>Neogobius rattan</i>) from Bulgarian Black sea“</p>
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Research Article

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Prague, Czech Republic, September 2-5, 2014

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association between prevalence of food taboos with weight gain status of the pregnant women ($p < 0.05$). Conclusion: The finding of this study shown that the prevalence of food taboos was high among Malay pregnant women and the main reason for adhering to food taboo was fear of abortion. Multipara women with higher number of pregnancy (>3) seem to observed food taboo more than those with lower number of pregnancy. There was no association between the prevalence of food taboo with status of weight gained in pregnant women.

P62. Dietary intake and nutritional knowledge of pregnant mothers in comparisons with RNI and food pyramid guidelines and association with their weight gain

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Background: Appropriate nutrients intake and acceptable gestational weight gain are very crucial during pregnancy. Low pre-pregnancy body mass index (BMI) and suboptimal gestational weight gain are directly associated with poor maternal and foetal outcomes. In order to maintain optimal health while avoiding deficiency or toxicity, pregnant women are recommended to consume according to the Recommended Nutrient Intake (RNI) and Malaysia Food Pyramid Guidelines. Based on the recommended weight gain range issued by Institute of Medicine (IOM) in 2009, optimal weight gain can be achieved by pregnant women.

Objectives: To determine the correlation between the dietary intakes of pregnant women and gestational weight gain, and also to compare the dietary intakes of pregnant women in Malaysia with the Recommended Nutrient Intake (RNI) for Malaysian 2005, and the Food Pyramid Guideline.

Methods: A cross-sectional study was conducted in a district health clinic in Malaysia in 2011. A total of 60 pregnant women who were in their third trimester of pregnancy were recruited by convenience sampling. Anthropometric assessment, self-administered questionnaire and 2-day dietary recall were used for data collection. Due to attrition, the data from only 55 pregnant women were included in the analysis.

Results: Mean pre-pregnancy BMI of the subjects was 23.2kg/m².

P63. Vitamin A, D₃ and E contents and polyunsaturated fatty acid composition of turbot (*Psetta maxima*), red mullet (*Mullus barbatus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea

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Introduction: Fish have always been seen as a food necessary for good health being a rich source of fat soluble vitamins as well as n-3 and n-6 long chain polyunsaturated fatty acids (PUFA). There is a lack of information for fat soluble vitamins and PUFA contents of traditionally consumed Black Sea fish species from Bulgarian waters.

Aim: To determine and compare the contents of retinol, cholecalciferol and alpha-tocopherol, and PUFA composition in some commercially important fish species of Bulgarian Black sea coast.

Material and methods: Edible tissue of three fishes - turbot (*Psetta maxima*), red mullet (*Mullus barbatus*) and goby (*Neogobius rattan*), were used for evaluation the fat soluble vitamins and PUFA content. All-trans-retinol (vitamin A), cholecalciferol (vitamin D₃) and alpha-tocopherol (vitamin E) were analyzed simultaneously using HPLC/UV/FL, equipped with RP analytical column ODS2 Hypersil™ 250 x 4,6 mm, 5u, with mobile phase composed of 97:3=MeOH:H₂O. The sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. The fatty acid (FA) composition was analyzed by Gas Chromatography with MS detector. Lipid extraction was done according to the Bligh and Dyer method.

Results: The content of fat soluble vitamins in fresh edible fish tissue were in the range: 0.9 ± 0.1 µg/100g - 15.7 ± 1.2 µg/100g for all-trans-retinol; 4.6 ± 0.5 µg/100g-4.9 ± 0.3 µg/100g for cholecalciferol, and 308.0 ± 23.4 µg/100g- 2836.8 ± 96.0 µg/100g for alpha-tocopherol. The PUFA content ranged from 0.41 g/100g (turbot) to 1.065 g/100g (red mullet). n-3 PUFA contents were higher compared to n-6 FAs for all analyzed species. The DHA level was always found higher than that of EPA. The sum EPA+DHA ranged from 0.22 ± 0.03 g/100g (turbot) to 0.396 ± 0.020g/100g (red mullet) due to its higher lipid content.

Conclusion: The present work indicates that 100 g portion of studied Black Sea fish species meets the recommended daily intake for cholecalciferol and EPA+DHA.

7.	<p>12th European Nutrition Conference, FENS 2015 October 20 -23, Berlin, Germany A. Merdzhanova, M. Stancheva and D. A. Dobрева <i>‘Nutritional quality of marine and freshwater fish species from Bulgaria’</i></p>
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12th European Nutrition Conference (FENS)

Berlin, Germany, October 20–23, 2015

Abstracts

Guest Editors

Heiner Boeing, Nuthetal

Helmut Oberritter, Bonn

Hannelore Daniel, Freising-Weihenstephan

on behalf of the German Nutrition Society

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lower temperature. Advantages also include shorter extraction times, higher yields, extract purity, and lower energy consumption.

Objectives: The aim of this study was to investigate the effect of HHP on the anthocyanin content in freeze-dried red grape skin pomace (cv. Teran). Optimization was carried out to obtain high extraction yields using varying high pressures (300, 400 and 500 MPa), time (3, 6.5 and 10 min), solvent type (ethanol vs. methanol) and solvent polarity (30,50 and 70%,v/v).

Method / Design: Experiments were designed as a full factorial randomized experimental design. Dependent variable was total anthocyanin content (TAC), while independent variables were high pressure, time, solvent type and polarity. Individual anthocyanins were analysed by HPLC UV/Vis and expressed as malvidin-3-glucoside equivalents with an external calibration.

Results: Higher solvent polarity and higher pressure resulted in higher recovery of TAC, while the optimal time for extraction of TAC was 3 min.

Conclusions: HHP has been shown to be an efficient method for TAC recovery from red grape skin and could have potential benefits for the use in different industries.

Keywords: (maximum 5): High hydrostatic pressure extraction, anthocyanins, grape skins, winery byproduct.

149/727. Nutritional quality of marine and freshwater fish species from Bulgaria

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Introduction: Many studies suggest that marine and freshwater fish are one of the most important dietary sources of essential polyunsaturated fatty acids (PUFA) and fat soluble vitamins (E, D3 and A). It is well known that the nutritional benefits of sea food consumption are mainly attributed to several potential cardio protective effects of omega-3 (n-3) PUFAs. Fat soluble vitamins are essential components of marine lipids and they control a variety of biologically important processes in the human body.

Objectives: The aims of the presented study were to compare the nutritional quality based on PUFA, fat soluble vitamins content; and lipid quality indices of three Black Sea fish: shad (*Alosa imaculata*), goby (*Neogobius melanogaster*) and turbot (*Psetta maxima*) and three freshwaters species: Common carp (*Cyprinus carpio*), catfish (*Silurus glanis*) and brown trout (*Salmo trutta fario*).

Method / Design: Total lipid (TL) content was determined according to Bligh&Dyer. Fatty Acid Methyl Esters were performed by GC/MS system. Vitamins A, D3 and E were analysed simultaneously using RP-HPLC system.

Results: Black Sea fish showed SEA>PUFA>MUFA distributions, while freshwater fish presented species-specific FA patterns. Marine

fish contained higher omega-3 PUFA levels compared to freshwaters. All analyzed fish contained over than 0.250g.100g-1wet weight EPA+DHA n-3.

The fat soluble vitamins content were in range: 3.1±0.2µg-41.3±1.6µg.100g-1ww (vitamin D3); 1.9±0.07µg-30.8±2.1µg.100g-1ww (vitamin A) and 461.5±0.07µg-3293.7±140.7µg.100g-1ww (vitamin E). Black Sea shad provides eight times higher amounts of vitamin D3 RDI.

Conclusions: All species are valuable sources of EPA+DHA n-3 and fat soluble vitamins. Marine fish and brown trout have better nutritional quality than common carp and catfish.

Keywords: (maximum 5): fatty acids, fat soluble vitamins, human health, lipid quality indices

149/729. Optimizing microwave-assisted extraction parameters for polyphenols recovery from sage (*Salvia officinalis* L.)

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Introduction: Medicinal and aromatic plants (MAPs) contain bioactive compounds (BACs) (e.g. polyphenols, carotenoids, organo-sulphur compounds) that can positively affect the nutritive, chemical, microbiological, and sensory quality of food products. Due to large structural diversity among different groups of BACs and their physical/chemical properties, it is important to identify/optimize the most effective extraction parameters required to isolate and/or separate BACs from other plant components. Sage (*Salvia officinalis* L.) is rich with polyphenols that are known for their antioxidative activity and positive influence on human health (e.g. risk reduction for cardiovascular disease, diabetes, obesity, hypertension etc.). Innovative extraction techniques as microwave-assisted extraction (MAE) is a process that uses microwave energy, along with solvent, to extract target compounds from various matrices. Highly localized temperature can cause selective migration of target compounds from the material at a faster rate, thus providing enriched extracts compared to conventional extractions.

Objectives: Aims of this study were to identify optimal MAE parameters for extraction of total phenols (TP) from sage.

Method / Design: Study was full factorial randomized design with independent variables: (i) time (3,5,7,9,10 min); (ii) temperature (30,50,60,80°C); (iv) solvent (30% ethanol, 30% acetone, water); and (v) acidity (addition or not of HCl 10%). The TP content was evaluated by the Folin-Ciocalteu colorimetric method, calibrated against gallic acid calibration curve as the reference standard. Data were analyzed with multivariate analysis at $\alpha=0.05$.

Results: Average amount of TP in study was 6945.81±121.03 mg/100 g d.m. Optimal extraction time was from 7-9 minutes, at

Изготвил:

/гл. ас. Диана Ат. Добрева, дх/