sciendo

10.2478/aucft-2018-0016

COMPARATIVE STUDY OF LIPOSOLUBLE VITAMINS AND FATTY ACIDS FROM SEA BUCKTHORN OIL, WHEAT GERM OIL AND FISH OIL

– Short communication –

Isabela CRĂCIUN*, ** [1]

**Polisano Pharmaceuticals SA, 156 Alba Iulia Avenue, 550052, Sibiu, Romania*
*** Faculty of Medicine,University Lucian Blaga of Sibiu, Str. Lucian Blaga, Nr. 2A , 550169, Sibiu, Romania*

Abstract: An important role in human nutrition is played by fats, both of plant and animal origin. Fats are a category of foods rich in liposoluble vitamins and fatty acids. They are widely used in nutrition, in cosmetics, in the content of creams as well as in pharmaceutical products, in the form of dietary supplements, such as capsules with oils rich in fatty acids and the addition of liposoluble vitamins. The objective of this paper was to study the content in vitamins and fatty oils of two vegetable oils (wheat germ and sea buckthorn oils) and an animal oil (fish oil). The results show that these oils are rich in liposoluble vitamins, omega-3 fatty acids and omega-6 fatty acids. Hence, these oils can be used successfully in daily nutrition as substitutes for synthetic vitamins.

Keywords: wheat germ oil, sea buckthorn oil, fish oil, lipid-soluble vitamins, fatty acids, nutrition.

INTRODUCTION

Daily human nutrition is based on three major categories of nutrients as proteins, carbohydrates and lipids, which provide the necessary caloric intake. Besides these macronutrients important roles in the optimal functioning of the body have micronutrients such as antioxidants, vitamins, minerals (Gunstone, 2011).

Nutrition determines a good health and a great capacity for hard work from the very beginning of the embryonic development. Also, nutrition influences the mental and emotional mood. It is said that "we are what we eat".

Nutrition generates the optimal function of metabolism. Thus, for the optimal growing and functioning of the human organism, foods should contain basic nutrient as proteins, lipids and sugars and micronutrients as vitamins and oligoelements). Also, the foods must be safe for consumption, in order to not endanger the human organs and the entire body with microbial infections and chemicals (Segal et al, 2002); an actual trend is to use different plant extracts as volatile oils with strong natural antimicrobial action (Georgescu & Mironescu, 2012; Georgescu & Mironescu, 2011).

In food, an important role is played by vegetable and animal oil due to their high content in lipid-soluble vitamins and fatty acids.

It was demonstrated that between the body needs and the daily intake of food is not always a balance. This balance is broken by a lot of factors. One of these factors is the deficiency of micronutrients. The energetic deficit expressed as a lack of macronutrients leads to a decrease in the intensity of metabolic processes and implicitly to health disturbances (Banu et al, 2007).

We mention that in developing countries is a deficiency of proteins that cause a low weight of the neonates. A very high protein deficiency causes poor functioning of the central nervous system as well as metabolism in general. Thus, results that in developing countries the emphasis is on ensuring optimal protein intake.

Then again, there are things in developed countries where increased living conditions have led to an overconsumption of foods rich in fats having a high caloric content but deficient in vitamins. This overconsumption associated with other reasons, eg lack of physical activity (sedentary) and stress, has drive to disorders of the metabolic reactions manifested in the occurrence of nutritional diseases (diabetes, cardiovascular diseases, hypertension and obesity) (Niac, 2004).

As a result of these manifestations, in the state of health of the population in the developed countries appears on the hand the necessity to limit the consumption of fats to 30 percent of

[1] Corresponding author. E-Mail address: isabela.craciun@polisanopharmaceuticals.ro

total energy intake and on the other hand the necessity of supplementing the usual diet with foods and dietary supplements rich in antioxidants and minerals (Tufeanu and Tita, 2016).
This supplementation helps a lot of people in the prevention of chronic maladies and in maintaining an optimal state of health.
Fats are important because they provide a large amount of calories (9 calories / g), provide unsaturated fatty acids and liposoluble vitamins that contribute to a good skin health (Akoh, 1998). For this reason, moderate fat consumption is beneficial while high fat consumption can be harmful to the body, resulting in nutritional diseases (cholesterol, diabetes) (Siraj et al., 2015).
Vegetable oils are obtained using different processes as mechanical extraction, cold pressing of seeds and fruits, extraction with supercritical fluids, etc.
Structurally, animal and vegetable lipids are glycerol esters of aliphatic hydrocarbons with at least 14 carbon atoms. The properties of vegetable and animal oils vary with the degree of unsaturation (Zielińska et al., 2017).
In sea-buckthorn (*Hippophae rhamnoides*) there are present many active ingredients including vitamin C, microelements, polyphenols, polysaccharides, flavonoids and fatty acids. The seeds (*Semen Hippophae*) and the fruits (*Fructus Hippophae*) are commonly used as ingredients in food and also in cosmetic products (Walczak-Zeidler et al., 2012).
Sea-buckthorn is also surnamed Siberian pineapple. It is presented as a spiny shrub from the family *Elaeagnaceae* that can grow up to 7 meters (Fu et al., 2014).
The sea-buckthorn is a bush, tolerant to moist, heliophilous, found in clusters or bushes stretched out on sands, rocky ribs and cliffs. It is found in geological formations of saliniferous regions. In Romania it is found on large areas in Moldavia, Muntenia and in the islands of Danube Delta. The sea-buckthorn is a plant which resist to cold and also to drought conditions (Georgescu et al. 2007).
Due to the high content in vitamins, sea-buckthorn fruits have general tonicity and are recommended for treatment of avitaminosis, anaemia, asthenia, diarrhea, liver toxicity, rheumatism, urticaria and neuroendocrine diseases (Brad et al, 2002).
Wheat germs are extracted using as raw material the common wheat (*Triticum aestivum ssp vulgare*), an annual, unisex-mono herbaceous plant, grown since ancient times, needing moderately warm and humid conditions. The wheat also resists to cold winter temperatures and high summer temperatures. The wheat fruit is a caryopsis. Wheat grains are rich in proteins, starch, fibbers, vitamins, fats.
Wheat germ oil is a yellow and oily liquid, tasteless and odourless. Wheat germ oil is used in mineralization of the body, in the treatment of skin diseases and to combat constipation. Wheat germ oil contains polyunsaturated fatty acids that cannot be synthesized by mammals. These fatty acids are essential to the body and are obtained from food (Kammoun et al., 2015).
Fish oil is extracted using various ocean fish (principally mackerel and sardines).
The fish oil is an oily, yellowish-coloured liquid with characteristic taste and smell. Fish oil contains highly amounts of liposoluble vitamins and unsaturated fatty acids (omega-3 and omega-6). Omega-3 fatty acids from fish regenerate the brain, the retina and decrease the risks of cardiovascular diseases.Omega-3 plays an important role in regulating the absorption of fatty acids. This can increase metabolism and reduce fat storage, helping to prevent corpulence (Byun et al, 2008).
Fish oil is an important source of highly polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have a major role in the prevention of different human diseases.
Diet supplementation with these fatty acids is increasingly recommended. For this reason, it is necessary to obtain concentrated supplements rich in polyunsaturated fatty acids (Maqsood et al., 2012).
This paper aims to perform a comprehensive study of two vegetable oils (wheat germ and sea buckthorn oils) and an animal oil (fish oil) in terms of their quality (vitamins and fatty oils content) towards their use as food and as dietary supplements.

MATERIALS AND METHODS

Materials

The sea buckthorn oil was extracted from dried sea buckthorn fruits. The fish oil used was encapsulated oil from farmacy. The wheat germ oil was extracted using wheat germ acquired from Boromir Company.
For the extraction of sea buckthorn and wheat germ oil, the raw materials were grinding using

a cryogenic Forplex hammer mill. The grinding process allows preserving vitamins and oils. A 7-site Retsch granulometry machine and a timer were used to prepare the extraction sample.
The oil content was determined using a Soxhlet extraction system As extraction solvent was used analytical purity ethyl ether.
The Soxhlet extraction method was used only to quantitatively analysis of oil. Oils used to quatify the lipid-soluble vitamins and fatty acids contents have been extracted using supercritical carbon dioxide extraction method.

Method for lipid-soluble vitamins extraction
Assay of vitamins A, D3 and E from the mentioned oils was performed using high performance liquid chromatography method.
The retinol, cholecalciferol and tocopherol preliminary extraction was performed by saponification of oils with a potassium hydroxide solution in methanol, followed by extraction into a suitable solvent. The assay of cholecalciferol, retinol and tocopherol was performed by high performance liquid chromatography (HPLC) with array diode detection.
Substances identification was made using the retention times against an external standard.
The standards used were the following;
• for vitamin A retinol acetate 1000000 IU from Sigma-Aldrich;
• for vitamin D3 cholecalciferol from Sigma-Aldrich;
• for vitamin E alpha-tocopherol from Merck.
The separation of retinol, cholecalciferol and tocopherol was performed using a Varian HPLC system consisting of a quaternary pump, degassing device, autosampler, diode array detector (PDA), and Varian software.
A Zorbax SB-C18, 150 x 4,6 mm column was used. The mobile phase used for isocratic elution consisted of distilled water - methanol (5: 95) (v/v).
The acquisition parameters used were: flow rate 1.0 mL/min, injection volume 20 µL, column temperature 25ºC and detection was performed at 325 nm for retinol; 280 nm for tocopherol and 265 nm for cholecalciferol.

Fatty acids extraction was made in two steps. The first step consisted in esterification of fatty acids to methyl esters and the second one in quantitation of methyl esters using gas chromatography.
For the preparation of methyl esters of fatty acids, the boron trifluoride method of ISO 5509/2000 "Animal and Vegetable Fats and Oils - Preparation of Methyl Esters of Fatty Acids" was used.
Fatty acids identification and assay was performed by flame ionization gas chromatography. The equipment used was a QP 5000 Shimadzu gas chromatograph equipped with split / splitless injector, flame ionization detector (FID) and Class 5000 software. The column used for separation of fatty acids was an Agilent 30m x 0.32mm x 0.15µm DB-WAX column.
Two standard blends of saturated and unsaturated C14-C22 fatty acids from Supelco were used to identify and measure fatty acids content.
Working parameters used were: carrier gas (nitrogen) flow 1ml/min; injection temperature 220°C; detection temperature 240°C; gradient column temperature. The gradient temperature was 160°C for 1 minute than rise the temperature to 250C with a rate of 3°C/minute and then maintains the temperature at 250°C for 10 minutes.

All measurements were performed in triplicate and the results were calculated as median value.

RESULTS AND DISCUSSIONS

Quantification of retinol, cholecalciferol and tocopherol was performed on the basis of external standard method against average of 6 peak areas of standard solution chromatogram. The results are shown in Table 1. The results obtained for vitamin A, vitamin E and vitamin D3 content represented against the daily recommended intake are presented in figures 1, 2 and 3, respectively.

Table 1. The lipid-vitamins content in analysed oils

Vitamin (mg/kg)	Oil		
	Sea-buckthorn	Wheat germ	Fish
Vitamin A	154	269	165
Vitamin E	2615	1282	2816
Vitamin D3	3	9	2

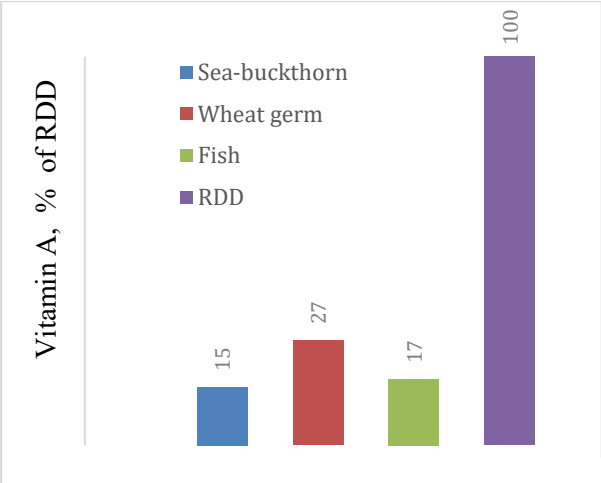

Figure 1. Vitamin A contain of sea-buckthorn, wheat germ and fish oils against recommended daily dose (RDD)

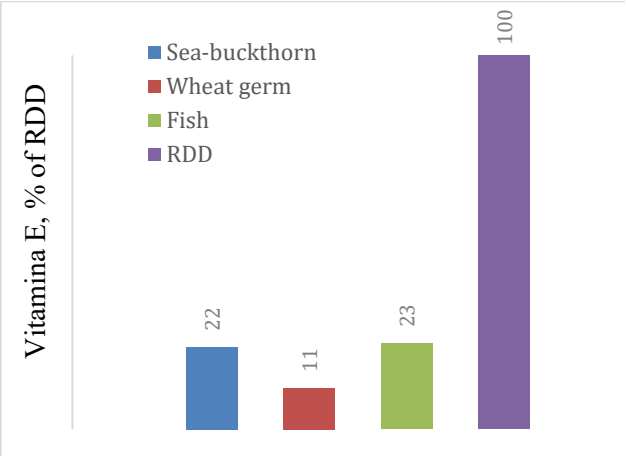

Figure 2. Vitamin E contain of sea-buckthorn, wheat germ and fish oils against recommended daily dose (RDD)

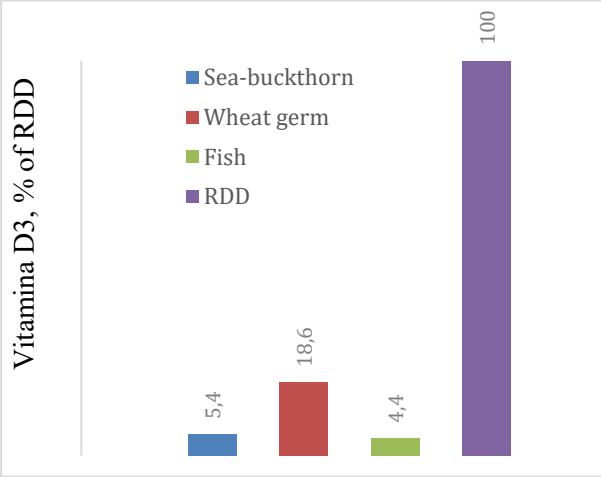

Figure 3. Vitamin D3 contain of sea-buckthorn, wheat germ and fish oils against recommended daily dose (RDD)

Analyzing data from Table 1 and Figures 1-3 it is noted the following:

- Wheat germ oil has the highest content of vitamin A covering 27 percents of recommended daily dose;
- Fish oil was richest in vitamin E covering almost 30 percents of recommended daily dose;
- The vitamin D3 content of those oils is not significant, because for covering the RDD is needed to consume a high quantity of oil (about 100 grams daily).

Fatty acids were identified based on the retention time according to the retention times of the compounds in the standard solution. The results of fatty acids composition of sea-buck, wheat germ and fish oils are summarized in Table 2. The data of the table 2 suggest the following aspects:

All these oils are rich in unsaturated fatty acids, on the top being the wheat germ oil followed by fish oil. The highest content of omega 6 fatty acids has the wheat germ oil (about 52 %) and the highest content of oleic acid is noticed in sea buckthorn oil (about 23%).

Table 2. The fatty acids content of analysed oils

Fatty acids, %	Oil		
	Sea-buckthorn	Wheat germ	Fish
Myristic acid (C 14:0)	0.26	ND	7.70
Myristoleic acid (C 14:1)	ND	ND	3.0
Pentadecanoic acid (C 15:0)	ND	ND	0.60
Palmitic acid (C 16:0)	35.01	22.76	19.56

Palmitoleic acid (C 16:1)	27.7	ND	10.75
Hexadecadienoic acid (C 16:2)	ND	ND	0.95
Hexadecatrienoic acid (C 16:3) - omega 3	ND	ND	2.00
Hexadecatetraenoic acid (C 16:4)	ND	ND	1.60
Margaric acid (C 17:0)	ND	ND	0.20
Stearic acid (C 18:0)	0.89	0.84	5.6
Oleic acid (C 18:1)	22.52	15.46	13.9
Cis-vaccenic acid (C 18:1)	8.12	1.14	ND
Linoleic acid (C 18:2) - omega 6	3.7	51.75	1.34
Linolenic acid (C 18:3) - omega 3	0.94	6.65	0.8
Arachidic acid (C 20:0)	0.17	1.40	0.1
Gadoleic acid (C 20:1)	ND	ND	0.7
Arachidonic acid (C 20:4) - omega 6	ND	ND	2.5
Eicosapentaenoic acid (C 20:5) - omega 3	ND	ND	14.87
Behenic acid (C 22:0)	0.25	ND	0.22
Clupanodonic acid (C 22:5) - omega 3	ND	ND	1.7
Docosahexaenoic acid (C 22:6) - omega 3	ND	ND	8.3
Lignoceric acid (C 24:0)	0.18	ND	ND
Saturated fatty acids	36.76	25.01	33.98
Unsaturated fatty acids • Monounsaturated • Poliunsaurated	62.0 58.3 3.7	75.0 16.6 58.4	64.19 29.43 34.76
Omega 6:omega 3 ratio	4:1	8:1	0.15:1

ND – Not detected

CONCLUSIONS

From the point of view of active principles, all the oils analyzed contain important amounts of liposoluble vitamins, these oils being a worthwhile natural source to supplement the deficiency of liposoluble vitamins.
Also, these oils are rich in unsaturated healthy fats and could successfully substitute the animal saturated fat in the daily nutrition. The ratio of unsaturated fatty acids versus saturated fatty acids in these oils is situated between 1.7 and 3 and from this point of view these oils can be included in the category of "Lipids with high biological value". In conclusion, the oils from wheat germ, sea buckthorn and fish can be introduced into daily food.

REFERENCES

1. Bourre, J., (2007). Dietary omega-3 fatty acids for women. Biomedical Pharmacology, 61, 2, 105 - 112.
2. Banu, C., Bărascu, E., Stoica, A., Nicolua, A., (2007). Suveranitate, securitate și siguranță alimentară, București, , Editura Asab.
3. Brad, I., Brad, L., (2002). Cătina albă - o farmacie într-o plantă, București, Editura Tehnică.
4. Byun, H. G., Eom, T. K., Jung, W. K., Kim, S-K., (2008). Characterization of Fish Oil Extracted from Fish Processing By-products, *Jurnal Food Science and Nutrition*, 13, 7-11. DOI: 10.3746/jfn.2008.13.1.007.
5. Fu, L., Su H., Li, R., Cui, Y. (2014). Harvesting technologies for sea buckthorn fruit. *Engineering in Agriculture*, 7(2), 64–69. DOI: 10.1016/j.eaef.2013.10.002.
6. Georgescu, C., Crăciun, I., Bratu, I., Turtureanu, A., (2007). The quantitative analysis of flavonoid compounds in the species of *Teucrium chamaerys* and *Hippophae rhamnoides. Journal of Agroalimentary Processes and Technologies,* XIII (1), 139-142.
7. Georgescu C., Mironescu M. (2012). Study of the action of some volatile oils on moulds isolated from citrus, Proceedings of the International Conference "Agricultural and Food Sciences, Processes and Technologies" Fifth edition, Sibiu, 341-346.
8. Georgescu C., Mironescu M. (2011). Obtaining, characterisation and screening of the antifungal activity of the volatile oil extracted from *Thymus serpyllum*. Journal of Environmental Protection and Ecology, 12, 4A, 2294-2302.

9. Gunstone, F. (2011). Vegetable Oils in Food Technology. Composition, Properties and Uses Retrieved March 17, 2011, from Wiley Blackwell https://onlinelibrary.wiley.com. DOI:10.1002/9781444339925
10. Kammoun, M., Rassaa, N., Mejri-Gaïda, M., Richel, A. 2015. Biochemical characterization of the oil of durum wheat germ (Tunisian variety). Journal of new sciences, Agriculture and Biotechnology, 19(6), 744-747. Retrieved July 1, 2015, from Journal of new sciences database on the World Wide Web: http://www.jnsciences.org/agri-biotech/27-volume-19/87-biochemical-characterization-of-the-oil-of-durum-wheat-germ-tunisian-variety.html.
11. Kostov, G., Shopska, V., Denkova, R., Ivanova, M., Balabanova, T., Vlaseva, R. (2016) Encapsulation of plant and animal oils used in dairy industry: a review. Acta Universitatis Cibiniensis Series E: FOOD TECHNOLOGY, XX (1), 21-40. DOI: 10.1515/aucft-2016-0002.
12. Maqsood, S., Kamal-Eldin, A., Benjakul, S. (2012). Extraction, Processing, and Stabilization of Health-Promoting Fish Oils. Recent Patents on Food, Nutrition & Agriculture, 4(2),141-147. DOI: 10.2174/221279841120402014.
13. Niac, G., (2004). Alimentație, nutrienți, alimente: Știința alimentației, tehnologii culinare, Deva, Editura Emia.
14. Segal, R., (2002). Principiile nutriției. Alimentele și sănătatea. Galați, Editura Academica.
15. Tifrea, A., Tița, O., Mathe, E., Ketney, O. (2013). Physicochemical parameters of probiotic yoghurt with bioactive natural products from sea buckthorn. Acta Universitatis Cibiniensis Series E: FOOD TECHNOLOGY, XVII (1), 27-37. DOI: 10.2478/aucft-2013-0003.
16. Tufeanu, R. Tița, O. (2016). Possibilities to develop low-fat products: a review. Acta Universitatis Cibiniensis Series E: FOOD TECHNOLOGY, XX (1), 3-19. DOI: 10.1515/aucft-2016-0001.
17. Walczak-Zeidler, K., Feliczak-Guzik, A., Nowak, I. (2001). Oleje roślinne stosowane jako surowce kosmetyczne – leksykon: Olej z rokitnika (pp. 101–105). Kostrzyn: Cursiva; 2012.
18. Zielińska, A. (2017). Abundance of active ingredients in sea-buckthorn oil. Lipids Health Dis, 16:95. DOI: 10.1186/s12944-017-0469-7.