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**FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY AND ENVIRONMENTAL PROTECTION**

# PhD Thesis

## Studies on biologically active substances used as food additives and nutrients to improve quality and food safety

## - Summary-

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# INTRODUCTION

The present paper "Studies on biologically active substances used as food additives and nutrients to improve quality and food safety" is divided into four chapters, set on 176 pages, containing 23 tables, 48 figures and 18 appendices. The text includes references to 159 sources of which more than 50% represent works published in the last 10 years. The results were disseminated in 7 works that are found in the list of published works.

In Chapter I, "PhD thesis objectives", I have approached the thesis objectives and reasons that led to the study presented in the thesis.

Chapter II, entitled "Documentary study", containe s general information about the oils studied, the raw materials they are produced of and the active principles they contain. It presents as well theoretical aspects of quality and safety characteristics of food and methods of identification and quantitative characterization of these features.

The third chapter , "Experimental Study", reveals the characteristics and origin of the raw materials used to perform experiments, the analytical methods used for their characterization in terms of quality and safety, details regarding the used equipment performance and the results obtained.

In Chapter IV, "Conclusions", I synthesized the theoretical and experimental results of this work, original studies and opportunities to address future research directions.

In the following pages are presented the main issues raised in each chapter of the thesis, the thesis being rigorously pursuied. The summary of the thesis follows the pattern of numbering the chapters, the figures, tables and bibliographic index of the doctoral thesis.

# I. PhD THESIS OBJECTIVES

Health and nutrition determine the ability to work as early as embryonic stage. Diet has a beneficial effect on the normal functioning of all organs including the brain, affecting the general wellness and mental health care. It is believed that what we eat today will walk and talk tomorrow. Nutrition determines the optimum development of metabolic processes. Thus, for the optimal functioning of the body and for a very good state of health, the food consumed must have nutritional value, expressed in quality and quantity of basic nutrients (carbohydrates, proteins, lipids) and biologically active substances (minerals, vitamins). Also, the food should not endanger the human body, should not contain harmful microorganisms nor chemicals (heavy metals, pesticide residues, mycotoxins, etc.), this characteristic beingexpressed by innocuity (hygienic quality).

Unfortunately there is not always an equilibrium between the needs of the body and the food consumed as this balance is disturbed by many factors among which the most important are the deficiency of biologically active substances such as vitamins and minerals, the deficiency of nutrients with plastic role (proteins) and therefore the energy deficiency lowers the metabolic processes and thus the intensity of health disorders.

For example, in the developing countries there is a deficit of substances with plastic (protein) that causes a small weight to the average of newborns. A large deficit of protein substances cause malfunctioning of the central nervous system and of the metabolism in general. It follows that developing countries focus on ensuring an optimal intake of protein.

In the developed countries, the rising living standards and the ease of purchasing goods lead to an overconsumption of food characterized inhigh calories but low in biologically active substances. This overconsumption associated with other factors such as physical inactivity (sedentariness), pollution, stress led to imbalances manifested by the appearance of adverse metabolic nutrition diseases (diabetes, cardiovascular disease, hypertension, obesity).

Following these events in the health of the population in developed countries it was necessary to supplement the normal diet and food supplements rich in antioxidants and minerals that can help people in the prevention of chronic diseases and diseases related to aging as well as maintain optimal health.

Another important condition that food must meet is safety, because otherwise the body it becomes a useful product for the consumer risk, a source of infection, in some cases with fatal effect.

Globalization of the food chain causes constant new challenges and risks to health and the interests of European consumers.The main objective of EU food safety policy is to achieve the highest possible degree of protection of human health and consumer interests in terms of food. In this respect, the EU trade in food commodities is respecting certain regulations relating to.

- Levels of pesticide residues in food (Regulation (EC) no. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal and amending Directive 91/414/EEC);
- The levels of heavy metals in food (Regulation (EC) no. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in food;
- The levels of radionuclides Regulation (EC) no. 733 from 15 July 2008 on conditions for imports of agricultural products originating in third countries following the accident at the Chernobyl nuclear power plant.

These regulations are complied with, guarantee food safety and ensure both the proper functioning of the internal market and Community.

Some food high in soluble vitamins and fatty acids are vegetable oils and animal fats. They are widely used both in food and in cosmetics and pharmaceuticals.

In the literature there are many studies on the active principles of vegetable oils but most of them deal with these oils in terms of the levels of fatty acids and tocopherols. In terms of safety data available in the literature, they refer to pesticide residues and mercury in fish oil.

Given these considerations, the objectives pursued in this thesis are:

- completely study of sea buckthorn oil, wheat germ oil and fish oil including quality and freshness indices and active principles in their composition (fat-soluble vitamins, unsaturated fatty acids-saturated, trace elements copper, zinc);
- correlation of the active principles found in these oils with recommended daily dose;
- study of sea buckthorn oil, wheat germ oil and fish oil in terms of their food safety by determining contaminants represented by heavy metals (lead, cadmium, mercury), residues of organochlorine pesticides and radioactive particles.

# II. DOCUMENTARY STUDY

## 1. Raw materials-general notions about sea buckthorn oil, wheat germ and fish

### 1.1. Sea buckthorn oil

Sea buckthorn oil is an oily liquid, of intense orange color, odorless, tasteless, which is separated from the aqueous phase resulting from pressing fresh fruit, containing large amounts of vitamin A, beta-carotene and vitamins D and E. Because of the important content of vitamins such as E and A, it is used with great success both internally and externally to treat vitamin deficiencies, immune system, to stimulate intellectual activity, to improve the heart's activity withthe elderly, combating the effects of stress, improving chemotherapy [19].

External use of sea buckthorn oil protects mucous membranes and skin of harmful action of radiation.Sea buckthorn oil is used to treat burns, frostbite, various wounds by daily anointing [148].Because of its antibacterial properties, sea buckthorn oil is recommended for skin infections, respectively in order to prevent or treat eczema and rheumatic pain, due to its anti-inflammatory and soothing effects.

Sea buckthorn oil can be obtained by different extraction procedures from sea berry fruits (Hippophae rhamnoides) that is part of the family Eleagnaceae.The scientific name of the genus derives from the Greek words „hippos", which means „horse" and phao-by murder.The name refers to the use of the fruits in the past to eliminate intestinal worms in horses. Following other authors would mean "shining horse." Sea berry is also known as the river sea buckthorn, sea buckthorn barbed river sea buckthorn, sea buckthorn blue, barberry, red sea buckthorn, hedge [21].

Sea buckthorn is a shrub bushy, amfitolerant moisture, heliofil, large shrubs found in clusters or on the sand and gravel, green land, rocky coasts, cliffs. The geological formations met salinifere in mountainous regions up to the coast. In our country they are met on large surfaces inWallachia and Moldavia Carpathians, between Olt and Siret, on the island ofthe Danube Delta. It is a plant resistant to both drought and cold and tough from the ground [20, 22].

Sea berry has branched stems 2-3 high, up to 6 m, bark dark brown color, Armalite lujerii scaly, silvery gray, lateral branches with numerous spines, with small buds, hairy, copper-colored, linear-lanceolate leaves, the 1-6 cm long, whole, with median rib obvious, short-petiolate, silvery gray on the underside with rusty scales, arranged alternate.The flowers

are yellow-rust, unisexual, dioecious, the male flowers grouped in globular and the female raceme [22]. Blossoming occurs in March-April. Fruits are false drupe, of 6-8 mm, oval, fleshy, orange, hard stone, branches remain over winter. Turned to 4-5 years. The fruit is harvested from August to first frost.

Its fruits are rich in: sucrose, organic acids, pectin, tannins, cellulose, oil, beta-carotene, vitamins (C, E, B1, B2, F, K, A), minerals (Ca, P, Mg, K). Due to their high content of active principles, the sea buckthorn fruits are also called natural multi-vitamins. They act as general tonic, have antiscorbutic action, are astringent, vermifuge, and antibacterial. It is recommended as treatment for the following diseases: beriberi, diarrhea, rheumatism, urticaria, neuroendocrine diseases, anemia, liver disease, circulatory diseases and spring asthenia [50].From the data collected from the literature [82, 83 157]

Sea buckthorn oil contains:

- vitamin E 1230 mg/kg;
- vitamin A 25mg/kg ;
- fatty acids: 0.23% myristic acid,11.6% palmitic acid, 5.41% palmitoleic acid, 0.23% heptadecanoic acid, 3.17% stearic acid, 17.4% oleic acid, 32.0% linoleic acid, 29.2% linolenic acid,0.46% peanut acid, 0.23% eicosenoic acid.

**1.2. Wheat germ oil**

Wheat germ oil is an oily bright yellow, tasteless and odorless liquid.Wheat germ oil is used to combat constipation, in body mineralization and external treatment of skin diseases [48].Wheat germ are obtained from common wheat (Triticum aestivum ssp vulgare).

Wheat is an annual herbaceous plant, unisexual, monoecious, cultivated since ancient times. It is widely spread from the tropics to the north to parallel 64, and to the south to the parallel 50. It occupies 90% of the global wheat cultivated surfaces. It is a plant that requires moderate warm and humid climate. It resists the low temperatures of winter and the high temperatures of the summer.The fruit is a cariopsa. Wheat grains contain protein, carbohydrates (starch), fats, vitamins A, B, E, K, D, PP. They contain large amounts of fiber. From wheat beans are extracted wheat germs which are used to extract wheat germ oil, rich in vitamin E [103].

From data collected from the literature [50] wheat germ oil contains:

- vitamin E 2150mg/kg;

-fatty acids: 16, 7%, palmitic acid, 0, 7% stearic acid, 14.6% oleic acid, 56.5% linoleic acid, 6.2% linolenic acid,0.2% eicosanoic acid, 1.5% gadoleic acid.

### 1.3. Fish oil

Fish oil is obtained from different species of marine fish (sardines, mackerel). Fish oil is an oily liquid, straw yellow coloured,with specific taste and odor. It is rich in vitamins A and E as well as omega-3 polyunsaturated fatty acids and omega-6 (Vitamin F). Fish oil contributes tothe structure and functions of cell membranes and produce hormone-like substances that have anti-inflammatoryand stimulating effect. Omega-3 fatty acids from fish regenerates a large part of the brain, the cerebral cortex and primary visual center of the eye, that is retina. Omega-3 fatty acids have different effects on the multivariate cardiovascular risk parameters [84]. Omega-3 fatty acids reduce the risk of heart attacks by reducing blood thickening and strengthening cell walls. It also reduces triglyceride levels and blood pressure by promoting vasodilatation [28, 8, 70]. Omega-3 plays an important role in regulating fatty acid absorption. This may increase metabolism and reduce fat storage, helping to prevent weight gain [77].

A report published in "Circulation Journal "American Heart Association," suggests that a diet rich in fish may decrease the level of leptin, the hormone that influences body weight and that such individuals can help control their appetite.The occurrences of both breast and prostate cancer are lower with people who consume more omega-3 fatty acids [4].The regularyassimilation of omega-3 fatty acids may have prophylactic effect on the two diseases of the eye leading to blindness: maculopathy and glaucoma. Also consumption of fish oil have benefits in treating dry eye.

General epidemiological studies show that with people who consume large amounts of fish or fish oil, the frequency of mental illness is reduced [107]. The studies done on elderly people with depressia, mental diseases or vascular dementia Alzhaimer revealed that these persons have a low content of omega-3 fatty acids.

High consumption of omega-3 fatty acids may slow cognitive process and can combat depression [122].

The data collected from the literature [112, 114]shows that fish oil contains:

- Vitamin E 1650 mg/kg;
- Fatty acids:8.5% myristic acid, 19.4% palmitic acid, 10.1% palmitoleic acid, 3.9% margaric acid, 5.4% stearic acid, 15% oleic acid, 3.5% linoleic acid,1.7% linolenic acid, 1.4% gadoleic acid,11.9% eicosapentaenoic acid (EPA), 2.5% clupanodonic acid,12.9% docosahexaenoic acid (DHA).

## 2. Food quality - general concepts

### 2.1. Quality features of oils:

- indices of freshness - acidity index, iodine index, saponification index, peroxide value
- beta carotene
- fat soluble vitamins A, D and E
- Fatty acids (saturated, unsaturated)

#### 2.1.1. Quality indices

**Acidity index**

The acidity index means the quantity of potassium hydroxide, expressed in milligrams, required to neutralize one gram of fat.

Value of this indicator helps in assessing the quality of fat, by determining the free fatty acids that occur as a result of partial hydrolysis in the presence of microorganisms and the degradation transformations in oxidizing process [49, 51].

**Iodine index**

The iodine index indicates the degree of unsaturation fatty acids from oils and fats and it is expressed in grams of iodine absorbed by 100g product.

**Saponification index**

Saponification index is directly proportional to the average molecular weight of the fatty acid composition of oils.

Saponification index gives information about the molecular weight of fatty acid constituents of fats analyzed. Free fatty acids in the analyzed material contribute to the saponification value of the index, while nesaponificabile components causes a decrease in its value [49].

**Peroxide value**

Peroxide value is an indicator of freshness of the fats and represent peroxides content and other oxidizing substances in a certain amount of product that oxidizes potassium iodide releasing iodine. Peroxide value is expressed in milliequivalents of peroxide per kilogram of product [51, 68 ].

### 2.1.2. Precursors of vitamin A, beta carotene

Carotene is the yellow-red pigment of carrots, widespread in green leaves as well , in flowers and fruits and in the animal body (blood serum, fat, milk).The human body uses beta carotene to make vitamin A in the liver as needed. The medical studies have proven that it is not toxic and can be consumed in any dose. Consumption of beta-carotene reduces the risk of cancer and cardiovascular accidents, improves the immune system, eyesight and even bone growth [62].

### 2.1.3. Fat soluble vitamins A, D and E

**Vitamin A**

Vitamin A has numerous and diverse functions in the body, but it is best known for the role it plays in preventing blindness and other eye problems, and thus ensuring the regeneration of rhodopsin visual acuity**.** It also helps to improve and maintain the immune system; it is essential for cell growth and development and it is necessary for a healthy skin and hair. Vitamin A, through its antioxidant role helps protect cells and tissues of the body against cardiovascular diseases and cancer. The optimal daily dose of vitamin A is 1,000 micrograms molded (1 mg) retinol equivalents or 5000 international units (IU).One may reach the dose of vitamin A by consuming mixed carotenoids. We recommend 6-15 mg mixed carotenoids (15 mg equivalent to 25 000 IU of vitamin A).Carotenoids have an activity similar to that of vitamin A but apparently do not produce toxicity [119, 152].

**Vitamin D**

Vitamin D is not similar to other vitamins. In fact, this substance does not meet the classic definition of a vitamin because the body can produce as much as it needs using the sun's ultraviolet rays. However, people who do not have adequate exposure to sunlight during the year, may need to extract vitamin D from dietary sources.

Vitamin D is a biologically active substance essential for maintaining bone density. In fact, without vitamin D, your body would not be able to use calcium for the building of bones. Vitamin D increases intestinal absorption of minerals in bone structure such as calcium and phosphorus.It also regulates blood levels of calcium and phosphorus. In its active form, vitamin D is involved in cell growth and maturation which are essential for healthy functioning immune system. A little-known role of vitamin D is to stimulate production of insulin, a hormone produced by the pancreas, with role of regulating blood sugar content.When there is vitamin D deficiency, the body may not be able to produce enough

insulin needed to use simple sugars. This may contribute to the development of diabetes [105].Optimal daily dose of vitamin D in men and women is 400 international units [152].

**Vitamin E**

Vitamin E is a powerful antioxidant that protects cells against oxidative effects by neutralizing molecules very unstable, known as free radicals.

Vitamin E helps protect cells by inhibiting the oxidation of phospholipids, fats contained in cell membranes, which are highly susceptible to free radical attack. Acting as an antioxidant, it protects the lungs against damage caused by air pollution, protects the whole body against free radicals and prevents tumors.Vitamin E also prevents oxidative damage of other nutrients, including vitamin A and C, and beta-carotene. Evidence suggests that vitamin E act synergistically with other antioxidants such as vitamins A and C. The required daily amount of vitamin E depends on each individual's body weight and the amount of polyunsaturated fat they consume because vitamin E protects the fat oxidation process. Due to these individual variations, it is difficult to estimate a value that is optimal for all people. However the recommended optimal daily dose is comprised between 100 and 400 international units [152]. Higher values are appropriate for anyone at high risk of atherosclerosis and coronary heart disease.

### 2.1.4. Fatty acids

Fatty acids are organic, low-acid character, which enters into the constitution of most lipids.

Together with glycerol, fatty acids form the most common kind, namely the fat triglycerides.

In nature we know over 300 such compounds that have been identified in microorganisms, plants, animals and humans [104].

**The importance of fatty acids**

Man, like all other creatures, synthesizes their own fat in most of the fat from food. Human body through metabolic means at its disposal, can not create carbon double bond beyond 9, therefore, polyunsaturated fatty acids should be necessarily introduced into the body through food.For this reason, they are called essential polyunsaturated fatty acids (EFAs) or vitamin F (G. NIACE). All fatty acids that man need, the steps can synthesize EFAs, therefore, saturated fatty acids are non essential substances for man and animals.Providing essential acids can be exclusively vegetable sources (oils), except for acid ω-3, which is more in fish and fish oil [24]. Saturated fats are not necessary for body but also brings health

disadvantages in that they get fat and increase cholesterol and trigliceridemia. Long-chain saturated fatty acids (palmitic, stearic), is digested and absorbed hard and can create various digestive problems.

If the healthy, fat should provide about 15% of calories. Of these lipids, it is recommended that over 66% should come from unsaturated fatty glycerides [27].

**The optimal ratio of fatty acids**

The optimal ratio of the three categories (monounsaturated fatty acids, polyunsaturated and saturated acids) should be 1:1:1.

In the AGE, it is important a ratio of omega 6 and omega 3. Between the two types of polyunsaturated fatty acids, there must be a relationship type:

ω-6/ω-3 = 5:1;

ω-6 = 5g/zi,

ω-3 = 1g/zi.

The conditions in our daily diet, the report is unfavorable, being around 10:1. For this reason it is recommended to increase the consumption of fish, especially marine species of salmonids (trout, salmon), which are rich in omega 3 [134 141]. Also for rebalancing report, indicate internal use offlex oil and fish oil.

### 2.1.5. Microelements

Minerals are inorganic substances that originate in water, soil and are absorbed by plants or eaten by animals, which in their turn are human food.

Minerals are biologically active substances, which, like vitamins are needed by the body in small amounts.Minerals may function as coenzymes, acting with enzymes to activate the body's biochemical processes. They also help the formation of strong bones and teeth (Ca, Mg) and are require to produce hemoglobin, which carries oxygen in blood. (Fe)

Minerals can be divided into macro-and microelements.

Macroelements are needed by the body in quantities greater than trace. Macroelements include calcium, chlorine, magnesium, phosphorus, potassium, sodium and sulfur.

Trace elements include copper, zinc, iron, chromium, fluoride, molybdenum and selenium.

**Zinc**

Although the human body contains only 2-3g of zinc, a trace mineral that is essential for growth and development.

Zinc is found in all body cells being highly concentrated in the eye, liver, bone, skin, hair and nails.

Zinc is considered a tonic for the immune system that can help reduce the frequency of colds and sore throat. Also, zinc may protect the body from the harmful effects of toxic metals such as lead and cadmium [100].Zinc is involved in the synthesis of genetic material contained in all cells, such as DNA and RNA.

Zinc can become harmful to the body only when consumed in large quantities, more than 2 g taken once.Also, excessive intake of zinc over 30 mg can lower copper content and may cause copper deficiency. Copper deficiency is dangerous because copper helps to build bone, hemoglobin and red blood cells and collagen [153].

**Copper**

Copper plays an essential role in the synthesis of hemoglobin, the blood component that carries iron. Also part of important enzymes, including those essential for the formation of bones, nerves and collagen production.

Copper plays an important role in maintaining the integrity of the myelin that covers nerves, maintain proper functioning of the heart and arteries, ensuring the health of the immune system.

Optimal daily dose of copper is between 1.5 and 3 mg. Nutritional studies show that the ordinary person fails to reach the optimal daily dose lower limit, with a maximum intake of 1.3 mg copper.

Copper intoxication occurs if the quantity exceeds 10 mg. Copper intoxication is rare in people with normal diet and not taking copper supplements [153].

## 3. Food safety - general concepts

Food safety is also known as hygienic quality or innocuity.

Innocuity can be:

- microbiological
- chemical

Biological safety - biological contamination of food can be:

• Bacterial

• Viral

• Parasitologycal

### 3.1. Chemical safety

The concept refers both to natural substances existing in different foods and food substances occurring pollution and contamination. There is a distinction between pollution and contamination because in the first case, the occurrence of toxic substances occurs accidentally, while in the latter process is permanent [11].

As a result of the effect of pollution in food occur:

• Antibiotics

• Radioactive substances

Contamination is responsible in particular for the passage of heavy metals (Cu, Fe, Sn, Pb, As, Cd, Hg) and pesticides in food.

Heavy metals and pesticides in food may come in three ways:

• As a result of spraying with insecticides and fungicides containing heavy metals compositional

• In the technological process

• At the storage of metal containers

#### 3.1.1. Pesticide Residues

The term pesticide includes all substances used in agriculture to combat different types of pests.

**Organochlorine pesticides**

The most important representatives of this class are: DDT, metoxichlor, keltan, hexachlorocyclohexane (HCH), pentaclornitrobenzene, aldrin, dieldrin, chlordane, heptachlor, heptaclorepoxide.

- organochlorine insecticides toxicological aspect consists of:
- action on liver function: organochlorine insecticides in mammals produce weight gain in relation to liver weight, protein synthesis are to grow by microsomes and especially lead to increased synthesis of oxido-reductase [7.33];
- endocrine action: estrogen action, disruption of testosterone metabolism by stimulating the action of liver enzymes and calcium metabolism changes;
- action teratogenic (embryo): placenta is easily permeable to pesticides, the fetus in the womb being able to concentrate the pesticide residues from the age of 4 weeks. The new born is contaminated with pesticides in breast milk [7, 11];
- carcinogenic action could not be demonstrated in all cases.

### 3.1.2. Heavy metals

The high concentrations of heavy metals have a harmful effect on the body, because it blocks the enzymatic reactions. Also heavy metals accelerate oxidation of vitamins A and E from food.

**Mercury contamination**

The level of mercury in food is low, the variations depending on the type of product, the level of mercury in the environment in which food is produced and the eventual use of mercury in agriculture. A high concentration of mercury in fish and shellfish occurs because of the high degree of pollution of seas and oceans.

Mercury accumulates in the liver, kidney, pancreas, bladder and can accumulate in the brain. Inorganic mercury is excreted by the kidneysthrough urine, the liver via the bile, the intestinal mucosa, salivary glands and sweat. Almost 90% of ingested mercury is excreted in stool.

**Cadmium contamination**

Among the most important food contaminated with cadmiumare fish, potatoes, milk, beer, eggs.

Although the amounts of cadmium in food are relatively low and absorption ingastrointesinal tract is reduced, a daily exposure for long life in the body leads to a very considerable accumulation of the metal.

Cadmium is toxic to almost every body system. Multiple manifestations whichhas cadmium among the the human body is Itai-Itai disease, which occurred in Japan due to consumption of

fish and shellfish contaminated with elevated cadmium and rice grown on land contaminated with cadmium.

Among the major effects reported for cadmium are mutagenicity and carcinogenicity of this metal. Cadmium is considered one of the most potent metal carcinogens known to date [15].

**Lead contamination**

Lead is not an essential element for humans, it is potentially toxic to all biological systems. Lead accumulates in human tissues in the liver, kidneys, bones. Food and water are the main sources of contamination by ingestion.

Bone retains the largest amount first colloidal formand then crystalline. The amount of lead present in the bone tissue is dependent on previous exposure time and varies from one individual to another, while the soft tissues are relatively constant concentrations. Saturni benign intoxication is accompanied by a slight reduction in nervous influx.

To those who have eaten food contaminated with lead,nerve lesions are related to the degree of anemia, and an excessive ingestion of lead causes an inflammation of the gastrointestinal tract [30, 32].

### 3.1.3. Radioactive pollution

Radioactive pollution is a natural pollution of the environment, whose components, air, water, soil and subsoil are contaminated at the same time.

Radioactive pollution is the most insidious and pervasive forms of aggression on the environment, "almost perfect" because it is invisible, colorless, odorless and does not cause immediate pain.

Radioactivity, that is the emission of alpha particles, beta and gamma can be:

- natural, natural radiation from the sun, from distant galaxies, the earth, rocks,watersof seas and oceans or the atmosphere. These are the natural background radiation to which human beings have adapted over time and is essential to normal biological activity.
- artificial, as a result of human activity, carried out the extraction and processing of radioactive minerals in nuclear reactors for research and production of electricity for detecting defects in industrial components (defectoscopy materials), the type X-ray equipment used in nuclear medicine, in experiments with nuclear weapons. The nuclear industry has a specific risk - ionizing radiation - which are found throughout

the work of prospecting, mining, processing and transport of nuclear fuel, proper operation of nuclear reactors, radioactive waste management, decommissioning nuclear plants [85]. Ionizing radiation are radiation capable to remove an electron orbital of an atom interacting with. Ionizing radiation are alpha, beta, gamma and X radiation.

## 4. Methods of analysis of quality characteristics

### 4.1. Methods for determining the quality indices

**The titrable acidity**

Determination of free acidity is done by titration with sodium hydroxide solution 0.1 N concentration after preliminary dissolution of a given quantity of oil in a mixture of alcohol and ethyl ether in a proportion of 1:2 neutralized to phenolphthalein.

**Peroxide value**

Determination of peroxide is made by titration with sodium thiosulphate solution of known titre of free iodine in the presence of a starch solution as indicator.

**Saponification index**

Determined by titration with hydrochloric acid solution with phenolphthalein in the presence of known titre as an indicator after a certain amount of oil saponification with alcoholic potassium hydroxide solution.

Iodineindex

Iodine index is determined by titration of excess iodine with sodium thiosulfate solution in the presence of starch as indicator.

### 4.2. Methods for determination of beta carotene and fat soluble vitamins A, D and E

#### 4.2.1. Spectrophotometric method for determination of beta carotene

Spectrophotometric method is based on the selective properties of substances to absorb electromagnetic radiation and is used for identification, purity and dosage determination.

Absorption spectra are obtained from continuous radiation beam passing through the test substance that can absorb some of its energy. The amount of energy absorbed depends on the

structure and the number of atoms and molecules interacting with substance beam of radiation.

### 4.2.2. Semiquantitative methods of identification and quantification of vitamins A, D and E by thin layer chromatography

Thin Layer Chromatography (TLC-Thin Layer Chromatography) is a technique that is based on a absorption and desorption of ***a*** mixture substances ***and a*** successive absorption of each component [72].

TLC analysis consistsin differential migration of a set of components subjected to separation. It is used to identify substances in mixtures.

### 4.2.3. Assay of vitamins A, D and E

**HPLC method (Hight Performance Liquid Chromatography*)***

HPLC method is a method of physico-chemical chromatographic separation where mobile phase is a liquid and the stationary phase contained in a column consists of a fine-grained solid or solid impregnated with a liquid or solid that organic groups are grafted.

Pressure liquid chromatography consists of following main parts:

-pumping system;

-injection system;

-column chromatography;

-detector;

-registration system;

### 4.2.4. Assay of fatty acids

**GC method (Gas Chromatography) or gas chromatography**

Of gas chromatography can be analyzed all mixtures of liquids, gases or solids which are or may be transferred in the gas phase without decomposition when heated in other chemical species. The usual way of analysis is to analyze solution when the mixture is a liquid which is made by evaporating gaseous.GC analysis using restriction refers to substances that have a vapor temperature above the decomposition temperature in this case being able to form other chemical compounds.

In these cases substances to be analyzed are derived in advance by specific chemical reactions of volatile compounds. In gas chromatography the sample is evaporated at the entry in column, to its end or în a special device at the entrance to the column.

Separation of the column (elution) is using a carrier gas which not react with the mobile phase but is designed to transport chemicals in the mixture through the column. After the type of column used, gas chromatography is divided into:

• gas chromatography stationarysolid phases (adsorption chromatography);

• gas chromatography stationary liquid phases (partition chromatography);

The adsorption chromatography retention of analyzed substances in the mixture shall be based on physical absorption on the inner wall of column chromatography, which interior diameter of tenths of millimeters and lengths of tens of meters.Because of the irreversible absorption this method is used rarely and only to substances with few molecules in the structure [62].

## 5. Methods of analysis used to determine the safety characteristics

### 5.1. Atomic absorbtion spectrometry (AAS)

Can be**:**

•AAS with graphite furnace

• flame AAS

The principle of analytical atomic spectroscopy is based on a property of atom to issueor absorb electromagnetic radiation specific item under certain physical conditions.Up to the last stage, it is necessary to deliver the items to be investigated in a sample of compounds, generally by absorbing energy and make them available as free particles.

Using mass spectrometry that charged particles can be separated and detected in a mass spectrometer by energy absorption.

Electromagnetic radiation which crosses the environment is provided by a special source called "hollow cathode lamp." Cathode lamp is made of this element to be determined. For each chemical element is therefore required specific lamp.

In atomic absorption spectroscopy, analyzed elements are converted into free atomic state in the atomization device by absorbing heat.These atoms are able to absorb radiation specific item.

To this end, a specific element lamp, hollow cathode made of the element to be investigated is introduced into the beam path in atomic absorption spectrometer atomization device and a detector [125].Depending on the concentration of the element to be determined

in the sample, part of the hollow cathode lamp radiation intensity absorbed by the atoms is formed.

Two photo-multiplier measures the intensity of unmitigated radiation and radiation after leaving atomization device while providing a sample solution.Element concentration in the sample can be calculated by the difference between the two intensities.

### 5.2. Gas-liquid chromatography (GLC)

In gas-liquid chromatography (GLC)the stationary phase is anon-volatile liquid immobilized on a solid support. Gas chromatography stationary phases is based on the distribution of liquid substance analyzed between gaseous mobile phase and a liquid stationary phase immobilized on the inner wall of column chromatography [102].

### 5.3. Spectrometric measurements of alpha, beta and gamma radiation

Energy absorbed in the substance, when crossing radiation lead to several changes, which can be used to measure and record radiation.

Thus, the flow of charged particles passing through a gas excitation occurs and / or ionization of atoms of that substance, processes leading to the formation of ion pairs and free electrons that alter the electrical resistance of the volume of gas considered.

At radiation passing through the substance, the type of particles,will occur electromagnetic interactions and / or nuclear.

In the detection processes that are only interested in the given energy of particles, leading to the most important energy loss.

In most cases, to detect nuclear radiation loaded using ionization or excitation processes of atoms or molecules detector environment.

Neutral particles or electromagnetic radiation to be detected must interact, in a first phase detector with substance or auxiliary substance to produce charged secondary particles (electrons, protons, and alpha particles) are detected in one of its second stage by means of charged particles [40].

#### 5.3.1. Global beta measurements

Global beta measurement requires simple and robustequipment, being the most used method in system of monitoring environmental radioactivity. For detection and measurement of beta

radiation can be used Geiger-Muller counters, scintillation detector, semiconductor detector or SSB and Si (Li) [40, 75, 83].

### 5.3.2. Global alfa measurements

The equipment used for measurements of global beta is not very different from that of global alpha measurements.

But due to the much more sharp absorption of alpha radiation in air, the detector window and selfabsorbtion of the sample, alpha measurements needs additional precautions: the sample is deposited in the thin layer, sample-detector distance is as small as possible, the thickness of the detector window measurements are corresponding to alpha.Alpha spectrum is narrower than the beta, so calibration can be a source of I-241 without special problems if are respected same conditions of geometry to measure the standard and the sample [40.83].

### 5.3.3. Gamma spectrometric measurements

The best way to determine concentrations of radioactive kernels is high-resolution gamma spectrometry, practiced with detector Ge (Li) or HPGe.

There can be determined by gamma spectrometry concentrations of radionuclides which issue pure beta radiation and pure alpha radiation. Most spread radionuclides in this situation are: Sr-89, Sr-90 Y-90, tritium, C-14 and especially plutonium isotopes.

Gamma spectrometry can be done with crystal scintillation detectors with Na (Ti).

## III. EXPERIMENTAL STUDY

### 1. Characteristics and origin of raw materials used

**Sea buckthorn oil** used for the tests was obtained from sea buckthorn fruitdried at 40 ° C in fluidized bed, being harvested from the Breaza, Prahova County.

**Wheat germ oil** was extracted from dry wheat germ from SC Boromir SA Sibiu.

**Fish oil** used is a fish oil sardine and mackerel obtained from the North Sea.

Raw materials used for the extraction of seaberry oil and wheat germ oil had baseline characteristics presented in Table 3

Table1. Baseline characteristics of raw materials

| Raw material | Water content,% | Fat content,% | Peroxide index,mmol KOH/100ml |
|---|---|---|---|
| Dried seaberry fruits | 10,5 | 13,2 | 0,7 |
| Dried wheat germs | 13,6 | 9,5 | 0,5 |

To extract the sea buckthorn and wheat germoilsprior was performed a shredding of material using a hammer cryogenic mill Forplex with a capacity up to 5 kg / h milled product.Grinding by this process helps keep the taste and flavor of raw materials which are made up of water and oils.For the preparation of sample extraction was used a Retsch apparatus for grading with7sites and timer.

Humidity of raw materials was determined by oven drying at a temperature of 100 ° C for 3 hours and oil content was determined using a five-seatSoxhlet installation like solvent using ethyl ether of analytical.

Soxhlet extraction methods was used only for quantitative assessment of the oil content of raw wheat germ and sea buckthorn because the extraction with solvent at boiling temperature and drying in oven at 105˚C to constant weight degrades the extracted oils and increase the peroxide index.

Oils which were determined characteristics of freshness, active ingredients and contaminants content was obtained from the same raw materials but their extraction method was performed by modern supercritical fluid extraction.

Extraction with supercritical gas is a modern technique that aims to replace traditional solvent technologies.

In the supercritical state at a certain temperature and pressure liquefied gases have properties that characterize them in the gas phase.

Oils are soluble in liquefied gases under pressure action then there is the relaxation, compression, extraction, separation of oil from liquid gas phase, the relaxation due to lower gas pressure and recirculation [29].

Currently trying to use a larger scale extraction efficiency of the process due to high oil purity remain no traces of solvent, low cost of gas in the supercritical state.

Supercritical fluids have density and solvent properties like liquids but also viscosity and diffusion coefficient close so quickly of gas causing oil separation plant product of origin [45].

As working parameters are used to temperature range 55 º C and pressure ratings up to 7 atmospheres.

Supercritical fluid extraction is superior then solvent extraction that it is achieved in a shorter time, it eliminates the risk of non-volatile solvent in the product and the risk of product degradation due to operating temperature and contact with air [87].

Extraction of sea buckthorn oil and wheat germ Superfluids installation was performed on a 5-3 separator SEP in three-stages. Installation is at the Food Industry Technical College in Sibiu.

SEP 5-3 Superfluids installation has the following characteristics:

• Extraction capacity of 5L;

• Two separators with a capacity of 1.5 l;

• A separator with a capacity of 0.3 l;

The installation also has two auxiliary units having role to maintain the extraction temperature areas such as:

- Cooling unit with glycol-water;

- A plant to produce hot water.

For extraction has worked with the following characteristics:

- in extraction process at the contact material and solvent, pressure was 240-250 bar and temperature of 50-60 ° C;
- for the second stage of separation the pressure was 70-80 bar and temperature of 30°C;
- in the third stage the separator pressure was 50-55 bar and temperature of 20 ° C..

## 2. Quantitative determination of quality characteristics

### 2.1. Determining quality indices

#### 2.1.1.Determination of free acidity

Determination of free acidity by titration was performed in the presence of indicators. Organic acid determination was performed, resulting in degradation of fatty of raw materials [34].

The amount of free fatty acids expressed as oleic acid is calculated using the formula:

$$\% \text{ oleic acid} = \frac{282 * V * C}{10 * m}$$

### 2.1.2. Determination of peroxide index

Product was treated in acetic acid medium and chloroform using a potassium iodide solution then titrated liberated iodinewith a solution of sodium thiosulphate titre known [34].
Peroxide value is calculated using the formula:

$$\text{Peroxide index (meq/kg)} = \frac{V_p - V_m}{m} * N * 1000 \text{(meq/kg)}$$

### 2.1.3. Determination of saponification index

Was determined by titration with hydrochloric acid solutionof known titer in the presenceof phenolphthalein as indicator after a certain amount of oil saponification with alcoholic potassium hydroxide solution [34].
Saponification index is calculated using the formula:

$$I_s = \frac{V_m - V_p}{m} * 28{,}05$$

### 2.1.4. Determination of iodine index

For determination of the iodine has been used Hanus method:
Iodine index is calculated using the formula:

$$\text{Iodine index} = \frac{12{,}69 * (Vm - V_p) * N}{m} \text{(g/100g)}$$

The results obtained for quality indicators analyzed are listed in Table. 4.

Table 2. Quality indices of sea buckthorn oil, wheat germ oil and fish oil

| Oil | Free acidity, % oleic acid | Peroxide index,meq/kg | Saponificationindex, mg KOH/g | Iodine value, g/100g |
|---|---|---|---|---|
| Buckthorn oil extracted by supercritical $CO_2$ | 0,30 | 0,38 | 200,3 | 71,5 |
| Wheat germ oil extracted by supercritical $CO_2$ | 0,97 | 0,56 | 248,3 | 110,7 |
| Fish oil | 0,40 | 0,43 | 187,6 | 80,2 |

**Conclusions:**

Analyzing the values in the table above was found the following:

- acidity oils analyzed do not show significant differences, which means that the oils were fresh (Figure 8);
- the higher acidity and peroxide index recorded in wheat germ oil is due to long storage time of raw material (wheat germ) before oil extraction;
- indices of the oils analyzed peroxide not significantly different and fall within the maximum prescribed by the existing rules of 12 meq / kg, a sign that the oils obtained were not degraded after extraction (Figure 9);
- comparing the values of the saponification index (Figure 10) that the average molecular weight fatty acids of wheat germ oil should be higher than the average molecular weight fatty acids in fish oil and sea buckthorn oil;
- comparing the values ofiodine indices it follows that the largest unsaturation degree haswheat germ oil, followed by fish and sea buckthorn oils;

**2. 2. Determination of quality features of sea buckthorn oil, wheat germ oil and fish oil**

**2.2.1. Determination of beta-carotene of sea buckthorn oil and wheat germ oil**

The method consists of extraction of beta-carotene with acetone and then petroleum ether or n-hexane, separation of the other coloring matter on a chromatographic column, then measuring the sample at a wavelength of 430 nm from petroleum ether or n-hexane.

For products containing large amounts of fats, previously is done afatty substances saponification with an alkaline solution of potassium hydroxide in 40% alcohol.

Standard curve was achieved using potassium dichromate standard solution. Were read each standard solutions absorbances then graph absorbance function of concentration [35].

For the calculation of the concentration of beta-carotene was used the formula:

B-carotene $= \frac{A \times F \times V}{M}$; mg/100g

After extraction, measurement and calculation results were obtained in the table 5.

Table3. Beta-carotene content of theanalyzed oils

| **Analyzed oils** | **Mass, g** | **β-carotene content, mg/100g** |
|---|---|---|
| Fish oil | - | - |
| Buckthorn oil | 3,9409 | 23,3 |
| Wheat germ oil | 34,3942 | 1,9 |

**Conclusions:**

- studying the data in table 5 shows that beta-carotene content from sea buckthorn oil is greater thanwheat germ oil.

Fish oil is an animal product anddoes not contain beta-carotene;

### 2.2.2. Determination of vitamins A, $D_3$ and E in the fish oil, sea buckthorn oil and wheat germ oil by high performance liquid chromatography

The method consists in extraction of retinol by saponification with potassium hydroxide solution in methanol or ethanol, followed by extraction in a suitable solvent, determination by high performance liquid chromatography (HPLC) with detection or fluorometrică (F) or photometric (UV), identification of substances based on retention times and determine the external standard method using peak areas or heights of peaks [139].

In this case we used the UV photometric detection.

After the steps described in operating mode were obtained four chromatograms in the standard solutions and one for the other three sample solutions. Every chromatogram

displayed peaks corresponding to specific vitamins, retention times and peak areas for standards and samples.

The results obtained after the calculations are summarized in Table. 6.

Table4. Vitamins A, $D_3$ and E from sea buckthorn oil, wheat germ oil and fish oil

| Oil | Vitamin A, mg/kg | Vitamin E, mg/kg | Vitamin $D_3$, µg/kg |
|---|---|---|---|
| Buckthorn oil | 153,5 | 2615,4 | 2,7 |
| Wheat germ oil | 268,6 | 1281,7 | 9,3 |
| Fish oil | 165,3 | 2815,9 | 2,2 |

**Conclusions:**

- The highest vitamin A content is found in wheat germ oil, followed by fish oil and wheat germ;
- Thehighest vitamin E content is found in fish oil, followed by wheat germ oil and buckthorn oil.
- Consumption of 1g dailyfrom fish oil covers 27% of RDA for vitamin A and 23, 5% of the RDA for vitamin E
- Consumption of 1g daily from sea buckthorn oil covering 15% of RDA for vitamin A and 21, 8% for vitamin E.
- Consumption of 1g daily from wheat germ oil covers 27% of RDA for vitamin A and 10, 7% of the RDA for vitamin E
- Vitamin $D_3$ content from analyzed oils is small; a rate of almost 20% of the RDA of vitamin $D_3$ consumption is covered by 100g wheat germ oil.

### 2.2.3. Determination of microelements (Cu, Zn)

Principle of the method consist în sample calcination at 450 ° C with gradual increase of temperature, ash dissolved in hydrochloric acid and evaporation to dryness the solution obtained, the residue dissolved in nitric acid 1:6 and final determination of metals by furnace atomic absorption spectrometry graphite [142].

After the steps described in operating mode and the calculations were obtained the results in Table No. 8.

Table5. Zn and Cu content from sea buckthorn oil, wheat germ oil and fish oil

| Oil | Zn, mg/kg | Cu, mg/kg |
|---|---|---|
| Buckthorn oil | 3,5 | 0,13 |
| Wheat germ oil | 5,2 | 0,58 |
| Fish oil | 2 | 0,07 |

**Conclusions:**

- The data presented in Table 8 shows that wheat germ oil is rich in Zn and Cu than sea buckthorn oil and wheat germ oil;
- Sea buckthorn oils, wheat germ oil and fish oil are not important sources of micronutrients Zn, Cu because to cover 34.7% from the recommended daily allowance for zinc and 38.7% of RDA for copper should consume 1 kg from oil wheat germ.

**2.2.4. Determination of fatty acid composition of sea buckthorn oil, wheat germ and fish**

Working method consists of two stages:

• Preparation of fatty acid methyl esters

• Analysis of fatty acid methyl esters by gas chromatography

After studyingobtained chromatograms results are shown in tables No. 9-11.

Table 6. Fatty acid composition of sea buckthorn oil

| Fatty acids | Content (%) |
|---|---|
| **Myristicacid C14:0** | **0,26** |
| **Palmitic acid C16:0** | **35,01** |
| **Palmitoleic acid C16:1** | **27,7** |
| **Stearic acid C18:0** | **0,89** |
| **Oleic acid C18:1** | **22,52** |
| **Cis-vaccenic acid C18:1** | **8,12** |
| **Linoleic acid C18:2 (omega 6)** | **3,7** |
| **Linolenic acid C18:3 (omega 3)** | **0,94** |
| **Peanutacid C20:0** | **0,17** |
| **Behenic acid C22:0** | **0,25** |
| **Lignoceric acid C24:0** | **0,18** |
| **Saturated fatty acids** | **36,76** |
| **Unsaturated fatty acids, of which:** | **62** |
| - **monounsaturated** | **58,34** |
| - **polyunsaturated** | **3,7** |
| **Report omega 6: omega-3** | **4:1** |
| **Average molecular weight** | **24889** |

Table 7. Fatty acid composition of fish oil

| Fatty acids | Content (%) |
|---|---|
| **Myristic acid C14:0** | **7,7** |
| **Myristoleic acidC14:1** | **3,0** |
| **Pentadecanoic acidC15:0** | **0,6** |
| **Palmiticacid C16:0** | **19,56** |
| **Palmitoleic acid C16:1** | **10,75** |
| **Hexadecadienoic acid C 16:2** | **0,95** |
| **Hexadecatrienoic acid C16:3 (omega 3)** | **2** |
| **Hexadecatetraenoic acid C16:4** | **1,6** |
| **Margaric acid C17:0** | **0,2** |
| **Stearic acidC18:0** | **5,6** |
| **Oleic acid C18:1** | **13,9** |
| **Linoleic acid C18:2 (omega 6)** | **1,34** |
| **Alfa linolenic acid C18:3 (omega 3)** | **0,8** |
| **Arachidic acid C20:0** | **0,1** |
| **Gadoleic acid C20:1** | **0,7** |
| **Arachidonic acid C20:4 (omega 6)** | **2,5** |
| **Eicosapentaenoic acid C20:5 (omega 3)** | **14,87** |
| **Behenic acid C22:0** | **0,22** |
| **Clupanodonicacid C22:5 (omega 3)** | **1,7** |
| **Docosahexanoic acid C22:6 (omega 3)** | **8,3** |
| **Saturated fatty acids** | **33,98** |
| **Unsaturated fatty acids, of which:** | **64,19** |
| - **monounsaturated** | **29,43** |
| - **polyunsaturated** | **34,76** |
| **Report omega 6: omega 3** | **0,15:1** |
| **Average molecular weight** | **24786** |

Table 8. Fatty acid composition of wheat germ oil

| Fatty acids | | Content (%) |
|---|---|---|
| **Palmitic acid** | **C16:0** | **22,76** |
| **Stearic acid** | **C18:0** | **0,84** |
| **Oleic acid** | **C18:1** | **15,46** |
| **Cis-vaccenic acid** | **C18:1** | **1,14** |
| **Linoleic acid** | **C18:2 (omega 6)** | **51,75** |
| **Linolenic acid** | **C18:3 (omega 3)** | **6,65** |
| **Eicosanoic acid** | **C20:0** | **1,40** |
| **Saturated fatty acids** | | **25,01** |
| **Unsaturated fatty acids, of which:** | | **75** |
| - **monounsaturated** | | **16,6** |
| - **polyunsaturated** | | **58,4** |
| **Report omega 6: omega 3** | | **8:1** |
| **Average molecular weight** | | **25922** |

**Conclusions:**

- the oil rich in unsaturated fatty acids is wheat germ oil with 75% followed by 64.19% fish oil and sea buckthorn oil by 62%;
- the richest oil in polyunsaturated fatty acids is wheat germ oil with a rate of 58.4%, followed by 34.76% fish oil and sea buckthorn oil with only 3.7%;
- linoleic acid (omega 6)highestcontent is in wheat germ oil at the rate of 51.75%;
- large amounts of oleic acid was found in sea buckthorn oil (22.52%), wheat germ oil (15.46%) and in fish oil (13.9%);
- is recommended to use these oils rich in oleic acid because maintain the level of HDL-cholesterol and prevent growth of triglycerides in the blood;
- sea buckthorn oils, wheat germ and fish, along with other vegetable oils should replace saturated fats in the diet;
- from a diet of 2,000 calories, fatty acids should be about 30%, of which about 7% to saturated fatty acids, 8-9%monounsaturated and 9-11% polyunsaturated; theratio polyunsaturated fatty acids / saturated fatty acids is better to be 1.25 to 1.5;
- by this requirement is closest fish oil with aratio polyunsaturated fatty acid / saturated fatty acids closed to 1;

- saturated fats in the diet contributes to increased LDL-cholesterol and total cholesterol in the blood to the detriment HDL-cholesterol;
- sea buckthorn oils, wheat germ oil and fish oil contain 62%, 75% and 64.2% unsaturated fatty acids and the ratio unsaturated fatty acids (UFAs)/ saturated fatty acids (SFAs) is 1.7%, 3% and 1.9% falling under based on these considerations into the class "Lipids with high biological value";

- iodine index indicating the degree of unsaturation of fatty acids of sea buckthorn oil 71.5, 80.2 and 110.7 fish oil in wheat germ oil, is directly proportional to the content of unsaturated fatty acids, respectively 62 % UFAsin sea buckthorn oil, 64.19% UFAs in fish oil and 75% UFAs in wheat germ oil;
- saponification index, indicating the average molecular weight from fatty acid composition of oils from fish, sea buckthorn,wheat germ is 187.6, 200.3 respectively 248.3, its value is directly proportional to molecular weight average, respectively 24 786 g / mol for fish oil, 24 889 g / mol to 25 922 sea buckthorn oil and wheat germ oil..

## 3. The safety features of sea buckthorn oil, wheat germ oil and fish oil

### 3.1. Determinations of heavy metals Pb, Cd, Hg

#### 3.1.1. Determination of heavy metals (Pb, Cd) by graphite furnace AAS

The principle of this method consists in sample calcination at 450 ° C with gradual increase of temperature, ash dissolved in hydrochloric acid and evaporation to dryness the solution obtained, dissolutionthe final residue in nitric acidsolution 1:6 with water and determination of metals by furnace atomic absorption spectrometry graphite [142].

After sample preparation and measurement were obtained spectral recordings for each element determined in part according to related spectral records.

#### 3.1.2. Determination of Hg content from sea buckthorn oil, wheat germ and fish by flame atomic absorption spectrometry

In atomic absorption spectrometry flaming radiation of wavelength specific items to investigate, which is absorbed by the atoms present in the ground state. Absorption is proportional to the concentration of atoms in the flame, so the concentration of the solution.

Atoms from sample solution are issued using flame produced by burning a gaseous fuel consists in an air-acetylene mixture, which prepared the sample solution is sprayed under review (flame temperature must be about 2500 ° C).

After the calculations were obtained from results table. 15.

Table 9. The contents of Pb, Cd and Hg from sea buckthorn oil, wheat germ oil and fish oil

| Oil | Pb, mg/kg | Cd,mg/kg | Hg,mg/kg |
|---|---|---|---|
| Buckthorn oil | 0,037 | 0,0027 | Sld |
| Wheat germ oil | 0,061 | 0,0033 | Sld |
| Fish oil | 0,042 | 0,0023 | 0,024 |

**Conclusions:**

After studying the results it finds that:

- Highest content of lead and cadmium was determined in wheat germ oil, a possible source of lead contamination could be the exhaust gases of the cars road transport because at present still widely used fuel treated with tetraethyl-lead to decrease the effect of detonating engines. In areas with intensive automobile traffic lead pollution of soil and vegetation is well known;
- Highest mercury content was determined in fish oil as a possible source of contamination of industrial because wastes are discharged into seas and oceans.
- World production of mercury exceeds the the number of 10,000 t / year. Sea water contains concentrations of the order of 30 mg / l at the surface, with a tendency to increase depth. A total Hg in seawater is estimated at 108 tonnes.
- Due to reduced biodegradationof its derivatives, Hg tends to focus on different types of organisms.

- For example, algae can accumulate in their cells over 100 times more than there is in water. Pelagic fish like tuna, captured at great distances from sources of pollution can accumulate up to 120 ppb Hg [5].
- Sea buckthorn oil is the least contaminated because the origin of raw material from which was extracted is an unpolluted mountain area, traces of lead and cadmium found can come from extraction equipment;
- Wheat germ oil, fish and sea buckthorn contain quantities of heavy metals below the maximum allowed by applicable law.

### 3.2. Determination of organochlorine pesticide residues contamination

**Gas chromatographic method for the determination of organochlorine pesticide residues**

By this method organochlorine pesticide residues from fat or etheric extract of the sample are trained selectively with acetonitrile.

In acetonitrile are reproduced with petroleum ether and purified by selective adsorption florisil column and here are extracted, all selectively, with an eluent composed of a mixture of petroleum ether, ethyl ether and ethyl alcohol [143].

Elution fluid concentrate is subjected to gas chromatographic determination. Individual residues, calculated and expressed in mg to 1 kg product (ppm) is fixed to a reference standard containing known amounts from pesticides to be investigated [145].

The method has two stages:

A. Extraction and purification

B. Determination of organochlorine pesticide residues from eluate obtained

Calculations for each sample for each type of organochlorine pesticide were performed in Excel.

The results are summarized in Tables No. 17-19.

Table 10. Organochlorine pesticide residues from sea buckthorn oil

| Organochlorine pesticide residues from sea buckthorn oil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Type | Sample area | Constant | $C_e$ | R | $M_p$ | Standard area | Conc.(ppm) | CMA |
| 2,4 DDT | 100728 | 5 | 1 | 82,1 | 3,1356 | 933387 | 0,0021 | 0,05 |
| 4,4' DDT | 81987 | 5 | 1 | 75,9 | 3,1356 | 829013 | 0,0021 | |

Table 11.Organochlorine pesticide residues from wheat germ oil

| Organochlorine pesticide residues from wheat germ oil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tip | Sample area | Constant | $_{Ce}$ | R | $M_p$ | Standard area | Conc. (ppm) | CMA |
| A-HCH | 400316 | 5 | 1 | 76,8 | 3,4415 | 3511942 | 0,0022 | 0,004 |
| B-HCH | 330559 | 5 | 1 | 80,6 | 3,4415 | 2877445 | 0,0021 | 0,003 |
| G-HCH | 120459 | 5 | 1 | 78,4 | 3,4415 | 1045534 | 0,0021 | 0,008 |
| Aldrin | 344358 | 5 | 1 | 79,2 | 3,4415 | 2851943 | 0,0022 | 0,006 |
| Dieldrin | 202232 | 5 | 1 | 81,4 | 3,4415 | 1900857 | 0,0019 | 0,006 |

Table 12. Organochlorine pesticide residues from fish oil

| Organochlorine pesticide residues from fish oil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tip | Sample area | Constant | $C_e$ | R | $M_p$ | Standard area | Conc.(PPm) | CMA |
| Aldrin | 304435 | 5 | 1 | 79,2 | 3,5103 | 2851943 | 0,0019 | 0,006 |
| Dieldrin | 202923 | 5 | 1 | 81,4 | 3,5103 | 1900857 | 0,0019 | 0,006 |
| 2,4DDT | 100728 | 5 | 1 | 82,1 | 3,5103 | 933387 | 0,0019 | 5 |
| 4,4DDT | 819874 | 5 | 1 | 75,9 | 3,5103 | 829013 | 0,0186 | |
| 4,4DDE | 680972 | 5 | 1 | 78,1 | 3,5103 | 688465 | 0,0180 | |
| 4,4DDD | 882803 | 5 | 1 | 77,5 | 3,5103 | 834320 | 0,0194 | |

**Conclusions:**

- in sea buckthorn oil organochlorine pesticide residues detected were those of DDT which are summarized below maximum permissible concentration (0.0042 about ten timessmaller then 0.05);
- in wheat germ oil were found residues of isomers (α, β, γ) HCH and aldrin and dieldrin residues that are smaller than the maximum permissible concentration in all cases;
- maximum permissible concentration was considered that fixed for fruit, vegetables and cereals by Order ANSVA / MS / MAFRD / ANPC nr.12/286/173/124 2006;
- in fish oil pesticide residues detected are aldrin, dieldrin, DDT isomers, 4-4 'DDE and 4-4' DDD which summarized are under the concentration of 5mg/kg fat fixed by the Codex Alimentarius;
- DDT is currently considered a "themain citizen of the world " because it was found throughout the Arctic Circle to the equator [113];

- concentrations of DDT and its metabolites showed that the affinity for fat storage is related to each chemical compound (Morgan and Roan, 1971) and increases in the order 4,4 'DDD <4,4'-DDT <4, 4 'DDE;
- it is estimated that the amount of DDT in the human body reaches about 6ppm. Because of its high toxicity and retention, DDT was banned in many countries, but was replaced by other organochlorine substances as toxic;
- presence of DDT in the environment is greater than total HCH's, similar with β-HCH isomer the regarding capacity of retention. However, itschronic harmfulness is smaller. This is why the CMA is higher (5 mg / kg fat to 0.003 mg / kg β-HCH in the case).
- in the case of fish oil is noted that on the chromatogram, with DDT, DDE appeared because after a period as the existence in the environment, DDT undergoes biotransformation processes in which the DDE is the main metabolite.
- Numerous studies have shown that the DDE has a much higher chemical stability as DDT, which causes in time to gradually decrease the proportion of DDT and to increase theDDE.
- In conclusion, it is considered that although in the investigated oils were found small amounts of residues of HCH, aldrin, dieldrin and DDT they do not risk human health, especially since oils are not consumed in large quantities.
- presence of pesticide residues in studied oils once again proves their affinity for fats and their ability to remanence long after restricting their use.

## 3.3. Determination of radioactive contamination

### 3.3.1. Determination of alfa-beta radioactive ontamination

Beta radiation are much easier and faster than alpha. They traveled by air and are more penetrating than alpha.

Neutral particles or electromagnetic radiation to be detected must interact, in a first phase with a detector substance or auxiliary substance to produce charged secondary particles (electrons, protons, alpha particles) which are detected in a second stage by specific methods of charged particles.

The detector used to measure beta radioactivity was a detector based on light emission of excited atoms or molecules (scintillation detector).

Samples did not require special training, measurements being made on the sample itself.

After connecting the device to power supply and starting, the background was initial measuredat 1000s, then source (Sr + Y) 90 at 3000s.

There were set the parameters for measuring the sample, namely time measurement at 3000s and then introduced a gram of sample in tray measuring which was introduced in the lead castle.

From the menu displayed on the screen was selected "Start measurement". After the measurements, were listed measurement reports containing the results of measurements in the time selected in relation to each type of sample measured.

From reports were centralized measurement results in Table 20.

Table 13. Beta activity of sea buckthorn oil, wheat germ oil and fish oil

| | **Buckthorn oil** | **Fish oil** | **Wheat germ oil** |
|---|---|---|---|
| Beta activity (Bq/g) | 1,0 | 1,0 | 1,0 |

**Conclusions:**

- samples analyzed have low global beta activity and does not indicate a beta radiation contamination and alpha activity is below the limit of detection equipment;

### 3.3.2. Determination of gamma radioactive contamination

The most used method for determining the concentration of radionuclides in the samples is gamma spectrometric measurement performed in the laboratory. This method is nondestructive for the sample and allows the obtaining in the same time of concentrations of many radionuclides.The method is based on gamma radiation interaction with substance.

Samples were transferred into working geometry, which consisted of PVC boxes of about 150 ml (Figure 44). These were sealed with paraffin and left for about a month for natural series radionuclides to reach equilibrium. They were then weighed.

The steps taken for the measuring of a sample were:

- energy calibration;
- efficiency calibration;
- laboratory background radiation measurement, which is subtracted from the sample spectrum;
- radiation spectrum of the sample collection;

- spectrum recording;
- spectrum analysis

The mass, the method of samples preparation, the measurement and geometry of the sample during the analysis are summarized in Table 21. After processing the spectral data obtained from records in Figures 46-48 there were obtained the following gamma activity values for the analyzed samples:

Table 14. Weight, time of analysis, the preparation and geometry of samples analyzed

| **Sample information** | **Buckthorn oil** | **Fish oil** | **Wheat germ oil** |
|---|---|---|---|
| Time of analysis | 61338s | 40394s | 31521s |
| Geometry | 150ml box | 150ml box | 150ml box |
| Sample preparation | Drying at 105°C | Drying at 105°C | Drying at 105°C |
| Sample wheight | 131g | 128g | 129g |

Table15. Natural radioactivity

| **U-238 Series** | **Buckthorn oil Activity, Bq/kg** | **Fish oil Activity, Bq/kg** | **Wheat germ oil Activity, Bq/kg** |
|---|---|---|---|
| Th-234 (U-238) | <6.36 | <34.1 | <9.27 |
| Ra-226 | <17.0 | 9.39± 3.1 | <17.8 |
| Pb-210 | <7.21 | <9.09 | <10.4 |
| Bi-214 | <2.36 | <2.77 | <3.06 |
| Pb-214 | <2.05 | <3.24 | <3.27 |
| | | | |
| U-235 | <1.04 | 0.51± 0.19 | <1.09 |
| | | | |
| | | | |
| Ac-228(Th-232) | <3.97 | <4.8 | <5.53 |
| Pb-212 | <1.62 | <2.03 | <2.34 |
| | | | |
| K-40 | <26.8 | <34.1 | 21.7± 10.6 |
| Be-7 | <8.49 | <11 | <12.6 |

It is assumed that U-238 is at equilibrium with Th-234 and Th-232 AC-228Tabelul nr. 16.

Table 23. Artificial radioactivity

| | Buckthorn oil | Fish oil | Wheat germ oil |
|---|---|---|---|
| Cs-137 | <1.22 | <1.54 | <1.69 |

**Conclusions:**

Studying the results from Tables 22 and 23, the following are to be mentioned:

- speaking of natural radioactivity, isotopes of Uranium series (U-238), thorium,actinouranium and radionuclides K-40 and Be-7 are below the detection limit of the equipment used;
- artificial radioactivity represented by Cs-137 radioactive isotope has similar values at the three oils analyzed, and small value as compared with the maximum value allowed, i.e 200Bq/kg for milk and drinking water and 500 Bq / kg for plant products, a sign that the effects of the Chernobil nuclear accident decreased considerably;
- The allowed maximum limits of the radionuclides concentrations in water and food are calculated so that the effective dose as a result of ingestion of radioactively contaminated water and food does not exceed 5 mSv per year;
- Also the low levels of the radioactivity of the studied oils show that the plants from its derived does not store any radioactive isotopes as lichens and tree buds.

## IV. Final conclusions, original studies, future research directions:

In the present paper I proposed to conduct a comprehensive study on sea buckthorn oil, wheat germ oil and fish oil.

I have approached this study from two perspectives: the quality of the analyzed oils and the food safety of these oils.

In terms of qualitative features I have found that:

- the freshness characteristics, acidity and peroxide indices of the analyzed oils were low, which proves that the raw materials from which they were extracted were fresh and the extraction methods did not affect their quality;
- saponification and iodine indices have high values, being closely connected with average molecular weight andrespectively with the unsaturation degree of fatty acids constituents;

- The main constituents of the analyzed oils are lipids, substances with high nutritional value containing saturated and unsaturated fatty acids with multiple roles in the body;
- these lipids provides a high energy value, knowing that 1 g fat equals 9.1 calories, 1 g carbohydrate, 1g protein respectively are equivalent to 4.1 calories each;
- the significant quantities of unsaturated fatty acids and the good report of omega-3 and omega-6 in these oils, classifies them as lipids with high biological value;
- as active principle, all analyzed oils contain significant amounts of fat soluble vitamins A, $D_3$ and E and sea buckthorn oil and wheat germ oil also contain a precursor of vitamin A, β-carotene; these oils are considered to be a worthy source to fill the deficit of fat-soluble vitamins;
- as representatives of the microelements in the analyzed oils there were found insignificant amounts of Cu and Zn, these oils should not be considered a source for Cu and Zn gap in the body.

In terms of food safety I have found that:

- by its chemical nature, the main constituents being lipids, these oils are not a favorable environment for growth of microorganisms, so they do not present microbiological risks;
- heavy metals presented in the analyzed samples were lead, cadmium and mercury, the quantities present in these oils is less than the maximum allowed;
- pesticide residues detected in theanalyzed oils were DDT, DDE's, HCH (isomers of α, β, γ), aldrin and dieldrin, all under the maximum allowed concentration;
- speaking of radioactive contamination, analyzed oils have low beta activity and speaking of gamma contamination,the radioactive isotopes from the natural series had values below the detection limit of the used equipment and the concentration of artificial isotope Cs-137 was below maximum allowed.

All these considerations argue in favor of the use of sea buckthorn oil, wheat germ oil and fish oil such as food and as food supplements.

The originality of the studies accomplished in this thesis is based on:

- The complete characterization of the analyzed oils;
- their analyses from the point of view of the radioactive contamination in the context of the Chernobyl accident consequences, Romania being one of the states under supervision, even

after a lapse of 15 years after the accident as well as the nuclear accident at Fukushima, Japan from March 2011.

The studies presented in this thesis are complex and fall within the general requirements of the current characterization of novel foods and food supplements in terms of quality and food safety.

PhD theme falls within the priority for the characterization of foods, conducted both food producers as well as authorized control institutions.

Studies proposed in this thesis are intended to complement the existing studies on sea buckthorn oil, wheat germ oil and fish oil and can be regarded as a starting point for the performance of complete studies of other types of foods and food supplements.

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