

Therapeutic Applications of Vegetable Oils and GC-MS Evaluation of ω -3, ω -6 and ω -9 Amounts in Six Oleaginous Plants

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*The medicinal importance of vegetable seed oil is highlighted by studying six oleaginous plants rich in nutrients essential for human health, meaning rose hip (*Rosa canina*), flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), amaranth (*Amaranthus caudatus*), safflower (*Carthamus tinctorius*), and camelina (*Camelina sativa*). The vegetable oils obtained by cold pressing are a direct source of essential polyunsaturated acids, like linolenic (ω -3) and linoleic (ω -6). For evaluating the fatty acids content in the seeds of the mentioned plants it was used the technique of gas chromatography coupled with mass spectrometry (GC-MS). The determined composition of the corresponding oils revealed a high content of polyunsaturated essential fatty acids ω -3 and ω -6. The 3:1 ω -6: ω -3 optimal ratio was observed in hemp oil, while flaxseed oil, rose hip oil, and camelina oil showed a higher content of ω -3 versus ω -6. On the other hand, safflower oil is richer in ω -6, while amaranth oil shows a low content of ω -3, but a high content in ω -6 and ω -9. The results are of interest for people and companies concern by a healthy way of life through the use of vegetable fatty acids.*

Keywords: vegetable oil, fatty acids, ω -3, GC-MS, PUFAn3

Vegetable oils therapy is a treatment and prophylaxis method for numerous disorders. From ancient times the ancestors knew a lot about the qualities of vegetable oils, as confirmed archaeological data. In some old-written documents, wise mans of ancient Egypt, Sumer, India, China, Greece, or Rome suggested the positive effect of various aromatic oils [1-3]. For example, Tibetan medical treatises contain descriptions of the use of vegetable oils for a wide range of diseases. Starting from the early twentieth century, the modern scientific research has demonstrated the importance of antiquity information related to the use of vegetable oils and ethereal in conjunction with the physiological effects on humans' health. Positive action on the human body is explained by the composition complexity and richness in mineral oils, giving them unique immune-stimulating properties and the capacity to eliminate toxins and restore the body's energy balance. In addition, vegetable oils are easily digestible (in a proportion of 95%), and have also the advantage that they can provide in a natural way essential fatty acids and fat-soluble vitamins (A, D, E, K).

Among fatty acids, ω -3 (linolenic acid) and ω -6 (linoleic acid), also define as PUFAn3 and PUFAn6 through the acronym PUFA that means poly-unsaturated fatty acids, are the essential lipids most abundant in brain (about 60%) and, as part of the cell membranes and neuronal membranes, have an important role in completing and normalization of unbalanced adults' or children's diets, and even anti-cancerous properties [4-6]. These polyunsaturated acids are produced exclusively by food intake because the body cannot synthesize them to restore the normal structure of the cell membranes (the dual lipoprotein layer), or to restore the mobility and functionality of receptor membrane and ion channels, for the optimal functioning of every cell of the human body [7-9].

Rose hip or dog rose (*Rosa canina*), member of *Rosaceae* family, is a perennial ancestor that grows near

the forests in Europe, North Africa and Asia. The fruits have the highest vitamin C concentration (0.30 g - 0.80 g%), which represent an amount up to 40 times greater than lemons and 10 times greater than black currants. The content in provitamin A, vitamins B1, B2, B6, P and K, carotenoids, antioxidants, sugars, tannins, fibres, minerals (potassium, calcium, manganese, cobalt, molybdenum, chromium, copper, sodium, phosphorus, magnesium) explains the fruit's therapeutic potential against various diseases. In addition, the content rich in alpha and beta tocopherol (vitamin E), lecithin, sugars, essential fatty acids (ω -3 and ω -6), volatile oil, and carotenoids confer its seeds anti-inflammatory, tissue regeneration, and anti-stress properties [10-12]. The rose hip seed oil, an excellent remedy for many diseases, is obtained by cold pressing, has a brownish-green colour, a slightly bitter taste and a characteristic odour. The polyunsaturated fatty acids (ω -3 and ω -6) make the rosehip oil to be used in improving the cardiovascular system's functions by anti-sclerotic action and for increasing the elasticity of the blood vessels. The rose hip seed oil boosts the immune system, the cell renewal and tissues regeneration, normalizes the intracellular metabolism, combats early aging, promotes rapid healing of wounds, prevents wrinkles and protect skin against UV radiations. It is also a good remedy for skin diseases and other diseases like psoriasis, eczema, inflammation, dermatitis, burns, varicose ulcer, colitis, and gastric ulcer (with effect on the digestive tract lining).

Safflower (*Carthamus tinctorius*) has been an important source for obtaining natural colours from antiquity to the early XVIII century due to the presence of pigments in flowers. Archaeological excavations carried out in Egypt showed that safflower was cultivated in ancient times and probably long before 1600 BC, especially as an undemanding plant that has managed to develop very well on poorer soils. At present, although in Romania it is less

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known, Safflower is grown in many other countries for the edible oil extracted from seeds.

Safflower seed oil obtained by cold pressing has a higher content of unsaturated fatty acids compared with other oleaginous plants, having therefore a high therapeutic potential, in particular in reducing the level of cholesterol and triglycerides in blood. Safflower oil has anti-inflammatory and antioxidant properties and prevents cardiovascular disorders, slows the early-aging process, reduces the appearance of inflammatory processes to lower C-reactive protein (a plasma protein synthesized by the liver and adipocytes, called marker of inflammation) [13,14]. It also has diuretic and toning properties, regulates the blood glucose by participating in lowering the haemoglobin glycation, helps in improving the insulin sensitivity of the diabetics, optimizes the proper functioning of mitochondria, and strengthens the immune system [15].

Hemp (*Cannabis sativa* L.) is generally accepted as one of the first plants cultivated by man. It is mentioned as cannabis in the oldest Chinese pharmacopeia, Shennong Bencaojing (also known as Shen-nung Pen-tsoo Ching or The Classic of Herbal Medicine), book attributed to the mythical Chinese sovereign Shennong who lived around 2800 BC. The Chinese people were using the plant's fibres for the manufacture of clothing, rope, paper, and in constructions, but also as a medicinal plant, reasons for which cannabis was placed in the group of *noble* or *upper herbs*.

On Romanian shores, hemp has been cultivated since ancient times as a textile plant as well as for oilseeds, the oil being used in alimentation before the appearance of sunflower oil and being known as *the fat that makes you lose weight*. Hemp seeds were used in Romanian folk medicine for external adhibit as an antibacterial milk obtained from vigorous mixing of crushed seeds with hot water, and for internal use in relieving rheumatic pain, to treat venereal diseases, congestion lung, vomiting, poisoning, cough, haemorrhoids, intestinal parasites (roundworms) [2].

In a recent review [16], *Cannabis sativa* is sumamed *the plant of the thousand and one molecules*, a very inspired and appropriate description that refers at the large number of phytochemicals (terpenes, cannabinoids, flavonoids, phenolic compounds etc.) found in hemp and to the huge potential of derived bioactive compounds.

Flax or linseed (*Linum usitatissimum*) is an herb used from ancient times, which originated in the Mediterranean region, Crimea, Caucasus, and Asia Minor, and which today can be found in cultures from Central Europe, Egypt, Algeria, Eastern India, and North America. Flax seeds are used as dietary purposes due to their content in several chemical components whose properties and mechanisms of action on the body are not fully understood. In studies that included overweight subjects it has been found that a diet rich in alpha-linolenic acid (ALA) for 4 weeks leads to improved arterial compliance [17]. Thus, oil linseed (*Oleum Lini*) administered internally combats the deficiency of vitamin F (Hypovitaminosis F), lowers the high cholesterol, reduces hypertriglyceridemia, decreases the risk of myocardial infarction and is a natural source of ω -3. Also, it had successfully treated constipation and liver disorders and was found to reduce the PMS symptoms and negative phenomena that accompany menopause [18,19].

Amaranth (*Amaranthus caudatus*) has its name from the Greek amaranth plant, where *amaranth* means *one that will not wither*. In ancient Greece it was considered a sacred plant, a symbol of immortality and eternal beauty. Amaranth cultivation history begins over 8000 years ago. Pre-Columbian peoples, the Aztecs and the Incas, were

the first to have found that Amaranth nutritional properties are far superior to corn and wheat. Amaranth was not only a basic food product, but also a remedy almost miraculous as reliever of haemorrhoidal pain, for treating colds, diarrhea, nerve and skin disorders, being also a great energizer.

Since the '70s, amaranth was rediscovered as a plant with great health benefits. It is a gluten-free, high value nutritious protein food, rich in lysine (an essential amino acid which can not be produced by the body, only obtained from food, and necessary for protein synthesis), fibres, A, C, E, D vitamins, B complex, essential amino acids and minerals (iron, magnesium, zinc, phosphorus, copper, manganese and especially calcium) [20]. Known also as a grain-drug, it showed beneficial effects in cardiovascular disease, hypertension, hypercholesterolemia, diabetes, cancer, inflammations, and contributes to immunity strengthen [21].

Camelina (*Camelina sativa* L.), an oleaginous plant of the *Brassicaceae* family, is native from Northern Europe and Central Asia, being cultivated today in Canada, United States, Slovenia and Italy [22]. In the recent years, this plant has aroused great interest as a rich source of polyunsaturated fatty acids ω -3 and other essential fatty acids, showing properties in prevention of heart disease, hypertension, arthritis, and cancer [23,24].

In the US and Canada, camelina seeds were approved as animal feed (for cattle), as well as nutrient-rich ingredient for chickens and turkeys (FDA, 2009) [25]. Also, the by-product obtained after oil extraction from Camelina seeds contains a significant amount of crude protein. For this reason, it was able to experimentally prove that, used in feeding turkeys, has increased significantly ($p < 0.01$) the concentration of ω -3 in meat (breast or thighs), compared with controls [26].

The presence of polyunsaturated fatty acids in vegetable oils prompted us to initiate a series of extensive studies for highlighting the oleaginous species rich in essential lipids useful for the preparation of dietary supplements with beneficial effects in a wide range of diseases and disturbances.

Experimental part

Materials and methods

The vegetable oils were obtained by cold pressing of the seeds of the studied plants: rose hip (*Rosa canina*), flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), amaranth (*Amaranthus caudatus*), safflower (*Carthamus tinctorius*), and camelina (*Camelina sativa*). The content of fatty acids in the corresponding vegetable oils was determined after derivatization to methyl esters by transesterification of triglycerides with methanol in acidic medium and using a Thermo Scientific GC-MS gas chromatograph coupled with a DSQ P 5000 mass spectrometer detector. It was used a Macrolog 2000 column, $\Phi = 0.25$ mm, $l = 30$ m, helium as carrier gas at a flow rate = 1 mL/min, injection temperature = 25°C, and column temperature = 25°C.

Results and discussions

The elution order and the GC-MS retention times (t_r) of fatty acids are presented in table 1, while the content in fatty acids for the six oleaginous plants is presented in table 2. A first observation is that GC-MS analyses revealed a high content of polyunsaturated fatty acids - ω -3 (linolenic acid) and ω -6 (linoleic acid) in the studied seed-oils. Some of the plants present a generally considered optimal ω -6: ω -3 ratio of 3:1, which makes the studied oleaginous plants to be valuable resources with high therapeutic potential, especially for the cardiovascular system, brain,

Fatty acid \ Seed-oil	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic
ROSE HIP	-	8.00	12.10	12.42	13.51	14.58	16.31
SAFFLOWER	6.51	8.48	12.61	12.95	13.92	15.12	16.92
HEMP	6.33	10.51	14.94	15.34	16.55	18.02	20.26
FLAX	6.71	8.65	12.79	13.16	14.27	15.42	17.16
AMARANTH	6.38	10.55	15.11	15.55	16.44	18.58	20.57
CAMELINA	-	8.46	12.60	12.94	13.86	15.16	16.92

Table 1
ELUTION ORDER
AND RETENTION
TIMES FOR THE
IDENTIFIED
FATTY ACIDS

Table 2

THE CONTENT OF SEED-OILS IN ω -3, ω -6,
AND ω -9 FATTY ACIDS DETERMINED BY GC-MS

Source of vegetable oil	Content in fatty acids, %			
	Linolenic (ω -3)	Linoleic (ω -6)	Oleic (ω -9)	Other fatty acids
ROSE HIP	48.13	17.95	21.57	12.35
SAFFLOWER	0.13	77.64	11.60	10.63
HEMP	9.76	54.89	21.95	13.40
FLAX	52.98	14.37	20.11	12.54
AMARANTH	1.07	47.44	25.57	25.92
CAMELINA	34.04	17.75	15.50	32.71

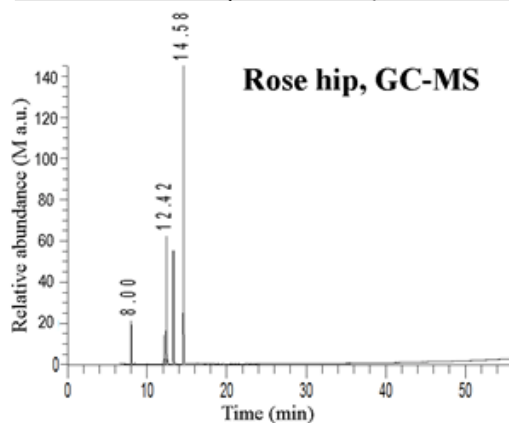


Fig. 1. GC-MS of rose hip (*Rosa canina*) seed oil

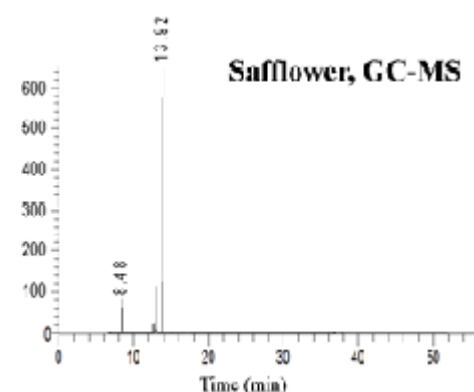


Fig. 2. GC-MS of safflower (*Carthamus tinctorius*) seed oil

immunity, mucosal tissue regeneration, and re-epithelialisation.

In figure 1 is shown the GC-MS analysis of the rose hip seed oil, while in table 2 are presented the retention times obtained for the representative fatty acids. From figure 1 and table 1 in can be observed that the most abundant fatty acid present in rose hip seed oil is linolenic acid (ω -6), and that the ω -6: ω -3 ratio is 2.7:1. In a similar study on the composition of unsaturated fatty acids in the seeds of several species of rose hip, the GC-MS method has evidenced a content of 45.38-54.58% linoleic acid (ω -6), 13.67-24.75% alpha linolenic acid (ω -3), 20.83% oleic acid (ω -9), 12.97% palmitic acid, 8.54% stearic acid, and 1.99% arachidonic acid [10]. The obtained results revealed that the oil from the seeds of rose hip contains polyunsaturated fatty acids ω -3 and ω -6, vegetable lipids essential for a normal, healthy diet.

Safflower seed-oil contains a relatively large amount of polyunsaturated fatty acids (to an extent of 79%) in comparison with other types of vegetable oils and unsaturated (13%) and saturated (8%) fatty acids. Other essential nutrients present in safflower seed-oil are proteins, mineral salts, conjugated linoleic acid, vitamin E

(a strong antioxidant which acts in decreasing the oxidative stress of the cells and in preserving the integrity of cell membranes), vitamin K, and a small amount of choline. In fact, safflower oil, a natural and easy assimilable nutrient, is a rich source of conjugated linoleic acid. In figure 2, linoleic acid appears as two adjoining and significant peaks around 13.9 min, the total calculated amount of ω -6 being 77.64%. The second and third fatty acids present in rose hip seed-oil, having a similar concentration around 11%, are oleic acid (ω -9) at 12.95 min and palmitic acid at 8.48. Regarding ω -3 presence, only small amounts (0.13%) were determined.

The conjugated linoleic acid (abbreviated CLA, the term referring to a group of linoleic acid isomers) is a fatty acid naturally found in beef, milk, and various seeds, and which possesses biological activity with positive action on reducing and even eliminating the body fat [27]. Through its high content of conjugated linoleic acid, safflower oil extracted from seeds by cold pressing (not exceeding 40°C) destroys fat cells and removes harmful compounds from the body. It is beneficial as a dietary supplement and reduces abdominal fat and the global absorption of fat, decreases the production of fat, accelerates fat burning (even during sleep), reduces cortisol levels (called *stress hormone* that is responsible for the accumulation and storage of fat in the abdomen), reduces appetite, and it is useful in diets that aim the reducing of the elevated blood

cholesterol, helping in this way to mitigate the development of atherosclerosis.

Hemp is an important bio-resource that provides balanced nutrition with proteins and unsaturated fatty acids, the essential amino acids helping digestion, stimulating the nervous system, improving and maintain memory at all ages, skin health and beauty. Hemp seed oil obtained by cold pressing of the seeds is a rich source of polyunsaturated fatty acids ω -6 and ω -3. This is the only plant oil that contains no saturated fatty acids, but contains about 90% unsaturated fatty acids, of which 50-60% linoleic acid (ω -6), 15-20% alpha-linolenic acid (ω -3) and about 2-6% gamma-linolenic acid (GLA), and plays an important role in the body, similar to vitamins [2, 28].

In figure 3 it can be observed that the main compounds present in hemp seed oil are linoleic acid (t_R at 16.55 min) and oleic acid (t_R at 15.34 min), followed by palmitic acid (t_R at 10.51 min), while from table 2 it can be calculated the ω -6: ω -3 ratio, this being 5.62:1. Hemp is a direct source of essential fatty acids ω -3 and ω -6 for all ages and is a valuable product that nourishes the brain, helping it to support the health of the body under stress and intense intellectual activity.

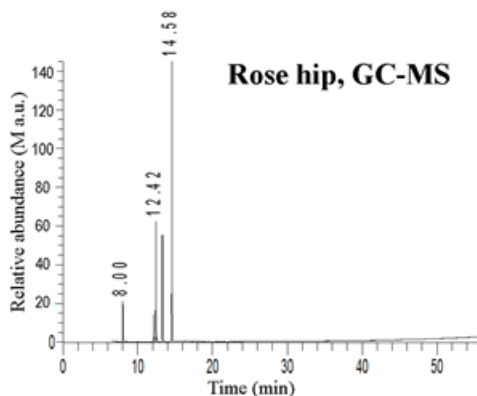


Fig. 3. GC-MS of hemp (*Cannabis sativa*) seed oil

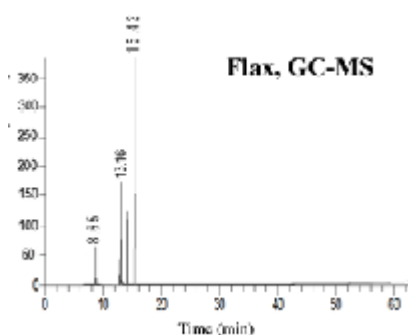


Fig. 4. GC-MS of flax (*Linum usitatissimum*) seed oil

Flaxseed contains carbohydrates, cellulose, proteins, salts of potassium and magnesium, oil, vitamin E, mucilages, galacturonic acid, rhamnase, galactose, xylose, arabinose, monounsaturated fatty acids (oleic acid) and polyunsaturated (linoleic and linolenic acid), cyanozidic glycosides etc. . Regarding the polyunsaturated fatty acids content in seeds, from figure 4 it can be seen that linolenic acid ($t_R=15.42$ min) prevails, with a percent of 52.98%, followed by oleic acid ($t_R=13.16$ min) with 20.11%. From table 2 it can be seen that for flaxseed, the ratio between linoleic acid (ω -6) and linolenic acid (ω -3) is reversed in favour of ω -3, the ratio ω -3: ω -6 being 3.69:1. For this reason flaxseed proves to be very beneficial for humans by bringing a surplus of ω -3 if the necessary diet requires more ω -3. It was evidenced that the oil obtained by cold pressing of linseed (called also *Oleum Lin*) contains polyunsaturated fatty acids (PUFA), including alpha-linolenic acid (ALA), which is converted by the body into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). For some people,

this transformation is rather weak because they lack the metabolic enzyme that has the required ability to complete this transformation. Numerous clinical studies have suggested that ω -3 polyunsaturated fatty acids have a beneficial effect on the anti-arrhythmia of the heart and stabilize the cell membranes [29]. Experimental studies in mice have shown that alpha-linolenic acid induces a protection against colon cancer [30].

Amaranth seed oil was found to contain 8% polyunsaturated fatty acids (PUFA), a high level of squalene (10.8%) compared with shark liver oil (1-1.5%), vitamin E, and beta-sitosterol. Squalene, a steroid precursor and a powerful antioxidant, induces healing properties to amaranth seed oil, a positive influence on cell regeneration, and even significant anticancer characteristics.

In figure 5 in can be seen that the main fatty acids in amaranth seed oil are linoleic acid ($t_R=16.44$ min), palmitic acid ($t_R=10.55$ min) and oleic acid ($t_R=15.55$), while the ω -6: ω -3 is in this case 44.34:1. Although amaranth has a low amount of ω -3, the consumption of amaranth oil leads to improved cardiovascular health, heart stimulation, decreases in bad cholesterol and triglycerides from blood, regulation of the blood pressure, and also helps prevent metabolic imbalances of calcium and iron, which generally occur at old age.

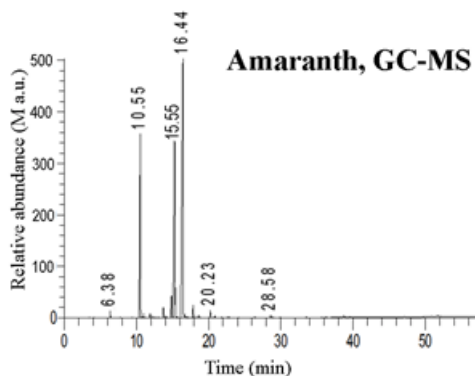


Fig. 5. GC-MS of amaranth (*Amaranthus caudatus*) seed oil

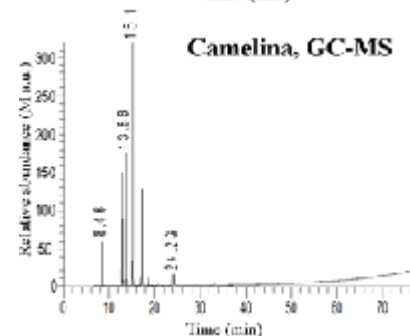


Fig. 6. GC-MS of camelina (*Camelina sativa*) seed oil

Camelina seed oil obtained by cold pressing was found to be rich in ω -3, ω -6, and vitamin E. In figure 6 can be identified as the main compounds linolenic acid (at 15.16 min), linoleic acid (at 13.86 min), stearic acid (at 12.60 min) and arachidic acid (at 16.92 min). The ω -3: ω -6 ratio in camelina seed oil was found to be 1.92:1, so, similar to rose hip and flax seed oils, ω -3 is the principal fatty acid present in the respective seed oil.

According to the opinion given by the EFSA Scientific Panel (27.11.2007), camelina oil is considered an important raw material, and is approved as an important source of antioxidants. Shukla et al. (2002) showed that the high content in polyunsaturated fatty acids (more than 50%) is beneficial to health, the camelina seed oil presenting distinguished content rich in natural antioxidants [31]. Moreover, Abramovic et al. (2006) have shown that phenolic extracts obtained from camelina oil and added to the lipid system during a certain period of time, significantly delays the oxidative processes due to the

Linolenic acid (Omega-3) content, %

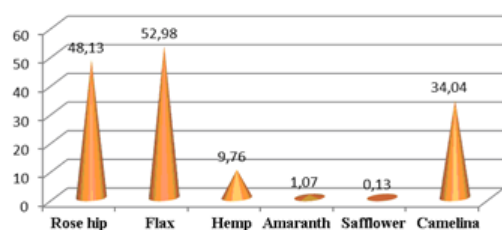


Fig. 7. Content in linolenic acid (ω -3) of the six oleaginous plants studied

Linoleic acid (Omega-6) content, %

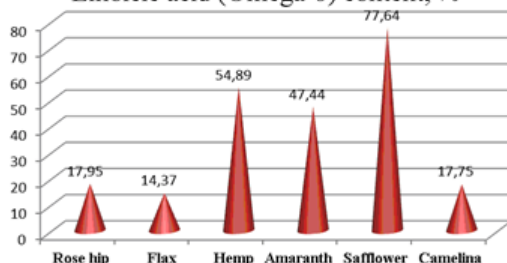


Fig. 8. Content in linoleic acid (ω -6) of the six oleaginous plants studied

Oleic acid (Omega-9) content, %

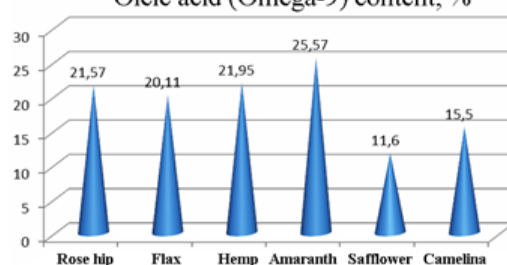


Fig. 9. Content in oleic acid (ω -9) of the six oleaginous plants studied

presence of vitamin E, also a powerful natural antioxidant [32]. In fact, camelina seed oil contains saturated fatty acids (7.9%), 34.7-38.1% mono-unsaturated fatty acids – like oleic and eicosenoic acids – and 16.9 to 57.4% polyunsaturated fatty acids like linoleic acid and alpha-linolenic acid.

A comparison between the content in ω -3, ω -6, and ω -9 of all six studied seed-oils is given in the figures 7, 8, and 9. This comparison can be used to balance the diet according to each situation (diet, supplements, bio-pharmacy) by choosing the right amount of seeds from each type of plant and mixing them in the desired ratio. This kind of mixtures could be used to enrich in fatty acid the meat of animals or poultry [33]. The figures 7 and 8 also show that three out of six plants are rich in ω -3 (rose hip, flax, and camelina), while the other three are rich in ω -6 (hemp, amaranth, and safflower). A proper mixing of seeds or plants from the two categories can lead to the desired vegetable supplement.

Conclusions

The studied seed-oils' composition revealed a high content of essential fatty acids ω -3 (alpha-linolenic acid), ω -6 (linoleic acid), and ω -9 (oleic acid). Depending on the ω -6: ω -3 ratio, the six oleaginous plants can be placed in two categories: plants with seed oil rich in ω -6, meaning hemp (*Cannabis sativa*), amaranth (*Amaranthus caudatus*), and safflower (*Carthamus tinctorius*), respectively plants with seed oil rich in ω -3, namely rose hip (*Rosa canina*), flax (*Linum usitatissimum*), and camelina (*Camelina sativa*).

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