Comparative Study Concerning the Composition of Certain Oil Cakes with Phytotherapeutical Potential

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Oil cake remaining after oil extraction from fatty seed caught the attention lately due to its valuable biologically active substances. This comparative study aims the characterization of oilseed cakes, such as Thistle (Milk thistle), Soybean (Glycine max), pumpkin (Cucurbita pepo), Sea Buckthorn (Hippophae rhamnoides), Rape (Brassica napus), Flax (Linum usitatissimum), Hemp (Cannabis sativa), safflower (Carthamus tinctorius). Comparative analysis of plant material was done after removing fatty oil from seeds. In this context the obtained cakes were characterized for the content of bioactive compounds: flavones, polyphenols, sugars, proteins and digestive enzymes such as amylase, protease lipase. Metals like: manganese, potassium, iron, zinc, calcium, magnesium were quantified. The existence / absence of heavy metals: lead, cadmium was noticed, too. Modern methods for plant material characterization such as atomic absorption spectrometry (AAS), UV-VIS spectrophotometery, and also standard biochemical methods in determining enzymes were used. The performed analyses of these oil cakes can be used in order to obtain food supplements.

Keywords: biologically-active compounds, oil cakes, biochemical methods, UV-Vis, AAS

In order to achieve rebalancing of the metabolic system, affected by various environmental harmful factors and unhealthy nutrition, studies were carried on concerning the natural organic oil cakes.

It is well known that cakes are by-products obtained after the extraction of oil from seeds. There are two cake types, edible and inedible. Edible cakes have a high nutritional value: especially having a protein content from 15 to 50%. Composition varies depending on the variety and the extraction methods [1].

The recent literature shows the composition of pumpkin seed cakes (*Cucurbita pepo*) compared to the soybean cakes (*Glycine max*). The pure proteins of soybean cakes was found in quantity of 59% compared to 12.4% in pumpkin cakes. Small amounts of polyphenols soybean cakes 0.2% compared to the pumpkin cakes 6.4%. In the soybean cakes was identified the existence of anti-nutritional factors and it is therefore recommended this product only in the mixture [2,3].

The main products of processing seabuckthorn fruits (*Hippophae rhamnoides*) are: sea buckthorn juice cold pressing fruit, sea buckthorn seed oil, sea buckthorn powder obtained after drying and crushing fruits, and the cakes left after pressing fruit. Seabuckthorn cakes contain about 15% protein and residues in fruits. Seeds contain over 190 valuable biologically active compounds in varying concentrations. These compounds include the fat soluble vitamins (A, K, and E), fatty acids, lipids, organic acids, amino acids, carbohydrates, vitamins C, B1, B2, folic acid, tocopherol, and flavonoids, phenols, terpenes and tannins. [4, 5]. The seeds and pulp of sea buckthorn contain oils what are considered the most valuable components due to composition soluble vitamins, and plant sterols [6-10]

It was noticed that the cakes of milk thistle (*Milk thistle*) have hepatoprotective effects, and are used in various food supplements or mixtures used for animal feed. The mixture of active compounds: silymarin flavolignans: silybin A, silybin B, isosilibin, silicristin, silidianin are responsible for effects. The hepatoprotective antioxidant properties, neuroprotective and anti-cancer [11]. The cakes obtained after removing oil contain 2.95 % silymarin and to a 18% protein concentration of cakes [6].

The chemical composition that gives the nutritional value of the rapeseed cakes (*Brassica napus*) is influenced by factors that are closely related to the processing technology and working conditions (type of oil press, temperature, humidity during the extraction process, etc.) and origin of the raw material. Therefore, the results of different studies are often controversial. Average in dry matter content of various samples was between 89.6% and 95.3%. Crude protein content ranged from 30.2 to 37.8%. Crude fat content varied from 10.3 to 15.1% [5, 6].

The seeds of the hemp plant (*Cannabis sativa*) contain carbohydrates, fat, fibers, calcium, magnesium, phosphorus, potassium, A, B1, B2, B3, B6, C, D, vitamins, especially vitamin E. The hemp protein has a complex structure very similar to proteins from the blood, which makes them easiesr to digest. This makes that in a mixture the hemp seed cake to be a valuable source of protein [12].

Flax seed cake (*Linum usitatissimum*) (before degreasing) showed 21.78% fat and 27.78% protein. The reported values from the literature regarding flax seed composition differs [13].

Safflower cake (*Carthamus tinctorius*) is mainly used as a protein ingredient for animal feed. Husking seeds improves crushing efficiency. Shelled seed cakes contain more than 40% protein. The safflower cakes quality is highly variable because it depends on the amount of foreign matter and the degree of extraction of oil [14].

It is known that enzymes are catalysts of biochemical compounds that speed up chemical reactions behaving like real „life drops". Current research studies have enabled the development of cellular molecular level, emphasizing the importance of enzymes in living organisms essential processes [15-19]. Oil cakes with high enzyme content

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represents a raw cheap material for dietary supplements industry.

This work proposes a comparative study concerning the biologically active compounds in existing oil cakes in order to obtain valuable natural food supplements.

Experimental part

The seeds of Thistle, Soybeans, Pumpkin, Seabuckthorn, Rapeseads, Flax, Hemp Safflower were obtained from organic cultures, constant dry weight and then cold-pressed by extrusion, resulting vegetable oil and oilseed cakes. The extraction of oil from the seeds by cold pressing took place under controlled temperature of 40°C, major constituents in particular plant enzymes from cakes being kept unaltered.

The total polyphenol acids content is expressed as caffeic acid equivalents and flavonoid derivatives expressed in rutin equivalent was determined by UV-VIS spectrophotometer using a JASCO 530. phosphotungstate reaction was used to determine total polyphenol acids and reading absorbance at 660 nm wavelength. For the determination of the flavone derivatives has been applied the method based on reaction with $AlCl_3$, at a wavelength of 430 nm and 10 mm glass cell [7, 8].

The sugars, fatty substances and proteins were determined by the usual standardized methods [21-22].

The determination of minerals was made by spectrometry absorption and the emission in flame technique using a spectrophotometer GBS Avanta PM equipped with computer and cathode lamp specific at each element. The samples were processed and analyzed by digestion with nitric acid, hydrogen peroxide and hydrochloric acid using a microwave Berghof MWS-2 digestion pressure system [21].

The enzymes present in plant material (amylase, protease, lipase) were highlighted by biochemical methods such as Wortington colorimetric methods of analysis.

-Amylase was determined by the α-amylase method dosage. The starch is hydrolyzed by the action of α-amylase in fragments with reducing groups which can be determined by the 3,5-dinitrosalicylic acid. The nitroaminosalicilic acid concentration is colorimetric measured at a wavelength of 540 nm, corresponding to enzyme activity. A unit consists of one micromole of reducing groups (calculated in equivalent maltose) per minute at 37° C [18, 19].

-Protease is dosed according to the endoproteinase classical method of dosing. This method consists in the determination of enzymatic activity in the cleavage of casein and is used as a substrate. One unit is the amount of enzyme which liberates acidosolubile fragments measured colorimetrically at a wavelength of 280 nm, which correspond to the enzyme activity per minute at 37° C and *p*H 7.8 [16-18].

-Lipase is volumetric dosed. Fatty acids resulting from the hydrolysis of triglycerides from olive oil used as a substrate in the lipase presence extracted with organic solvents is titrimetrically dosed with an alcoholic solution of NaOH in the presence of phenolphthalein - as indicator. A lipolytic activity unit is defined as the amount of enzyme which under the reaction conditions indicated 1μmol fatty acid per minute at 37° C [20,21].

Results and discussions

The plant material obtained after removal of the fatty oil was analyzed for the protein content, residual lipids, polyphenols, flavones, enzymes-biological interest compounds (table 1).

The data obtained are remarkable for the high protein content of analysed cakes between (14-30%), which makes these by products to be edible and which allow various combinations and associations for the food industry. In combination with different medicinal and aromatic plant extracts the cakes can be used to obtain new natural supplements with certain phytotherapic properties. Such combinations can diversify the types of proteins and amino acids from the diet, helping to stimulate and support biosynthetic processes in liver cells and thus cell regeneration process.

Residual fatty substances cakes analysed after extraction of oil from seeds from selected plant, shows values between 3.61% at thistle to 7.18% at rape seed. The studies reveal that there is a close correlation between proteins and lipids, due to the ability to facilitate the transfer of proteins from phospholipids cell membranes. It is therefore important to have a equilibrate ratio between proteins and lipids, and one considered to be optimal for the human body is 20-25% protein and 3-5% fat [23].

In terms of content in polyphenol acids (in caffeic acid equivalent) it were observed amounts ranging from 0.01% for pumpkin cake to 1.05% and hemp cake. They were identified amounts of flavones (expressed in rutin equivalent) 0.90% for rape seed cake and the absence (0%) in soybean cake.

The obtained results were identified conservative values regarding carbohydrate content of analyzed cakes between 9.61% at safflower cake to 24.92% at soybean cake. The largest amount of flavonoid derivatives (expressed as rutin) is found in rapeseed cake (0.9%), followed by the seabuckthorn and milk thistle (0.34% and 0.32%), and below 02% at hemp and flax seeds.

Small amounts of the flavonoid compounds are found in pumpkin and safflower cakes and were not found in soybeen cake.

From analyses we can see that by their complex composition in bioavailable substances, the analysed cakes satisfy both nutritional properties and cell regeneration, representing a cheap raw material.

No.	Plant material analyzed	Determination				
		Fatty substances, [%]	Polyphenol acids (expr. Caffeic acid), [%]	Flavones (expr. in routin),[%]	Proteines, [%]	Total Carbohidrates, [%]
1.	Thistle	3.61	0.41	0.32	15.64	16.10
2.	Soybean	4.71	0.05	0.00	31.72	24.92
3.	Pumkin	5.92	0.01	0.02	29.39	15.88
4.	Seabuckthorn	4.56	0.80	0.34	16.59	18.44
5.	Rape	7.18	0.15	0.90	14.08	17.00
6.	Flax	6.83	0.26	0.12	14.40	16.26
7.	Hemp	5.18	1.05	0.16	24.86	15.04
8.	Safflower	6.94	0.09	0.08	29.02	9.61

Table 1
BIOLOGICALLY ACTIVE COMPOUNDS IN OIL CAKES

No.	Plant material analyzed	Microelements (mg/100 g)									
		Ca	Mg	Na	K	Mn	Fe	Zn	Cu	Pb	Cd
1	Thistle	120	520	30	160	3.5	11.0	3.0	4.2	ND	ND
2	Soybean	37	200	36	1500	2.5	15.0	7.0	3.0	ND	ND
3	Pumkin	14	400	23	1000	2.6	13.0	8.5	1.8	ND	ND
4	Hemp	30	800	20	840	22.0	3.5	2.2	1.5	ND	ND
5	Safflower	50	400	3	450	3.2	5.0	5.0	2.0	ND	ND

Table 2
MINERAL CONTENT IN VARIOUS OIL CAKES

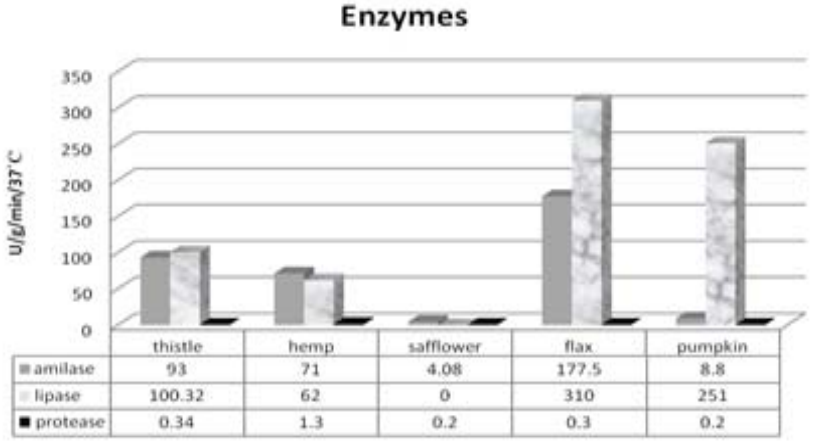

Fig. 1 Enzymatic activity in the oil seed cakes studied

The cakes containing a balanced ratio between the fat and protein were selected for mineral analyses (table 2).

Experimental study using the atomic absorption spectrometry (AAS), doesn't identify heavy metals (lead, cadmium) in studied cakes. The metals have a harmful effect on the body by blocking the enzyme reactions and accelerating the oxidation of fats and of the fat-soluble vitamins from food.

Also it was shown that all byproducts can bring a beneficial mineral intake in the body, such as manganese, potassium, iron, zinc, calcium, magnesium. From the obtained results it was observed that the greatest amount of calcium is found in milk thistle cake and the lowest value was found in safflower cake. The largest amount of magnesium was found in hemp and the lowest value to soybean cake. The greatest value for sodium was found in soybean cake and the lowest at safflower cake. The greatest value for potassium was found in soybean cake and the smallest to thistle cake. Manganese greatest value was found in hemp cake and the smallest soybean cake. Iron greatest value was found in pumpkin seed cake and the smallest cake at the hemp seed cake; It have been identified small amounts of zinc and copper in all analysed cakes. [24,25]

There have been done a number of enzymatic determinations including, specific amylase for hydrolysis of starch, glycogen, polysaccharides, specific lipase for lipid hydrolysis and proteolytic enzymes characteristic for breaking peptide bonds. The results are shown in figure 1.

The following results indicate enzymatic activity in decreasing order:

- tlax seeds cake> Pumpkin seeds cake> Thistle seeds cake> Hemp seeds cake> Safflower seeds cake

- for protease enzyme activity the values are low.

Conclusions

The study performed the main composition of some products obtained from food industry (oil cakes) for their superior capitalization in food industry and phytotherapy.

-The oil cakes, obtained after removal of oil, were analysed for the content of protein, residual fat acids, polyphenol acids, flavones, enzymes, and mineral complex.

- In food industry it can be used as a valuable source of protein in the different mixtures, taking into account the overall composition of the analysed plant material.

It was identified a high protein content in analysed plant material that range between 14-30%, which makes that cakes to be edible and which allow various combinations and associations for the food industry and in combination with different herbs and extracts with phithoterapeutic properties, getting new herbal supplements.

The determinations have highlighted the presence of remarkable quantities of polyphenols and flavanols (especially in seabuckthorn, hemp and rapeseed, thistle cakes) which recommende them in dietary supplements with antioxidants and hepatoprotective properties.

Acknowledgement: This paper is supported by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the project number POSDRU/159/1.5/S/134378. And by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132397.

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Manuscript received: 12.09.2014