CHARACTERIZATION OF PUMPKIN SEEDS OIL EXTRACTED WITH SUPERCRITICAL CO_2

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Abstract: Considering that the supercritical CO_2 extraction is a new alternative technology which has many advantages, it was studied the CO_2 extraction of *Cucurbita pepo* seeds.
The analyzed pumpkin seeds had high lipids content - 47.3%, making pumpkin seeds a potential source of vegetable oil. The results showed that the sum of unsaturated fatty acids is 81.8%. The beneficial effects of unsaturated fats on blood lipids and the impact of omega-3 polyunsaturated fats are to prevent the cardiovascular deaths, arthritis and asthma. Because pumpkin seeds oil is rich in both oleic and linoleic acids, it can be used as edible oil for cooking and salads or sauces.
Key words: supercritical CO_2-extraction, pumpkin seeds, vegetable oil, fatty acids

Introduction

Currently, nutritionally speaking, there is a growing interest for seed lipids content and composition of edible vegetable oils obtained from them. Pumpkin seeds are rich in lipids (20-40%) as well as soybean, cottonseed or corn germ. Due to the high content in fatty compounds, pumpkin seeds oil is an excellent source of energy (100 g of pumpkin seeds has 547 calories). The benefits are expected from monounsaturated and polyunsaturated fatty acids, which play an important role in modulating plasma cholesterol and prevention of coronary heart disease risk.

CO_2 extraction is a relatively new technology, based on extraction with supercritical carbon dioxide. The efficiency of the extraction process depends on several parameters: the degree of grinding of raw materials, raw material moisture content, temperature, pressure and duration of the extraction process. [1]

A successful application of this method in order to obtain biologically active compounds extracted with pumpkin seed oil would allow the optimal combinations and qualities of these substances. It should be considered that such a method like CO_2-extraction applied in commercial area would have significant benefits for the food, pharmaceutical and cosmetic industries.

Since Moldova has favorable soil and climate conditions for growing pumpkins, and annual production is increasing (from 32625 t in 2005 to 63412 t in 2010), and the quantity of seeds produced increases, it is possible to obtain annually large quantities of pumpkin seed CO_2-extracts. [2]

Pumpkin seeds

Pumpkin seeds contain great concentrations of vitamins (vitamin E, K, ß-carotene, B_1, B_2, B_3, B_5, B_9), minerals (magnesium, manganese, iron, copper, phosphorus, zinc) and phytosterols. They are an important source of protein, unsaturated fatty acids and fiber, but they have a low quantity of carbohydrates. [3, 4, 5] Pumpkin seeds kernel contain a high percentage of crude protein (39.22 ± 2.46%) and oil (43.69 ± 3.92%), which makes pumpkin seeds a potential source of oil and vegetable protein. [6]

Often pumpkin seeds that remain after cooking are thrown with the pumpkin peel. But even our ancestors knew that they are very good for health, so they dried and used them as a medicine. [7]

Rich in vitamins and minerals, pumpkin seeds are both an ingredient and a healthy and delicious snack. With a high content of protein, are perfect appetizers for regaining energy. From seed it can prepare different powders, tinctures, infusions, poultices and decoctions. The infusion and decoction of crushed seeds are used to improve digestion and lactation. [7] The pumpkin seeds can be added to salad dressing in cereals with milk or yogurt in cakes or in various types of food. [8] These are used to produce oil and bread.

The humidity of the pumpkin seeds is relatively small which represents an advantage in terms of shelf life of seeds and for supercritical CO_2-extraction.

Materials and methods

To achieve the research it was used the local raw material pumpkin seeds *Cucurbita pepo, Bulgarian* variety from the company CP "Agro-Grebleşti" Grebleşti village, Straseni, Republic of Moldova. For research were used both whole pumpkin seeds and kernels. The dried seeds were dehulled manually to remove the kernels, and then were mixed. The flours were stored in the fridge at at 4 °C.

It was determinate the lipid content in pumpkin seeds by Soxhlet method.

It was made the organoleptic characteristic of CO_2-extracts of pumpkin seeds.

It was made the physicochemical characterization of CO_2-extracts by the following indicators: refractive index, specific gravity, iodine value, saponification value, free fatty acid, acid value, peroxide value and fatty acid composition.

Results and discussions

Determination of lipids content (Soxhlet method) in pumpkin seeds and pumpkin seed meal obtained after CO_2-extraction

The amount of lipid compounds was calculated according to the formula of Soxhlet method. Thus, it was determined that the content of lipids in pumpkin seeds is around 36.72%. Since lipids are concentrated in the kernel and not in shell, in pumpkin seeds kernel the lipid compounds are approximately 47.51%. It can be concluded that pumpkin seeds are an important source of fatty substances because about half of substances that are in the kernel of the seeds are lipids and the CO_2-extraction of these will have a higher yield.

After the CO_2-extraction, it was determined the lipid content by Soxhlet method in the pumpkin seed meal and pumpkin seed kernels meal. Following calculations established that the lipid content of pumpkin seed meal is 5.83% and the amount of fatty compounds remaining in the meal is about 7.08%. Based on the data obtained, it can be concluded that in CO_2-extraction process the most lipids are involved with supercritical carbon dioxide and in this way decreases the lipid content from 36.72% to 5.83% in pumpkin seeds and from 47.51% to 7.08% in pumpkin seeds kernel.

Organoleptic characteristic of CO_2-extracts of pumpkin seeds

The lipids were extracted from pumpkin seeds (*Cucurbita pepo*) by supercritical carbon dioxide and were obtained CO_2-extracts in 2 different separators.

It was performed the organoleptic analysis of CO_2-extracts from both separators, I (fig. 1) and II (fig. 2).

Fig. 1. Pumpkin seed CO_2-extract, I Separator

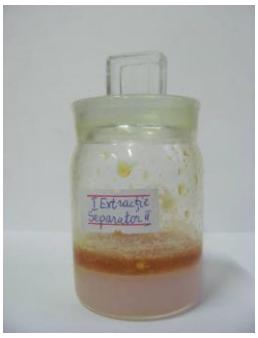

Fig. 2. Pumpkin seed CO_2-extract, II Separator

The specific characteristics of each sample: aspect and consistency, color, taste and odor are included in Table 1.

Table 1. Organoleptic characteristic of CO_2-extracts

Organoleptic indices	**Characteristics of pumpkin seed extracts**	
	I Separator	**II Separator**
Aspect and consistency	Transparent, the presence of sediment. Slight opalescence and the occurrence of greenish white flakes when refrigerated at t = 4 °C, which disappear at room temperature during (over a) 2 hours.	Consisting of two well separated phases: aqueous phase and the lipid phase. Less transparent, presence of sediment. White flakes green appearance when refrigerated at t = 4 °C, which disappear at room temperature for 2 hours.
Color	Dark amber yellow, slightly reddish tinted green.	Soluble phase - milky white. Liposoluble phase - yellow-brown (amber) closed slightly reddish in hue and reflections of green.
Taste and odor	Characteristic type of pumpkin seed oil, odorless, taste and bitterness.	Without stranger odor and taste or bitterness

The CO_2-extract of I Separator has a much lower water content 0.08% than the CO_2-extract of II Separator - 69.33%. It can be concluded that CO_2-extract of I separator is a lipid substance and it can be used as oil.

Also, according to the Technical Regulation "Uleiuri vegetale comestibile" for crude oil the mass fraction of moisture and volatile matter is 0.20% maximum. So this confirms that pumpkin seed CO_2-extract of I Separator meets the standards established by technical regulations.[9]

Physico-chemical characteristics of the pumpkin seed oil

As the extract of II Separator is not a homogeneous substance the physicochemical properties were determined only at the extract of I Separator. The physicochemical properties of pumpkin seed oil are presented in Table 2.

Table 2. Physico-chemical characteristics of the pumpkin seed oil

Physical and chemical indices	Unit of measure	Average value
Refractive index at 25°C		1,4718
Specific gravity at 20°C	g/cm^3	0,915
Iodine value	g I_2/ 100g oil	136,9 137,0
Saponification value	mg KOH/g oil	176.9

The determination of refractive index was made according to GOST 5482-90. From the data obtained it is noted that the refractive index is equal to 1.4718 for oil obtained by CO_2-extraction method, the amount that covers the range from 1.470 to 1.475 from the data of the literature. [6]

The determination of specific gravity with the hydrometer was made according to STAS 145-67. Following analyzes it was observed that the specific gravity of pumpkin seed oil obtained by CO_2-extraction is 0.915 g/cm^3. According bibliographic study, the specific gravity of pumpkin seed oil varies between 0.916 to 0.920 g/cm^3, so the analyzed oil is included in the given interval.

The saponification value in pumpkin seed oil was determined according to GOST 5478-90 and the iodine value was determined according to GOST 5475-69.

The analysis established that the calculated saponification value is equal to 176.9 mg KOH/g of CO_2-extracts. This index shows that the average molecular weight acids in pumpkin seeds oil are high.

According to literature sources, the iodine value in pumpkin seeds oil varies between 116 and 133 g I_2/100 g oil, but CO_2-extracts had a highest value, approximately 137 g I_2/100 g oil, that reflecting a high degree of unsaturation. [6]

The free fatty acid and the acid value were determined according to GOST 5476-80.

The peroxide value in pumpkin seed oil was determined according to GOST 26593-85.

In view to aver the stability of CO_2-extract it was studied and compared the evolution of the acid value and the peroxide value of it with the oil obtained by the Soxhlet method extracted with hexane.

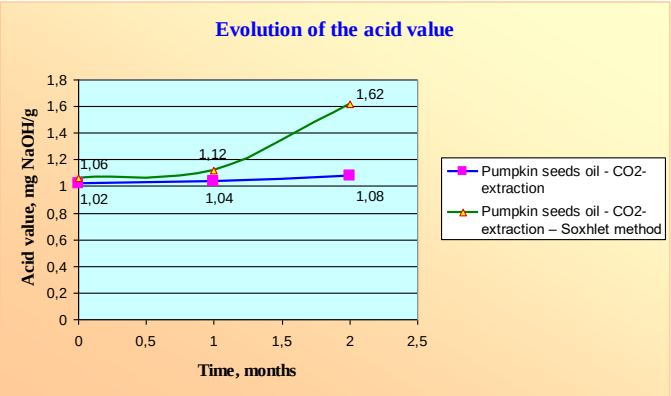

Fig. 3. Evolution of the acid value

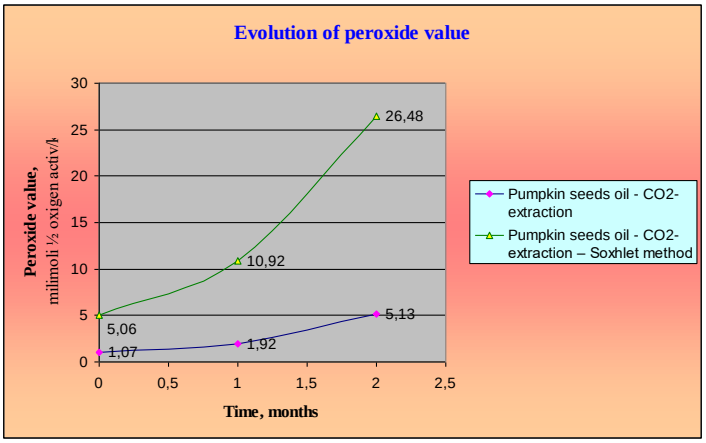

Fig. 4. Evolution of the peroxide value

In pumpkin seed oil extracted with supercritical carbon dioxide it was determined that the oil acid value of pumpkin seed kernels obtained by CO_2- extraction is low 1.02 mg NaOH/g, and respectively the free fatty acid, which is directly proportional to it, has a low value - 0.513% oleic acid. The CO_2-extract was stored at 4 °C, and the acid value increased for one month to 1.04 mg NaOH/g and after two months reached 1.08 mg NaOH/g. It can be said that, according to the acidity value, the degradation of oil occurs very slowly.

The acidity value of the pumpkin seed oil extracted with hexane by Soxhlet method is also rather low - 1.06 mg NaOH/g but during the storage was observed a faster increase trend of this indicator than in the case of the CO_2-extract.

The analyzed oil has a very good acid value because their values are lower than the norm for oils established technical regulation "Uleiuri vegetale comestibile" approved by Government Decision No.434 of May 27, 2010. This Technical Regulation which lays down minimum quality vegetable oils requires that for the unrefined oils the norm is 4.0 mg NaOH/g.[9]

From the facts obtained it is observed that initially the peroxide value of pumpkin seed oil obtained by CO_2-extraction is 1.07 ½ mmol active oxygen/kg, increasing to 1.92 ½ mmol active oxygen/kg after the first month of storage in the refrigerator, and reaching 5.13 ½mmol active oxygen/kg at the end of the two month period.

Peroxide limit for oils established technical regulation "Uleiuri vegetale comestibile" unrefined oils is 10.0 ½ mmol active oxygen/kg. Analyzing the data obtained shows that pumpkin seed oil extract by CO_2-extraction is an index that includes the peroxide value in the limits, and also this shows that CO2-extracts are stable. [9]

The pumpkin seed oil obtained by Soxhlet method extracted with hexane after a month of storage has a peroxide value higher than the limit laid down in the technical regulations, from 5.06 initially to 10.92 mmol ½ active oxygen/kg. It shows a fast evolution of the peroxide value, reaching 26,48 mmol ½ active oxygen/kg in two months.

The determination of fatty acid composition of CO_2-extract was made according to GOST 30418-96. The results are presented in Table 3.

Table 3. Fatty acids composition of pumpkin seeds oil

No.	Fatty acids	Content in CO_2-extract, %	Content in pumpkin seeds oil, GOST 30623-98 [10]
1	($C_{14:0}$) Myristic acid	0,1	-
2	($C_{16:0}$) Palmitic acid	5.9 -12.0	15.50
3	($C_{18:0}$) Stearic acid	2.64	3.0 to 6.0
4	($C_{18:1}$) Oleic acid (ω-9)	40.11	24.0 to 47.0
5	($C_{18:2}$) Linoleic acid (ω-6)	41.65	26.0 to 57.0
6	($C_{18:3}$) α-linolenic acid (ω-3)	marks	max. 9.0
7	Σ Saturated fatty acids	18.24	
8	Σ Unsaturated fatty acids	81.76	
9	Unsaturated/saturated fatty acids	4.48	
10	$C_{18:1}/C_{18:2}$	0.96	

As shown in table 3, even if the oleic and linoleic acid are in the limits of GOST 30623-98, the CO_2-extract has a larger amount of palmitic acid, and a insignificant amount of myristic acid which missing in GOST, and the α-linolenic acid is found only in trace amounts.

The amount of saturated fatty acids is 18.24% which it's represented by the palmitic acid 15.50%, stearic acid 2.64% and by the myristic acid 0.1%. The amount of unsaturated fatty acids is 81.76% and consists of 40.11% oleic acid and 41.65% linoleic acid. The linoleic acid is the major fatty acid followed by oleic, palmitic and stearic acid. The presence of great quantities of essential linoleic acid suggests that pumpkin seed oil is highly nutritious. Because pumpkin seed oil is rich in both oleic and linoleic acid, it can be used as edible oil for cooking and salads or for making margarine. [11]

According GOST 30623-98, pumpkin seed oil is part of the category oils with mass mostly linoleic acid of 26-82%. It can be confirmed that the analyzed CO_2 pumpkin seed extract belongs of this group of oils.

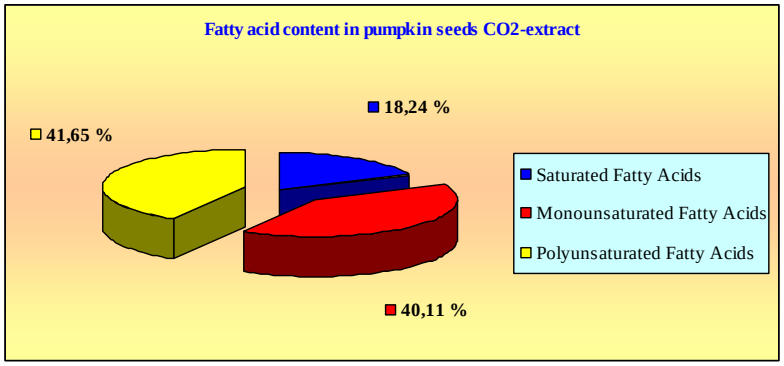

Fig. 5. *Fatty acid content in pumpkin seeds CO_2-extract*

In fig.5 it observed that the amount of saturated acids is much smaller than the unsaturated fatty acids, 18.24% versus 81.76%. According to the literature, it is recommended that more than 66% of total lipids consumed daily, should come from

unsaturated fatty acids formed. So, the fact is that pumpkin oil is an important source of unsaturated fatty acids that provide a healthy diet.

The total content of monounsaturated fatty acids is approximately 40.11% and polyunsaturated fatty acid is approximately 41.65%.

Report unsaturated fatty acids/saturated (ratio of the amount of unsaturated fatty acids to saturated fatty acids amount) has a low value - 4.48, which indicates that the shelf life should be long.

Conclusions

1. The CO_2-extract from I separator is a homogeneous liquid of fatty compounds that meets the standards established by technical regulations.
2. Pumpkin seeds extract have an important content of polyunsaturated fatty acids, including omega 6 and omega 9, which gives it therapeutic qualities.
3. The amount of unsaturated fatty acids (81.76%) is higher than that of saturated fatty acids (18.24%), and among the basic fatty acids includes oleic, linoleic, palmitic and stearic acid.
4. After two months of storage the storage CO_2-extract of pumpkin seeds was quite stable.

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