Determining the color of bio-oils from berry seeds obtained through supercritical extraction using UV-Vis spectrophotometry

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Introduction

Oils are an important part of a human's diet. Over 90% of the worldwide production of oils from natural plant, animal and sea materials is used as food or food elements. A special role is played by so-called bio-oils (also referred to as special oils). These are complex mixtures which contain a number of bioactive lipid compounds such as: phytosterols, tocopherols, tocotrienols, carotenoids, squale, polyunsaturated acids. In the majority of cases, these compounds are not synthesized by the human body; however, because of their various physical, chemical and physiological properties, their impact and help in ensuring proper physiological processes is very broad. Polyunsaturated acids are part of cell membranes, brain and retina structures; they take part in creating hormones, protect against the development of atherosclerosis, decrease the intensity of inflammations, lower the level of oxidative stress [4]. Carotenoids are natural antioxidants and constitute a significant factor in human health. β- carotene together with α-carotene and cryptoxanthin are precursors of vitamin A; lutein and zeaxanthin are carotenoids of the yellow spot of the eye where they accumulate and are responsible for its protection against photooxidation [5]. Tocopherols have a strong anti-oxidizing effect; they protect the body against the destructive influence of free radicals, preventing DNA damages or the appearance of degenerative diseases (atherosclerosis, infarctions, heart strokes).

The source of bio-oils are nuts (almonds, peanuts, hazelnuts, walnuts, pistachios), seeds (borage, pumpkin, sea-buckthorn, sesame, evening primrose), grains (amaranth, oats), as well as fruit and vegetables (cloudberry, tomato). In Poland, a valuable source of bio-oils may be berry seeds which can be found in byproducts of processing fruit for juice and beverages in so-called pomace.

One of the quality determinants which characterize oils is their color. The appearance of food is the first stage of evaluating the product's quality. The color influences the perception of food's taste and provides general information on the state and quality of the product. The color of the product which is unacceptable by the customer causes a loss of interest in that product.

The color of oils is the effect of natural pigments present in the initial materials, which are transferred to the product during oil extraction. In technologies of obtaining fats and oils from plant materials two groups of pigments are present most often: carotenoids and chlorophylls. The amount of both these groups depends on the type of material and its maturity, while the manner of their extraction influences the final content in the end product and decides about the obtained pigment of the oil. The interest in the content of pigments in oils results especially from the pro - or anti-oxidizing activity which influences the life of oils [3].

Carotenoids are a group of over 600 polyene pigments made of 8 isoprene units. They allow for obtaining a color from yellow to orange and red. They accompany chlorophyll in chloroplasts, give yellow color to fruit and flowers and are also present in seeds and underground parts of certain plants. They are considered the most effective, natural singlet oxygen quencher which improves the life of oils [3]. In products containing oxidizing lipids conversions of carotenoids are strictly related to the processes of autoxidation of unsaturated fatty acids. Free radicals and peroxides created during this process increase the rate of carotenoid degradation; on the other hand, pigments hamper the photooxidation of fatty acids. A number of studies have shown that, depending on the surrounding conditions, carotenoids may present a pro- or anti-oxidizing activity [3]. The pro-oxidizing activity is influenced mainly by the following factors: concentration of oxygen, carotenoids, light. The anti-oxidizing effect is supported by a synergistic reaction with tocopherols, a chemical structure containing functional groups, 9 or more double bonds, an acyclic chain structure, as well as the color of carotenoid and model of the lipid matrix [3].

Chlorophyll pigments are porphyrin pigments in which the basic particle skeleton constitutes a unit of four pyrrole rings with a central magnesium atom. Chlorophyll pigments are photosensitizers. In conditions of oil storage photochemical reactions with their participation activate the conversion of triplet oxygen into reactive singlet oxygen which initiates oxidative conversions of unsaturated fatty acids and carotenoids, among others [3÷5].

Experimental part

Characteristics of the method for obtaining bio-oils

Despite recognizing a critical point already in the 20-ties of the 19th century, the practical application of supercritical liquid properties was possible only after a considerable technological advance. Currently, an intensive development is observed of supercritical extraction processes in many industry branches - food, pharmaceutical, cosmetic, fodder, fuel, to name but a few.

The Institute of Fertilizers has been dealing with research on supercritical liquids for over 10 years. One of the main research directions is the application of supercritical liquids in processes of obtaining valuable substances from plant and animal materials through extraction. As a result of conducted studies, a technology of hop extraction has been developed and implemented. An industrial installation was constructed for producing hop extract for brewery purposes [6].

Currently, a technology is being developed for obtaining special oils from berry seeds through supercritical extraction using carbon dioxide. Research studies are conducted with the use of a ¼ technical installation, with an extractor capacity of approx. 20 dm^3, with a 3-stage product separation and a closed carbon dioxide circuit. The installation allows for performing tests in a broad range of extraction pressures (up to 400 bars) and a temperature up to 100°C, with the flow of the extracting gas up to 150 l/h.

The use of this method allows for obtaining bio-fuels without their exposure to oxidizing changes or thermal degradation. This method is fully safe for people and the environment. This is especially important taking into consideration the target use of the final product which must fulfill rigorous cleanliness standards: in the pharmaceutical, cosmetic and food industries. Moreover, this method is characterized by high output and selectivity with regard to active elements.

During research studies aimed at developing a technology for obtaining special oils, considerable color changes of the obtained oil were observed in the case of certain plant species, depending on the duration of the extraction. Thus, studies were undertaken on the qualitative perspective of the observed changes.

Material for color studies

The material for studies included bio-oil samples obtained during testing the kinetics of the bio-oil supercritical extraction from berry seeds from the 2010 crop. The parameters of oil extraction from seeds may have a significant impact on the color of the sample; thus, the experiments were conducted under the same conditions of pressure and temperature (320 bars and 50°C) for all three seed types (raspberry, chokeberry, strawberry) in order to obtain a comparable material for studies.

Instrument and analysis method

The tests were performed with the use of a double-beam UV-Vis spectrophotometer, model V-650.

The tests on bio-oil samples were performed through the spectrophotometric method, based on the Polish standard PN-A-86934 from November, 1995 [7]. The method involved measuring the size of oil samples' absorbance after their diluting in n-hexane, at a double length of waves in a visible spectrum:

for carotenoid pigments $\lambda = 442$ nm and for chlorophyll pigments $\lambda = 668$ nm. The obtained absorbance values are summed up and expressed in the form of integers, in accordance with equation (1).

$$B = 1000 \cdot (A_{442} + A_{668}) \quad (1)$$

The measurement of the absorbance value at double wave lengths also allowed for determining the impact of both pigment groups on the final color of the product.

Analysis of results

The stability of oils depends on the contents of natural pigments; thus, specifying the color of the oil constitutes very useful information. During the development of the seed extraction technology using supercritical carbon dioxide the oil color allows for specifying the time for obtaining an extract of the highest desirable pigment content.

Oil from raspberry seeds

Three oil samples obtained at different extraction times were subjected to color assessment. The color of the oil from raspberry seeds does not show significant changes in the extraction time and it may be specified as straw-colored. The obtained results of color parameters performed using the spectrophotometric method are presented in table 1.

Table 1

Color of bio-oil from raspberry seeds

Extraction time, min	Oil color
15	639
60	602
90	644

The analysis of individual pigment groups (carotenoids and chlorophylls) shows that carotenoids are the dominating group in the oil from raspberry seeds – their amount is 5 times bigger than the amount of chlorophylls.

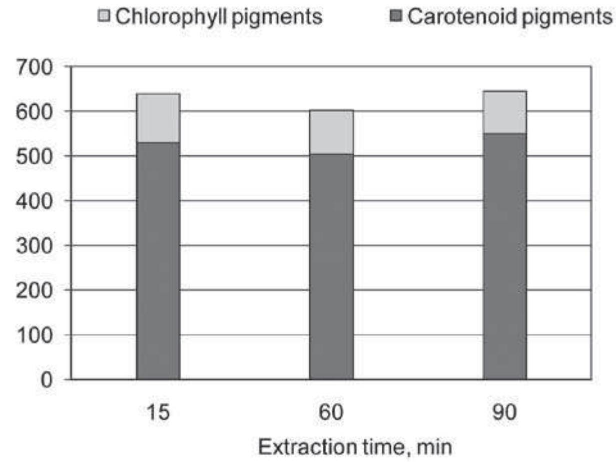

Fig. 1. Participation of pigments in the color of bio-oil from the raspberry seeds

Oil from chokeberry seeds

Four oil samples obtained at different extraction times were subjected to color assessment. The oil was orange. The obtained results of color parameters performed using the spectrophotometric method are presented in table 2.

Table 2

Color of bio-oil from chokeberry seeds

Extraction time, min	Oil color
15	1995
30	1947
75	1063
90	1003

The analysis of the participation of individual pigment groups shows that carotenoids dominate in the oil from chokeberry seeds; their amount is even 7 times bigger than chlorophyll pigments at the initial stage of extraction.

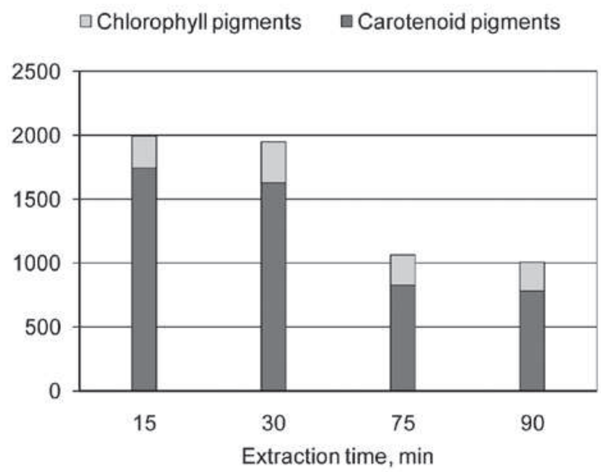

Fig. 2. Participation of pigments in the color of bio-oil from the chokeberry seeds

Oil from strawberry seeds

The obtained bio-oil showed a large variability of color during the extraction process - from light green through straw-colored to dark green. For a full analysis of the process of extracting pigments from strawberry seeds, the measurement of the obtained oil's color was performed in detail for extracts at a 15-minute time interval.

The obtained results are presented in table 3.

Table 3

Color of strawberry seeds bio-oil

Extraction time, min	Oil color	Extraction time, min	Oil color
0	1625	135	297
15	456	150	211
30	363	165	266
45	362	180	1155
60	349	195	1889
75	364	210	2177
90	330	225	2827
105	347	240	3299
120	460		

Straw-colored oil was obtained within 15÷165 minutes of extraction. Oil after compression (time 0) and in the final stage of extraction (extraction time above after 165 minute) is green and dark green.

The analysis of the participation of individual pigment groups shows that the variability of individual extracts' color results from a change in proportion of both pigment groups (Fig. 3).

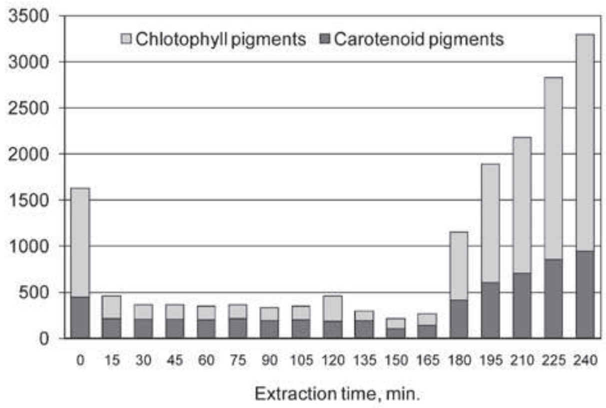

Fig. 3. Participation of pigments in the color of bio-oil from the strawberry seeds

In the bio-oil samples from the first extraction stage (extraction time 15÷165 minutes) the dominating group are carotenoids - approx. 30% more than chlorophylls. Oil samples after compression and from the final stage contain a majority of chlorophylls - over 2 times more than carotenoids.

A comparison of the obtained results with the extraction process curve leads to the conclusion that the change of oil color occurs at the moment of a decreased extract secretion (Fig. 4).

From the list of obtained color results related to the process output it stands that 78% of oil obtained through supercritical extraction from strawberry seeds constitutes oil of a weighted average color approx. 347; the remaining 22% is green oil of a color approx. 1945 with a prevailing content of chlorophyll pigments.

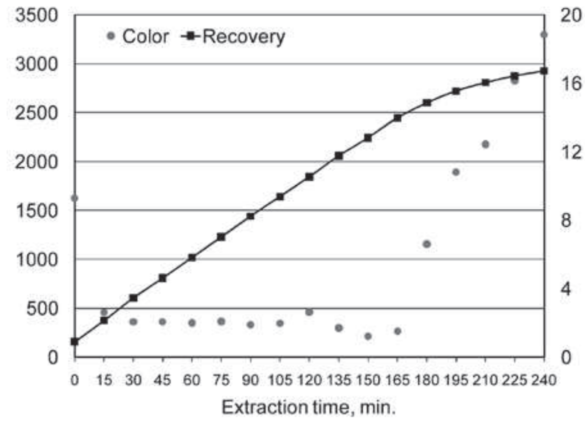

Fig. 4. Comparison of the color obtained from strawberry bio-oil with the kinetic curve of oil extraction

Examples of absorption spectra

In the tests on color absorbance measurements were performed for a specified wave length; however, for a couple of tested solutions full characteristics were gained of the UV-Vis spectrum of obtained oil extracts by performing a measurement of full absorption spectrum. The presented results pertain to oil samples from strawberry seeds prepared as for marking chlorophyll pigments, i.e. with a 1:1 dilution v/v in n-hexane (Fig. 5).

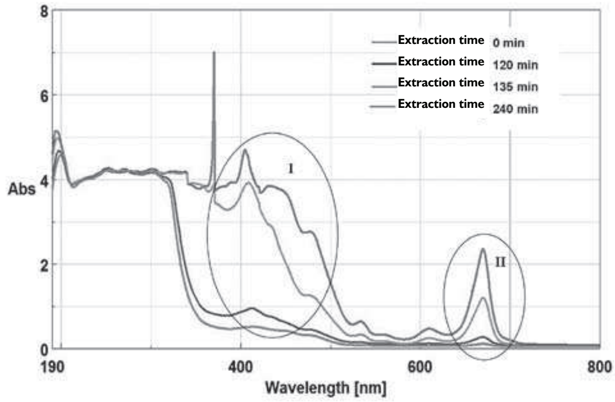

Fig. 5. The absorption spectra of bio-oil samples from the strawberry seeds (at 1ml oil / 1 ml n-hexane)

Absorption bands for carotenoid (I) and chlorophyll (II) pigments are clearly visible.

Summary and conclusions

Oil color is one of the characteristic physical and chemical parameters, dependent on the species and maturity degree of the plant from which the oil is extracted, as well as on the method of obtaining. The presented studies are a first analysis of this type pertaining to bio-oils obtained through supercritical extraction. The applied method of a spectrophotometric marking of color allowed for assessing the color of oils from three species of berry plants. The analysis of mutual absorbance relations of both color groups of carotenoid and chlorophyll compounds allows for a qualitative assessment of the obtained oil.

For example, the absorbance value for chokeberry oil obtained in the 75th minute of extraction is 1,063; a similar absorbance value is obtained for oil from strawberry seeds in the 180th minute of extraction – 1,155, although visually oil from chokeberry is orange and oil from strawberry – green.

In light of the carried out tests there is a considerable advantage of supercritical extraction over classic methods of obtaining oils (cold pressing, solvent extraction). The obtained results will contribute to developing technological guidelines which would allow for optimizing the duration of the extraction and the manner of separating the oil extract in such a way so as to obtain a product of the most desirable color to customers. This will also allow for reducing the content of chlorophyll compounds in the final product, thanks to which it will no longer be required to additionally clean the obtained oil of pigment compounds, and the stability of the product over time will improve. At the same time, the obtained data indicates that it is possible to control the contents of pigments in oils produced using an industrial installation by installing a suitable control and measurement instrument.

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World-class Polish metrology

Polish research into geometric probes for CNC machine tools are part of the international work on various issues related to measurements on machine tools. Dr. Eng. Adam Woźniak and his team of graduate students began to implement development grant funded by the National Centre for Research and Development for the amount of nearly PLN 800 thousand.

In an interview with PAP the scientist spoke of his research and compared the scientific work in Poland and abroad. The project concerns a new trend in global research - measurements of made objects directly on the CNC machines (called on-machine measurement).

Work of Dr. Woźniak is associated with a continuous search for new ways to increase accuracy and speed of measurements. For his PhD he investigated the CMM pulse probes. He analysed the metrological characteristics of existing measurement devices and their most important components - pulse probes, as well as the latest generation of active and passive scanning probes used to locate points of the measured object in the measuring space.

In his habilitation thesis defended with honours in March of this year, he proposed a new, original method of determining the adjusted points in scanning measurements, significantly reducing measurement errors. The solution raised interest of international manufacturers of measurement equipment and software (e.g., Quality Vision International, Inc. and Origin International Inc.), as well as the industry (e.g., Pratt & Whitney Canada). In this regard, Dr. Woźniak is also a co-author of a patent filed in the United States.

Research conducted by his international team will contribute to the development of modern measuring techniques of high-tech machines and equipment. Their results were published in May in the prestigious scientific journals, Precision Engineering and International Journal of Advanced Manufacturing Technology.

Dr. Wozniak's experience allows to compare research work in Poland and abroad. He worked as a researcher at the university in Canada, he also observed research work in Japan and the United States. He did not notice, however, as is commonly believed in Poland, a civilisation gap.

In his view, modern science projects require a broad interdisciplinary knowledge beyond the capabilities of one man. Therefore, their implementation requires forming a team of involved people focused on the project, and not working on and off. To make this possible, it is necessary to offer the people who work for the benefit of science appropriately salaries. Otherwise a capable programmer of electronic, who would earn much more in a private company, cannot be motivated to work in for a research project.

To illustrate he added that after his return from Canada to the Poland his co-operation with Canadian professors did not weakened. Since then they have done a lot of joint research and wrote a number of joint publications. Much of this research has been conducted on the equipment in his laboratory at the Warsaw University of Technology.

Coordinate Measuring Technology Laboratory of the Warsaw University of Technology headed by Dr. Eng. Adam Woźniak is currently equipped with high-end coordinate measuring machines from Carl Zeiss. In 2010 it purchased additional modern equipment for funds received from the Ministry of Science and Higher Education in an amount more than PLN 1.3 million, including equipment for the contact-free measurement using a laser scanner.

This year, next purchase of equipment for nearly PLN 2 million is planned, including computerized tomography for engineering applications. It will be one of the first such machines in Poland. Such equipment allows to pursue interesting scientific topics and work with the best research centres in the world.

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