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Physicochemical characteristic of seed oils of Bulgarian species pumpkin and melon

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Tocopherol, carotenoid and chlorophyll content of three pumpkin species (*Cucurbita moschata, Cucurbita pepo, Cucurbita maxima*) and three melon species (*Honeydew, Dessert 5* and *Hybrid 1*) grown in southern part in Bulgaria were investigated. Highest content of tocopherols was found to be in *Cucurbita moschata* and in *Honeydew* glyceride oils - 417 mg kg^{-1} and 828 mg kg^{-1}, respectively. Chlorophyll content was detected in melon species but this pigment was not observed in pumpkin species. In *Cucurbita maxima* and melon species *Hybrid 1* oils were established higher quantities of β-carotene - 1222.33 ppm and 35.97 ppm, respectively. The green components predominates in the oils of all melon varieties, while there are differences in pumpkin seed oils - from highly dominant red component in *Cucurbita pepo* to less pronounced red color in *Cucurbita moschata* and to a clearly dominant green component in *Cucurbita maxima*. Fluorescence spectra of three wavelength in visible region (λ=370 nm, λ=395 nm, λ=425 nm) and in ultraviolet region (λ=305 nm) were obtained. Correlation between intensity of fluorescence maximum at λ=384 nm and γ - tocotrienol content was found in different species from melon seed oils. There was correlation between intensity of fluorescence maximum at λ=675 nm and chlorophyll content, too. Other fluorescence maxima were found at λ=634 nm, λ=688 nm, λ=480 nm and λ=500 nm, which were connected with presence of different pigments. On the other hand, these fluorescence maximums were indicators of passing process of oxidation.

Key words: *pumpkin and melon seed oils, tocopherols, carotenoids, chlorophyll, fluorescence, colour parameters.*

INTRODUCTION

The *Cucurbitaceae* is a family of annual and perennial herbaceous vines, distributed in tropical, subtropical and temperate areas. It includes about 800 species in 125 genera. The *Cucurbitaceae* family includes gourds, melons, watermelons, pumpkins and squashes. They are characterized by their fleshy fruits.

The chemical content of the pumpkin family has been the object of a number of studies and it has been established that the content of the basic chemical components of the fruit vary in large ranges depending on the sort, the climatic conditions and the way of cultivation [1]. The dry substance of the fruit is within the limits from 6.0 % to 20.0 % out of which between 2.0 % and 14.0 % are sugars. Relatively high are the contents of starch (from 2.0% to 7.0%), fats and proteins (from 1.3 % to 1.5%) of the total fruit weight. They are a rich source of mineral salts of potassium, phosphorus, sodium, magnesium, etc.

Pumpkin seeds contain proteins from 25.0 % to 51.0 %, crude oils from 34.5 % to 60.0 % and in the fatty acid profile dominate linoleic acid – over 40.5 % and oleic acid – over 46.9 %, fibres – around 13.8 %, mineral substances in the range from 4.45 % to 5.00 % [2,3,4]. In the literature, information about the lipid content of oils from seeds of various sorts of the family *Cucurbitaceae* is based on research on the physico-chemical properties and the fatty acid contents of the oils, and the data obtained varied depending on the type of the seeds and the area of cultivation. Data on the content of tocopherols can be found in the literature in the fruit, in the peel, and in the seeds and the latter is the greatest in pumpkin. The content of γ-tocopherols in the seeds was 66.85 mg kg^{-1} and of the α-tocopherols was 25.74 mg kg^{-1} raw weight. The content of β-carotene is the highest in the peel of pumpkin (68.3 mg kg^{-1}), while in the seeds [5] it is 7.15 mg kg^{-1}. The studies on the lipid content of the seeds of local sorts of pumpkin are rather scanty. Cold pressed pumpkin oil is commercially available but it is still not widely popular due to its dark green color.

Melon seeds are characterized by no smaller values and fat contents is 25.0 % - 45.2 % with a high nutritional value, due to the high level of

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polyunsaturated fatty acids (linoleic from 51.0 % to 69.0 % and oleic 12.1 % to 31.0 % acids) [6-11]. Melon seed oil has still found no use, and there lack data on the lipid content.

The objectives of this work are to lead investigations about tocopherol composition of different pumpkin and melon seeds grown in Bulgaria and to examine the physico-chemical characteristics of the oils as color parameters and fluorescence in UV-VIS range.

EXPERIMENTAL

Samples. The pumpkin species *Cucurbita moschata*, *Cucurbita pepo* and *Cucurbita maxima*, and melon species *Honeydew*, *Dessert 5* and *Hybrid 1* from fam. *Cucurbitaceae*, were grown and obtained from the region of South Bulgaria, crop 2013.

Isolation of glyceride oil and determination of oil content. The seeds (50 g sample) were air-dried and ground to powder and the oil was extracted with n-hexane in *Soxhlet* apparatus for 8 h. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content [12].

Analysis of tocopherols. *T*ocopherols were determined directly in the oil by HPLC on a "Merck-Hitachi" (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000. The operating conditions were as follows: mobile phase of n-hexane:dioxan 96:4 (by volume), flow rate 1.0 ml/min, excitation 295 nm, emission 330 nm [13]. 20 μl 1 % solution of oil in hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual tocopherols. Reference tocopherol homologues were purchased from Merck (Darmstadt, Germany). The tocopherol content was calculated on the basis of tocopherol peak areas in the sample versus tocopherol peak area of standard α-tocopherol solution.

Colour parameters. CieLab colour parameters are measured directly by using spectrophotometer (Lovibond Tintometer PFX 195, UK). The content of β-carotene and chlorophyll are determined using special software.

The fluorescence of the samples was studied by exciting them with light emitting diodes (LEDs) emitting at 370 nm, 395 nm and 425 nm and in UV range at λ=305 nm. A 90 degree geometry of light detection in 10x10 mm cuvette were used. Samples were studied without any preliminary solution. For UV illumination the samples are fixed between two quartz plates. Fluorescence and scattering spectra are recorded using fiber-optic spectrometer *Avantes 2048* with a spectral sensitivity within the 250-1100 nm range.

RESULTS AND DISCUSSION

The investigated pumpkin *Cucurbita moschata, Cucurbita pepo* and *Cucurbita maxima* seeds from Bulgarian origin contain high quantities of glyceride oil (45.1 % - 51.5 %) which is rich in poly- and monounsaturated fatty acids especially linoleic acid (35.6 % - 50.8 %) and oleic acid (21.8 % - 35.9 %) and have low amounts of saturated fatty acids [14]. The oil content in the seeds of melon (*Honeydew, Dessert 5* and *Hybrid 1*) varied from 41.6 % to 44.5 % and the major fatty acid in total lipid was linoleic (51.1 % - 58.5 %), followed by oleic acid (24.8 % - 25.6 %) [15].

Tocopherol content and tocopherol composition of the seed oils are presented in Table 1.

Table 1. Total tocopherol content and tocopherol composition of different pumpkin and melon seed oils

Tocopherols	**Pumpkin**			**Melon**		
	Cucurbita moschata	*Cucurbita pepo*	*Cucurbita maxima*	*Honeydew*	*Dessert 5*	*Hybrid 1*
α – tocopherol, %	1.6±0.1	7.1±0.2	2.4±0.1	2.9±0.2	19.7±0.2	6.2±0.1
β – tocopherol, %	-	-	-	1.7±0.1	-	-
γ-tocopherol, %	88.4±0.4	71.7±0.5	58.1±0.2	91.5±0.5	71.4±0.4	78.5±0.2
γ – tocotrienol, %	10.0±0.1	21.2±0.2	39.5±0.4	3.9±0.1	8.9±0.2	15.3±0.3
Total tocopherol content, mg kg^{-1}	417±15	292±10	233±12	828±20	435±10	731±11

The contents of tocopherols were from 233 to 417 mg kg^{-1} and 435 - 828 mg kg^{-1} for the pumpkins and the melon, respectively. The highest content of tocopherols in pumpkin seed oils was found to be in variety *Cucurbita moschata* (417 mg kg^{-1}); and in melon seed oils - *Honeydew* and *Hybrid 1* (828 and 731 mg kg^{-1}, respectively). The content of tocopherols was found to be in *Cucurbita pepo* and *Cucurbita maxima* seed oils (292 - 233 mg kg^{-1}, respectively). According Ardabili et al. [16] and Gemrot et al. [17] the total tocopherol content in oil of pumpkin seeds (*Cucurbita pepo*) was 882.65 mg kg^{-1} and in the seeds was 107.0 mg 100 g^{-1}. Azhari et al. [18] reported for the total tocopherol content in *Cucumis melo* var. *tibish* seed oil was 43.20 mg 100 g^{-1}, which was similar to tocopherol content of oils from *Dessert 5*.

γ-Tocopherol (from 58.1 % to 88.4 %) was dominated component in *Cucurbita maxima, Cucurbita pepo and Cucurbita moschata* pumpkin seed oils followed by γ-tocotrienol (10.0 % - 39.5 %). In the oil from seeds of *Cucurbita pepo* was found a higher content of α-tocopherol (7.1 %) than the other two varieties (1.6 % - 2.4 %). M.Y. Kim et al. [5] reported that the seeds of *Cucurbita pepo* and *Cucurbita moschata* have significantly higher quantity of γ - tocopherol (61.65 mg kg^{-1} - 66.85 mg kg^{-1} in raw weight) than in *Cucurbita maxima* seeds (28.70 mg kg^{-1} raw weight). According to them the content of γ - tocopherol in oil from seeds of *Cucurbita pepo* and *Cucurbita moschata* was 2-3 times higher than α-tocopherol. In another reports in the oil of twelve pumpkin cultivars (*Cucurbita maxima*) α-tocopherol ranged from 27.1 μg g^{-1} to 75.1 μg g^{-1} in the oil, γ-tocopherol from 74.9 μg g^{-1} to 492.8 μg g^{-1} and δ - tocopherol from 35.3 μg g^{-1} to 1109.7 μg g^{-1} [19].

γ - Tocopherol predominated in all melon seed oils (71.4 % - 91.5 %). α - Tocopherol content in the oil from *Dessert 5* was highest (19.7 %), followed by *Hybrid 1* (6.2 %) and *Honeydew* (2.9 %). The content of γ-tocotrienol was found to be 15.3 % in *Hybrid 1*, 8.9% in *Dessert 5* and 3.9% in *Honeydew*. β-Tocopherol was detected in *Honeydew* oil in small amounts (1.7%).

The colour parameters of the considered oils were studied. The results are presented in Table 2.

Chlorophyll content was detected in oils from melon species (0.02-0.04 ppm) but in oils from pumpkin species this pigment was not observed. In *Cucurbita maxima* and melon species *Hybrid 1* oils were established higher quantities of β-carotene - 1222.33 ppm and 35.97 ppm, respectively.

Of all the studied pumpkin oils *Cucurbita pepo* is characterized by lowest luminosity value while for the rest luminosity is comparable. The observations for melon oils are analogous – lowest luminosity are of the *Hybrid* 1 sort, while the highest luminosity is of the oil from the seeds of *Dessert* 5.

In melon seed oils dominant is the green (*a<0.0*) and yellow (*b>0.0*) components. This is not valid for the pumpkin seed oil - it is red (*a>0.0*) for two sorts, while for *Cucurbita maxima* the green components dominates (*a<0.0*).

The fluorescence spectroscopy in the visible and ultraviolet region gives an opportunity for distinguishing of four main fluorescence peaks connected respectively with: the presence of tocopherols at λ= 346 nm and 384 nm; the presence of oxidation products at λ= 514 - 520 nm; the presence of chlorophyll at λ= 675 - 678 nm; the presence of pigments at λ= 620 nm and 700 nm.

Table 2. Colour parameters of oils from seeds of different sorts of melon and pumpkin

Type of oil	Pumpkin seed oil						Melon seed oil					
	Cucurbita moschata		*Cucurbita pepo*		*Cucurbita maxima*		*Honeydew*		*Dessert 5*		*Hybrid 1*	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Luminosity (L)	66.42	0.02	22.31	0.02	60.8	0.00	76.7	0.94	91.78	0.06	48.29	1.83
a	0.61	0.00	16.49	0.02	-7.31	0.01	-4.04	0.1	-5.21	0.01	-3.51	0.21
b	71.63	0.05	35.69	0.05	48.18	0.02	25.4	0.05	22.05	0.05	49.44	1.22
Chlorophyll, ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.02	0.00	0.02	0.00
β-carotene, ppm	72.91	2.32	62.84	4.78	1222.33	0.72	8.73	0.71	6.58	0.03	35.97	4.31

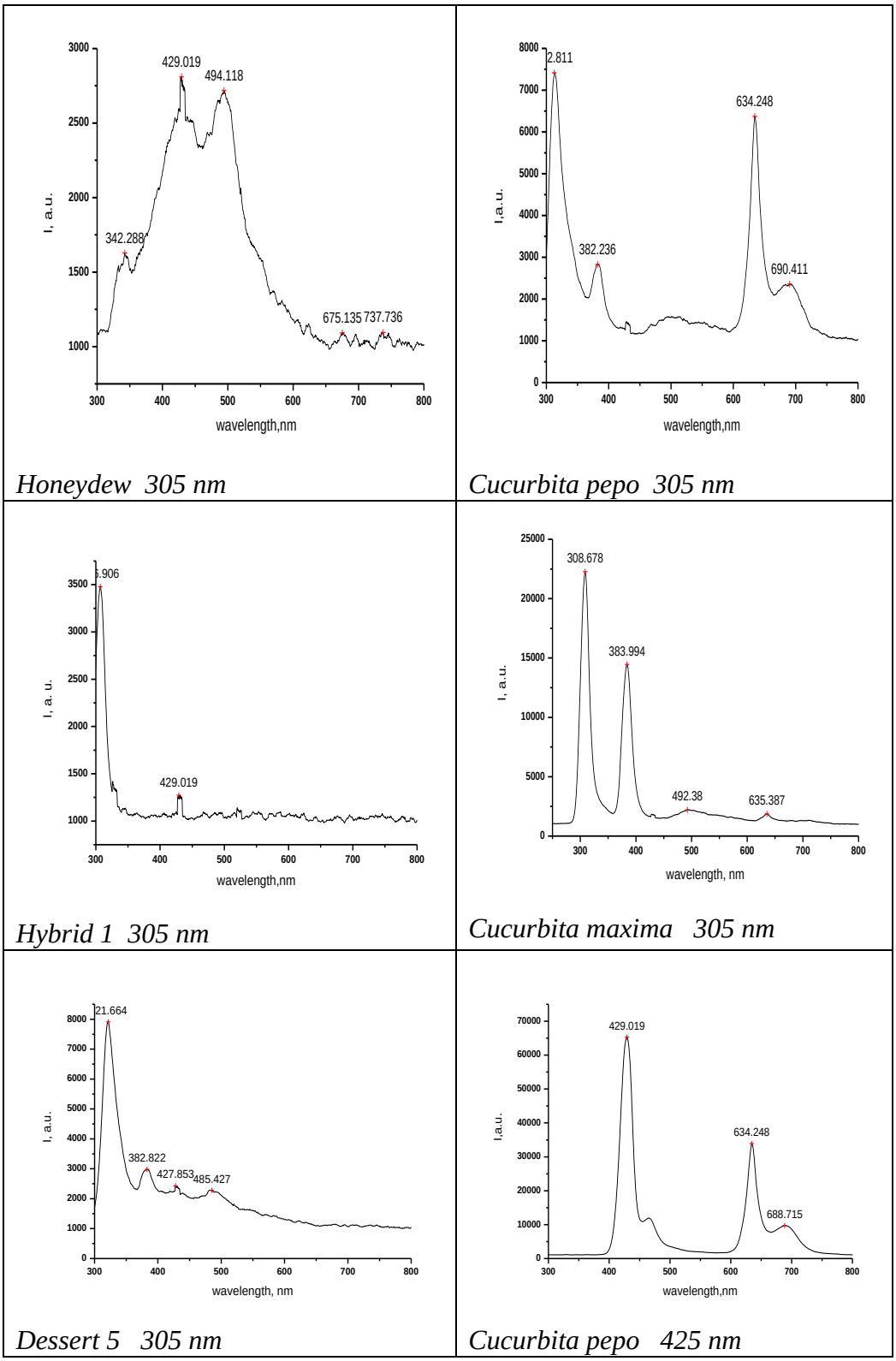

Fig. 1. The fluorescence spectra in the ultraviolet and visible ranges of oils from seeds of Bulgarian sorts of pumpkin and melon.

On analyzing the obtained spectra the best ratio of the fluorescence to the intensity of the excitation source and most fluorescence peaks are obtained at λ = 305 nm and λ = 425 nm. Fig. 1 presents fluorescence spectra of the considered oil samples of pumpkin and melon seeds in the visible and UV ranges at the indicated wavelengths.

The intensive peak at about 342 nm is observed only for oil from melon seeds of the *Honeydew* sort, when it is excited by a LED at λ = 305 nm.

The last fact can be explained with the highest total content of tocopherols from all oils obtained from different sorts of melons.

It is well known that the pigments of the chlorophyll groups are observed with oils extracted directly from seeds of different cultures prior to refining. Since melon oil of the *Honeydew* sort is the richest in chlorophyll (0.04 ppm), a strong fluorescence peak is observed at 675 nm when excited at 305 nm. For the rest of the melon oils no such peaks are observed because their chlorophyll content is comparatively lower (0.02 ppm). Similar fluorescence maxima have been observed for other vegetable oils such as rape seed, soy bean etc [20].

For all sorts of pumpkin oil chlorophyll content is 0.00 ppm and the observed fluorescence maxima around 634 nm and 688 nm are related to the existence of pigments [20] different from chlorophyll.

The maxima of fluorescence radiation are about 480 nm and 500 nm are related to the presence of oxidation processes in oils. From the intensity of this maximum it can be concluded that the presence of the oxidation products is the weakest for melon oil of the type *Hybrid 1*, followed by that of *Dessert 5*. Similar fluorescence maxima are between 500 nm and 520 nm for edible vegetable oils are found in [21]. According to the fluorescence maximum oxidation products exist in the pumpkin seed oil of the type *Cucurbita maxima*.

CONCLUSION

The melon seed oils have higher content of tocopherols than the pumpkin seed oils. The connection between tocopherol content, products of oxidations, chlorophyll, β-carotene and presence of pigments and the fluorescence maxima at different wavelength in UV-VIS were observed. It has been investigated small amounts of β-carotene and chlorophyll in these oils; the exception was the oil from *Cucurbita maxima* in which β-carotene was 1222.33 ppm. Because of the comparatively high content of tocopherols and lower content of oxidation products pumpkin and melon seed oils are very valuable sources of biologically active components and can be use for human consumption, added in foods and cosmetics. This is the first study about physicochemical characteristics of pumpkin and melon seed oils grown in Bulgaria and could take a place in further investigations on these oils and their application in food industries, cosmetics and medicine.

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ФИЗИКОХИМИЧНИ ХАРАКТЕРИСТИКИ НА МАСЛА ОТ СЕМЕНА НА БЪЛГАРСКИ СОРТОВЕ ТИКВА И ПЪПЕШ

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(Резюме)

Изследвани са токоферолния, каротеноидния и хлорофилния състав на семена от три сорта тиква (*Cucurbita moschata, Cucurbita pepo, Cucurbita maxima*) и три сорта пъпеш (*Медена роса*, *Десертен 5* и *Хибрид 1*), отгледани на територията на Южна България. Най-високо съдържание на токофероли е определено в маслата от *Cucurbita moschata* и *Медена роса* - 417 mg/kg и 828 mg/kg, съответно. Съдържание на хлорофил е открито в маслата от изследваните сортове пъпеш, но наличие на този пигмент в маслата от различни сортове тиква не е установено. В тиквеното масло от сорт *Cucurbita maxima* и в пъпешовото масло от сорт *Хибрид 1* са определени високи количества на β-каротен (1222.33 ppm и 35.97 ppm). Зелената компонента преобладава в маслата от всички изследвани сорта пъпеш, докато в маслата от тиква се наблюдават различия – от преобладаваща червена компонента в *Cucurbita pepo*, през по-слабо изразен червен цвят в *Cucurbita moschata* и до ясно доминираща зелена компонента в *Cucurbita maxima*. Получени са и флуоресцентните спектри при различни дължини на вълните във видимия (λ=370 nm, λ=395 nm, λ=425 nm) и в ултравиолетовия спектър (λ=305 nm). Представена е и зависимостта между флуоресцентния максимум при λ=384 nm и съдържанието на γ - токотриенол в маслата от семена на различните сортове тиква и пъпеш. Установено е и съществуването на зависимост между интензитета на флуоресцентния максимум при λ=675 nm и съдържанието на хлорофил. Други флуоресцентни максимуми са получени и при λ=634 nm, λ=688 nm, λ=480 nm и λ=500 nm, които са свързани с наличието на различни пигменти. От друга страна, тези максимуми са и индикатори за протичащи окислителни процеси в маслата.