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EVALUATION OF THE PROFILE AND CONTENT OF CHLOROPHYLL PIGMENTS AND ACIDITY IN SELECTED COLD PRESSED OILS

***Monika Bartosiak*[1], *Karol Mińkowski*[2]**

[1] ORCID:0000-0002-9617-3103
[2] ORCID:0000-0001-6217-9403
[1,2] Meat and Fat Technology Department
Institute of Agricultural and Food Biotechnology, Warsaw, Poland

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Abstract

The work evaluated the profile and content of chlorophyll pigments in cold pressed oils from rapeseed, flax, camelina, hemp, safflower, pumpkin, milk thistle, and from olive and avocado fruits, as well as the degree of hydrolysis of the oils examined. Content of chlorophylls *a* and *b* and their derivatives, being pheophytin *a*, pheophytin *b*, pyropheophytin *a* and pyropheophytin *b* were simultaneously determined in one sample, by modified and validated reversed phase HPLC method. The degree of hydrolysis of the oils was determined by their acid value. Market cold pressed oils have a very different content of chlorophyll pigments, regardless of whether they are from seeds or fruits. Considerable amounts were found in hemp oil (79.82 mg kg^{-1}) and pumpkin oil (57.55 mg kg^{-1}), and small quantity in flax oil (1.08 mg kg^{-1}).The content of chlorophyll pigments in extra virgin olive oil was an intermediate level (15.44 mg kg^{-1}). Chlorophyll derivatives dominate in the profile of chlorophyll pigments it is pheophytin *a*, pheophytin *b*, pyropheophytin *a* and pyropheophytin *b*. Extra virgin olive oil had the highest share of pheophytins, on average 82%. A statistically significant correlation (r = 0.6509) was found between the percentage share of pheophytins in the total content of chlorophyll pigments and the acid value of extra virgin olive oil. It was noted that the percentage share of pyropheophytins in the total of pheophytins and pyropheophytins can be an indicator of the bioconversion of chlorophylls during storage of oils. There was a statistically significant negative correlation (r = –0.8836) between the percentage share of pyropheophytins in the total of pheophytins and pyropheophytins and the length of the period remaining until the expiry date of extra virgin olive oil.

Address: Karol Mińkowski, Institute of Agricultural and Food Biotechnology, ul. Jubilerska 4, 04-190 Warsaw, Poland, phone: +48 (22) 509 70 23, e-mail: Karol.Minkowski@ibprs.pl

Introduction

Chlorophylls are commonly green plant pigments. The major chlorophylls in plants include chlorophyll a and b, which occur approximate ratio 3 : 1 (SCHWARTZ and VON ELBE 1983). Chlorophyll pigments are natural components of oilseeds and fruits. It is known, that significant differences in contents of chlorophyll pigments in them occur depending on degree of maturity, which is determined by region of cultivation, form (winter or spring) and term and conditions of harvest (GANDUL-ROJAS et. al. 2016, ROCA et. al. 2016). Contents of chlorophyll pigments depend also on drying and storage conditions (KANAI et. al. 2010). They are included in all vegetable oils, in quantities depending on their content in raw material, pre-treatment parameters, pressing and purification (CRIADO et. al. 2007). The chlorophyll molecule consist of a central magnesium atom surrounded by a nitrogen-containing structure called a porphyrin ring; attached to the ring is a long carbon-hydrogen side chain known as a phytol. Chlorophyll is readily degraded when exposed to heat, light, oxygen, acids, and enzymes. Chlorophyll can degrade to undesirable grey-brown compounds such as pheophorbide and pheophytin. This degradation is mediated by acid and the enzyme chlorophyllase. Pheophorbide can be further metabolized to colorless compounds in metabolically active tissue. The effect of elevated temperature causes formation of pyro-compounds (HEATON and MARANGONI 1996, ROCA et. al. 2016). The hydrolytic, structural and thermal changes can have a significant influence on the content and composition of chlorophyll pigments (GANDUL-ROJAS et. al. 2016). Green color losses in processed and minimally processed fruit and vegetable products are associated with decrease in of quality of such products (HEATON and MARANGONI 1996). The presence of chlorophyll pigments in cold pressed oils has a negative effect on their taste, smell, color, clearness, thermal stability and shelf life. Those compounds are strong photosensitisers in oxidation processes of unsaturated bonds of lipids, that negatively impacts the oxidative stability of oil during storage (SYMONIUK et. al. 2018). Chlorophylls are photosensitizers that allow oxygen to transform into a singlet form that initiates the oxidation of unsaturated fatty acids (CHOE and MIN 2006). This is especially important when choosing the packaging and oil storage conditions (GARGOURI et. al. 2015). Cold pressed oils represent a small fragment of market food oils, but an increase in consumer interest in these products due to high nutritional properties has been observed (BRŰHL and MATTHÄUS 2006). They are obtained without changing the nature of the oil, by mechanical means, e.g. by expelling or pressing, without the use of heat. This category also includes virgin oils, i.e. oils and fats

obtained as a result of mechanical action and the possible use of thermal processing in the technological process. They can be cleaned only by applying water, sedimentation, filtration or centrifugation (*Codex Alimentarius* 2015). They are not refined, and therefore they are a rich source of antioxidants such as tocopherols, polyphenols and squalene (TUBEROSO et. al. 2007). These also contain polyunsaturated fatty acids from the n-3 and n-6 groups as well as sterols, which exert a bioactive effect (CHOO et. al. 2006, RACZYK et. al. 2016, RĘKAS et. al. 2016, TEH and BIRCH 2013). Chlorophylls and its derivatives play an important role in human nutrition, as anti-cancer factor, having antioxidant and anti-mutagenic activities (FERRUZZI and BLAKESLEE 2007). The content and profile of chlorophyll pigments plays an important role in oil stability (PSOMIADOU and TSIMIDOU 1998). Important parameter of cold-pressed oils is their acidity, which has significant influence on chlorophyll pigments profile (HEATON and MARANGONI 1996). Cold pressed oils were subject to numerous research, in which content of chlorophyll pigments was determined in total, after recalculation to pheophytin *a* (CHOO et. al. 2006, RACZYK et. al. 2016, RĘKAS et. al. 2016, SYMONIUK et. al. 2018, TEH and BIRCH 2013). In some research, knowledge of profile of chlorophyll pigments was used to determine quality and authenticity of olive oils (ANNIVA et. al. 2006, LAZZERINI et. al. 2016). For identification and quantitative determination of chlorophyll pigments mostly spectroscopic and high-performance liquid chromatography techniques were used (*AOCS Official Method* 2017, ISO 2014:AMD 1:2016, LAZZERINI et. al. 2016, PSOMIADOU and TSIMIDOU 1998).

Test materials were market oils popular among Polish consumers, that differed by term of shelf-life, acidity and content of chlorophyll pigments. It was taken into consideration that hydrolytic and structural changes that occur during their storage have significant influence on bioconversion of chlorophyll pigments. Hydrolytic changes and an increase in oil acidity have a direct impact on the pheophytinization of chlorophylls. Water in oil, involved in the hydrolysis of triacylglycerols, has only an indirect effect and therefore has not been determined. Chlorophylls are involved in the oxidation of vegetable oils in the presence of light. The kinetics of unsaturated lipids oxidation is influenced by a number of pro and antioxidant factors. Chlorophylls, in addition to metal ions such as Fe, Cu and enzymes, are an important pro oxidative factors. Oils also contain a number of other compounds with antioxidant activity, such as: tocopherols, sterols, phenolic compounds, squalene and corotenoids. The course of lipid oxidation process is also influenced by the type of packaging and storage conditions. It was found that with such multidirectional influence of various factors on the course of oxidation of market cold pressed oils, it will

be difficult to link these processes, measured only by the peroxide value, with the profile of chlorophylls. According to SYMONIUK et. al. (2018) total chlorophylls content is only weak correlated with the induction time measured in the Rancimat test.

The purpose of the work was evaluation of the profile and content of chlorophyll pigments and acidity in selected cold pressed oils. The work was also aimed at determining the relationship between profile of chlorophyll pigments and the degree of hydrolysis of oil and the time till the end of shelf-life.

Materials and Methods

Materials

The test materials were oils, from seeds and fruits, delivered to the retail network by various producers. The oils were cold pressed according to declaration of producers. Oils were purchased in one of Warsaw's supermarkets. The oils came from the seeds of: rapeseed, flax, camelina, hemp, safflower, pumpkin, milk thistle and from olives and avocado fruits. In total, 45 oil samples were tested. All oils were in their shelf-life. The period from testing time to the end of the shelf-life was determined basing on the dates printed on the packaging. The oils were packed in glass bottles, light, brown or green, with a capacity from 0.25 to 1 dm^3 (Tab. 1). Chemical analyzes were performed within 10 days of purchase of oils. During analysis the samples of oils were stored in freezing conditions.

Table 1

Kind of oil and their origin, volume and color of packaging and periods up to the end of shelf-life

Kind of oil	No.	Brand name / Country of origin	Color of glass of bottle	Bottle volume [dm^3]	Best for period [month]
1	2	3	4	5	6
Rapeseed	1	Oleofarm / Poland	brown	0.5	6
	2	Semco / Poland	brown	0.25	6
	3	Kruszwica / Poland	green	0.5	8
	4	Olvita / Poland	clear	0.5	10
	5	Olandia / Poland	clear	0.5	10
Flax	1	Semco / Poland	green	0.25	3
	2	Kruszwica / Poland	green	0.25	1
	3	Eurolen / Poland	brown	0.5	2

cont. Table 1

Camelina	1	Semco / Poland	brown	0.25	4
	2	Olvita / Poland	brown	0.25	4
	3	Olandia / Poland	green	0.25	3
Hemp	1	Oleofarm / Poland	brown	0.25	9
	2	Olvita / Poland	brown	0.5	8
	3	Olini / Poland	brown	0.25	10
Safflower	1	Oleofarm / Poland	brown	0.25	4
	2	Olvita / Poland	clear	0.25	8
	3	Perlo / Poland	brown	0.5	6
Pumpkin	1	Oleofarm / Poland	brown	0.25	9
	2	Kruszwica / Poland	green	0.25	8
	3	Monini / Italy	green	0.25	6
Milk thistle	1	Oleofarm / Poland	brown	0.25	2
	2	VitaCorn / Poland	green	0.25	5
	3	Herbanordpol / Poland	brown	0.5	4
Olive extra virgin	1	Carras Queno/ Spain	clear	0.25	2
	2	Los Raigones / Spain	brown	0.5	15
	3	OlivaOro / Spain	brown	0.5	5
	4	Carrefour / Tunisia	green	0.25	10
	5	Goccia D˙oro / Italy	clear	1	12
	6	La Espanola / Spain	clear	0.75	5
	7	La laguna / Spain	green	0.5	17
	8	Carras Queno / Spain	clear	0.5	12
	9	Pons / Spain	green	0.5	17
	10	Cirio / Italy	brown	1	13
	11	Melissa Primo Gusta / Greece	brown	0.5	21
	12	Goccia D˙oro / Italy	clear	1	19
	13	Olivari / Portugal	green	0.5	30
	14	Olitalia / Italy	green	0.5	12
	15	Ondoliva / Spain	green	1	10
	16	Monini Delicato / Italy	green	1	19
	17	Monini Classico / Italy	green	0.5	31
	18	Salvadori / Italy	clear	0.5	12
	19	Di Carlo / Italy	clear	0.5	9
Avocado	1	Olvita / Kenya	brown	0.25	5
	2	Frontier Foods / Mexico	green	0.25	5
	3	Oleofarm / South Afrika	brown	0.25	7

Methods

Content of chlorophylls a and b and their derivatives, it is pheophytin *a*, pheophytin *b*, pyropheophytin *a* and pyropheophytin *b* were simultaneously determined in one sample, by modified and validated reversed phase HPLC method (ISO 29841:2014/AMD 1:2016) The analysis of chlorophyll pigments was carried out with using reference substances. Both chlorophylls: *a* (chl *a*) and *b* (chl *b*) were purchased from Sigma Aldrich (Poznan, Poland). Pheophytin *a* (phy *a*) and pheophytin *b* (phy *b*) were prepared from corresponding chlorophylls by acidification ether solutions with 13% hydrochloric acid (SCHWARTZ and VON ELBE 1983). Pyropheophytin *a* (pyr *a*) and pyropheophytin *b* (pyr *b*) were prepared from corresponding pheophytins dissolved in pyridine, by heat treatment at 110°C (PENNINGTON et. al. 1964). The HPLC-grade solvents, methanol and acetone, were obtained from Avantor (Gliwice, Poland), and diethyl ether from Sigma Aldrich (Poznan, Poland). De-ionized water was made using a Milli-Q purification system from Millipore (Bedford, MA, USA). Other chemicals were of analytical-reagent grade and were used without further purification. Hydrochloric acid (purity ≥ 37%) and sodium sulfate anhydrous (purity ≥ 99.0%) were purchased from Avantor (Gliwice, Poland) and pyridine anhydrous (purity ≥ 99.8%) were obtained from Sigma Aldrich (Poznan, Poland). Mobile phases were filtered through a Millipore 0.22 μm membrane filter before usage. An HPLC system (Agilent Technologies Series 1100, Santa Clara, CA, USA) composed of a G-1379A vacuum degasser, a G-1311A quaternary pump, a G-1313A autosampler, a G-1316A column oven and G-1321A fluorescence detector. Agilent Chemstation for LC and LC/MS systems software was used. Vortex mixer was from JWE-electronic, Warsaw, Poland. The C18 column Aeris PEPTIDE XB (3.6 μm, 250 mm length x 4.6 mm ID.) were from Phenomenex Corp. (Torrance, CA, USA). FLD detector (excitation wavelength λ_{ex} = 430 nm and emission wavelength λ_{em} = 670 nm) was employed, and program elution with 0.8 ml/min flow rate at temperature 25°C was used. The injection volume was 20 μL. The mobile phase was a gradient prepared from water : methanol : acetone 4 : 76 : 20 (solvent A) and methanol : acetone 30 : 70 (solvent B). Initial conditions: 100% A by 3 min, next decrease of share of A to 0% by 10 min, holding that condition by 18 min, after which increase share A to 100% by 22 min and stabilization of system in initial conditions by 30 min. Identification of chlorophylls and derivatives was carried out on the basis of spectral analysis using the UV-VIS spectrophotometer at wavelengths from 400 to 700 nm. Calibration curves were prepared using calibration standard solutions. Limits of detection (LOO_s) and limits of quan-

tification (LOQ_s) were determined. Content of chlorophylls and derivatives was calculated, using equations of standard curves, experimentally determined for examined ranges of concentrations. 2 g of oil was weighed into a glass tube and acetone was added to volume of 10 ml. The sample was vortex mixed for 1 min.

The acid value (AV) of oils was determined according to the ISO 660:2010.

Statistical analysis. All analyses were made in triplicates. Statistical analysis was performed using Statistica 13, TIBCO Software Inc. (2017). Evaluation of significance of deviations of average contents of chlorophyll pigments in oils was done using one way analysis of variance and Tukey`s post hoc tests. Correlation coefficients were also determined, to show the relations between percentage share of pheophytins, in total content of chlorophyll pigments and acid value, and between percentage share of pyropheophytins in sum of pheophytins and pyropheophytins and the amount of time till the expiry date. All tests were done assuming significance level of 0.05.

Results and Discussion

The market characteristics of the oils are given in Table 1. It shows that all cold pressed oils were packed in glass bottles. More than 20% of the tested oils were improperly packaged in clear glass bottles. In oils packed in clear glass, due to the access of light, accelerated oxidation takes place initiated by photochemical reactions catalyzed by chlorophyll pigments (ROCA et. al. 2016). Unfavourable changes can be reduced by using the right type of packaging, such as dark glass bottles and metal cans or as well as by reducing the air content in the packaging by blowing inert gases and forming a cushion of these gases above the oil surface (GARGOURI et. al. 2015, WRONIAK et. al. 2016). The cold-pressed oils were characterized by very different shelf life declared by producers – from seeds between 3 (flax) and 24 months (safflower) and from fruits between 12 (avocado) and 36 months (olive extra virgin). Time periods from date of analysis up to the end of shelf-life products, differs – from seeds, between 1 and 10 months and from fruits, between 2 and 31 months (Tab. 1, col. 6). This could have some influence on the results obtained (GARCIA-GONZÁLEZ et. al. 2008, VUJASINOVIC et. al. 2010). The results of our research are presented in Table 2 (for seeds), and 3 (for fruits). There was a significant variation in the content of chlorophyll pigments depending on the kind of oil.

Table 2

Content of chlorophyll pigments and acid value of oils derived from seeds

Kind of oil	No.	Content of chlorophyll pigments [mg kg^{-1}]							AV [mg KOH g^{-1}]
		chl *a*	phy *a*	pyr *a*	chl *b*	phy *b*	pyr *b*	total	
Rapeseed	1	0.73	0.32	0.24	n.d.	0.09	0.06	1.44	1.05
	2	0.41	1.76	0.31	n.d.	0.18	0.22	2.88	2.57
	3	0.59	0.12	n.d.	n.d.	0.06	0.02	0.79	1.12
	4	0.46	0.70	0,26	n.d.	0.12	0.14	1.68	1.33
	5	0.55	1.95	0.34	n.d.	0.20	0.28	3.32	2.89
	$\bar{x}$ ± SD	0.55^{a} ± 0.12	0.97^{a} ± 0.84	0.23^{a}± 0.12	n.d.	0.13^{a}± 0.06	0.14^{a}± 0.11	2.02^{a}± 1.05	1.79 ±0.87
Flax	1	0.49	0.11	n.d.	n.d.	0.07	nd.	0.67	1.01
	2	0.44	0.05	n.d.	n.d.	0.05	n.d.	0.54	0.97
	3	0.47	1.19	n.d.	n.d.	0.27	0.11	2.04	1.94
	$\bar{x}$ ± SD	0.47^{a}± 0.03	0.45^{a}± 0.03	n.d.	n.d.	0.13^{a}± 0.12	0.04^{a}± 0.05	1.08^{a}± 0.83	1.31± 0.55
Camelina	1	0.51	2.51	0.27	n.d.	0.46	0.50	4.25	2.05
	2	0.48	0.60	0.37	n.d.	0.18	0.15	1.78	1.27
	3	0.26	1.37	0.36	n.d.	0.27	0.20	2.46	1.46
	$\bar{x}$ ± SD	0.42^{a}± 0.14	1.49^{a}± 0.14	0.33^{a}± 0.06	n.d.	0.30^{a}± 0.14	0.28^{a}± 0.19	2.83^{a} ± 1.26	1.63 ±0.45
Hemp	1	0.69	38.02	3.89	3.02	9.68	7.78	63.08	3.01
	2	0.88	61.54	6.82	n.d.	25.98	15.17	110.39	3.59
	3	0.99	41.17	5.87	2,19	8.78	6.98	65.98	2.26
	$\bar{x}$ ± SD	0.85^{a}± 0.15	46.91^{b}± 12.77	5.53^{c} ± 1.49	1.74± 1.27	14.81^{b}± 9.68	9.98^{b}± 4.52	79.82^{b}± 26.52	2.95 ±0.67
Safflower	1	0.44	0.14	0.21	n.d.	0.09	0.03	0.91	1.15
	2	0.49	0.11	0.42	n.d.	0.19	0.28	1.49	1.22
	3	0.20	0.24	0.18	n.d.	0.15	0.05	0.82	1.11
	$\bar{x}$ ± SD	0.38^{a}± 0.16	0.16^{a}± 0.07	0.27^{a}± 0.13	n.d.	0.14^{a}± 0.05	0.12^{a}± 0.14	1.07^{a}± 0.36	1.16 ± 0.06
Pumpkin	1	17.94	12.98	1.77	n.d.	31.63	8.76	73.08	1.17
	2	10.23	6.99	2.11	n.d.	23.82	4.01	47.16	3.31
	3	16.01	7.98	2.18	n.d.	21.00	5.23	52.40	2.01
	$\bar{x}$ ± SD	14.73^{b}± 4.01	9.32^{a}± 3.21	2.02^{b}± 0.22	n.d.	25.48^{b}± 5.51	6.00^{b}± 2.47	57.55^{b}± 13.71	2.16 ±1.08
Milk thistle	1	0.50	0.94	0.39	n.d.	0.41	0.23	2.47	1.27
	2	0.44	0.89	0.28	n.d.	0.30	0.11	2.02	1.04
	3	0.31	1.15	0.27	n.d.	0.27	0.15	2.15	1.34
	$\bar{x}$ ± SD	0.42^{a}± 0.10	0.99^{a}± 0.14	0.31^{a}± 0.07	n.d.	0.33^{a}± 0.07	0.16^{a}± 0.06	2.21^{a} ± 0.23	1.22 ±0.16

Values (means ± SD) bearing different superscripts are statistically significantly different ($p < 0.05$); n.d. – not detected; chl *a* – chlorophyll *a*; phy *a* – pheophytin *a*; pyr *a* – pyropheophytin *a*; chl *b* – chlorophyll *b*; phy *b* – pheophytin *b*; pyr *b* – pyropheophytin *b*; AV – acid value

The most chlorophyll pigments in oils from seeds contained hemp oil of an average of 79.82 mg kg^{-1}. These are amounts typical for oil pressed from mature seeds (TEH and BIRCH 2013*)*. The rich source of chlorophyll pigments was also pumpkin seed oil (an average of 57.55 mg kg^{-1}). It is clearly higher than data earlier presented (SYMONIUK et. al. 2018). Considerably less chlorophyll pigments were found in other oils. The total content of chlorophyll pigments in rapeseed oil was on average 2.02 mg kg^{-1} (from 0.79 to 3.32 mg kg^{-1}), which is in agreement with previously published data (GHAZANI et. al. 2014, RĘKAS et. al. 2016). The higher level of chlorophyll pigments in cold pressed rapeseed oil is presented by YANG et. al. (2013). Content of these compounds in flax oil was on average 1.08 mg kg^{-1} (range from 0.54 to 2.04 mg kg^{-1}). These quantities do not differ from the literature data (CHOO et. al. 2006, RACZYK et. al. 2016) but are clearly smaller than other presented by TEH and BIRCH (2013). The content of chlorophyll pigments in camelina oil ranged from 1.78 to 4.25 mg kg^{-1}. Smaller variability was presented by SYMONIUK et. al. (2018). The content of chlorophylls in milk thistle oil was on average 2.21 mg kg^{-1} and do not differ from the literature data (MALEKZADEH et. al. 2011). In extra virgin olive oil, the average content of chlorophyll pigments was 15.44 mg kg^{-1} (variability from 2.16 to 37.94 mg kg^{-1}). These are the content typical for this product (GANDUL-ROJAS et. al. 2016). Avocado oil contained significant amounts of chlorophyll pigments (average 73.56 mg kg^{-1}), many times exceeding the literature data (WONG et. al. 2010). This can be caused by the immaturity of the fruits and technological process. The process for recovering oil from avocados is mechanical extraction, similar to olive oil extraction, with the additional step of removing the skin. Skin is abundant source of chlorophylls and when the technological process has been incorrectly carried out it remains in the raw material subjected to cold pressing (WONG et. al. 2011). Chlorophylls a and b were a small fragment of a pool of chlorophyll pigments in analyzed oils. The proportion of chlorophyll a varied from 0.5% in olive oil to 27.2% in rapeseed oil. A significant, unusual share of chlorophyll a was in flax oil (43.5%) may be due to seed immaturity. Chlorophyll b was absent in rapeseed, flax, camelina, milk thistle and safflower oils. The proportion of chlorophyll *b* in other oils ranged from under 0.1% in avocado oil to 2.2% in hemp oil. The lack or small content of chlorophylls *a* and *b* in oils indicates their profound changes to derivatives that took place from the raw material to the point of analysis. The oils were dominated by pheophytins a and b. Share of pheophytins in total content of chlorophyll pigments varied from 53.7% (flax oil) to 77.3% (hemp oil). The exception was safflower oil, in which deep bioconversion of pheophytins to pyropheophytins occurred. Share of phe-

ophytins in total content of chlorophyll pigments was high in fruit oils, 72.9% in avocado oil and 81.5% in extra virgin olive oil. In case of extra virgin olive oil, the significant amount of pheophytins is typical for technological process applied. The fruits of the olives after crumbling are subjected to malaxation, i.e. slow mixing of the oil paste with the addition of warm water. During malaxation, in the presence of organic acids from the fruits, at reduced pH, the chlorophylls are pheophytinized, i.e. the process bioconversion in the corresponding pheophytins (CRIADO et. al. 2007). A similar process can also take place in seeds with high water content, if they are stored for too long before drying. Then the hydrolysis of triacylglycerols and the increase in the content of FFAs took place, which promotes the conversion of chlorophylls to pheophytins (GARCIA-GONZÁLEZ et. al. 2014). Other derivatives of chlorophylls found in the oils were pyropheophytins, a and b. Pyropheophytins criterion showed good performance as indicator of overall quality and freshness of oils as well as highlighting any problems during the storage of the product (GUILLAUME et. al. 2014). The percentage of pyropheophytins in the total content of pheophytins and pyropheophytins can be a useful indicator of these undesirable changes. In the range of 0–20%, considered appropriate by ANNIVA et al. (2006), there were oils: rapeseed, flax, hemp, pumpkin and olive extra virgin. In the range of 20–30%, oil was found to be excessive: camelina, milk thistle and avocado. Safflower oil was characterized by a very high proportion of pyroforms ranging from nearly 50% to almost 90%. Basing on data from Table 1 and Table 3 (for 17 out of 19 samples) it was stated that statistically significant, negative correlation exists between % share of pyropheophytins in sum of pheophytins and pyropheophytins (y) and the amount of time till the expiry date of extra virgin olive oil (x) ($r = -0.8836$), with the following equation of regression:

$$y = -0.525x + 26.34.$$

Thus, during storage of extra virgin olive oil pheophytins are converted into pyropheophytins and the closer to the expiry date there are more pyro-derivatives. Correlation of $r = -0.8836$ suggests a strong, negative relationship between studied variables. Degradation of pheophytins to pyropheophytins in olive oil is visible already after 3 months of storage (GALLARDO-GUERRERO et. al. 2005). Seeds oils showed various acid value varying from 1.16 mg KOH g^{-1} for safflower oil to 2.95 mg KOH g^{-1} for pumpkin oil. In any case acid value did not exceed acceptable value of 4 mg KOH g^{-1} (*Codex Alimentarius* 2015). Similar results were also reported (TEH and BIRCH 2013, YANG et. al. 2013). During storage of oil, as a result of progressive hydrolysis (enzymes, traces of water) its acidity grows,

which stimulates bioconversion of chlorophylls to pheophytins (VUJASINOVIC et. al. 2010, YANG et. al. 2013). Basing on data from Table 2 a correlation was determined ($r = 0.7379$) between percentage share of pheophytins in total content of chlorophyll pigments (y) and acid value of seeds oils (x), with the following equation of regression:

$$y = 17.187x + 26.71.$$

Table 3

Content of chlorophyll pigments and acid value of oils derived from fruits

Kind of oil	No.	Content of chlorophyll pigments [mg kg^{-1}]							AV [mg KOH g^{-1}]
		chl *a*	phy *a*	pyr *a*	chl *b*	phy *b*	pyr *b*	total	
Olive extra virgin	1	0.46	2.99	3.76	n.d.	0.30	1.19	8.70	0.89
	2	n.d.	27.60	5.36	n.d.	2.90	2.08	37.94	1.82
	3	n.d.	1.25	0.62	n.d.	0.07	0.22	2.16	0.71
	4	0.49	5.37	0.82	n.d.	0.43	1.16	8.27	0.77
	5	n.d.	9.50	1.29	n.d.	0.37	1.76	12.92	1.01
	6	n.d.	4.14	1.29	n.d.	0.37	0.90	6.70	0.94
	7	0.44	12.29	2.01	n.d.	0.61	1.12	16.47	1.44
	8	n.d.	17.45	3.82	n.d.	1.48	1.34	24.09	1.54
	9	n.d.	9.06	1.52	n.d.	0.73	0.85	12.16	1.04
	10	n.d.	9.00	1.65	n.d.	0.80	1.15	12.60	1.11
	11	n.d.	17.10	0.30	2.45	0.60	1.70	22.15	1.39
	12	n.d.	15.10	0.25	1.05	0.60	1.70	18.70	1.29
	13	n.d.	22.90	0.60	0.75	1.80	1.25	27.30	1.52
	14	n.d.	9.75	1.70	n.d.	0.60	1.25	13.30	1.13
	15	n.d.	6.70	1.55	n.d.	0.55	0.90	9.70	0.67
	16	n.d.	16.75	0.30	n.d.	0.65	1.75	19.45	1.23
	17	n.d.	18.85	0.25	n.d.	0.80	1.40	21.30	1.40
	18	n.d.	12.65	n.d.	n.d.	0.70	n.d.	13.35	1.18
	19	n.d.	6.05	n.d.	n.d.	n.d.	n.d.	6.05	0.65
	$\bar{x}$ ± SD	0.07± 0.17	11.82± 6.85	1.43± 1.42	0.22± 0.59	0.76 ±0.65	1.14± 0.65	15.44± 8.38	1.14 ±0.32
Avocado	1	1.02	61.49	5.92	n.d.	25.21	13.13	106.77	0.98
	2	1.26	18.61	12.66	0.03	5.03	8.65	46.24	0.76
	3	0.18	33.21	7.31	0.07	17.22	9.67	67.66	0.80
	$\bar{x}$ ± SD	0.81± 0.46	37.77± 17.80	8.63± 2.91	0.03± 0.03	15.82± 8.30	10.48± 1.91	73.56± 25.06	0.85 ±0.10

Values (means±SD); n.d. – not detected; chl *a* – chlorophyll *a*; phy *a* – pheophytin *a*; pyr *a* – pyropheophytin *a*; chl b – chlorophyll *b*; phy *b* – pheophytin *b*; pyr *b* – pyropheophytin *b*; AV – acid value

This correlation was not statistically significant though, with significance level of 0.05. The lack of significant correlation between the studied variables could result from several reasons. The oils came probably from seeds of varying quality and were probably stored under different conditions. As it can be assumed, it influenced their acidity. The differences in the shelf life of oils and the color of the glass bottle indicate different rates of lipid oxidation, which could also contribute to changes in the profile of chlorophyll pigments. Acidity of oils coming from fruits was lower than seeds oils. Average acid value of extra virgin olive oil was 1.14 mg KOH g^{-1} (range from 0.65 to 1.82 mg KOH g^{-1}) and avocado oil 0.85 mg KOH g^{-1} (range from 0.76 to 0.98 mg KOH g^{-1}). Basing on data from Table 3 a correlation was determined ($r = 0.6509$) between percentage share of pheophytins in total content of chlorophyll pigments (y) and acid value of extra virgin olive oil (x), with the following equation of regression:

$$y = 16.421x + 59.22.$$

It was confirmed that higher acidity fosters pheophytinization of chlorophylls. Correlation of $r = 0.6509$ suggests a moderate, positive relationship between studied variables. The obtained results could also be influenced by the differences in the technologies used. In the case of seeds, after cleaning, they are directly pressed on a screw press. In the process of obtaining extra virgin olive oil, an additional malaxation process is used, in which chlorophylls largely convert into the appropriate pheophytins before pressing.

Conclusions

Market cold pressed oils have a very different content of chlorophyll pigments, regardless of whether they are from seeds or fruits. Considerable amounts were found in hemp oil (79.82 mg kg^{-1}) and pumpkin oil (57.55 mg kg^{-1}), and small quantity in flax oil (1.08 mg kg^{-1}). The content of chlorophyll pigments in extra virgin olive oil was an intermediate level (15.44 mg kg^{-1}). Chlorophyll derivatives dominate in the profile of chlorophyll pigments i.e. pheophytin *a*, pheophytin *b*, pyropheophytin *a* and pyropheophytin *b*. Extra virgin olive oil had the highest share of pheophytins, on average 82%. The acidity of extra virgin olive oil influences the profile chlorophyll pigments. Statistically significant correlation ($r = 0.6509$) was observed, between percentage share of pheophytins in total content of chlorophyll pigments and acid value of extra virgin olive oil. The percentage of pyropheophytins in the total content of pheophytins

and pyropheophytins can be an indicator of the transformation of chlorophylls during storage of extra virgin olive oil. Statistically significant negative correlation ($r = -0.8836$) exists, between percentage share of pheophytins in sum of pheophytins and pyropheophytins and length of the period which was left till the expiry date of extra virgin olive oil.

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References

ANNIVA C., GRIGORIADOU D., PSOMIADOU E., TSIMIDOU M.Z. 2006. *Pheophytin a degradation product as useful indices in the quality control of virgin olive oil.* J. Am. Oil Chem. Soc., 83: 371–375.

AOCS OFFICIAL METHOD. 2017. *Chlorophyll and chlorophyll-related pigments in vegetable oil. Spectrometric method.* AOCS Method Cc 13k-13.

BRŮHL L., MATTHÄUS B. 2006. *Sensory assessment of virgin rapeseed oil.* Eur. J. Lipid Sci. Technol., 110: 608–610.

CHOE E., MIN D.B. 2006. *Mechanisms and factors for edible oil oxidation.* Comp. Rev. Food Sci. Food Saf., 5: 169–186.

CHOO W.S., BIRCH J., DUFOUR J.P. 2006. *Physicochemical and characteristics of cold-pressed flaxseed oils.* J. Food Comp. Anal., 20: 202–211.

CODEX ALIMENTARIUS 2015. Codex Stan. 210–1999. *Codex standard for named vegetable oils.* Amendment 2005, 2011, 2013, 2015.

CRIADO M.N., ROMERO M.P., MOTILVA M.J. 2007. *Effect of the technological and agronomical factors during olive oil extraction.* J. Agric. Food Chem., 55: 5681–5688.

FERRUZZI M.G., BLAKESLEE J. 2007. *Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives.* Nutr. Res., 27 (1): 1–12.

GALLARDO-GUERRERO K., GANDUL-ROJAS B., ROCA M., MINGUEZ-MOSQUERA M.I. 2005. *Effect of storage on the original pigment profile of Spanish virgin oil.* J. Am. Oil Chem. Soc., 82: 33–39.

GANDUL.-ROJAS B., ROCA M., GALLARDO-GUERRO L. 2016. *Chlorophylls and carotenoids in food products from olive tree.* In: *Products from olive tree.* Ed. Boskou D. IntechOpen, DOI:10.5772/64688: 67–97.

GARCIA-GONZÁLEZ D.L., APARICIO-RUIZ R., APARICIO R. 2008. *Virgin olive oil – chemical implications on quality and health.* Eur. J. Lipid Sci. Technol., 110: 602–607.

GARGOURI B., ZRIBI A., BOUAZIZ M. 2015. *Effect of containers on the quality of Chemlali olive oil during storage.* J. Food Sci. Technol., 52: 1948–1959.

GHAZANI S.M., GARCIA-LLATAS G., MARANGONI A.G. 2014. *Micronutrient content of cold-pressed, hot-pressed, solvent extracted and RBD canola oil: implications for nutrition and quality.* Eur. J. Lipid Sci. Technol., 116: 1–8.

GUILLAUME C, GERTZ CH., RAVETTI L. 2014. *Pyropheophytin a and 1,2 di-acyl-glycerols over time under different storage conditions in natural olive oils.* J. Am. Oil Chem. Soc., 91: 697–709.

HEATON J.W., MARANGONI A.G. 1996. *Chlorophyll degradation in processed foods and senescent plant tissues.* Trends in Food Science & Technology, 7(1): 8–15.

ISO 660. 2010. *Animal and vegetable fats and oils – Determination of acid value and acidity.*

ISO 29841:2009/AMD 1:2016. *Vegetable fats and oils – Determination of the degradation products of chlorophylls a and a' (pheophytins a, a' and pyropheophytins)* – Amendment 1.

KANAI G., KATO H., UMEDA M., OKADA K., MATSUZAKI M. 2010. *Drying condition and qualities of rapeseed and sunflower.* J.A.R.Q., 44: 173–178.

LAZZERINI C., CIFELLI M. DOMENICI V. 2016. *Pigments in extra-virgin olive oil. Authenticity and quality.* In: *Products from olive tree.* Eds. D. Boskou, M.L. Clodoveo, IntechOpen, DOI: 10.5772/61902, pp. 99–114.

MALEKZADEH M., MIRMAZLOUM S.I., MORTAZAVI S.N., PANAHI M., ANGORANI H.R. 2011. *Physicochemical properties and oil constituents of milk thistle (Silybum marianum Gaertn., cv. Budakalászi) under drought stress.* J. Med. Plants Res., 5: 2886–2889.

PENNINGTON F.C., STRAIN H.H, SWEC W.A, KATZ J.J. 1964. *Preparation and properties of pyrochlorophyll a, methyl pyrochlorophyllide a, pyropheophytin a, and methyl pyropheophorbid a derived from chlorophyll by decarbomethoxylation.* J. Am. Chem. Soc, 86: 1418–1426.

PSOMIADOU E., TSIMIDOU M. 1998. *Simultaneous HPLC determination of tocopherols, carotenoids, and chlorophylls for monitoring their effect on virgin olive oil oxidation.* J. Agric. Food Chem., 46 (12): 5132–5138.

RACZYK M., POPIS E., KRUSZEWSKI B., RATUSZ K., RUDZIŃSKA M. 2016. *Physicochemical quality and oxidative stability of linseed (Linum usitatissimum) and camelina (Camelina sativa) cold-pressed oils from retail outlets.* Eur. J. Lipid Sci. Technol., 118: 834–839.

RĘKAS A., WRONIAK M., SZTERK A. 2016. *Characterization of some quality properties and chemical composition of cold-pressed oils obtained from different rapeseed varieties cultivated in Poland.* Pol. J. Natur. Sc., 31 (2): 249–261.

ROCA M., CHEN K., PÉREZ-GÁLVEZ A. 2016. *Chlorophylls.* In: *Handbook on natural pigments in food and beverages.* Eds. R. Carle, R. Schweiggert, Elsevier, Dublin, Ireland, pp. 125–158.

SCHWARTZ S.J., VON ELBE J.H. 1983. *Kinetics of chlorophyll degradation to pyropheophytin in vegetables*. J. Food Sci., 48: 1303–1306.

SYMONIUK, E., RATUSZ K., OSTROWSKA-LIGĘZA, E., KRYGIER K. 2018. *Impact of selected chemical characteristics of cold-pressed oils on their oxidative stability determined using the rancimat and pressure differential scanning calorimetry method.* Food Anal. Methods, 11: 1095–1104.

TEH S.S., BIRCH J. 2013. *Physicochemical and quality characteristics of cold pressed hemp, flax and canola seed oils.* J. Food Comp. Anal., 30: 26–31.

TUBEROSO C.I.G., KOWALCZYK A., SARRITZU E., PAOLO CABRAS P. 2007. *Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use.* Food Chem., 103: 1494–1501.

VUJASINOVIC V., DJILAS S., DIMIC E., ROMANIC R., TAKACI A. 2010. *Shelf life of cold-pressed pumpkin (Cucurbita pepo* L.*) seed oil obtained with a screw press.* J. Am. Oil Chem. Soc., 87: 1497–1505.

WONG M., REQUEJO-JACKMAN C., WOOLF A. 2010. *What is unrefined extra virgin cold-pressed avocado oil?* Inform, 21: 198–201.

WONG M., ASHTON O., MCGHIE T., REQUEJO-JACKMAN C., WANG Y., WOOLF A. 2011. *Influence of proportion of skin present during malaxing on pigments composition of cold pressed avocado oil.* J. Am. Oil Chem. Soc., 87: 1373–1378.

WRONIAK M., FLOROWSKA A., RĘKAS A. 2016. *Effect of oil flushing with nitrogen on the quality and oxidative stability of cold-pressed rapeseed and sunflowers oils.* Acta Sci. Pol. Technol. Aliment., 15: 79–87.

YANG M., ZHENG C., ZHOU Q., HUANG F., LIU C., WANG H. 2013. *Minor components and oxidative stability of cold-pressed oil from rapeseed cultivars in China.* J. Food Comp. Anal., 29: 1–9.