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EFFECT OF EXTRACTION AND REFINING OF RAPESEED OIL ON PROFILE AND CONTENT OF CHLOROPHYLL PIGMENTS

S u m m a r y

The objective of the research study was to determine the effect of industrial extraction and refining of rapeseed oil on the profile and content of chlorophyll pigments, where those processes were carried out under industrial conditions with the use of a modernized production line. The material to be analysed were samples of oil collected at various stages of the rapeseed oil extraction and refining processes. The content of chlorophyll pigments was determined using a reversed phase high-performance liquid chromatography with fluorometric detection. It was found that the industrial method of extracting rapeseed oil affected the profile and content of chlorophyll pigments. The content of chlorophyll pigments in the extracted oil was 40 % higher than that in the pressed oil. The chlorophyll pigments analysed mainly contained pheophytin and phyropheophytin, and only small amounts of chlorophylls a and b. The thermal treatment of oil performed prior to the refining caused the chlorophylls to fully transform. The phyropheophytin to pheophytin ratio changed from 0.7 to 3.8 : 1. The crude oil contained chlorophylls derivatives only. The acid-degumming and neutralisation processes of oil caused the total content of chlorophyll pigments to slightly decrease and their profile to change a little. The effectiveness of reducing chlorophyll pigments after bleaching was 98.5 %. Compared to the chlorophyll b derivatives, the chlorophyll a derivatives were more easily removed from the oil with the use of bleaching clay. In some batches of the bleached oil the phyropheophytin b was still present and its amount was of 0.09 mg/kg.

Key words: chlorophyll pigments, rapeseed oil, extraction, refining, HPLC

Introduction

Chlorophylls are commonly found plant pigments, which play a crucial role in photosynthesis. They have a backbone porphyrin structure with the magnesium ion placed in the centre of the particle and a long phytol group placed on the side [21]. The basic chlorophyll pigments are chlorophylls a and b; in plants their ratio is mostly 3:1 [18]. Apart from them, a wide range of various derivatives is also found; the differ-

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ences among them are that in a centre of the particle, the magnesium ion or hydrogen cation is placed as are different substituent groups. In the structure of chlorophyll particles, there is found the conformation of sequential double and single bonds or the conformation of conjugated bonds. Thanks to that chlorophylls have the ability to absorb sunlight.

Those compounds are not produced by human organism, so they must be supplied with food. Owing to their antioxidant and antimutagenic activities, chlorophylls and its derivatives play an important role in human nutrition as an anti-cancer agent [8]. The technological importance of chlorophyll pigments lies mostly in that they take part in creating the colour of product and in its oxidative activity. On the other hand, it has been shown that chlorophyll and its derivatives are powerful prooxidants (photooxidation) when exposed to light. Thus, the chlorophyll pigments in rapeseed oil are highly undesirable [2].

Chlorophyll pigments are found in all plant oils and their quantities depend on such factors as: variety and maturity of raw material, environmental conditions, year of production, and extraction and refining technology [3, 17]. Rapeseed and canola seeds are a raw material where chlorophyll pigments are found in substantial amounts [4]. In the full-grown seeds chlorophylls a and b are found along with small amounts of methylpheophorbid [17]. If on an industrial scale, the technology of extraction and refining of oil may have a significant effect on the content and composition of chlorophyll pigments because of the parameters applied (changes in pH, destruction of cell structure and activity of chlorophyllase, higher temperature, organic solvents or occurrence of oxygen and metals). Pheophytin is formed in a weak acidity environment and after replacing the magnesium ion with two hydrogen cations. Pheophorbid is formed in a strong acidity environment after replacing the magnesium ion with two hydrogen cations and after hydrolysis of the ester bond and break of phytol group. Chlorophyllides are formed as a result of the activity of chlorophyllase or weak alkali after selective hydrolysis of the ester bond and break of the phytol group. The application of a higher temperature results in the separation of carboxymethoxy group and in the formation of phyro-compounds [18, 21].

In view of the rising customer requirements as regards the bright clear colour and high oxidative stability of oil, there is a need to completely remove chlorophyll pigments from oil [4, 12]. Small amounts thereof are removed in a super-degumming and neutralisation processes, however their substantial amount is eliminated during the bleaching process with the use of activated absorbents [17]. Bleaching is important and it is a critical stage in the refining of the rapeseed oil. This process creates lots of problems, it is energy consuming, substantial losses of raw material occur and numerous ecological problems arise. Because of the specific bleaching process parameters, biologically active substances are lost and, next, they are found in oils [5, 9]. A substantial

effect on the chlorophyll pigments could be achieved through the modification of technological process that consists in the thermal treatment of oil before refining. Therefore, the knowledge on transformation of chlorophylls may be of high value in order to optimise the process of their removal using, also, alternative chemical or enzymatic methods [6, 22].

The objective of the research study was to determine the effect of industrial extraction and refining of rapeseed oil on the profile and content of chlorophyll pigments, where those processes are carried out under industrial conditions with the use of a modernized production line.

Material and methods

The material consisted of samples of oils taken from an industrial, modernized technological line in one of the Polish oil processing plants. The extraction and refining of oil was a continuous process and they run according to the scheme as shown in Fig. 1. In the seeds processing department, the following operations took place: pre-heating of seeds, flaking, conditioning, pre-pressing, solvent extraction, thermal treatment of extraction oil and water-degumming. A new process added was thermal treatment of the extraction oil at 100 ºC during the temporary 24 h storage. Chemical reactions occurred prior to the refining of oil as did thermal transformations of accompanying substances present in the extracted oil in increased amounts. This facilitated the refining process.

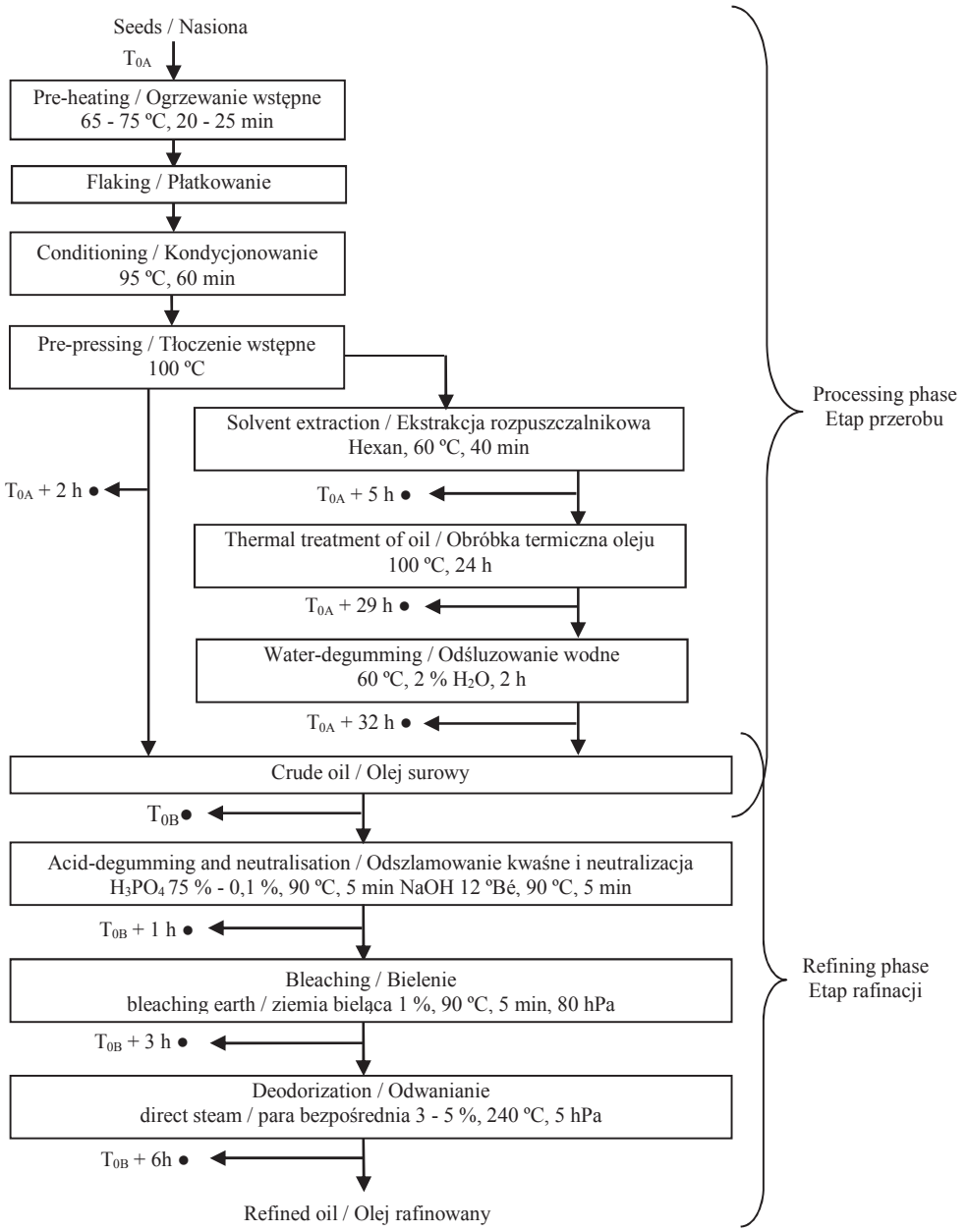
The authors did not have any possibility to conduct the research on the industrial scale prior to the implementation of thermal treatment of the extraction oil. Checked were six production batches of oils; they were obtained after the seeds from two different suppliers were processed. The following oils were analysed: pressed, extracted, extracted after thermal treatment and degummed oil. The samples were collected directly after each of the processes from the collecting points within the technological line – the sampling points and the time of collecting the samples are marked on the scheme (Fig. 1).

In the refinery, the following processes were completed: acid-degumming, neutralisation, bleaching and deodorization. The acid-degumming and neutralisation processes were mutually coupled, however it was possible to collect samples after each of them. Six production batches of oils from the refinery were checked. The following oils were analysed: crude, acid-degummed, neutralized, bleached and deodorized oil. The samples were collected directly after each of the processes from the collecting points within the technological line – the sampling points and the time of collecting the samples are marked on the scheme (Fig. 1).

During the research work, the samples were kept at temperatures between -18 and -22 ºC, away from the light. The analysis of chlorophyll pigments was carried out with

the use of reference substances. The two chlorophylls: a (chl a) and b (chl b) were purchased in the Sigma Aldrich Company (Poznan, Poland). The pheophytin a (phy a) and pheophytin b (phy b) were prepared from the corresponding chlorophylls through acidifying ether solutions with 13 % HCL [20]. The phyropheophytin a (pyr a) and phyropheophytin b (pyr b) were prepared from the corresponding pheophytins dissolved in pyridine by means of the heat treatment at 110 ºC [16]. The HPLC-grade solvents, methanol and acetone were obtained from Avantor (Gliwice, Poland) and diethyl ether from Sigma Aldrich (Poznań, Poland). The deionised water was made using a Milli-Q purification system from Millipore (Bedford, MA, USA). Other chemicals were of analytical-reagent grade and they were applied without further purification. The hydrochloric acid (purity ≥ 37 %) and sodium sulphate anhydrous (purity ≥ 99.0 %) were purchased in Avantor (Gliwice, Poland) and the pyridine anhydrous (purity ≥ 99.8 %) in Sigma Aldrich (Poznań, Poland). Before usage, the mobile phases were filtered through a Millipore 0.22 μm membrane filter. Furthermore, the following equipment was utilized: HPLC system (Agilent Technologies Series 1100, Santa Clara, CA, USA) composed of G-1379A vacuum degasser, G-1311A quaternary pump, G-1313A autosampler, G-1316A column oven, and G-1321A fluorescence detector; Agilent Chemstation for LC and LC/MS systems software; Vortex mixer from JW Electronic (Warsaw, Poland) and C18 column Aeris PEPTIDE XB (3,6 μm, 250 mm length × 4,6 mm I.D.) from Phenomenex Corp. (Torrance, CA, USA).

The content of chlorophylls a and b and their derivatives, i.e. pheophytin a, pheophytin b, phyropheophytin a and phyropheophytin b was simultaneously determined in one sample by a modified and validated reversed phase HPLC method [7]. A stationary phase with a smaller particle size was applied; the composition of solvent B of the mobile phase and the elution program were changed and the speed of flow of the mobile phase was reduced. An FLD detector (excitation wavelength λ_{ex} = 430 nm and emission wavelength λ_{em} = 670 nm) was employed; an elution program was used with 0.8 ml/min flow rate at a temperature of 25 ºC. 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). The gradient scheme was as follows: initial conditions 100 % A for 3 min; next the percent amount of A was decreased to 0 %, the percent amount of B was increased to 100 % for 10 min; those conditions were maintained for 18 min and thereafter the percent amount of B was decreased to 0 % and the percent amount of A was increased to 100 % for 22 min; the system was stabilised under those initial conditions for 30 min.



Explanatory notes / Objaśnienia:
T_{0A} – starting time of seeds processing / czas rozpoczęcia przerobu nasion, T_{0B} – starting time of crude oil refining / czas rozpoczęcia rafinacji oleju surowego, ● – point of sampling / miejsce pobrania próbki

Fig. 1. Scheme of technological processes of extraction and refining of rapeseed oil
Rys. 1. Schemat procesów technologicznych wydobywania i rafinacji oleju rzepakowego

The chlorophylls and their derivatives were identified by comparing their retention times with the retention times as set in the respective standards. The calibration curves were developed using calibration standard solutions. The content of chlorophylls and derivatives was calculated using the equations of standard curves that were experimentally determined for the analysed ranges of concentrations. 2 g of oil was placed in a glass tube and acetone was added to fill up to 10 ml. The sample was mixed in a vortex mixer for 1 min. The analyses were made in triplicates.

The statistical analysis was performed using a Statistica 13 software (Statsoft, Poland). The significance of deviations of average chlorophyll pigments content in oils was evaluated using a one way analysis of variance and Tukey's post hoc tests. In the case the assumptions of parametrical analysis of variance were not fulfilled, a non-parametrical Kruskal-Wallis test and multiple comparisons of appropriate averages rang were performed. All the tests were performed with the adopted assumption that the significance level was 0.05.

Results and discussion

Effect of extraction of rapeseed oil on profile and content of chlorophyll pigments

The analysis results of the oil samples are shown in Tab. 1. The evaluation of the significance of differences between average content of particular chlorophyll pigments showed the following dependencies:

- chl a – the content of this pigment in the pressed oil significantly differed from its content in the extracted oil. The content of this pigment in the oils after heat treatment and water-degumming was similar, however it significantly differed compared to those in the pressed and extracted oils;
- phy a – no significant differences were found in the content of this pigment between the pressed and other oils. The content of this pigment in the extracted oil significantly differed from its content in the oils after heat treatment and water-degumming, which were similar to each other;
- pyr a – no significant differences were found in the content of this pigment between the pressed and extracted oils, and between the oils after heat treatment and water-degumming, however those two groups significantly differed from each other as regards the content of this pigment;
- chl b – no significant differences were found in the content of this pigment between the pressed and extracted oils, and between the oils after heat treatment and water-degumming, however those two groups significantly differed from each other as regards the content of this pigment;

Table 1. Content of chlorophyll pigments during extraction of rapeseed oil
Tabela 1. Zawartość barwników chlorofilowych w czasie wydobywania oleju rzepakowego

Kind of oil Rodzaj oleju	Batch Partia	Content of chlorophyll pigments [mg/kg] Zawartość barwników chlorofilowych						
		chl a	phy a	pyr a	chl b	phy b	pyr b	total
pressed tłoczony	1	1,39	3,09	1,35	1,03	0,15	0,61	7,62
	2	1,16	2,79	1,27	0,90	0,10	0,57	6,78
	3	1,06	2,41	1,40	0,77	0,10	0,57	6,30
	4	0,64	3,48	2,90	0,05	0,41	1,50	8,93
	5	0,86	3,64	3,16	0,25	0,40	1,60	9,91
	6	0,73	3,37	3,14	0,05	0,43	1,58	9,30
	$\overline{X}$ SD	$0,97^{b}$ 0,28	$3,13^{ab}$ 0,46	$2,20^{a}$ 0,95	$0,51^{a}$ 0,20	$0,27^{a}$ 0,16	$1,07^{a}$ 0,54	$8,14^{a}$ 1,46
extracted ekstrakcyjny	1	0,83	3,60	1,86	1,02	0,40	0,84	8,54
	2	0,80	3,86	1,84	1,05	0,46	0,93	8,95
	3	0,66	2,59	1,74	0,97	0,26	0,73	6,96
	4	0,52	5,80	4,49	0,35	1,15	2,71	15,02
	5	0,68	5,92	4,36	0,55	1,08	2,66	15,24
	6	0,62	5,42	4,01	0,43	0,95	2,32	13,75
	$\overline{X}$ SD	$0,69^{a}$ 0,12	$4,53^{b}$ 1,37	$3,05^{a}$ 1,36	$0,73^{a}$ 0,32	$0,72^{a}$ 0,39	$1,70^{a}$ 0,96	$11,41^{a}$ 3,67
after heat treatment po obróbce termicznej	1	bdl	0,91	4,88	bdl	0,10	1,48	7,37
	2	bdl	1,65	4,19	bdl	0,18	1,37	7,39
	3	bdl	0,84	4,63	bdl	0,07	1,40	6,94
	4	bdl	2,27	12,50	bdl	0,65	5,15	20,57
	5	bdl	2,72	10,72	bdl	0,66	4,74	18,84
	6	bdl	3,70	8,55	bdl	0,95	3,84	17,04
	$\overline{X}$ SD	-	$2,02^{a}$ 1,11	$7,58^{b}$ 3,54	-	$0,44^{a}$ 0,37	$3,00^{a}$ 1,78	$13,03^{a}$ 6,44
water- degummed odśluzowany	1	bdl	0,84	4,83	bdl	0,11	1,47	7,26
	2	bdl	1,50	4,28	bdl	0,18	1,41	7,37
	3	bdl	0,81	4,64	bdl	0,12	1,42	6,97
	4	bdl	2,19	12,53	bdl	0,86	5,43	21,01
	5	bdl	2,71	10,96	bdl	0,85	5,05	19,58
	6	bdl	3,35	8,51	bdl	0,99	3,96	16,81
	$\overline{X}$ SD	-	$1,90^{a}$ 1,03	$7,63^{b}$ 3,57	-	$0,52^{a}$ ± 0,42	$3,12^{a}$ ± 1,91	$13,17^{a}$ ± 6,68

Explanatory notes / Objaśnienia:
chl a – chlorophyll a / chlorofil a; phy a – pheophytin a / feofityna a; pyr a – phyropheophytin a / pirofeofityna a; chl b – chlorophyll b / chlorofil; phy b – pheophytin b / feofityna b; pyr b – phyropheophytin b / pirofeofityna b; bdl – below detection limit / poniżej granicy wykrywalności.
$\overline{X}$ – mean value / wartość średnia; SD – standard deviation / odchylenie standardowe; n = 6; a, b – mean values in columns and denoted by different letters differ statistically significantly ($p \leq 0.05$) / wartości średnie w kolumnach oznaczone różnymi literami różnią się statystycznie istotnie ($p \leq 0,05$).

- phy b – no significant differences the in content of this pigment were found in the oils analysed ($p = 0.195$, so $p > 0.05$);
- pyr b – no significant differences in the content of this pigment were found in the oils analysed ($p = 0.088$, so $p > 0.05$);
- in summary – no significant differences were found in the content of the pigments in the oils analysed ($p = 0{,}305$, so $p > 0.05$).

The content of chlorophyll pigments was in total 40 % higher in the extracted oil than in the pressed oil. In this instance, the additional crushing of cake and the application of hexane at 60 ºC resulted in a deeper extraction of chlorophyll pigments. The results achieved in this research study agree with those as reported by Ghazani et al. [10].

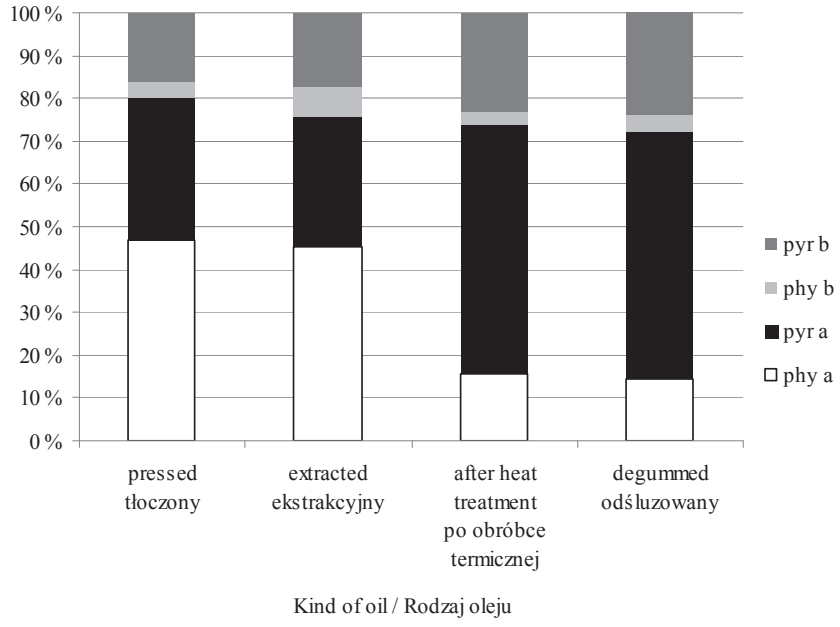
Small amounts of the chlorophylls a and b were present in the pressed and extracted oils, in 2 : 1 and 1 : 1 proportions, respectively. Those proportions significantly differ from the proportion occurring naturally in rapeseeds (3 : 1) [18]. This shows that the chlorophyll a is thermally less stable than the chlorophyll b. The chlorophyll a has a methyl group at C-3 carbon, while a formyl group is bonded to the same carbon atom in chlorophyll b. In addition to the structural differences, their thermal stabilities are also different. It also shows that the transformation of chlorophyll a in the extraction process is far stronger than that during pre-pressing. It may be a result of the additional thermal processing and usually of a higher content of free fatty acids in the extracted oil.

The pheophytin a and phyropheophytin a were dominant in the pressed and extraction oils, in contrast to the seeds, where the chlorophylls a and b were found [1]. The pyr a : phy a proportions in the pressed and extraction oils were the same (0.7). In both of them the content of b-forms was several times lower than that of a-forms. The pyr b : phy b proportion was 4 : 1 in the pressed oil compared to 2 : 1 in the extracted oil. Consequently, the phy b shows a higher vulnerability to technological conditions than the phy a. Different factors could have an effect on the content and profile of the chlorophyll pigments in both oils; above all, a higher temperature is such factor.

In the processing phase whole seeds, flakes, cake and miscella underwent the thermal treatment (Fig. 1). As reported by Kralijc et al. [13], the conditioning of seeds results in the double or triple increase in the chlorophyll content in oil. Under the given conditions, the chlorophyllase enzyme, a catalyst for the hydrolysis of chlorophyll, is inactivated [15]. Its activity is present mostly during the mild drying of rape seeds [11]. Prior to refining, the extracted oil was subjected to heat treatment at 100 ºC for 24 h. As a result, it was reported that the content of chlorophyll pigments increased 14 %, probably because of their deeper extraction from the chlorophyll-protein complexes [19]. The thermal processing of oil caused the profile of chlorophyll pigments to change. The chlorophyll a and b were completely transformed, the content of pheo-

phytins was lowered, while the content of pyr a rose 2.5 times and that of pyr b 1.8 times. The pyr a : phy a proportion changed radically from 0.7 to 3.8 : 1.

The next stage in the technological process was water-degumming of extraction oil after its thermal treatment. In the authors' own research, it was performed in an oil mill plant. Mild conditions of the process did not cause any significant quantitative changes in the content of chlorophyll pigments. Fig. 2 displays the degree of pheophytins transformation to phyropheophytins that occurred during the extraction and initial processing of oil. The method of oil extraction (pre-pressing, solvent extraction) did not have any significant effect on the percent content of particular pheophytins and phyropheophytins in the total content of those compounds. The transformation of the highest degree took place as a consequence of the thermal treatment of oil during the temporary-storage phase. The water-degumming process did not result in any significant changes in this matter. While analysing the occurring transformation, there should be considered substantial differences in the content of chlorophyll pigments between the batches of oils. Most probably those differences appeared because the rapeseed batches processed were derived from different suppliers.



Explanatory notes as in Tab. 1. / Objaśnienia jak pod tab. 1.

Fig. 2. Changes in percent content of pheophytin a and b, and phyropheophytin a and b in the total of those compounds in oils during extraction

Rys. 2. Zmiany udziału feofityny a i b oraz pirofeofityny a i b w sumie tych związków w olejach podczas wydobywania

It is known that significant differences occur in the content of chlorophyll pigments in the rape seeds depending on the maturity degree of seeds, which is determined by the region of cultivation, harvesting season (winter or spring) and harvesting conditions. The content of chlorophyll pigments depends also on drying and storage conditions [1, 11].

Effect of refining of rapeseed oil on profile and content of chlorophyll pigments

The analysis results of the samples are shown in Tab. 2. The evaluation of the significance of differences in the average content of particular chlorophyll pigments proved the following dependencies:

- phy a – the content of this pigment in crude oil significantly differed from its content in the rest of oils. No significant differences were found in the content of this pigment between the acid-degummed and neutralized oils. However its content differed significantly compared to the bleached oil;
- pyr a – the content of this pigment in the crude, acid-degummed and neutralized oils significantly differed from its content in the bleached oil;
- phy b – the content of this pigment in the crude oil differed significantly from its content in the rest of oils. No significant differences in the content of this pigment between acid-degummed and neutralized oils were found. However its content differed significantly compared to the bleached oil;
- pyr b – the content of this pigment differed significantly in all the oils analysed;
- sum – the content of pigments in the crude oil differed significantly from its content in the rest of oils. No significant differences were found in the content of pigments between the acid-degummed and neutralized oils. However its content differed significantly compared to the bleached oil.

The crude oil was a blend of the pressed and extracted oils; their proportion was, approximately, 3:1. In the crude oil there were found only the following chlorophyll derivatives: phy a, phy b, pyr a and pyr b. The chlorophylls a and b transformed to derivatives during extraction and initial processing of oil. There were found the derivatives of type a; their amount was 78.6 % of the total content, with the dominant pheophytin (5 times more than the amount of the form b) and phyropheophytin a (29.5 % of the total amount). Ward et. al. [23] reported similar data. The crude oil was subjected to the acid-degumming process (removal of non-hydratable phosphatides). As a result of the process the content of chlorophyll pigments decreased 5.7 %. The pyr a : phy a ratio (0.64) was slightly higher than that in the crude oil (0.60). This indicates that the acid-degumming process slightly affected the content of chlorophyll pigments.

In the next stage, the oil was neutralized (free fatty acids were removed). This process caused the total content of chlorophyll pigments to decrease 3.4 %. The

pyr a : phy a ratio increased from 0.64 to 0.73. This is a proof for further transformation of phy a to pyr a.

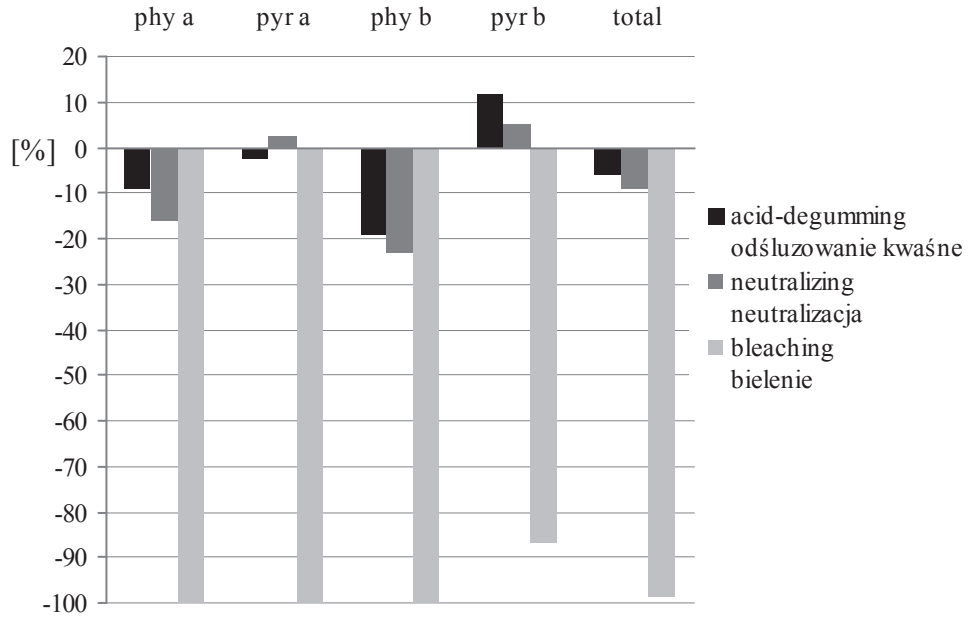
Table 2. Content of chlorophyll pigments during refining of rapeseed oil
Tabela 2. Zawartość barwników chlorofilowych w czasie rafinacji oleju rzepakowego

Kind of oil Rodzaj oleju	Batch Partia	Content of chlorophyll pigments [mg/kg] Zawartość barwników chlorofilowych				
		phy a	pyr a	phy b	pyr b	total
crude surowy	1	2,76	1,54	0,55	0,61	5,46
	2	2,81	1,59	0,51	0,64	5,55
	3	2,74	1,60	0,54	0,59	5,47
	4	2,71	1,54	0,53	0,60	5,38
	5	2,25	1,56	0,50	0,61	4,92
	6	2,32	1.55	0,49	0,59	4,95
	$\overline{X}$	$2,60^{b}$	$1,56^{a}$	$0,52^{b}$	$0,61^{a}$	$5,29^{b}$
	SD	0,25	0,03	0,02	0,02	0,28
acid-degummed odszlamowany	1	2,44	1,61	0,45	0,69	5,19
	2	2,31	1,52	0,41	0,70	4,94
	3	2,39	1,55	0,44	0,71	5,09
	4	2,35	1,49	0,45	0,69	4,98
	5	2,36	1,44	0,41	0,65	4,86
	6	2,34	1,51	0,40	0,64	4,89
	$\overline{X}$	$2,37^{a}$	$1,52^{a}$	$0,43^{a}$	$0,68^{c}$	$4,99^{a}$
	SD	0,05	0,06	0,02	0,03	0,13
neutralized po neutralizacji	1	2,20	1,54	0,42	0,64	4,80
	2	2,19	1,61	0,39	0,66	4,85
	3	2,21	1,59	0,41	0,65	4,86
	4	2,18	1,59	0,42	0,64	4,83
	5	2,15	1,61	0,38	0,63	4,77
	6	2,17	1,63	0,37	0,65	4,82
	$\overline{X}$	$2,18^{a}$	$1,60^{a}$	$0,40^{a}$	$0,65^{b}$	$4,82^{a}$
	SD	0,02	0,03	0,02	0,01	0,03
bleached bielony	1	bdl	bdl	bdl	bdl	bdl
	2	bdl	bdl	bdl	bdl	bdl
	3	bdl	bdl	bdl	bdl	bdl
	4	bdl	bdl	bdl	bdl	bdl
	5	bdl	bdl	bdl	0,09	0,09
	6	bdl	bdl	bdl	0,09	0,09
	$\overline{X}$	-	-	-	0,04	0,04
	SD				0,00	0,00

Explanatory notes as in Tab. 1. / Objaśnienia jak pod tab. 1.

The next process was the bleaching of oil using activated adsorbents in order to fully remove the chlorophyll pigments. Based on the data in Tab. 2, it was found that after bleaching in some oil batches the pyr b was still present; its amount was 0.09 mg/kg. This proved that the chlorophyll derivatives of type a were more easily re-

moved with the use of absorbent than those of type b. Kreps et al [14] reported that the total content of chlorophylls after bleaching was twice as high. After deodorization no chlorophyll pigments were found in the oil batches analysed. According to Przybylski [17], through the alkaline refining and bleaching it is possible to remove the chlorophyll pigments from the crude oil up to a level of 0.05 mg/kg. Ghazani et al. [10] found several times higher amounts of chlorophyll pigments in the fully refined canola oil.



Objaśnienie symboli jak pod tab. 1 / Meanings of symbols as in Tab. 1.

Fig. 3. Changes in content of chlorophyll pigments in rapeseed oil after refining processes
Rys. 3. Zmiany zawartości barwników chlorofilowych w oleju rzepakowym po procesach rafinacji

On Fig. 3 changes in the content of chlorophyll pigments after refining are shown. There were changes in pH of oil resulting from the treatment with phosphoric acid in the acid-degumming process and subsequently from the treatment with caustic soda in the neutralisation process at a higher temperature; those changes in pH of oil caused the total content of chlorophyll pigments to decrease. After acid-degumming the content of chlorophyll pigments decreased 5.7 %, and after those two processes 8.8 %. The bleaching proved to be the main process in removing chlorophyll pigments. After those three processes the degree of removal of the chlorophyll pigments was 98.5 %. In the research study by Wroniak et al. [24] the degree of removal was slightly lower.

Conclusions

1. The industrial method of extracting rapeseed oil affects the profile and content of chlorophyll pigments.
2. The content of chlorophyll pigments in the extracted oil was 40 % higher than that in the pressed oil. Those two kinds of oil contained only small amounts of chlorophylls a and b.
3. The thermal treatment of oil before refining results in deep transformations of the quality and quantity of chlorophyll pigments. The crude oil contains chlorophylls derivatives only. Among them the pheophytin a and the phyropheophytin a are dominant.
4. The acid-degumming and neutralisation of oil caused only the total content of chlorophyll pigments to slightly decrease and their profiles to change relatively little.
5. The bleaching is a process, during which the chlorophyll pigments are almost completely removed from the oil. The derivatives of chlorophyll a are more easily removed from oil using the bleaching earth than the derivatives of chlorophyll b. In some batches of the bleached oil the phyropheophytin b was still found and its amount was 0.09 mg/kg.

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WPŁYW WYDOBYWANIA I RAFINACJI OLEJU RZEPAKOWEGO NA PROFIL I ZAWARTOŚĆ BARWNIKÓW CHLOROFILOWYCH

Streszczenie

Celem pracy było określenie wpływu wydobywania i rafinacji oleju rzepakowego w warunkach przemysłowych, na zmodernizowanej linii technologicznej, na profil i zawartość barwników chlorofilowych. Jako materiał doświadczalny zastosowano próbki oleju pobierane na różnych etapach procesów jego wydobywania i rafinacji. Zawartość barwników chlorofilowych oznaczono metodą wysokosprawnej chromatografii cieczowej w układzie faz odwróconych, z detekcją fluorymetryczną. Stwierdzono, że przemysłowa metoda wydobywania oleju rzepakowego ma wpływ na profil i zawartość barwników chlo-

rofilowych. Olej ekstrakcyjny zawierał o 40 % więcej barwników chlorofilowych niż olej tłoczony. Zawierały one głównie feofitynę a oraz pirofeofitynę a i tylko niewielkie ilości chlorofili a i b. Obróbka termiczna oleju przed rafinacją powoduje całkowitą transformację chlorofili. Proporcja pirofeofityna a/feofityna a zmieniła się z 0,7 na 3,8 : 1. Olej surowy zawierał tylko pochodne chlorofili. Kwaśne odszlamowanie oraz neutralizacja oleju spowodowały niewielkie zmniejszenie zawartości barwników chlorofilowych ogółem oraz względnie małe zmiany ich profilu. Efektywność usunięcia barwników chlorofilowych po bieleniu wynosiła 98,5 %. Pochodne chlorofilu a były dużo łatwiej usuwane z oleju za pomocą ziemi bielącej niż pochodne typu b. W niektórych partiach oleju po bieleniu pirofityna b w ilości 0,09 mg/kg była nadal obecna.

Słowa kluczowe: barwniki chlorofilowe, olej rzepakowy, ekstrakcja, rafinacja, HPLC