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Changes to the composition of colorants caused by the temperature of drying rapeseed

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A b s t r a c t. The pigment content in both seeds and oil has been an essential indicator of the oil quality, in particular for the cold pressed oil. The chlorophyll content in the seeds depends upon many agents, among which harvesting before full maturity, in case of one-step harvesting and harvesting before technical maturity, in case of two-step harvesting are the most significant ones. The chlorophyll quantity in seeds designed for future processing should not exceed a value of 25 mg kg^{-1}. Where this occurs, a remarkable quantity of chlorophyll pigments will occur both in the seed cover as well as in the oil extracted from non – mature seeds. The high level of chlorophyll content has a negative influence on oil quality (smell) and also oil stability due to its pro-oxidative action. Another indicator of negative chlorophyll influence is the visual darkening of the oil. The high temperature of seed drying and the presence of defective seeds also exert a negative influence on oil colour. Apart from the chlorophyll pigments in rape seeds, carotenoid pigments occur in quite considerable amounts. The presence of carotenoid in the oil is very important mainly due to its antioxidant properties (lipid protection) and vitamin-forming action (dietary purposes). The aim of these studies was the examination of those agents responsible for the pigment content in rapeseed.

K e y w o r d s: rapeseed, oil, temperature, drying, chlorophyll, carotenoid

INTRODUCTION

Drying rapeseed represents one of the most important elements in the complexity of actions defined as the 'post-cropping' process. The efficiency of this process will have an impact not only on the costs of production but also on the quality of oil and post-extraction meal produced from the seeds.

Research conducted by Rybacki *et al*. [20], has demonstrated that 24% of the facilities used for drying rapeseed in the Kujawy Pomorze area was purchased before 1970, while only 45% after 1990. This relates to both the technical ability and the method of controlling and registering the temperature of the drying medium. Such a situation poses the question of the final quality of the raw material to be dried in such conditions. Another element of risk for the technological value of the rapeseed is the need to apply different drying parameters for rapeseed and grain. This derives mainly from the chemical composition of seeds and the porosity of their shell. This directly affects the speed of drying, which in the case of rapeseed is substantially lower. It is basically caused by the higher pressure of air passing through the thick layer of seeds. The pressure (resistance) even increases with the greater number of impurities contained in the air-blown sample of seeds [14,15]. In such a case, the ventilation fan – usually operated on parameters set for grain – creates higher static pressure at lower volumes of air, which makes it likely for the temperature of the air delivered to the container to increase. Most of the time, this is the case – in the opinion of the facility controlling organs. The over-drying of seeds, in general, deteriorates the quality of the oil in respect of its acid and peroxide value [25].

Another relevant indication of the quality of the oil, particularly for cold-pressed oil, is the presence of colorants. The colorants affect the quality (odour) and stability of the oil [10,11,18]. This relates in particular to chlorophyll, which acts in a pro-oxidative manner and darkens the oil [19]. The presence of chlorophyll in the seeds is a function of several factors of which the crucial one is cropping the seeds before full maturity – at one-stage-cropping and before full technical maturity – at two-stage-cropping [26]. According to Daun [9], the amount of chlorophyll in the seeds for

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processing should not exceed 25 mg kg^{-1}. Under such conditions a substantial amount of colorant sets aside in the seed-coating and in the immature seeds themselves [16]. The number of broken seeds has also a negative impact on the colour of the oil [18]. Apart from the chlorophyll colorants, there are also other colorants present in the seeds of rape, i.e., the carotenoid group colorants also appear in significant amounts there. However, they are much more present in the oil. The presence of β-carotene in the oil is important in respect of their anti-oxidative and pro vitamin qualities [22,23].

The carotenoid colorants are chemically tetraterpenes, consisting of two hydrocarbon units containing 20 atoms of carbon and modified by substitutive groups [7]. They are present in animal and plant tissue as well as in blood plasma. In the case of vegetable plants, they are a component of the photo-system and their role is to absorb the quantum of light and to transfer energy to chlorophyll colorants to enable photosynthesis [26]. They are also a stabilising protein in the photo-system [13]. They also perform other biological functions, of which the most important – in the case of food products rich in it – is the protection they afford against attacks of free radicals. The consumption of highly or thermally processed foods increases the level of free radicals [2,12]. Therefore, the presence of carotenoids – in their natural form or artificially added – is highly desirable in food. The protective role of carotenoids is reflected by quenching the triplet state of light sensitive particles (usually proteins and lipids) as well as singlet oxygen. The protective role of the carotenoids becomes even more important in the case of lipids rich in polyunsaturated fatty acids which are particularly sensitive to attacks from free radicals. So, all the oils with a high level of carotenoids are potentially better to human health than those having little or none. Furthermore, β-carotenoids are the precursors of pro-vitamin vitamin A [6]. Being well soluble in fats and scarcely if at all soluble in water, they represent the perfect oil base for vitamins in human nutrition.

The presence of carotenoid colorants in oil is also important due to their richness in other important components such as lutein and zeaxanthin. These two polar carotenoids were found in the lipid membranes of the *macula lutea* of the mammal eye [3–5]. Their physiological role has not been so far fully understood but it is assumed that they play an important role in protection against excessive UV radiation reaching the eye as well as in the quenching of free radicals.

Other natural vegetable colorants in food products belong to the group of polyphyrine (chlorophylls) and flavonides, which – like carotenoids – take part in the process of photosynthesis [13]. Once the presence of carotenoids becomes highly desirable, the presence of chlorophyll colorants in food products seems to be harmful for purely aesthetic reasons and the problems with its industrial processing (unpleasant fragrance and colour). This relates probably to their structure and location within the lipid membrane, which makes them sensitive to heating (more difficult technologically to processes mainly in refining) and sunlight (problems with storage).

The aim of the paper was to define the content of the colorants present in the oil extracted from seeds under different drying temperature treatments.

MATERIALS AND METHODS

Research determining the impact of the drying temperature of rapeseed on the colour of oil extracted therefrom was carried out on five varieties of winter seeds (Kana, Marita, Lisek, Lirajet and Bristol). The seeds of each variety underwent the drying process in a laboratory dryer at the following temperatures: 80, 100, 120, 150 and 180°C. The oil of seeds dried at room temperature was used as a control point. The starting humidity was 13%. The seeds were spread in a thin layer – 0.5 cm – and dried in a metal container with a perforated base, covered with four layers of glued seeds (to avoid direct contact of the seeds with the metal). The process took 20 min. After drying, the seeds were conditioned in a warehouse at 20°C and 7% humidity for 10 days in order to level up the humidity ranges.

Afterwards, the seeds were crushed under hydraulic press (temperature of the seeds 20°C and efficiency of the process – 70%) in order to extract the oil. The oil was then collected in a light protective container filled with nitrogen. The seeds were immediately examined for the presence of colorants.

The method for examining carotenoid and chlorophyll colorants in food products was based on absorbance spectra analysis. Additionally, analysis of the infrared spectra could be applied. This, however, requires several (prototype) control samples or standards and may prove difficult due to the presence of water in the biological material (food) and environment.

Absorption of light is a process in which the intensity of electromagnetic radiation decreases along the passage through some material substances. Absorption depends on the length of the radiation wave, the thickness of the sample and the characteristic extinction coefficient at a given wavelength. The change in the intensity of the out coming radiation is described by Lambert-Beer's law:

$$I = I_0\, 10^{-\varepsilon(\lambda)cx} \tag{1}$$

where: I_0, I – intensity of light coming in and out of the sample; $\varepsilon(\lambda)$ – molar coefficient for absorption (extinction); c – molar concentration of substance; x – thickness of sample (cm).

The measure of the absorption is an exponent of the above equation, which we call absorbance ($A = \varepsilon(\lambda)cx$).

The spectra of the analysed samples presented in this paper are the absorbance spectra.

The determination of carotenoid, chlorophyll and general colours was based on measuring the absorbance of oil – acetone diluted samples at wavelengths of 460 and 666 nm, this according to the Sector Norm BN 868050-30.

In order to establish a proper general colour for oil and, at the same time, determine the concentration of carotenoid colorants, a sector norm was applied in a standard way and additionally corrected in respect to 'Rayleigh' scattering by lipids in the short-wavelength region (c.a. 350 nm) [23].

The samples of oil were diluted according to recommendations enclosed in the norm. Also calculations of the general, carotenoid and chlorophyll colours were made according to the formulas contained in the norm.

The content of chlorophylls a and b (g ml^{-1}) was determined for the samples of oils diluted in the acetone according to the procedure [26] based on the following formulas:

$$C_a = 11.24\, A_{661.6} - 2.04\, A_{644.8} \quad (2)$$

$$C_b = 20.13\, A_{644.8} - 4.19\, A_{661.6} \quad (3)$$

The spectra were measured by means of a dual-beam UV-Vis spectrophotometer Cary 300 Bio from Varian company, equipped with quartz cuvettes, each of 2 ml capacity in 1 cm of optic path-length. The measure was taken on three independent samples from each sort of oil diluted 5, 10 and 15 times. The accuracy of the measure was 0.5 nm. The spectra were collected at 20±0.1°C; the temperature was stabilised by the Peltier element.

The data from the spectrophotometer was analysed by means of Grams software from the Galactic Company and presented as graphs by means of Grapher 2.0 software.

RESULTS AND DISCUSSION

Research conducted on the colour of oil coming from rapeseeds treated under different drying temperatures indicates the relevant temperature impact on the amount of chlorophyll and carotenoids in the oil (Table 1). The application of higher temperatures effected an increase of chlorophyll as well as carotenoid pigments. The lowest amount of colorants was present in oil coming from the control seeds. The noted level of absorbance (times 1000) for the carotenoids measured, accounting for scattering, was from 92 – for Kana variety to 122 – for Marita. Higher values of absorbance occurred in the case of measurements done according to the sector norm and were respectively: 123–206. The absorbance for chlorophyll was from 55 (Marita) to 147 (Kana). Significant differences with respect to different varieties have been noticed. The oil extracted from the Marita variety contained a high amount of carotenoid colorants and a low amount of chlorophyll. The results were quite different in the case of oil coming from the Kana variety (much chlorophyll, little carotenoid).

The calculated content of the chlorophyll a varied in the samples examined from 0.86 to 1.76 μg ml^{-1} of oil, while the chlorophyll b from 0.09 to 0.54 μg ml^{-1}. Only in the case of the oil coming from the Kana variety was the amount of chlorophyll b substantially higher – 2.16 μg ml^{-1}. The amount of this colorant was greatly reduced during the drying process until it completely diminished. The 'increase' in colorants was noticed while drying at a temperature of 120°C. For the oil from the seeds dried at this temperature, the level of absorbance was in the following amounts: for carotenoids – from 202 to 315, accounting for scattering, while if measured according to the sector norm – it was respectively from 252 to 440. For chlorophyll it was from 91 to 200. The increase in the drying temperature (150 and 180°C) only decreased these values. It is noticeable that there were no carotenoids observed at the highest drying temperature as the scattering caused by products of lipid, chlorophyll and carotenoid degradation disturbed any proper reading of the result.

The reported increase in the number of colorants after treating the seeds with higher temperature is problematic. Zadernowski *et al.* [27] also points to this correlation in his papers. Particularly doubtful is the increased content of chlorophyll in the dried seeds, as it is known that chlorophyll is very sensitive to light and increased temperature. It is only after a detailed analysis of deterioration of chlorophylls that we were able to give an explanation to this phenomenon. It is however obvious that any increased temperature (above 120°C) has a negative impact on the colour of oil and most probably, due to the formation of free radicals, has a harmful impact on the health of consumers.

Figure 1 represents exemplary absorbance spectra (UV-Vis) of the oil aceton solutions from the seeds of 5 varieties dried at different temperatures: 80, 100, 120, 150 and 180°C, as well as in the oil coming from the control seeds in the respective solutions of: 5 times (panel B, C, D, E) or 10 times (panel A and F). Different solutions were made so as to show on the spectra the outstanding ranges of absorption coming from both, the carotenoid and chlorophyll colorants. It is indicated, on the dotted line in panel B, that the spectrum coming from refined oil (Kujawski of first press) is free of carotenoids and chlorophyll, so this is a scattering of light coming from fatty acids. There is a particular deformation occurring to maxims in the spectrum of absorption for the carotenoid colorants in oil extracted from seeds treated in temperatures above 120°C [7]. Examining the phenomenon of the disappearance of carotenoid absorbance with respect to temperature for ranges of 400–500 nm, we can see that the number of carotenoids dramatically decreases above the temperature of 120°C. This contributes to the fact, that high temperature during the drying of seeds causes carotenoid degradation. It appears from the analysis of the absorption spectra that the same decrease is true for the absorbance of chlorophylls, meaning that they also deteriorate.

T a b l e 1. Content of chlorophyll and carotenoid colorants in oils coming from rapeseeds at different drying temperatures

Drying temperature	Variety	Carotenoids A_{460}		Chlorophyll A_{666}	General colour		Chlorophyll	
		R	N		R	N	a (g ml^{-1})	b (g ml^{-1})
Control	Kana	92±2	123±10	147±10	238±10	270±10	1.76±0.20	2.16±0.20
	Marita	122±10	206±5	55±5	177±10	261±5	0.86±0.30	0.20±0.10
	Lisek	98±5	205±5	69±5	167±5	274±5	1.48±0.20	0.09±0.02
	Lirajet	96±10	160±5	60±5	156±10	220±10	1.42±0.10	0.54±0.10
	Bristol	115±5	194±5	103±5	228±5	297±10	1.99±0.10	0.30±0.05
	M	104.6	177.6	86.8	187.2	264.4	1.50	0.66
80°C	Kana	158±10	201±10	194±10	352±10	395±10	2.97±0.50	4.01±0.50
	Marita	159±10	208±10	92±5	251±6	300±10	1.61±0.30	0
	Lisek	202±8	275±5	107±5	309±8	382±10	1.92±0.20	0
	Lirajet	217±10	300±10	115±10	332±10	415±10	2.12±0.20	0
	Bristol	211±10	277±10	129±10	340±10	406±10	2.52±0.15	0
	M	198.0	252.2	127.4	325.4	379.6	2.23	
100°C	Kana	223±10	308±10	97±5	320±10	405±10	2.14±0.20	0
	Marita	208±10	315±10	85±5	293±10	400±10	1.55±0.40	0.03±0.02
	Lisek	270±5	399±10	145±5	415±10	544±10	3.02±0.40	0
	Lirajet	255±10	377±10	125±6	380±10	502±10	2.58±0.10	0
	Bristol	288±10	422±10	130±8	418±10	552±10	2.52±0.30	0
	M	248.8	364.2	115.8	365.2	480.6	2.36	
120°C	Kana	202±10	253±10	200±10	402±10	453±10	3.39±0.40	2.85
	Marita	272±10	362±8	92±5	364±10	454±10	1.77±0.40	0
	Lisek	315±10	418±10	91±5	406±10	509±10	1.62±0.40	0
	Lirajet	275±10	407±10	175±10	450±10	582±10	3.63±0.40	0
	Bristol	295±10	440±10	120±5	415±10	560±10	2.45±0.20	0
	M	282.0	395.8	135.6	415.6	511.6	2.57	
150°C	Kana	135±10	267±10	81±5	216±10	348±10	1.56±0.20	0
	Marita	167±5	310±5	120±5	287±10	430±10	2.41±0.20	0
	Lisek	230±10	442±10	132±10	362±10	574±10	2.31±0.20	0
	Lirajet	155±10	306±10	120±10	275±10	426±10	2.12±0.40	0
	Bristol	206±10	428±10	145±10	351±10	574±10	2.82±0.20	0
	M	178.6	350.6	119.6	298.2	470.4	2.24	
180°C	Kana	–	195±10	121±10	–	316±5	1.52±0.30	0
	Marita	–	194±10	71±5	–	265±10	1.44±0.30	0
	Lisek	–	251±10	79±10	–	330±10	1.52±0.20	0
	Lirajet	–	194±10	79±10	–	282±5	1.52±0.20	0
	Bristol	–	261±10	95±10	–	336±10	1.68±0.30	0.31±0.30
	M	–	219.0	89.0	–	305.8	1.54	

R – carotenoids and general colour including the Raleigh distraction in lipids, N – carotenoids and general colour calculated according to the Sector Norm.

It can be seen, that along with the increase in the drying temperature there is an increase in the absorbance in the short wavelength range (ca. 350 nm), which indicates the creation of free radicals in the process of drying seeds [12].

Changes in the absorbance of oils at 350 nm, in respect of changes in the temperature of drying, have been presented on Fig. 2. The level of absorbance at 120°C is twice as high as it is at control temperature (25°C). It increases already up to six times as high, for a temperature of 180°C. It appears from the analysis of short-wavelength light scattering which can be an indication for free radicals forming, that up to 120°C, the level of free radicals marginally increases, while above this, it involves a significant deterioration of products thus giving less nutrition in the oil.

So, the temperature of 120°C seems to be the top limit for the drying of rapeseeds. Any excess, involves a thermal generation of a high amount of free radicals. Furthermore, the oil loses substantial amounts of carotenoids being precursors to vitamins, thus losing nutrition.

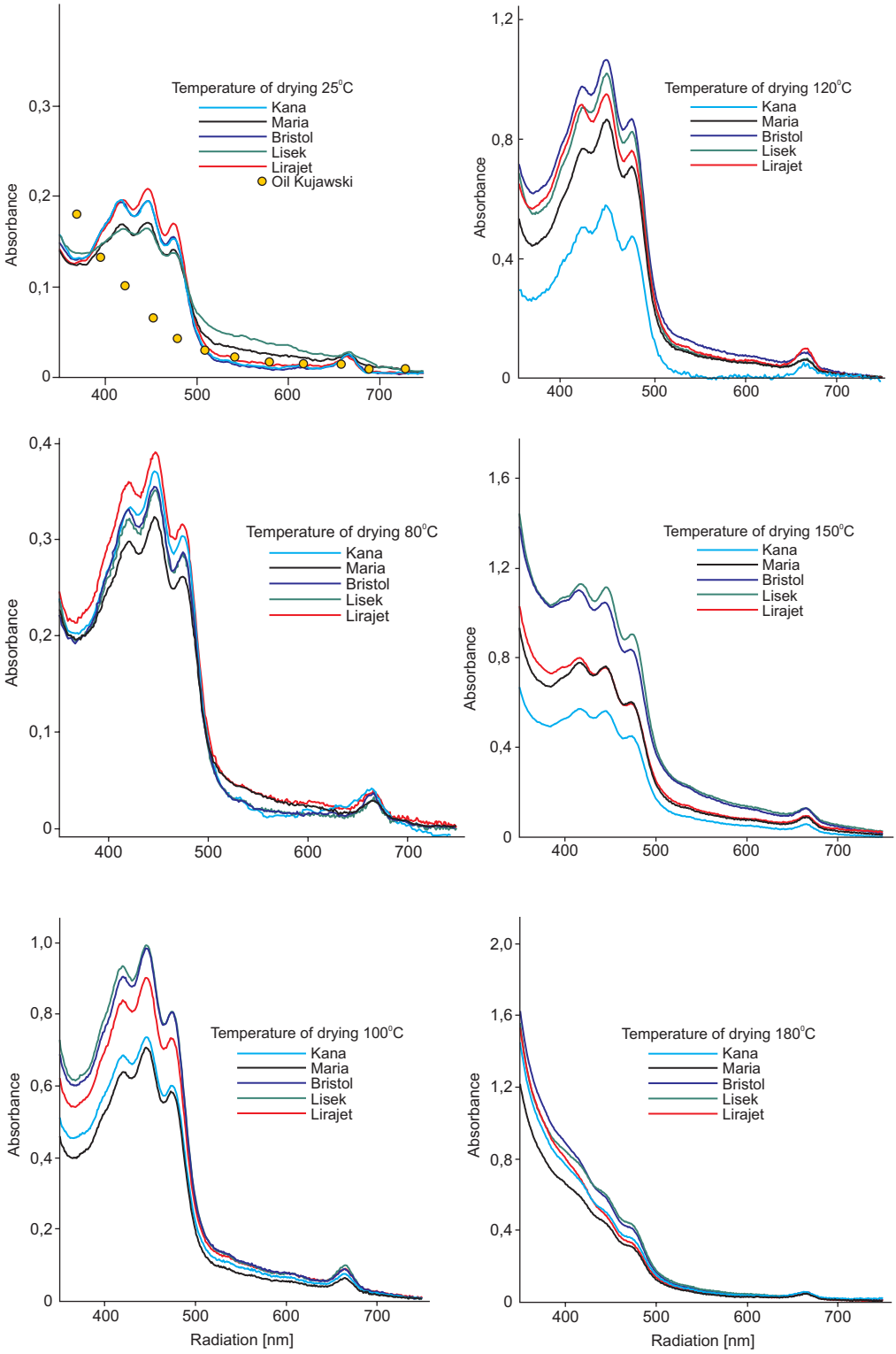

Fig. 1. Examples of absorption spectra recorded in the range of 350–750 nm from oil samples dissolved in acetone. In order to be measured, the oil samples were diluted 5–10 times. Different panels correspond to different drying temperatures (25–180°C), indicated.

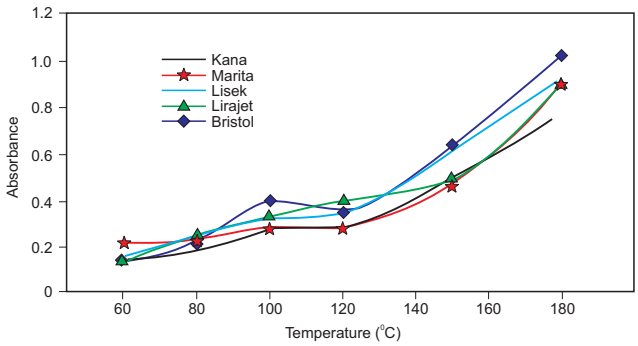

Fig. 2. Changes to the absorbance at 350 nm of oils in respect of changes to the drying temperature.

CONCLUSIONS

1. The varieties of seeds examined demonstrate significant differences in their chlorophyll and carotenoid content. The best, in the sense of the composition of colorants (much carotenoids and little chlorophyll) is oil extracted from the seeds of Marita, while the worst is from Kana.

2. The increase in the temperature of drying rapeseeds has a negative impact on the colour of the oil. The most adverse changes occurred in seeds dried at a temperature above 120°C.

3. The general colour of the oils examined was improved by heating to a temperature of 120°C. Thereafter, only a systematic decline was observed.

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