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Influence of drying and storage on lipophilic antioxidants content in rapeseed*

Wpływ suszenia oraz przechowywania na zawartość lipofilnych przeciwutleniaczy w nasionach rzepaku

Key words: rapeseed, drying, tocopherols, HPLC

During storage, lipids contained in seeds undergo adverse chemical and biochemical processes. The range of these transformations is conditioned mainly by the presence of water and also by the content of natural antioxidants. Among the lipophilic antioxidants occurring in the seeds of oil plants — also rape — are: tocochromanols (-T), plastochromanol-8 (PC-8) and β-carotene. In our climatic zone, the harvested rape seeds require additional drying until their moisture content drops to below 7%. The moisture content of 5–6% is considered the optimum due to high mechanical resistance of the stored seeds, resistance to microorganisms etc. Also the influence of these processes upon the native lipohilic antioxidants significantly determining the stability of lipids in the stored seeds, is of importance. The aim of this study was to investigate the influence of preliminary seed drying — in high as well as in low air temperatures — on the content of natural lipophilic antioxidants immediately after the harvest and after one-year storage. Two rape varieties were considered — Lisek and Kronos (00). Drying temperatures ranged from 60 up to 120°C and also some low air temperatures were applied. After reaching ca 6% moisture content, the seed samples were stored for a year at 18±1°C in darkness. After 12 months, tocopherols, PC-8 and β-carotene were determined again. Qualitative separation and quantitative determination of antioxidants in seeds were carried out by HPLC. It was noted that drying after the harvest resulted in ca 10% total tocopherol loss, and a year storage caused further loss of 50% of these compounds. Similar values were determined for PC-8 (ca 55% in relation to the original content). The smallest loss was determined for β-carotene, and its content in both varieties, Lisek and Kronos, did not exceed 35% and was the lowest in seeds dried at 60°C.

Słowa kluczowe: rzepak, suszenie, tokoferole, HPLC

Podczas magazynowania stwierdzono, że lipidy nasion roślin oleistych, w tym rzepaku, ulegają niekorzystnym procesom chemicznym i biochemicznym. Zakres tych przemian uwarunkowany jest przede wszystkim obecnością wody, a także zawartością naturalnych przeciwutleniaczy. Do lipofilnych antyoksydantów obecnych w nasionach roślin oleistych, w tym rzepaku, zaliczamy tokochromanole (-T), plastochromanol-8 (PC-8) i beta-karoten. Zazwyczaj po zbiorze, w naszych warunkach klimatycznych, nasiona rzepaku wymagają dosuszania do poziomu poniżej 7% wilgotności.

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Uważa się, że wilgotność 5–6% jest optymalna z uwagi na wysoką wytrzymałość mechaniczną przechowywanych nasion, odporność na drobnoustroje itp. Nie bez znaczenia jest również wpływ tych zabiegów na natywne lipofilne przeciwutleniacze, które w znaczący sposób determinują stabilność lipidów w magazynowanych nasionach. W pracy przebadano jaki jest wpływ suszenia nasion — wysoko- i niskotemperaturowego — na zawartość naturalnych lipofilnych antyoksydantów zaraz po zbiorze i rocznym przechowywaniu. Przebadano dwie odmiany rzepaku — Lisek i Kronos (00). Zastosowane temperatury suszenia to 60, 80, 100 i 120°C oraz niskotemperaturowe suszenie powietrzem. Próby nasion po dosuszeniu do około 6% wilgotności przechowywano przez rok w temperaturze 18±1°C bez dostępu światła. Po 12 miesiącach magazynowania ponownie wykonano oznaczenia tokoferoli, PC-8 oraz β-karotenu. Jakościowo i ilościowo przeciwutleniacze w nasionach oznaczano przy użyciu HPLC. Stwierdzono, że suszenie po zbiorze powoduje około 10% sumaryczny ubytek tokoferoli, a roczne magazynowanie dalsze około 50% straty. Podobne zależności określono dla PC-8 (około 55% w stosunku do początkowej zawartości). Najmniejszy ubytek oznaczono dla beta-karotenu, przy czym jego zawartość w obu badanych odmianach Lisek i Kronos nie przekraczała 35% i była najniższa w nasionach suszonych w temperaturze 60°C.

Introduction

During the last five years, the rapeseed cultivation in Poland was constantly increasing, and also the forecast for the coming period up to the year 2010 predicts further increase of both — yield as well as the cultivation area. Rapeseed, cultivated not only as a raw material for oil industry, should be characterized by high quality and also considerable durability. The seeds are biological material in which various biochemical processes occur during post-harvest processing (purifying, drying, and storing). The extent of these transformations is conditioned, first of all, by the presence of water, and also by the content of natural antioxidants. In our climatic zone, the rapeseeds usually require drying just after harvesting aiming at reaching the level of less than 7% moisture content. The moisture content of 5–6% is considered most favourable because of high mechanical resistance of the stored seeds as well as their resistance to microorganisms. Improper temperature of post-harvest drying significantly affects the technological quality of seeds, particularly the peroxide and free fatty acid content in the triacylglycerol fraction (Krasucki et al. 2002, Tys and Rybacki 2001). In seeds, oxidation processes initiated during post-harvest treatment proceed chain-like during further stages of seed processing, for instance — during oil refining. Taking into consideration the quality of seeds and also some economic aspects, specialists suggest to take up studies concerning low temperature drying of seeds cultivated in our climatic zone (Gawrysiak-Witulska et al. 2005). The goal of the study was to investigate the influence of rapeseed drying in high as well as in low temperatures on the content of native lipophilic antioxidants immediately after the harvest and after one year of storage.

Material and methods

Material for the study

Variety Lisek and hybrid variety Kronos, both of double improved quality — were investigated. Before the experiment the rapeseeds were moistened with a specified amount of water and left for 24 h at 8ºC. After this procedure, the moisture content of seeds was 12–13%. Both kinds of seeds were dried in high as well as in low temperatures until ca 6% moisture content was obtained. The moisture content of the stored material was determined using a Sartorius MA 30 balance (Gawrysiak-Witulska et al. 2005). Samples were stored for one year at 18±1^{o}C in total darkness.

Seed drying conditions

Low temperature drying of rapeseeds in a thick, motionless layer was carried out on a specially designed and constructed stand (Gawrysiak-Witulska i Ryniecki 2001). The seeds were dried on a stand built of segments — each 0.1 m high — all together forming a 1.2 m thick layer. Apparent velocity of the air flowing through the rapeseed layer was the same in all experiments and amounted to 0.14 m/s. A relative humidity and the temperature of air sucked in by the fan changed at random as in typical low temperature air drying. In order to avoid moistening of seeds, a very simple, electronic humidistat was applied to monitor air heating and keep the relative humidity of the air blown into the seed mass at the level not exceeding 40%. Every 8 hours the segments were weighed to determine changes in seed moisture content in layers 2 and 12. Drying continued until 6% moisture content was reached in layer 12. The time of experiments ranged from 48 to 56 hours.

High temperature drying proceeded in a laboratory drier. The rapeseeds were dried at 60, 80, 100 and 120ºC, in a thin layer of ca 0.5 cm, on a fine sieve. Laboratory drier was controlled by a computer program which calculated the current seed moisture content taking, as a basis the mass loss and the indicated initial moisture content. Drying continued until 6% seed moisture content was obtained. Drying time was 8–10 min. for 120°C, 12–14 min. for 100°C, 17–20 min. for 80°C and 35–40 min. for 60°C.

Tocopherol, plastochromanol-8 and beta-carotene determination

In order to determine the alpha-, beta-, gamma- and delta-tocopherols (α-, β-, γ-, δ-T) plastochromanol-8 (PC-8) and beta-carotene contents, samples of rapeseeds were saponified using 60% NaOH. After saponification, the nonsaponifiable substances were extracted three times using the peroxide free diethyl ether

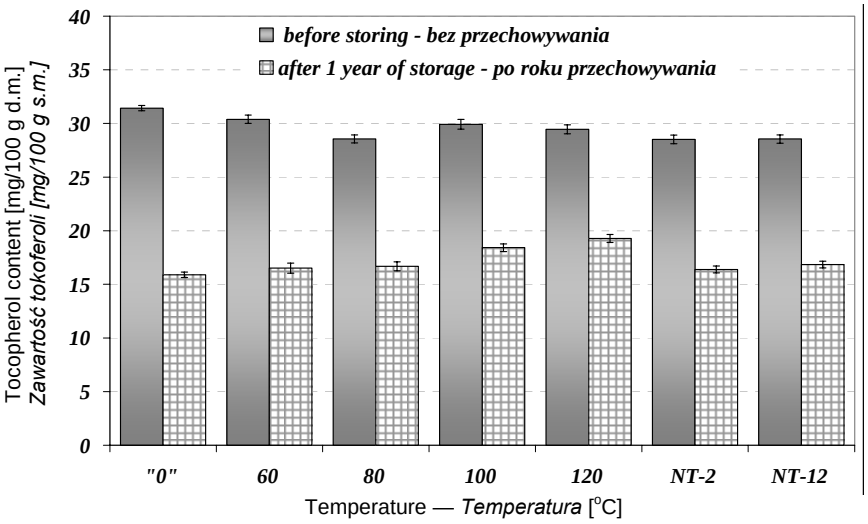
(Dunphy et al. 1966). Then, ether was distilled off and the residue was dissolved in *n*-hexane. The qualitative identification and quantitative determination of homologous tocopherols were carried out by HPLC (Gogolewski et al. 2000, Nogala-Kałucka at al. 2002). The tocopherols were analysed on the HPLC (Waters 600, Milford, MA, USA) and Waters Millenium 32 data acquisition system equipment with LiChrosorb Si 60 column (250 × 5 mm; 5 μm) and precolumn LiChrospher Si 60. The mobile phase was *n*-hexane and 1,4 dioxane (97 : 3 v/v). The tocopherols were monitored by a fluorimetric detector (295, 330 nm). The contents of individual tocopherol homologues was calculated on the basis of calibration curves made for pure standards of these compounds. Standards of alpha-, beta-, gamma- and delta-tocopherols (α-T, γ-T, β-T, δ-T) (99%) were purchased from Merck (Darmstadt, Germany).

Statistical analysis

The results obtained were subjected to statistical analysis. Results are represented as means ± standard deviation from three replicates of each experiment. One-factor analysis of variance and post-hoc Tukey's tests for the significance level $p < 0.05$ were carried out using a statistical package program Statistica (version 6.0).

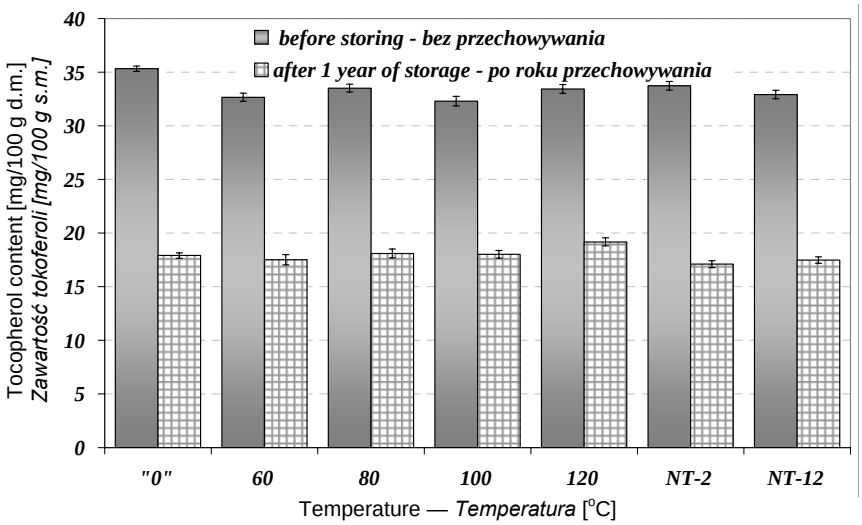
Results and discussion

Earlier investigations revealed that rapeseeds intended for sowing should be dried in air driers at a specific temperature. Sowing material should not be heated over 35ºC (Wałkowski et al. 2006). However, during harvest, it is sometimes necessary — e. g. because of the weather — to dry quickly big seed batches which are not intended for reproduction but, for instance, for some industrial application. The present study concerned model drying on a specially constructed low temperature drying stand (Gawrysiak-Witulska et al. 2005) and comparing the influence of this process and that of high temperature drying on the content of native antioxidants. Water content was determined in the seeds after harvest: in Lisek it amounted to 12.5% whereas in Kronos — to 11.3%; both varieties were taken as zero samples. The results of drying of these two varieties are presented in Figures 1–6. All biologically active compounds with antioxidant properties — tocopherols, beta-carotene and PC-8 — were determined in all samples right after the harvest and preliminary drying and then after a year of storage. The quantitative determination of the content of individual compounds was compared to the "0" sample of rapeseed of the same variety harvested and not subjected to drying. In the investigated varieties, total tocopherol content in Lisek amounted to 31.4 mg/100 g d.m., whereas in Kronos — it was 35.3 mg/100 gd.m. Both varieties



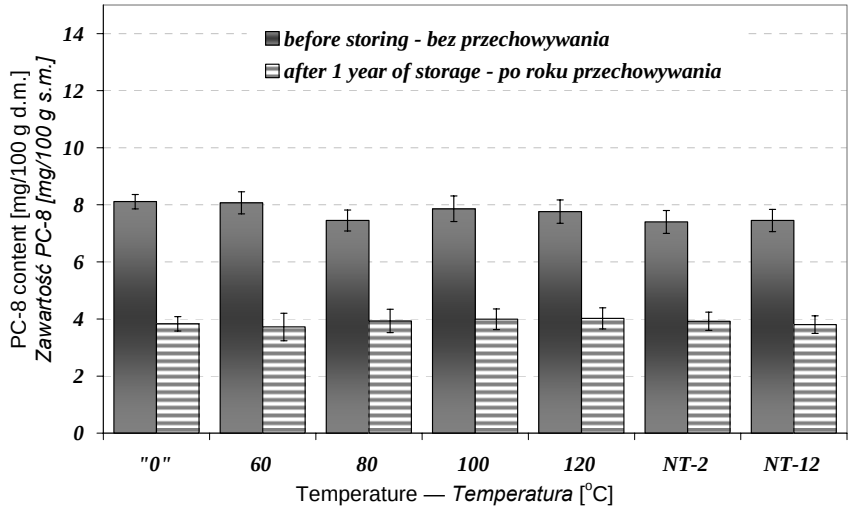
NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 1. Total tocopherol content in rapeseed – seeds of Lisek variety — *Zawartość sumy tokoferoli w nasionach rzepaku – nasiona odmiany Lisek*



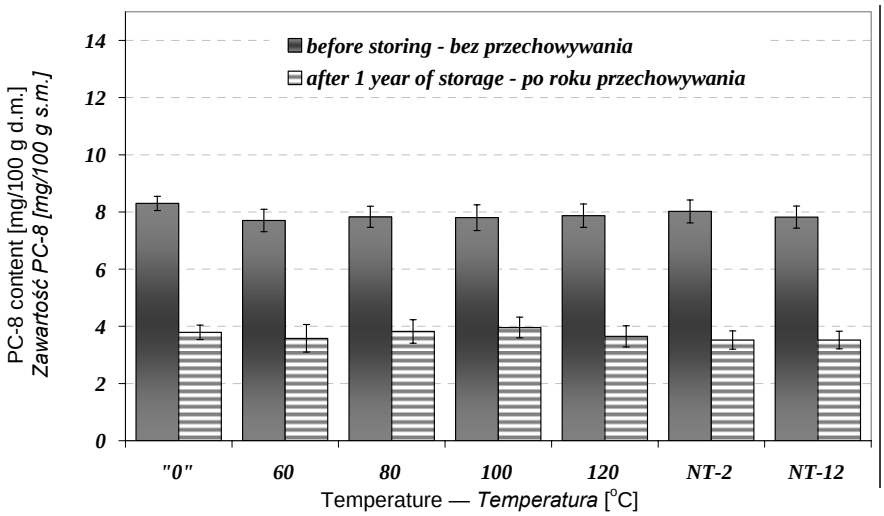
NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 2. Total tocopherol content in rapeseed – seeds of Kronos variety — *Zawartość sumy tokoferoli w nasionach rzepaku – nasiona odmiany Kronos*



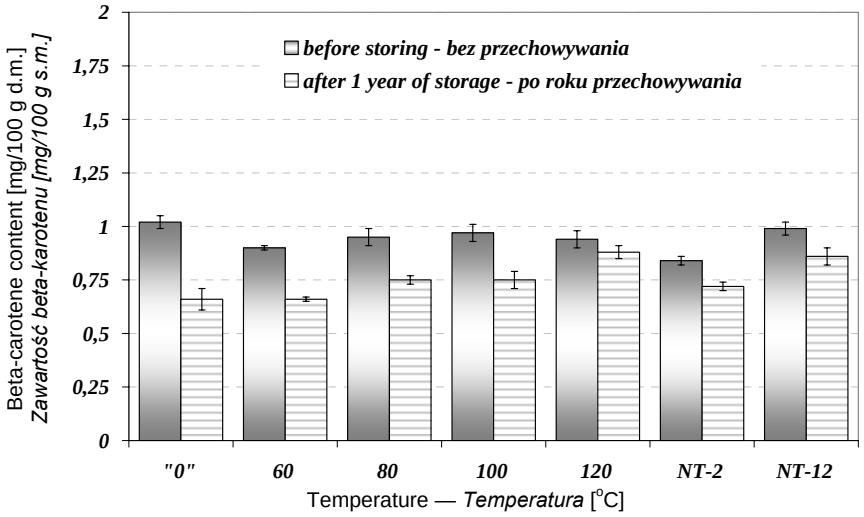
NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 3. PC-8 content in rapeseed – seeds of Lisek variety — *Zawartość PC-8 w nasionach rzepaku – nasiona odmiany Lisek*



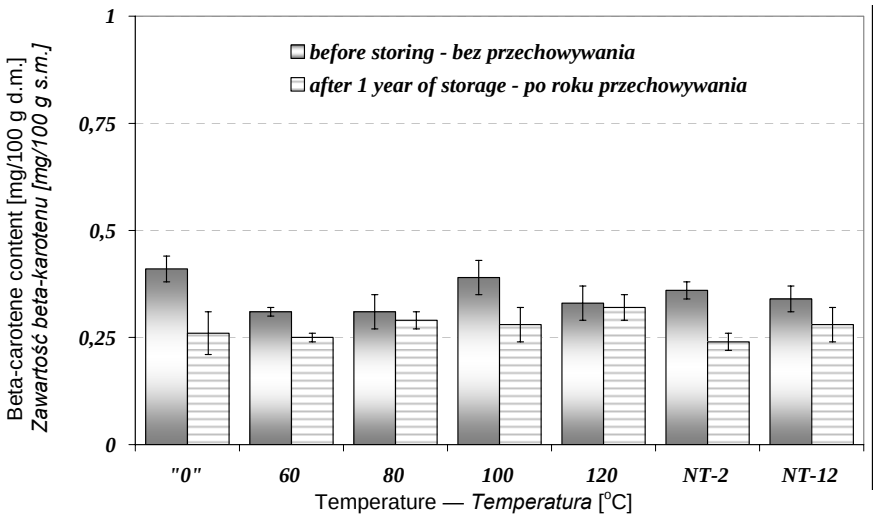
NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 4. PC-8 content in rapeseed – seeds of Kronos variety — *Zawartość PC-8 w nasionach rzepaku – nasiona odmiany Kronos*



NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 5. Beta-carotene content in rapeseed – seeds of Lisek variety — *Zawartość β-karotenu w nasionach rzepaku – nasiona odmiany Lisek*



NT-2 — low temperature drying layer 2 — *suszenie niskotemperaturowe segment 2*
NT-12 — low temperature drying layer 12 — *suszenie niskotemperaturowe segment 12*

Fig. 6. Beta-carotene content in rapeseed – seeds of Kronos variety — *Zawartość β-karotenu w nasionach rzepaku – nasiona odmiany Kronos*

contained more alpha-T (α-T) (16.8 mg/100 g d.m. — Lisek and 18.3 mg/100 g d.m. — Kronos) than gamma-T (γ-T) (14.1 mg/100 g d.m and 16.5 mg/100 g d.m, respectively). After drying to 6% moisture content, the tocopherol level slightly decreased. In Lisek the loss ranged from 3.3% (temp. 60°C) to 9.3% (low temperature segment 2), while in Kronos, it varied from 4.5% (low temperature layer 2) to 8.6% (temp. 100°C). During storage, further loss of tocopherols took place with γ-T undergoing decomposition more promptly than α-T. Post harvest processing also affected the content of PC-8 and beta-carotene. Depending on the drying temperature and the variety, the loss of PC-8 ranged from 1 to 9% (Figs. 3–4). After one year of storage 42 to 49% of the original PC-8 content remained in the seeds of investigated varieties. The greatest decomposition of beta-carotene occurred after drying at 60°C (Figs. 5–6). The loss of beta-carotene in rapeseeds ranged from 15 to 39% depending on the variety and drying temperature. Summing up the obtained results, it can be concluded that preliminary drying of rapeseeds to ca 6% moisture content influenced native antioxidants content. These losses amounted from 10% (tocopherols and PC-8) and 25% (beta-carotene). Greater losses were noted during a year of rapeseed storage (up to 50% of the initial content on average). After 12 months, the loss of γ-T was by 10% greater than that of α-T.

It is difficult to discuss the obtained results due to the lack of relevant literature data. It was noted that temperatures applied during high temperature drying did not cause greater losses of total tocopherols than those applied in low temperature process. Also, temperatures of 60, 80, 100 and 120ºC did not bring about losses of individual homologues, which can be explained by the stability of tocopherols in the biological material subjected to short time heating. Low temperature drying caused slightly bigger losses of total tocopherols after post- harvest drying reaching almost 10% in Lisek. These losses can be attributed to the longer drying process and the influence of water phase on tocopherols in rapeseeds (Wałkowski 2006). Seed drying at low temperature requires a long time. Therefore, the presented study was only preliminary and requires continuation. It is not possible to compare the obtained results to any other research because of the lack of the latter.

Conclusions

1. Post- harvest seed drying of the Lisek and Kronos rapeseed varieties caused a few per cent loss of the content of native antioxidants (up to ca 10%). One year seed storage caused further loss of tocopherols reaching 50% of their initial content.

2. Losses of plastochromanol-8 were comparable to tocopherol losses in seeds undergoing preliminary, post- harvest drying and then stored. They amounted to ca 55% when compared to the initial content of PC-8 in seeds of both investigated varieties.
3. In Lisek and Kronos, quantitative losses of beta-carotene were smaller than those of tocopherols and PC-8. After a year of storage, they amounted to 35% in seeds subjected to the preliminary, low temperature drying at 60ºC.

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