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ORIGINAL PAPER

Changes in Tocochromanol Content in Seeds of *Brassica napus* L. During Adverse Conditions of Storage

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Abstract Tocopherols and plastochromanol-8 were evaluated in seeds of *Brassica napus* L. during adverse conditions of storage at different temperatures (25 and 30 °C) and moisture levels (10, 12.5 and 15.5%). Both temperature and moisture content of seeds had a significant effect on the hydrolysis of triacylglycerols in rapeseed oil and on the contents of tocopherols and PC-8. The biggest losses of tocopherols (a drop by 14.4% after 18 days) were recorded for seeds with a moisture content of 15.5% and stored at a temperature of 30 °C. Losses of the α-T homologue were bigger than those of γ-T. The loss of PC-8 ranged from 4 to 24% depending on storage conditions and it was almost two times bigger than the loss of tocopherols.

Keywords Rapeseed · Tocopherol · Plastochromanol-8 · Degradation of tocopherols · Postharvest

Introduction

Rapeseed oil is considered to be one of the most valuable plant fats. It is a rich source of mono- and polyenoic acids [1] and natural antioxidants—tocopherols, plastochromanol-8 (PC-8), phenolic compounds and sterols [2]. Tocochromanols and PC-8 determine lipid stability in stored seeds [3]. Oxidation of edible oils containing polyenoic fatty acids is a considerable problem for the food industry due to the direct relationship with economic, nutritional, flavor, and storage factors. Oxidation products formed in the course of this process (free radicals, lipid peroxides, aldehydes, ketones, etc.) affect human health [4]. Types and amounts of individual oxidation products in oils depend on the fatty acid composition, storage conditions, particularly temperature, availability of oxygen, and light. The main factor limiting lipid oxidation is the presence of natural antioxidants [5, 6].

An important group of native antioxidants found in rapeseed comprises tocopherols. Four homologues, i.e., α-, β-, γ- and δ-, of tocopherol (T) are present in rapeseed, but α-T and γ-T contents amount to 800 mg/kg oil, while the other two are found in trace amounts [7, 8]. Antioxidant properties of tocochromanols depend on their concentration, type of substrate, solvent, light and temperature as well as other chemical compounds exhibiting a pro-oxidation action (ions of transition metals) and synergistic action (phospholipids, ascorbic acid, polyphenols) [9]. This has been shown by numerous studies conducted using different fat substrates under different conditions (temperature, light, oxygen) [10–12].

Gopalakrishnan et al. [13] investigated the effect of storage (room temperature and cold room) on chemical changes in lipids, including contents of tocopherols in comminuted rapeseeds. Gawrysiak-Witulska et al. [14] in their studies determined the effect of post-harvest procedures (near-ambient drying) on changes in tocopherol contents and plastochromanol-8 in rapeseeds. In turn, Goffman and Möllers [15] investigated the effect of temperature (5, 20 and 40 °C) and availability of oxygen during storage on contents of tocopherols and plastochromanol-8 in intact rapeseeds and in oil pressed from these seeds.

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Inadequate rapeseed preservation may contribute to a decrease in the contents of these compounds. Under the Canada Grains Act, the maximum moisture at which canola can be sold as straight grade (dry) is 10% moisture [16]. For storage longer than 5 months, canola should be binned at a maximum of 8% moisture [17]. The Australian Oilseed Federation (AOF) Standards Committee has recently introduced a maximum moisture concentration at seed delivery of 8% [18]. Generally in Poland, seeds after harvest are dried to a moisture content of 7%, which is considered safe in terms of storage conditions [19]. However, there are cases where, during storage of the seeds, moisture migrates in silos or from the heating of warehouse walls with southern exposure. As a consequence, warmer seed layers are dried slightly, while colder layers are repeatedly wetted. The moisture level and temperature of the product will influence events that occur during storage and may sometimes lead to spoilage and self-heating. Regular monitoring is required because rapidly respiring seeds produce heat and moisture favoring the growth of storage molds. Mold growth and respiration produces additional heat and moisture, further increasing the temperature within the seed bulk [20]. In the course of self-heating biologically active compounds may also be degraded. However, there are no literature data concerning the effect on tocochromanols found during storage of seeds with increased moisture content. The objective of the present study was to investigate the loss of tocochromanols in intact seeds during adverse storage conditions caused by different temperature (25 and 30 °C) and moisture (10, 12.5 and 15.5%) treatments in rapeseed. Moreover, the effect of these storage conditions on the free fatty acids content in oil extracted from these seeds was determined.

Materials and Methods

Materials

The material used for tests was canola cv. *Californium* obtained directly after harvest from the Zlotniki Experimental Station owned by the Poznan University of Life Sciences, Poland. Prior to the onset of the experiment, rapeseed was processed to obtain the assumed storage conditions (three moisture contents of approximately 10, 12.5 and 15.5% wet mass basis (w.b.) and two temperatures of 25 ± 1 and 30 ± 1 °C) by spraying seeds in batches of 4 kg with a specific amount of distilled water. The amount of water required to obtain seeds with the assumed moisture content was determined using a mass balance. Seeds after being moistened in order to equalize moisture content throughout the seed bulk were packaged in polyethylene bags and conditioned for 24 h at 5 °C.

Seed Storage

In the bulk of stored seeds, between kernels and the air found in the interseed spaces, constant migration of water occurs until a state of equilibrium is reached. Seed storage in the atmosphere with equilibrium relative humidity (RH), corresponding to the assumed seed moisture content at a given temperature, prevents migration of water between kernels and the air in the interseed spaces, which facilitates the maintenance of the seed moisture content at a constant level. The experimental seeds, after being moistened, were stored in a thermostatic chamber equipped with three hygrostatic devices that allow maintenance of a constant level of relative humidity in interseed spaces of seed, corresponding to the assumed seed moisture content of approx. 10, 12.5 and 15.5%. Relative humidity corresponding to the assumed seed moisture contents at both temperatures was determined based on Halsey's equation [21]

$$\mathrm{RH} = \exp\left[\frac{\exp(3.489 - 0.010553 \cdot T)}{(MC_D)^{1.86}}\right]$$

where RH is the relative humidity, T is the temperature in °C, MC_D is the dry-basis moisture content.

The constant level of equilibrium relative humidity in ambient air surrounding seeds placed in containers was maintained with the use of a saturated solutions of salts, i.e. NaCl, KCl and $BaCl_2$, placed in cells of the hydrostatic devices. Hygrostatic devices were equipped with fans, producing a constant air flow from above the salt solution through the seed layer. This provided a uniform distribution of the assumed RH in interseed spaces. The relative humidity in the containers with seeds was monitored using relative humidity probes with capacity sensors. Temperature in the seed bulk was monitored using Cu-Konstantan thermocouples (type EE21-FT6B53/T24). Relative humidity in the interseed spaces and the temperature were measured on-line using the I-7018 data acquisition system by ICP-CON and the ICP computer software for the recording, visualization and storage of data. Rapeseeds with a moisture content of 10, 12.5 and 15.5% were stored in a thermo-hygrostatic chamber for 18 days at a temperature of 25 and 30 °C. Samples for analyses were collected every 6 days during seed storage.

Determination of Seed Moisture Content

The seed moisture content was determined using an electronic moisture analyzer (MA150 Sartorius Mechatronics, Poland). The moisture analyzer used a reference standard prepared by drying a 5 g sample at the temperature of 115 °C to constant mass. The measuring accuracy of the analyzer was 0.05% w.b. (wet basis). The moisture

analyzer was verified using the oven method according to the Current Protocols in Food Analytical Chemistry [22], using the prepared reference as stated above.

Soxhlet Extraction

Seeds were ground in a universal mill (M-20 IKA®-Werke GmbH & CO. Germany) and then the lipids were extracted with petroleum ether in an automatic Soxhlet Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland) for 6 h. The extraction of lipids was carried out in low-boiling petroleum ether (a mixture of pentanes and hexanes with a boiling point of 35–40 °C). After extraction, the petroleum ether was evaporated at 35 °C, under reduced pressure using a rotary evaporator R-215 (Büchi Labortechnik AG, Flawil, Switzerland).

Determination of Acid Value

Samples of 5–6 g of oil were taken for analysis. The acid value was expressed as the amount of KOH (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil. Free fatty acids in a sample were measured as recommended in the Current Protocols in Food Analytical Chemistry [23].

Tocochromanol Contents

Samples of seeds (2 g) were saponified using 60% KOH (2 ml), ethanol (20 ml) and pyrogallol (0.5 g). The saponification was carried out at the ethanol boiling point temperature (78 °C) for 30 min. After saponifications, unsaponifiable substances were extracted using 50 ml *n*-hexane/ethyl acetate (90:10 v/v). Tocopherols and plastochromanol-8 (PC-8) were qualitatively and quantitatively identified using HPLC (Waters 600 Asc. Milford, MA, USA). A LiChrosorb Si60 column (250 × 4.6 mm; 5 μm) and a LiChrospher Si60 precolumn were used. The mobile phase consisted of *n*-hexane and 1,4-dioxane (97:3 v/v). Flow rate was 1.5 ml/min. The fluorometric detector (Waters 474 Asc. Milford, MA, USA) worked at an excitation of $\lambda = 290$ nm and an emission of $\lambda = 330$ nm for tocochromanols and PC-8 [24]. Standards of α-, β-, γ- and δ-tocopherol (α-T, γ-T, β-T, δ-T) (99%) were purchased from Merck (Darmstadt, Germany) and used to determine retention times and for quantification of tocopherol.

Statistical Analysis

Results are presented as means ± standard deviation from three replicates of each experiment. A P value < 0.05 was used to denote significant differences between mean values determined by the analysis of variance (ANOVA) with the assistance of Statistica 7.0 (StatSoft, Inc., Tulsa, OK) software.

Results and Discussion

In all the tested rapeseed samples stored under the assumed moisture and temperature conditions, the contents of free fatty acids increased (Fig. 1) with increasing moisture content of seeds. In seeds with a moisture content of 10 and 12.5%, stored at a temperature of 25 °C, the contents of free fatty acids after the completion of the experiment were 1.08 and 1.63 mg KOH/g, respectively. Seeds with a moisture content of 15.5% had an acid value of 6.4 mg KOH/g, which exceeded the admissible level of 3 mg KOH/g (Fig. 1). In seeds stored at a temperature of 30 °C, an even bigger increase was observed in the content of free fatty acids. The acid values of 1.45, 2.01, and 10.6 mg KOH/g were observed in rapeseed stored at 10, 12.5, and 15.5%, respectively (Fig. 1). Appelqvist and Loof [25] stated that good quality seeds with a moisture content of 7% may be stored for 3 years, showing only a slight increase in the content of free fatty acids, while in moldy seeds under identical conditions the amount of free fatty acids increased to 18%.

The contents of tocochromanols and plastochromanol-8 were analyzed in samples collected in the course of the experiment. Changes in the contents of α- and γ-tocopherol homologues and the total content of tocopherols in rapeseeds stored at 25 and 30 °C are presented in Figs. 2 and 3. The initial total content of tocopherols in the tested rapeseeds was 548.8 mg/kg. In rapeseed, the content of tocochromanols depended to a considerable degree on environmental conditions [26]. The percentage composition of the tocopherol fraction was characteristic of rapeseed. The dominant tocopherol was the γ-T homologue (291.1 mg/kg), which accounted for 53% total contents of tocopherols. The content of the α-T homologue was 251.1 mg/kg (46%), while that of homologues β-T and δ-T in the tested samples amounted to 1.5 and 5.1 mg/kg, respectively. Marwede et al. [27] showed a similar dependence concerning individual tocopherol homologues, where γ-T and α-T accounted for 64 and 35%, respectively, while β-T and δ-T were less than 1% of the total. According to the same authors, the α-T: γ-T ratio in rapeseed ranges from 0.54 to 1.70. In seeds collected for storage, the α-T/γ-T ratio was 0.86. Conducted analyses showed that during rapeseed storage under adverse conditions the total content of tocopherols decreases (Figs. 2, 3), with the rate of losses for these compounds being affected by both storage temperature and seed moisture content. In seeds with a moisture content of 10 and 12.5%, stored at 25 °C, losses of tocochromanols at the completion of the

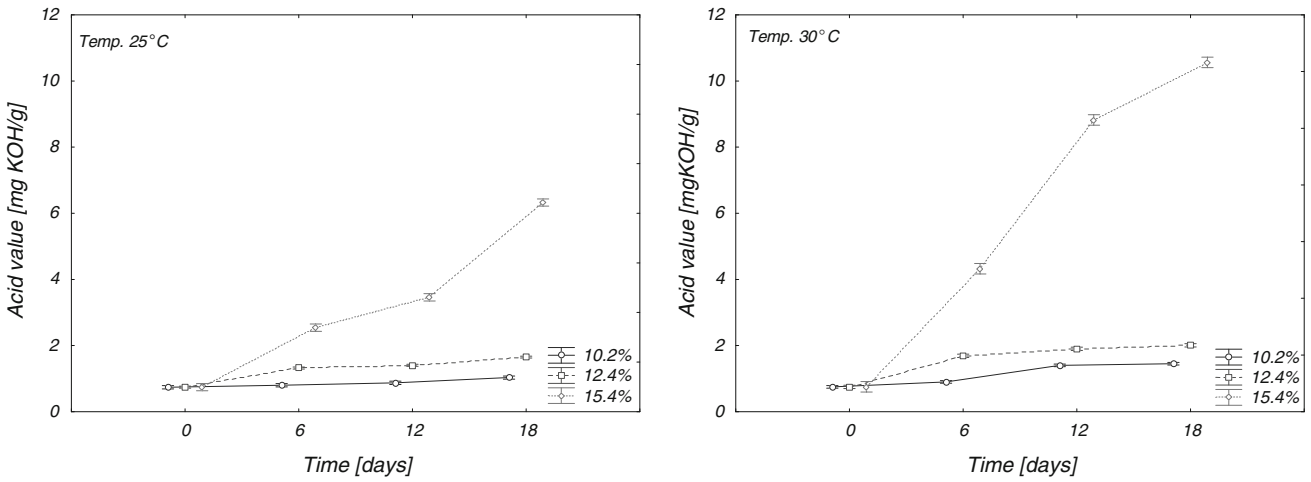

Fig. 1 Acid value in oil extracted from rapeseeds with different moisture contents at 25 and 30 °C

experiment were similar, amounting to 3.7–4.7%, respectively. Seeds with moisture content of 15.5% lost 8.2% of the tocochromanols. An increase in storage temperature to 30 °C considerably increased losses of these compounds. In seeds with a moisture content of 12.5%, losses amounted to 6.4%, whereas in seeds with a moisture content of 15.5% it was 14.4%.

The study also comprised an analysis of the effect of seed storage conditions on individual tocochromanol homologues. In the case of α-T, the storage of seeds with a 10% moisture content for 18 days resulted in the degradation of this homologue by 2–3% at both temperatures (i.e. 25 and 30 °C). Losses of α-T amounting to 5% at 25 °C and 7.5% at 30 °C were observed in seeds with 12.5% moisture content. Storage of seeds with a moisture content of 15.5% resulted in losses of α-T amounting to 9.4 and 15.8% at 25 and 30 °C, respectively. An increase in seed moisture content from 10 to 12.5% caused an almost twofold greater loss of α-T at 25 °C and threefold at 30 °C. Smaller losses were recorded for homologue γ-T. Seeds with a moisture content of 10% after 18 days of storage lost from 1.5 to 3% of the initial γ-T content. Increasing seed moisture content to 12.5% resulted in losses of 4–5%, while for a 15.5% moisture content losses of 6 and 12% were recorded (for 25 and 30 °C, respectively). The faster degradation of the α-T homologue than that of γ-T is illustrated by changes in the α-T/γ-T ratio. In seeds with a moisture contents of 12.5 and 15.5% and stored at 25 °C, this ratio decreased from 0.86 to 0.85 and 0.83, respectively. In the case of 30 °C, this index decreased to 0.84 (moisture content of 12.4%) and 0.82 (moisture content of 15.4%). Smaller changes were observed in seeds with a moisture content of 10%, where this ratio during storage at 25 °C did not change, while at a temperature of 30 °C it decreased slightly from the initial value of 0.86–0.85. Gopalakrishnan et al. [13], when investigating the effect of storage of ground rapeseed on the content of tocopherols, stated a 50% reduction of total tocopherols after a 10-day storage at room temperature (20 °C) and after 30 days at 5 °C. Goffman and Möllers [15] did not report such a loss of tocopherols in intact rapeseeds at different temperatures (5, 20 and 40 °C). However, they [15] observed losses of tocopherol contents only at a temperature of 40 °C. They also observed that the α-T homologue throughout the experiment (24 weeks) remained unchanged at all of the tested temperatures. In contrast, the content of γ-T decreased by 20% after 24-week storage, which was also illustrated by the α-T/γ-T ratio [15]. Also investigations conducted by Gawrysiak-Witulska et al. [14] showed that during storage of seeds with a moisture content of 7% at a temperature of 10 ± 2 °C the value of the α-T/γ-T ratio increased. This means that during storage of seeds with an appropriate moisture content a faster degradation occurred for homologue γ-T than α-T. In contrast, this study indicates that adverse storage conditions (elevated moisture content and temperature) significantly affect an accelerated degradation of the homologue α-T.

Rapeseeds are also characterized by the presence of plastochromanol-8 (PC-8), which is a derivative of gamma-tocotrienol [28]. Results concerning this compound are presented in Fig. 4. An increase in moisture content as well as temperature of rapeseed storage resulted in a decrease of PC-8 content. This compound turned out to be less stable under adverse storage conditions than tocopherols. The PC-8 losses were 4 and 8% for temperatures of 25 and 30 °C, respectively, for rapeseed stored 18 days at 10% moisture. An increase in moisture content to 12.5% resulted in a three times greater loss of PC-8 at 25 °C (a decrease of 9.4%). At 30 °C losses of PC-8

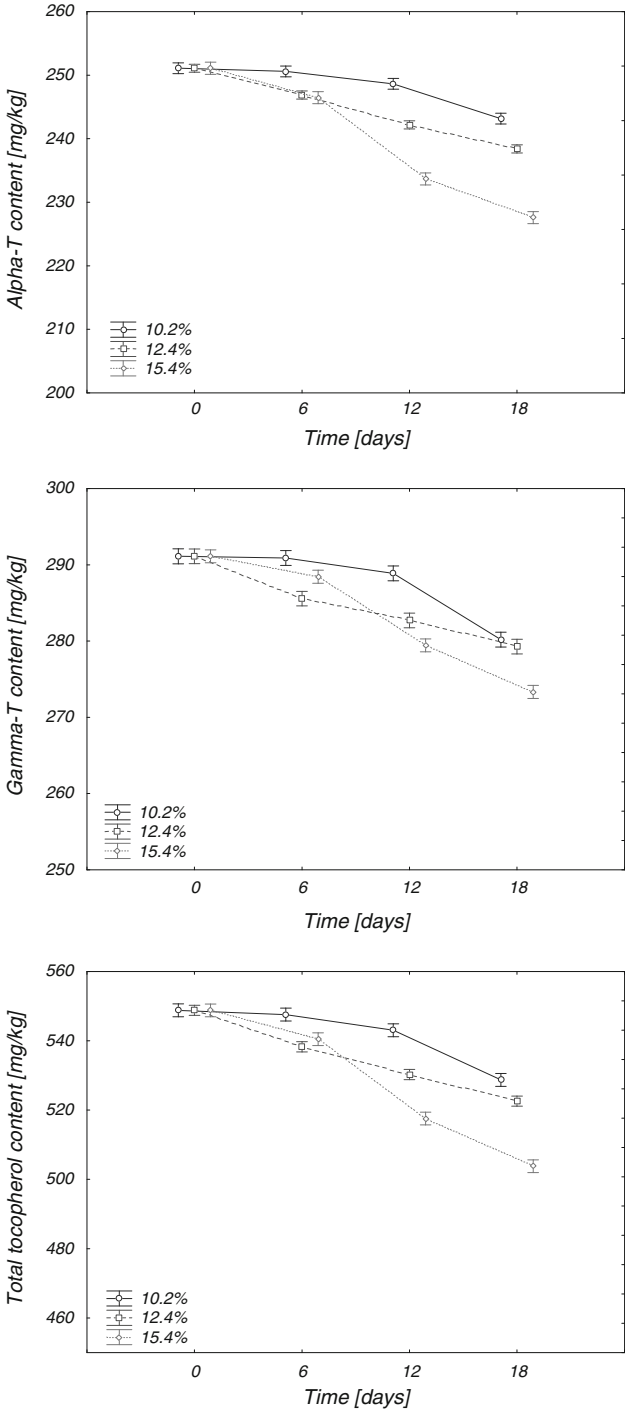

Fig. 2 Tocopherol content in rapeseeds with different moisture contents stored at 25 °C

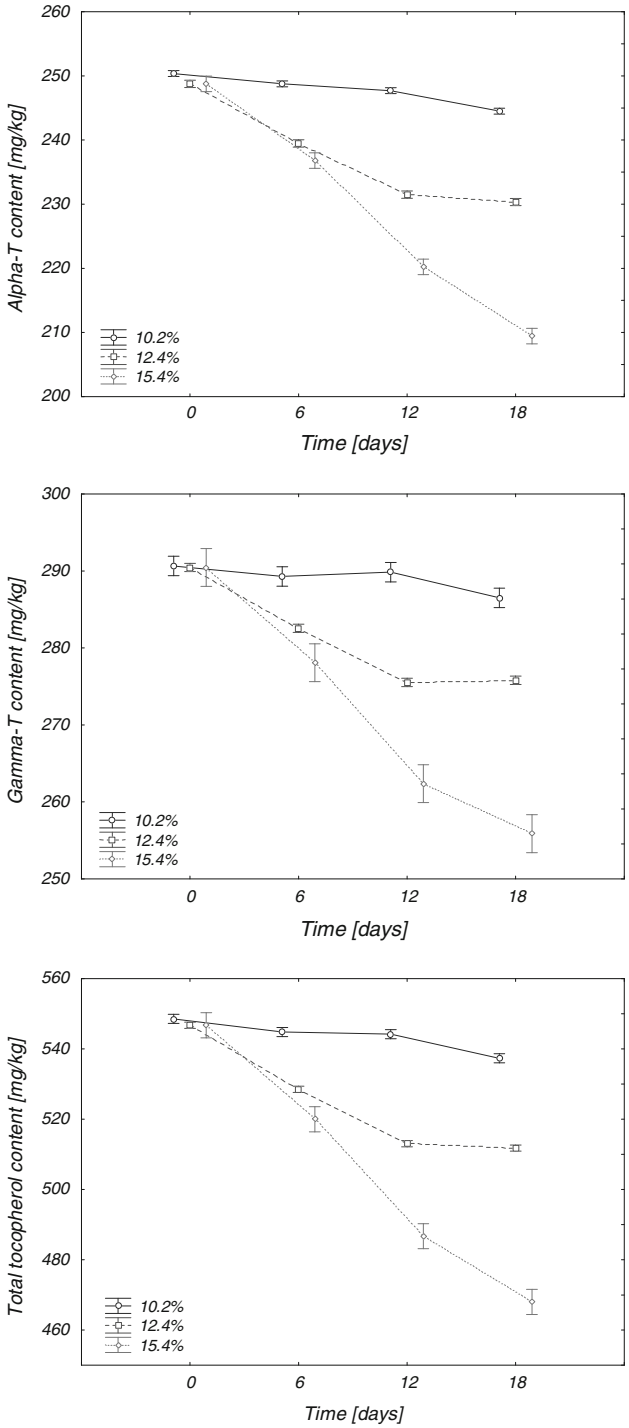

Fig. 3 Tocopherol content in rapeseeds with different moisture contents stored at 30 °C

increased to 12%. During storage of seeds with a 15.5% moisture content, the PC-8 content decreased by 14.5 and by 24% at a temperature of 25 and 30 °C, respectively. Also Goffman and Möllers [15] showed that PC-8 was degraded much more rapidly than tocopherols. In their study they reported a 45% decrease in the contents of PC-8 during a 24-week storage at a temperature of 40 °C. They also showed that the most significant and the fastest losses of this compound occur in the first 4–6 weeks, while after that time the rate of degradation decreased. No degradation of this compound was observed at a temperature of 5 or 20 °C [15].

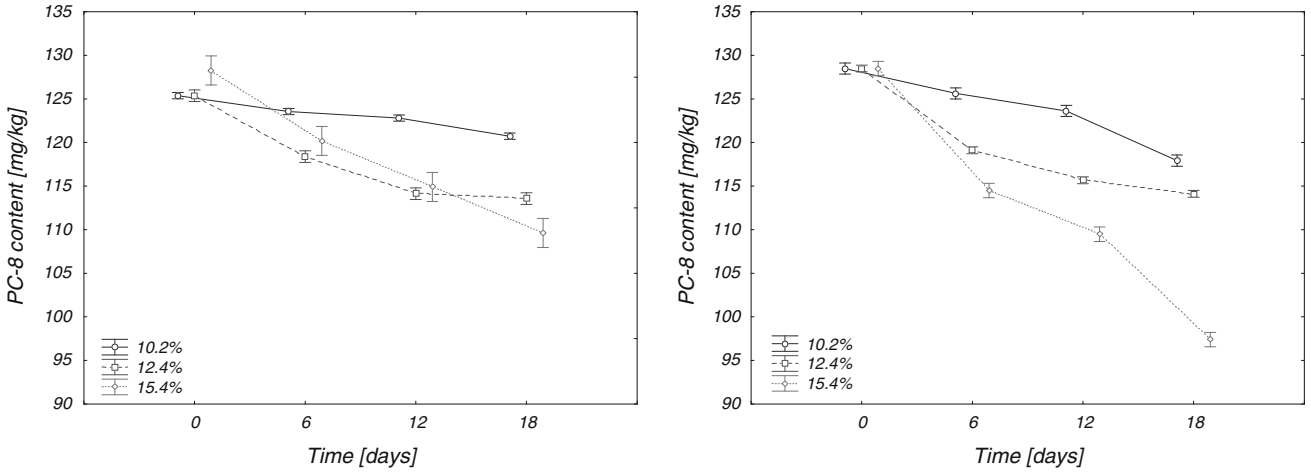

Fig. 4 PC-8 content in rapeseeds with different moisture contents stored at 25 and 30 °C

Conclusion

Experiments conducted within this study, concerning inappropriate storage conditions of rapeseed, clearly indicate that a too high moisture content as well as storage temperature have an adverse effect on contents of phytochemicals, such as native tocochromanols and plastochromanol-8. In rapeseed oil produced from the seed, contents of native tocopherols have a significant effect both with respect to the nutritive value (as a natural source of vitamin E) and from the technological point of view (as they inhibit autoxidation of fatty acids, thus extending shelf life of oil). By meeting respective parameters of postharvest procedures we may significantly affect the rate of degradation of tocopherols contained in rapeseed. Insight into the scale of changes in tocopherol contents in rapeseed for different storage conditions may be of significant importance for the optimization of seed preservation.

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