The Effect of Freezing on the Analysis of Chlorophyll Content of Canola Seed (*Brassica napus* L.)

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High levels of chlorophyll in harvested canola seed result in loss of revenue to producers and problems for processors. Studies on chlorophyll degradation often require plant material to be stored for some time prior to measuring the chlorophyll content. Storage of unripe canola seed in a freezer for up to one month prior to measuring the chlorophyll content did not alter the chlorophyll level in the seed. Seeds were frozen while still in the pods as well as after removal with no change in chlorophyll content over time.

KEY WORDS: *Brassica napus*, canola, chlorophyll, chlorophyll analysis, chlorophyll degradation, freezing, green seed, oilseed rape, pigments, storage.

High levels of chlorophyll in harvested canola seed cause numerous problems during processing. The chlorophyll pigments are extracted into the oil, where they promote oxidation upon exposure to light and result in rancidity (1). They impair hydrogenation reactions by acting as a catalyst poison (2), and they result in products with unacceptable color (1).

When sampling plant material, it is often necessary to store the material for some time prior to analysis. Canola seed with a high moisture content readily undergoes chlorophyll breakdown. Larsson and Gottfridsson (3) stored rapeseed at various stages of maturity for four weeks at temperatures of −5 to 25°C. Significant chlorophyll breakdown occurred, particularly for seed with a high moisture content at temperatures above 10°C (3). Therefore, a method of seed storage that prevents chlorophyll destruction is needed for analytical work. In this study, unripe canola seed was stored in the freezer for various lengths of time prior to measuring the chlorophyll content to determine whether chlorophyll degradation occurs in the frozen seed.

MATERIALS AND METHODS

Seed samples were obtained from field plot trials conducted to evaluate chlorophyll degradation rates in a number of oilseed rape (*Brassica napus* L.) cultivars. The plants were sampled when at least 50% of the plot had reached physiological maturity, as indicated by a color change of the seeds in the lower pod on the main stem from green to partially brown [Harper and Berkenkamp (4), growth stage 5.4]. The cultivar regent was sampled for the first seeding date, and the cultivar Westar was sampled for the second and third seeding dates.

Approximately 30 plants were sampled from each plot. The pods were removed from the main stems and mixed together to give a uniform sample. This sample was then divided in two. One-half was further subdivided into four treatment groups—fresh, frozen two days, frozen one week and frozen one month. The pods were then frozen in sealed plastic bags below −15°C in a dark freezer for the appropriate length of time. In the other half of the sample, seeds were removed from the pods, placed in sealed vials and subdivided into the same four treatment groups—fresh, frozen two days, frozen one week and frozen one month. Following each treatment, the pods or seeds were placed in a freezer-drier for approximately 48 h. Seeds were then removed from the pod material. The seeds from each treatment (1–8) were analyzed for chlorophyll content.

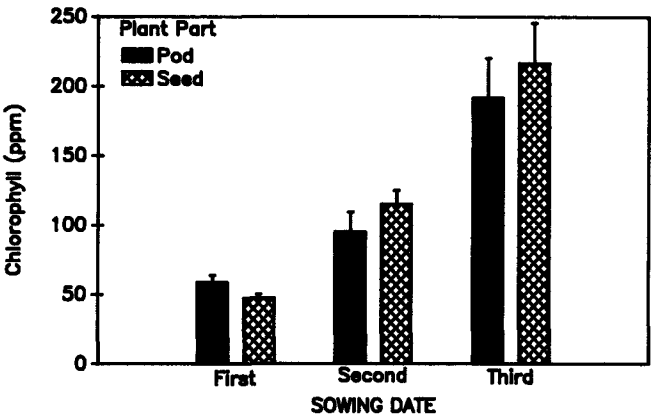

FIG. 1. Mean chlorophyll levels (ppm) in seeds frozen in pods (Pod) compared to seeds frozen after removal (Seed); $P = 0.05$.

Chlorophyll analysis was carried out by extraction and absorbance measurement on a Spectronic 1001 spectrophotometer (Bausch & Lomb, Rochester, NY) according to ISO method 10519 (5). One-gram samples of the freeze-dried seed were weighed and placed in stainless steel test tubes with ball bearings and 30 mL of isooctane/ethanol (3:1). Samples were shaken for one hour, filtered, and absorbance readings were measured. Three wavelengths, 625.5, 665.5 and 705.5 nm, were used, to measure the absorption peak for chlorophyll, with corrections on either side. This method is calibrated against chlorophyll a, but will measure "apparent chlorophyll," which includes the group of related pigments with absorption maxima near 665 nm, chlorophyll a and b and their respective degradation products. Three extractions and measurements were made on each set of seeds, and the results were averaged.

RESULTS AND DISCUSSION

An analysis of variance was run on the data for each sowing date. There were no differences between the mean chlorophyll levels in the seeds frozen in the pods compared to those frozen after removal at the $P = .05$ level of significance (Fig. 1). There also were no differences in chlorophyll content between the treatment groups of fresh and frozen for 2, 7 and 30 days (Fig. 2).

The ability to freeze seed while still in the pods saves considerable time during the sampling period. The seeds also can be removed much more rapidly after the pods have been freeze-dried.

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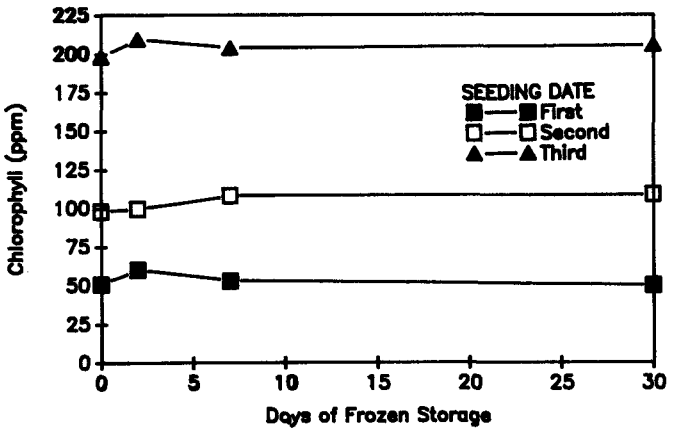

FIG. 2. Comparison of mean chlorophyll levels in seed sampled from three sowing dates (first, second and third) and sampled fresh or frozen for 2, 7 and 30 days.

The results of this study indicate that seed for chlorophyll analysis can be frozen in the pods and stored in the freezer for up to one month prior to analysis without a significant change in chlorophyll levels.

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REFERENCES

1. Clear, K.M., and J.K. Daun, in *Research on Canola Seed, Oil Meal and Meal Fractions*, 8th Progress Report, Canola Council of Canada Winnipeg, 1987, pp. 277-279.
2. Abraham, V., and J.M. deMan, in *J. Am. Oil Chem. Soc. 63*:1185 (1986).
3. Larsson, R., and K. Gottfridsson, in *Proceedings of the 4th International Rapeseed Conference*, Giessen, Germany, 1974, pp. 261-266.
4. Harper, F.R., and B. Berkenkamp, *Can. J. Plant Sci. 55*:657 (1975).
5. International Standards Organization, Geneva, 1992, Method 10519, in press.

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