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# Dry Matter, Lipids, and Proteins of Canola Seeds As Affected by Germination and Seedling Growth under Illuminated and Dark Environments

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The effect of germination and growth under illuminated and dark environments on canola seed reserves was investigated. Depletion of proteins and lipids in whole seedlings and their top (leaf/cotyledons) and bottom parts (stem/roots/seed coat) was independent of light, whereas the protein solubility increased at a faster rate under an illuminated environment than in the dark. A rapid increase in free fatty acids but a net decrease of dry matter content in seedlings grown in the dark environment was observed. The dry matter content of seedlings grown in the illuminated environment increased due to photosynthetic biomass accumulation.

**KEYWORDS: Oilseeds; canola; germination; seed reserves; free fatty acids; gas chromatography**

## INTRODUCTION

Canola is a member of the Cruciferae family and is an important cash crop in Canada (*1*). At present, canola is used mainly as a source of vegetable oil for food and industrial applications, and the meal after oil extraction is used in animal feed formulations. Canola seed contains both nutrients, such as lipids, proteins, and vitamins, and antinutrients, such as phytic acid and glucosinolates. The major obstacle to incorporating substantial amounts of whole canola seeds and/or seed fragments in human food is the presence of these aninutritional factors. It is known that during the process of germination canola seed can metabolize a substantial amount of glucosinolates, resulting in seedlings with low glucosinolate content. Therefore, an understanding of the mobilization and utilization of major seed reserves during the process of seed germination and seedling growth is important to explore the possibility of employing germination as a means of reducing antinutrients while maintaining or increasing the content of nutritionally important constituents. Antinutrients in plant materials are no longer considered to be unimportant because these substances are known to confer beneficial health effects in humans, and they are now termed as non-nutrient components. Changes in non-nutrient components in canola during germination have been researched and are out of the scope of our study. Although non-nutrient factors are important in terms of health benefits, it is the amount and proportions of seed reserves that determine the nutritional value of whole seeds used for human and animal consumption. Similarly, the amount and proportion of nutrients are the factors that determine the overall nutritional quality of germinated seeds and seedlings. Effects of germination on major components of seeds from several oilseed crops have been investigated (*2*−*6*). For instance, changes in the cell wall polysaccharides, lipids, and proteins of white and dark mustard and rapeseed have been reported (*7*−*11*).

In this study, the main objective was to investigate the effect of germination stage and light on major seed reserves, namely, lipids and proteins, of germinated canola seeds and seedlings. In addition, attempts were made to understand the changes in lipid and protein reserves in the leafy parts (seedling tops) and stem/root parts (seedling bottoms) of the seedlings at various stages of growth under illuminated and dark conditions.

## MATERIALS AND METHODS

**Materials.** The seeds of canola (*Brassica napus* L.) variety Q2 summer rape were a donation of Agricore United, Calgary, AB, Canada. Authentic C18:1 acylglycerol standard mixture, dihydrocholesterol, and bis(trimethylsily)trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) (99:1) were procured from Sigma Chemical Co., St. Louis, MO, whereas hexane was from Fisher Scientific Co., Nepean, ON, Canada.

**Sample Preparation and Dry Matter Analysis.** Five grams of canola seeds was soaked in 200 mL of distilled water for 4 h. After draining excess water, three seed samples were used as the control and the others were allowed to germinate in Petri dishes lined with moistened filter paper and paper towels. The germination was carried out in the presence and absence of light in a greenhouse at 20 °C (*12*). For the former, light was provided for 16 h/day, whereas for the latter, the dish was covered with aluminum foil to block the light. The light was supplied with 400 W high-intensity sodium bulbs. The germinating seeds and seedlings were watered twice daily using tap water (mineral content not determined) until the seedlings were harvested at intervals of 2, 5, 10, 15, and 20 days of germination and subjected to analysis. Portions of 10-, 15-, and 20-day samples were dissected into two sections, and the leafy top portion was labeled “seedling tops” whereas the remaining portion (stem, roots, and seed coat) was labeled “seedling bottoms”. Whole seedlings were also subjected to analysis. The

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harvested samples were frozen in liquid nitrogen, lyophilized, and then weighed. The dry matter loss during germination was determined using weight difference.

**Determination of Oil Content.** Oil content was determined gravimetrically. Approximately 2 g of sample was ground to powder in a coffee grinder. The oil was extracted by refluxing the sample with hexane for 6 h using a Goldfisch apparatus according to AACC method 30-20 (*13*). After the extraction was completed, the mixture was transferred into a preweighed glass tube. The extracted oil was then recovered by evaporation of the solvent under a stream of nitrogen in a water bath at 40 °C in a fume hood. The oil and glass tube were weighed to determine the oil content of the sample. The weight of the oil was recorded and the sample stored at −18 °C under nitrogen in an amber glass vial until used. The defatted meal was also kept at −18 °C in an amber glass container until used.

**Determination of Lipid Classes: Derivatization and GC Conditions.** The content of monoacylglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG), and free fatty acids (FFA) of oil samples was determined. Approximately 1 mg of oil and 0.1 mg of dihydrocholesterol internal standard were derivatized in 0.25 mL of pyridine and 0.25 mL of BSTFA containing 1% TMCS at 70 °C for 30 min. The mixture was then dissolved in hexane and analyzed by gas chromatography. A DB-5HT fused silica capillary column (J&W Scientific, 30 m × 0.25 mm i.d., 0.1 $\mu$m film thickness, Agilient Technologies) in a Varian 3400 gas chromatograph (Walnut Creek, CA) equipped with a flame ionization detector and a Varian 8100 autosampler was used. Helium was the carrier gas used at a head pressure of 25 psi. Chromatograms were recorded, and the peak integration was carried out using Shimadzu Class-VP software (version 4.2, Shimadzu Scientific Institutes, Inc., Columbia, MD). An initial column temperature of 60 °C was maintained for 2 min, ramped first at 30 °C min$^{-1}$ to 140 °C and then at 5 °C min$^{-1}$ to 235 °C, which was maintained for 7 min. The column temperature was then ramped to 350 °C at 5 °C min$^{-1}$ and maintained for 3 min before ramping at a rate of 10 °C min$^{-1}$ to a temperature of 380 °C, which was maintained for 14 min. An initial injector temperature of 70 °C was maintained for 0.1 min and then ramped to 370 °C at 150 °C min$^{-1}$, and this temperature was maintained for 69 min. The detector temperature was set at 370 °C. Authentic standards of FFA and MAG, DAG, and TAG of C16:0, C18:1,and C20:0, respectively, were run along with the same batch as samples for qualitative and quantitative analyses.

**Qualitative GC Analysis of Lipid Classes.** Canola oil is a very complex mixture, containing >100 components of various lipid classes. During germination, the breakdown of some of the components further increases the complexity, resulting in poor resolution of peaks. The carbon chain length of 99.5% of fatty acids in canola oil is between 16 and 20 carbons and, therefore, this study used authentic C16 and C20 fatty acids and acylglycerols to identify different lipid classes. The unidentified components with retention times less than that of C16 fatty acids were also grouped as "volatiles". Almost 95−99% of components in the samples were identified using this approach.

**Quantitative GC Analysis of Lipid Classes.** Quantification of each group of lipids was carried out using relative response factor (RRF) for internal standards of fatty acids from the C18 family and C18:1 acylglycerol standard mixture (containing equal amounts of FFA and MAG, DAG, and TAG). All calculations were based on the formulas

$$\text{RRF} = (\text{weight of AS} \times \text{area of IS})/(\text{weight of IS} \times \text{area of AS})$$

$$\text{weight of X} = \text{RRF} \times \text{weight of IS} \times \text{area of X/area of IS}$$

where RRF, AS, IS, and X are the relative response factor, authentic standard, internal standard, and target compound, respectively.

**Determination of Protein Content and Solubility.** The total (crude) protein content of the defatted meal was calculated by multiplying the nitrogen content with a conversion factor of 6.25. The nitrogen content was determined using a nitrogen analyzer (model FP-428, Leco Instruments Ltd., Mississauga, ON, Canada). The solubility of proteins was assessed after 0.7 g of defatted meal had been dissolved in 30 mL of distilled water. The mixture was shaken for 2 h at room temperature (20 °C) and then centrifuged at 1090*g* for 15 min. The supernatant and the residue were collected in preweighed plastic tubes, lyophilized in a freeze-dryer, and weighed. The protein content in each fraction was determined using the same method explained for total protein content. The protein solubility was calculated on the basis of the dry weight of soluble and insoluble fractions.

**Statistical Analysis.** All trials were performed in triplicate, whereas proximate and microanalyses were carried out in duplicate. Analysis of variance (ANOVA) of the data was performed using the General Linear Model procedure of SAS statistical software, version 8 (*14*). The effect of germination, light, and seedling part on the content and composition of all components was determined at $p \leq 0.05$.

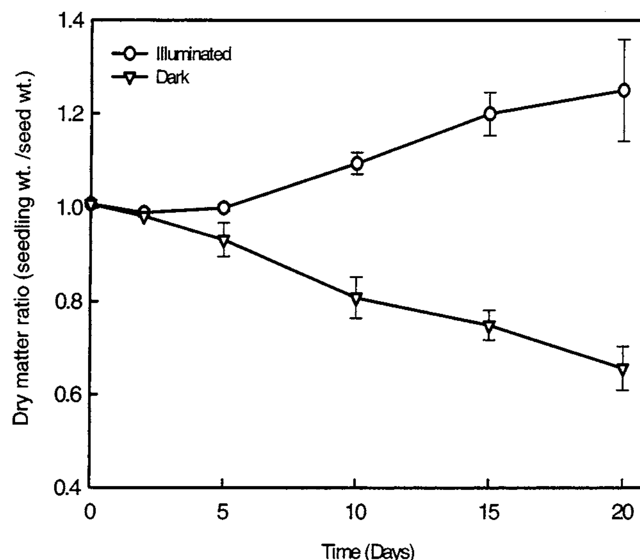


**Figure 1.** Effect of germination and growth environment on dry matter ratio of seedlings.

## RESULTS AND DISCUSSION

**Changes in Dry Matter Content. Figure 1** shows the significant ($p < 0.05$) changes in dry matter content of germinating seed/seedling in the presence and absence of light. The ratio of dry matter (seedling wt/seed wt) in the presence of light slightly decreased through day 2 and then increased to 1.25 on day 20 of seedling growth, whereas that in the absence of light decreased from 1.00 on day 0 to 0.66 on day 20. These results indicate the accumulation of biomass in the presence of light and may be attributed to the synthesis of structural carbohydrates through photosynthesis after the emergence of first leaves. The decrease in the ratio of dry matter observed for seedlings grown in the dark is consistent with other studies (*15*, *16*), which may be attributed to the absence of photosynthesis and consumption of carbohydrates in the course of respiration and utilization of food reserves by the growing seedling. Under illuminated conditions, a slight decrease in dry matter on day 2 may be attributed to the seed respiration and leaking of certain substances during 4 h of soaking before germination. By day 5, the roots have already emerged and started taking up minerals from water. Furthermore, the cotyledons were capable of photosynthesizing, causing the accumulation of biomass. On day 5, the accumulation was not enough to compensate the prior loss; therefore, the ratio of dry matter was still slightly lower than 1.

**Changes in Oil Content. Figure 2** depicts the oil content of canola seeds and seedlings during the process of germination in the presence and absence of light. There was a drastic decrease ($p < 0.05$) in the lipid reserves of seedling during the first 10 days of germination under both illuminated and dark conditions (**Figure 2A**). Oil content remained stable up to day

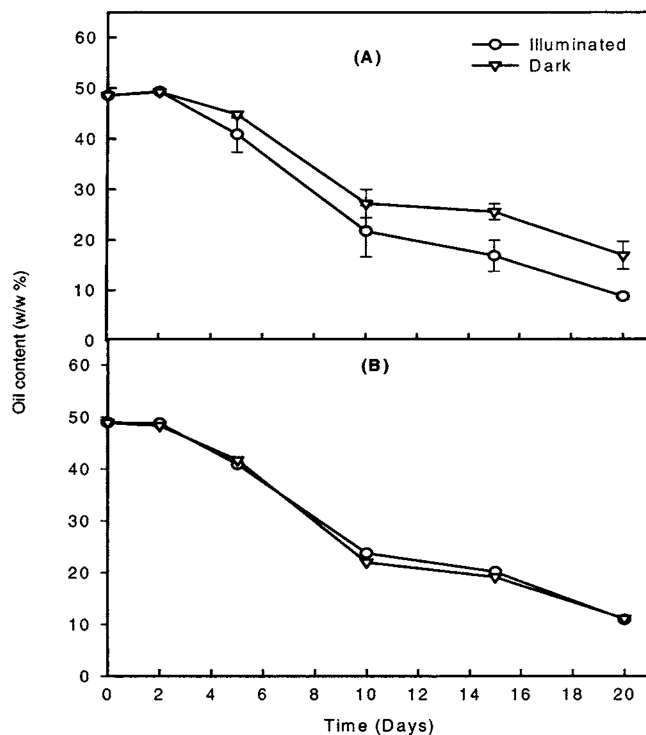


**Figure 2.** Effect of germination and growth environment on oil content on seedling basis (**A**) and seed basis (**B**).

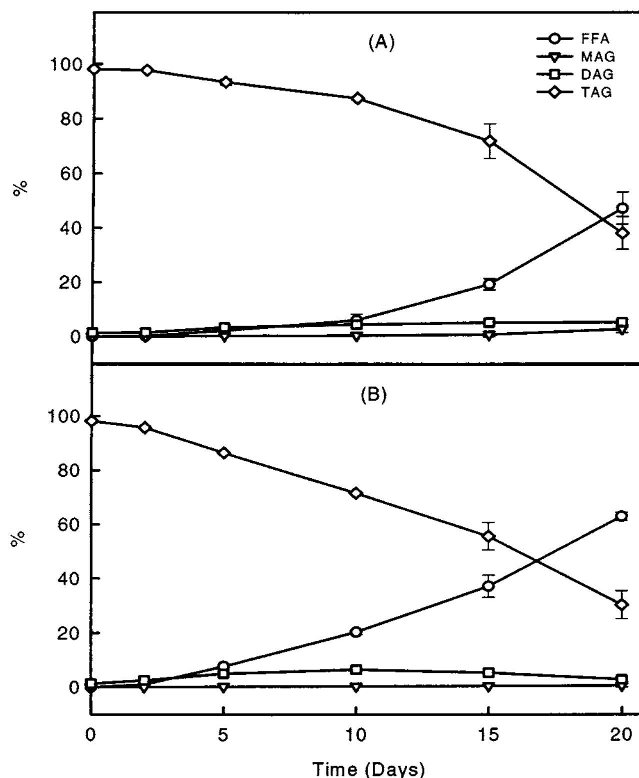


**Figure 3.** Effect of germination and growth under illuminated (**A**) and dark (**B**) conditions on various lipid classes of seedlings.

2, when it started to deplete rapidly from ∼50% (w/w) to ∼10−20% (w/w) on day 20. A decrease in total lipid content during canola germination has been reported in the literature (*3*, *15*−*17*). The difference between the oil content (seedling dry wt basis) in seedlings grown under illuminated and dark conditions may be attributed to differences in dry matter content. For this reason, changes in oil content during germination are best expressed on the basis of initial seed mass (**Figure 2B**). On the basis of these results, light is not a crucial factor ($p > 0.05$) in terms of the breakdown of lipids. In addition, there was no change in oil content up to 2 days of germination. This implies that the breakdown of lipid does not occur at early stages of germination.

Seedlings with developed stem and leaves were obtained on day 10 and onward. Under both illuminated and dark conditions, oil content in seedling tops decreased from ∼25% (w/w) to ∼11−17% (w/w) through days 10 and 15 with a marginal increase after day 15. In contrast, the seedling bottoms showed an opposite trend. This may be due to the translocation of lipids from cotyledons to stems/roots before consumption by the growing seedlings. During the day 10−20 period under illuminated conditions, the oil content in seedling tops decreased from ∼25% (w/w) to ∼14% (w/w), whereas the decrease in oil content under dark conditions was from ∼25% (w/w) to ∼19% (w/w). In contrast, the oil content in seedling bottoms under both illuminated and dark conditions reached ∼9% (w/w) on day 20 of the trial. This shows that the stems and roots were highly dependent on the original lipid reserves as compared to the relatively low dependency of leaves and buds.

**Changes in Different Lipid Classes. Figure 3A** depicts different lipid classes as a percentage of total lipids extracted from seedlings grown under illuminated conditions. In seeds, the predominant lipid class was TAG (98%), whereas only traces of FFA and MAG were present. Contribution of DAG to total lipids was ∼1.4%. Results suggest the heavy dependence of seedlings on TAG ($p < 0.05$) regardless of the growing conditions. FFA content dramatically increased ($p < 0.05$) from trace to ∼47% under light (**Figure 3A**) and to ∼63% in the dark (**Figure 3B**). For both illuminated and dark conditions, the changes in MAG and DAG contents were also significant ($p < 0.05$) but not as dramatic as in the content of FFA and TAG. Huang and Grunwald (*15*) and Chung et al. (*16*) reported similar observations. This may be due to coordinated mobilization and hydrolysis of lipid reserves by the growing seedlings. The effect of the main factor of light is not significant ($p > 0.05$). The predominant fatty acid found in the FFA fraction was 18:1.

Changes in lipid classes in 10-, 15-, and 20-day-old seedling tops and bottoms were also monitored for both illuminated and dark growing conditions (**Figure 4**). Changes were very similar to those observed for whole seedlings. Fifteen days after germination under illuminated conditions, a rapid increase of FFA and a concurrent decrease of TAG in seedling tops and bottoms were observed (**Figure 4A**,**B**). Under dark conditions, however, a relatively high amount of FFA was evident by day 10 of the trial (**Figure 4C**,**D**).

Lipid is the major food reserve in canola seeds and is stored in the form of lipid bodies, which consists of TAG surrounded by a membrane (*18*, *19*). The biochemistry of lipid reserve mobilization in many oil-rich seeds has been studied in detail. Lipases, located in the surrounding membranes, hydrolyze TAG and liberate fatty acids, which are metabolized through $\beta$-oxidation. The acetate generated then enters the glyoxylate cycle (*20*, *21*). Hydrolysis of TAG caused FFA levels in oil extracted from the seedlings to rise from almost zero at the beginning to 50−60% at the end of the 20-day period. One negative aspect of this increase in the free fatty acid levels is the potential generation of rancid odor in the oil. However, germination may provide an alternative, less expensive, environmentally friendly natural means of manufacturing FFA for food and nonfood uses

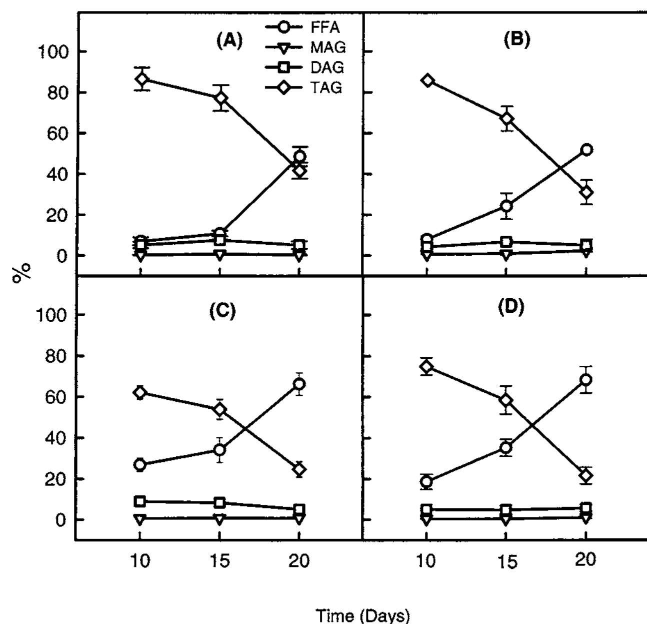


**Figure 4.** Effect of germination and growth environment on lipid classes of seedling tops and bottoms: (**A**, **C**) effect on seedling tops under illuminated and dark conditions, respectively; (**B**, **D**) effect on seedling bottoms under illuminated and dark conditions, respectively.

because the process of germination does not require externally added enzymes or chemicals for TAG hydrolysis.

**Changes in Protein Content and Solubility.** As shown in **Figure 5A**, the crude protein content of seedlings grown under illuminated conditions showed a slight decrease during the first 5 days, remained stable up to day 15, and then depleted rapidly until day 20. These observations are somewhat different from those of other studies (*3*, *16*, *22*), in which increased protein content was reported. A similar decreasing trend ($p > 0.05$) was observed for the crude protein content in seedlings grown under dark conditions (**Figure 5B**). The only difference was that the protein was depleted within the first 10 days, then remained constant for 5 days, and then decreased again. For seedlings grown under both illuminated and dark conditions, insoluble proteins were the major fraction of total proteins and contributed the most to the total protein depletion, whereas soluble proteins remained stable during the whole study period under both conditions (**Figure 5A**,**B**).

As shown in **Figure 6**, the solubility of proteins gradually increased in seedlings grown under illuminated conditions. This increase also occurred at a slower rate in the seedlings grown under dark conditions until day 10 and then started to decrease. Similar to our observation, Mahajan et al. (*23*) observed an increase in solubility of proteins of meal of germinated rapeseed.

For the tops and bottoms of seedlings grown under illuminated conditions, the crude and insoluble protein content decreased while the soluble proteins remained stable (**Figure 7A**,**B**). Under dark conditions, however, an increase in crude proteins and soluble proteins in seedling tops was observed, whereas the proportion of insoluble to soluble reached an almost equal amount on day 20 (**Figure 7C**). A similar trend was observed for the seedling bottoms grown under dark conditions (**Figure 7D**). In general, the solubility of proteins for parts obtained from seedlings grown under dark conditions (∼31−46%, w/w) was higher than that from seedlings grown under illuminated conditions (∼24−33%, w/w).

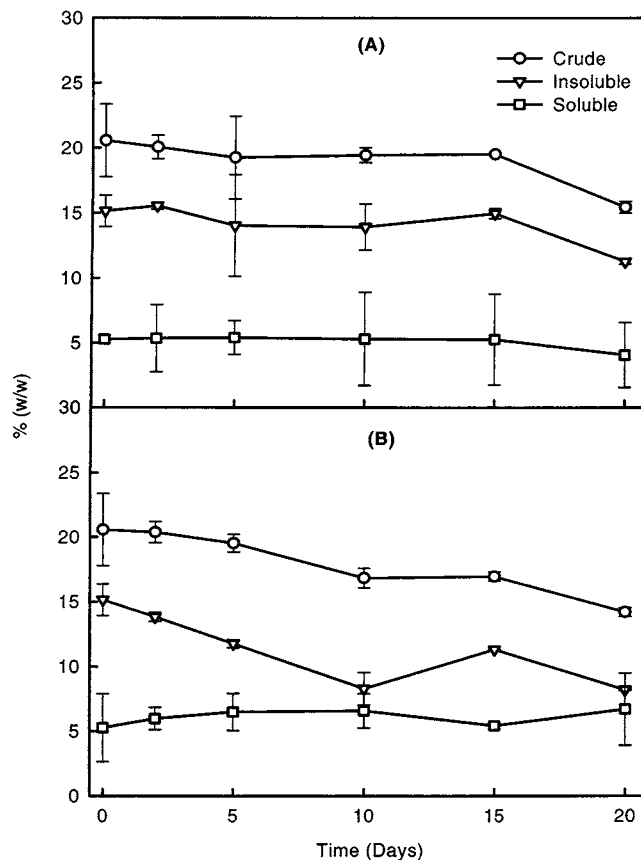


**Figure 5.** Effect of germination and growth under illuminated (**A**) and dark (**B**) conditions on protein classes of seedlings on seed dry weight basis.

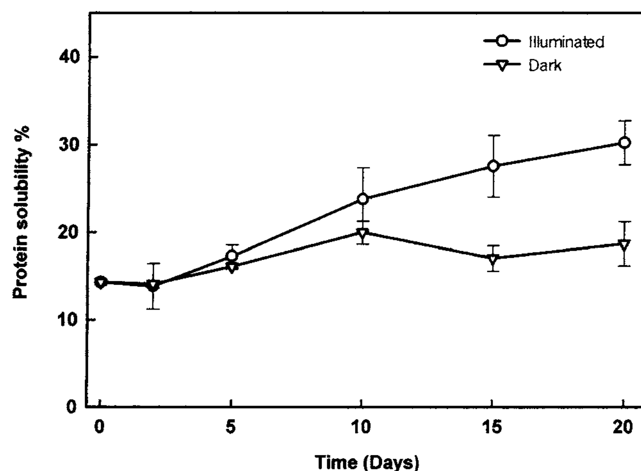


**Figure 6.** Effect of germination and growth environment on solubility of proteins in whole seedlings.

During the early stages of the seedling, embryonic tissues obtain energy for growth from mobilized seed reserves (*24*). The function of seed storage proteins is to provide free amino acids to the growing seedlings. Amino acids are translocated from the membrane-bound aleurone bodies to the growing axis, where they are used for the synthesis of enzymes and structural proteins (*25*). It has been reported that certain amino acids are deaminated and used for the synthesis of non-nitrogen-containing compounds or further metabolized to generate energy (*24*, *25*). In addition to improving the nutritional quality of canola due to increased bioavailability of proteins during

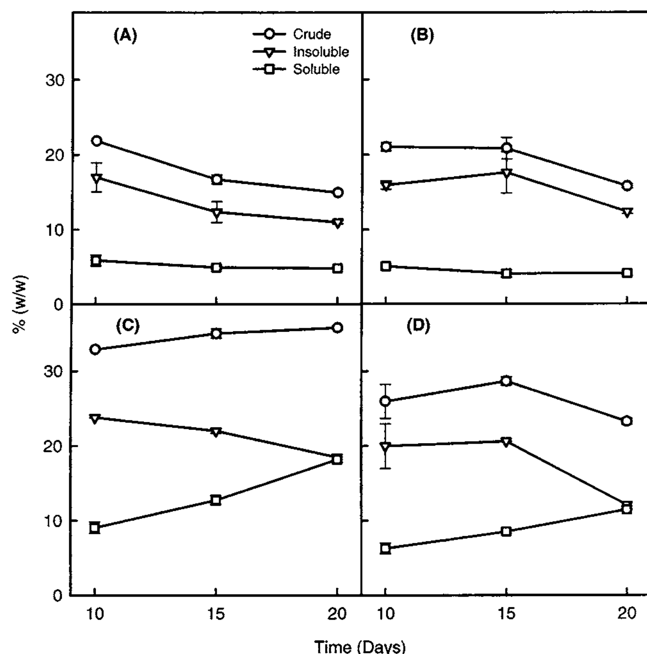


**Figure 7.** Effect of germination and growth environment on protein classes of seedling tops and bottoms: (**A**, **C**) effect on seedling tops under illuminated and dark conditions, respectively; (**B**, **D**) effect on seedling bottoms under illuminated and dark conditions, respectively.

germination, increased protein solubility may also find potential applications in food products, in which gelation, emulsification, and foaming properties are required.

## ABBREVIATIONS USED

FFA, free fatty acids; BSTFA, bis(trimethylsily)trifluoroacetamide; TMCS, trimethylchlorosilane; GC, gas chromatography; MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; RRF, relative response factor; IS, internal standard.

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