ANNALS OF THE UNIVERSITY OF CRAIOVA

Series: ✓ *Biology*
✓ *Horticulture*
✓ *Food products processing technology*
✓ *Environmental engineering*

Vol. XXIX (LXV) - 2024

QUANTITATIVE ANALYSIS OF NUTRITIONAL COMPOUNDS IN IRRIGATED AND NON-IRRIGATED LINSEED (*LINUM USITATISSIMUM* L.) CULTIVAR ALEXIN

Simion Enuță, Popoviciu Dan Răzvan*, Miron Liliana
„Ovidius" University of Constanţa, Faculty of Natural Sciences and Agricultural Sciences, Constanţa, Romania
* *Correspondence author. E-mail:* dr_popoviciu@yahoo.com

Keywords*: linseed, carotenoids, phenolic compounds, ascorbic acid, sugars, oil*

ABSTRACT

Flax from cultivar Alexin was grown on three different water regimes: non-irrigated, half-dose and full-dose irrigated. Seeds were harvested and taken for biochemical analyses.

They were found to contain low amounts of carotenoids, 7.57-12.79 mg/kg, with notable amounts of lycopene, lutein and zeaxanthin, but no ***detectable*** *β-carotene. Phenolic inventory was also low, but consistent with values usually found in ungerminated linseed: 3,106-7,211 mg/kg, dominated by flavonoids (37-51%; of which minor amounts of flavanols and anthocyanins) and hydroxycinnamic acids (23-37%). Coumarins and stilbenes had low concentrations.*

Oil content ranged from 21.3% in non-irrigated plants to over 29% in fully irrigated ones. Ascorbic acid content showed little variation, with 985-1,187 mg/kg, while soluble sugars were 2,482-4,073 mg/kg.

The concentrations of all these compounds, except for cryptoxanthin, were positively correlated to water dosage. The most affected by irrigation regime were phenolic compounds and sugars. The least influenced was ascorbic acid.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a common crop in many temperate areas of the world. An annual, herbaceous plant, is is mostly grown for textile fibers, but also for its seed.

The main product of flax seens is linseed oil, a mixture of fats that can reach 26-45% of seed dry weight. Apart from fatty acids (mostly α-linoleic acid), these seeds are known to contain other valuable organic nutrients, such as lignans, flavonoids and hydroxycinnamic acids, compounds that are antioxidant, antimicrobial, antidiabetic and support cardiovascular system health (Popa et al. 2012; Gai et al. 2023)

The objective of this paper was to determine the way different irrigation regimes affect the concentrations of several nutritional compounds and compound classes in linseed.

MATERIAL AND METHODS

Flax (cultivar Alexin) was grown on the experimental farm of the "Ovidius" University of Constanța, under three types of hydric conditions: non-irrigated, irrigated with half dose and irrigated with full dose.

Experimental field was prepared for cultivation by removing any plant wastes and leveling soil.

Due to the high temperatures within the growth period, irrigation was done by aspersion when soil humidity approached the minimal level.

The three experimental variants of irrigation were zero dose (as control), half dose (250 m^3/ha) and full dose irrigation (500 m^3/ha). Harvesting was done at the end of July, when qualitative analyses were done. Seeds were collected and taken for laboratory analyses. Seeds were ground and extracted in 70% ethanol at 10% final concentration.

Total carotenoids were determined by extract dilution in 80% acetone and spectrophotometric absorption reading (ONDA UV-21 spectrophotometer) at 470 nm (Popoviciu et al. 2023). Among individual carotenoids, β-carotene, lycopene, lutein (and lutein esters), cryptoxanthin and zeaxanthin were determined by dilution in acetone:hexane:petroleum ether, petroleum ether, ether, acetone, respectively ethanol and determining absorbance at 445, 446, 453, 505, 645, 663 nm according to Braniša et al. 2014, Sujith et al. 2010, Biehler et al. 2009, Butnariu et al. 2014.

The total amounts of phenolic compounds were determined by dilution in methanol and Folin-Ciocâlteu reaction. Absorbance was read at 765 nm against a gallic acid calibration curve (Popoviciu et al. 2023).

Flavonoids were determined by precipitation with formaldehyde and hydrochloric acid, and measuring non-flavonoid phenolic content by Folin-Ciocâlteu reaction. Flavanols (catechins), by dilution in 70% ethanol and reading at 280 nm (Yaneva et al. 2020). Anthocyanins, by dilution in 70% ethanol and reading at 520 and 700 nm (Braniša et al. 2014).

Phenolic acids (hydroxybenzoic and hydroxycinnamic) were analyzed by dilution in ethanol-hydrochloric acid mixture and spectrophotometry at 220, 275 325, 345 and 380 nm (Paula et al. 2017). Tannins were determined after thermal hydrolysis in water-hydrochloric acid mixture and reading at 550 nm (Moutari et al. 2018). Stilbenes, by dilution in 70% ethanol and reading at 304 nm (Bancuta et al. 2015). Coumarins, by dilution in 80% methanol and reading at 275 nm (Soares e Silva et al., 2012).

Total ascorbic and dehydroascorbic acid were determined by ethanol dilution, reaction with ammonium molybdate and sulfuric acid, and spectrophotometric reading at 494 nm (Riscahyani et al. 2019). Total soluble carbohydrates were determined by reacting extracts with sulfuric acid and phenol (5%) and reading at 490 nm (Agrawal et al. 2015). Lipids were determined by petroleum ether dilution and gravimetry (Orphanides et al. 2011).

RESULTS AND DISCUSSIONS

The concentrations of various classes of compounds with bioactive potential are shown in Figures 1-4.

Most types of compounds were positively correlated with irrigation dosage. Average total carotenoid content, for instance was 7.57 mg/kg in non-irrigated plants and 12.79 mg/kg in fully-irrigated ones. Among carotenoids, β-carotene was found in amounts below detection limit for all samples. Lycopene ranged from 0-1.80 mg/kg, lutein, 0.78-1.63 mg/kg, zeaxanthin, 0.71-0.88 mg/kg. Cryptoxanthin ranged from 0.19-0.26 mg/kg and was the only compound inversely proportional to water dosage.

Carotenoid fraction in linseed seems to be highly variable, from 0.5-2 mg/kg lutein and 0.1-0.2 mg/kg β-carotene (Tavarini et al. 2019) to 50-517 mg/kg just β-carotene (the higher amount being found during germination; da Silva et al. 2018).

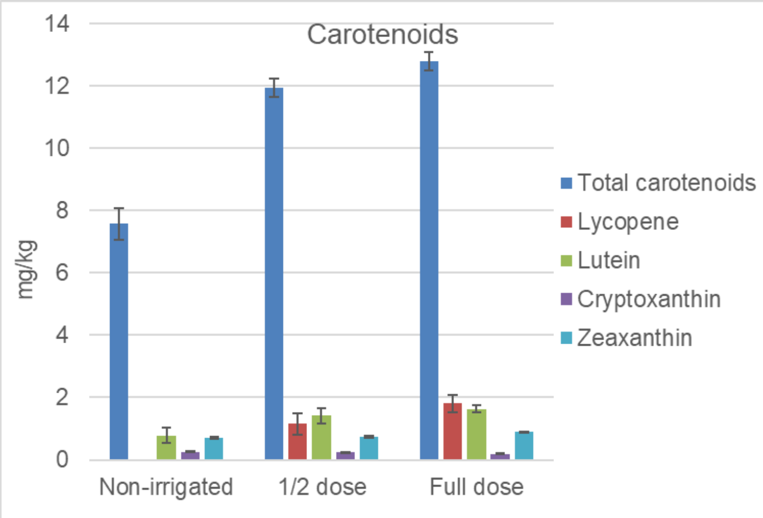

Figure 1. Concentrations of total and some individual carotenoids in linseed (mg/kg FW).

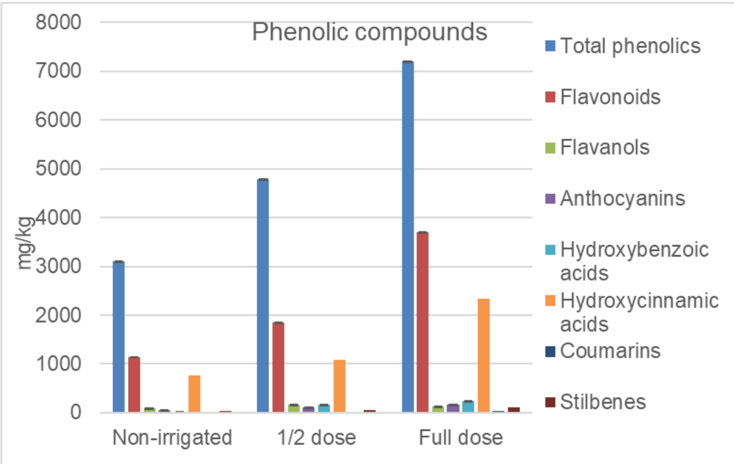

Figure 2. Concentrations of total phenolic compounds and some subclasses in linseed (mg/kg FW).

Average total phenolic content was highly dependent upon irrigation, with 3,106-7,211 mg/kg. Of these, flavonoids were a major component, with 1,143-3,701 (37-51%). Among flavonoids, flavanols (85-125 mg/kg) and anthocyanins (50-167 mg/kg) formed only minute fractions (3-9%, respectively 4-6%). Among phenolic acids, only hydroxycinnamic acids were found in major amounts: 764-2,340 mg/kg (23-37% of the phenolic inventory), while hydroxybenzoic ones were only 11-246 mg/kg). Small fractions of coumarins (4-31 mg/kg) and stilbenes 28-104 mg/kg) were found.

Linseed is known to contain rather low amounts of phenolic compounds, for comparison da Silva et al. (2018) found around 2,000 mg/kg in ungerminated seeds, Tavarini et al. (2019) – 3,500-5,000 mg/kg (of which 1,000-1,500 flavonoids). High amounts of hydroxycinnamic acids are also common (Gai et al. 2023).

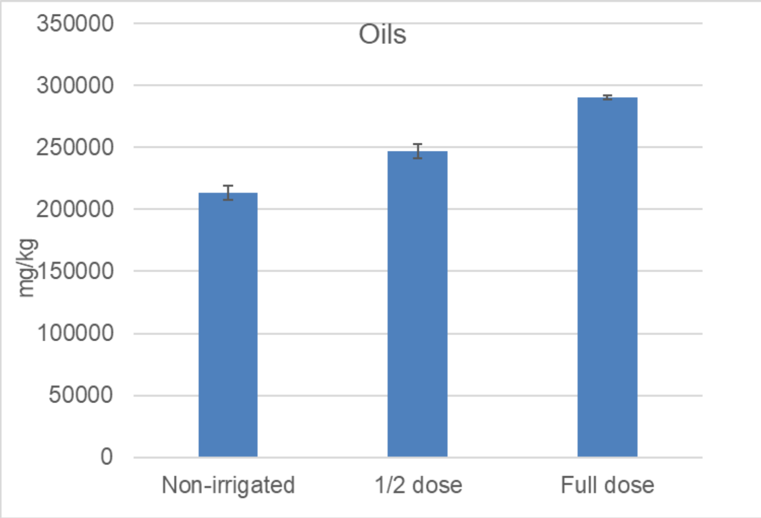

Figure 3. Concentrations of oils in linseed (mg/kg FW).

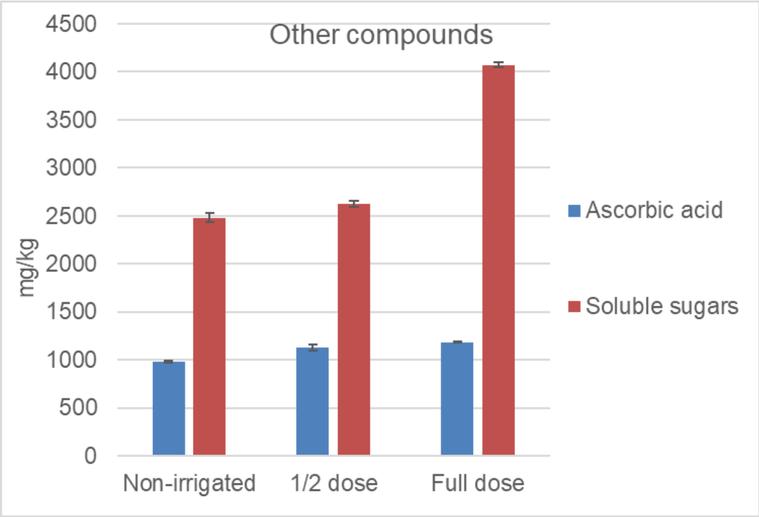

Figure 4. Concentrations of other nutritional compound classes in linseed (mg/kg FW).

Oil is one of the most important components of linseed. The amounts varied from 21.3% in non-irrigated plants to over 29% in fully irrigated ones, values that are within the normal range (Popa et al. 2018). Ascorbic acid concentration was 985-1,187 mg/kg, while soluble sugars – 2,482-4,073 mg/kg (with a major increase in fully-irrigated plants).

CONCLUSIONS

Flax seeds from cultivar Alexin were found to contain low amounts of carotenoids, 7.57-12.79 mg/kg, with notable amounts of lycopene, lutein and zeaxanthin, but no detectable β-carotene. Phenolic inventory was also low, but consistent with values usually found in ungerminated linseed: 3,106-7,211 mg/kg, dominated by flavonoids (37-51%; of which minor amounts of flavanols and anthocyanins) and hydroxycinnamic acids (23-37%). Coumarins and stilbenes had low concentrations.

Oil content ranged from 21.3% in non-irrigated plants to over 29% in fully irrigated ones. Ascorbic acid content showed little variation, with 985-1,187 mg/kg, while soluble sugars were 2,482-4,073 mg/kg.

The concentrations of all these compounds, except for cryptoxanthin, were positively correlated to water dosage. The most affected by irrigation regime were phenolic compounds and sugars. The least influenced was ascorbic acid.

REFERENCES

Agrawal N., Minj D.K., Rani K. 2015. Estimation of total carbohydrate present in dry fruits. IOSR J. Environ. Sci. Toxicol. Food Technol., 1(6): 24-27.

Bancuta O.R., Chilian A., Bancuta I., Ion R.M., Setnescu R., Setnescu T., Gheboianu A., Lungulescu M. 2015. FT-IR and UV-VIS characterization of grape extracts used as antioxidants in polymers. Rev. Roum. Chim., 60(5-6): 571-577.

Biehler E., Mayer F., Hoffmann L., Krause E., Bohn T. 2009. Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. J. Food Sci., 75(1), doi: 10.1111/j.1750-3841.2009.01417.x.

Braniša J., Jenisová Z., Porubská M., Jomová K., Valko M. 2014. Spectrophotometric determination of chlorophylls and carotenoids. An effect of sonication and sample processing. J .Microbiol. Biotech. Food Sci., 3(2): 61-64.

Butnariu M., Rodino S., Petrache P., Negoescu C., Butu M. 2014. Determination and quantification of maize zeaxanthin stability. Dig. J. Nanomater. Biostructures., 9(2): 745-755.

Da Silva S.D.S., Goularte-Dutra F.L., Manica-Berto R., Borges C.T., Zambiazi R.C., Helbig E., Bonilla-Lemos M.R. 2018. Bioactive compounds present in linseed (*Linum usitatissimum* L.) and millet (*Panicum miliaceum* L.) seeds under different germination times reveal important sources of antioxidants for human health and nutrition. Int. J. Med. Sci. Health Res., 2(4): 21-34.

Gai F., Janiak M.A., Sulewska K., Peiretti P.G., Karamać M. 2023. Phenolic compound profile and antioxidant capacity of flax (*Linum usitatissimum* L.) harvested at different growth stages. Molecules, 28(4), doi: 10.3390/molecules28041807.

Moutari S.K., Abdoulkadri A.M., Boulhassane A.S., Rabani A., Khalid I. 2018. Determination contents of total phenolic pigments and spectrophotometric characterization of crude extracts of ten tinctorial plants of Niger which is usable in solar energy. Eur. Sci. J., 14(33): 389-407.

Orphanides A., Goulas V., Chrysostomou M., Gekas V. 2011. Recovery of essential oils from carobs through various extraction methods. In Mastorakis N., Mladenov V., Lepădătescu B., Karimi H.R., Helmis C.G. (eds.), Recent Advances in Environment, Energy Systems and Naval Science, WSEAS Press, Athens, 219-224.

Paula V.B., Estevinho L.M., Dias L.G. 2017. Quantification of three phenolic classes and total phenolic content of propolis extracts using a single UV-vis spectrum. J. Apic. Res., 56(5): 569-580.

Popa V.M., Gruia A., Raba D.N., Dumbrava D., Moldovan C., Bordean D., Mateescu C., 2012. Fatty acids composition and oil characteristics of linseed (*Linum Usitatissimum* L.) from Romania. J. Agroaliment. Processes Technol., 18(2): 136-140.

Popoviciu D.R., Pricop S.M., Radu M.D. 2023. Quantitative analysis of nutritional and bioactive compounds in bitter gourd (*Momordica charantia* L.) fruits. Ann. Univ. Craiova, Ser. Biol. Hortic. Food Prod. Process. Environ. Eng., 28(64): 21-26.

Riscahyani N.M., Ekawati E.R., Ngibad K. 2019. Identification of ascorbic acid content in *Carica papaya* L. using iodimetry and UV-Vis spectrophotometry. Indones. J. Med. Lab. Sci. Technol., 1(2): 58-64.

Soares e Silva L., Santos da Silva L., Larissa Brumano L., Stringheta P.C., de Oliveira Pinto M.A., Dias L.O.D., de Sá Martins Muller C., Scio E., Fabri R.L., Castro H.C., da Penha Henriques do Amaral M., 2012. Preparation of dry extract of *Mikania glomerata* Sprengel (guaco) and determination of its coumarin levels by spectrophotometry and HPLC-UV. Molecules, 17(9), doi: 10.3390/molecules170910344.

Sujith A.M.A., Hymavathi T.V., Devi P.Y. 2010. Supercritical fluid extraction of lutein esters from marigold flowers and their hydrolysis by improved saponification and enzyme biocatalysis. Int. J. Nutr. Food Eng., 4(1): 74-83.

Tavarini S., Castagna A., Conte G., Foschi L., Sanmartin C., Incrocci L., Raneri A., Serra A., Angelini L. 2019. Evaluation of chemical composition of two linseed varieties as sources of health-beneficial substances. Molecules, 24(20), doi: 10.3390/molecules24203729.

Yaneva Z., Ivanova D., Beev G., Besheva K. 2020. Quantification of catechin in *Acacia catechu* extract by non-derivative, first derivative UV/Vis spectrophotometry and FT-IR spectroscopy. Bulg. Chem. Commun., 52: 41-47.