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Chemical composition, fatty acid content and phenolics (bioactive) compound on linseed (*Linum usitatissimum* L.) harvested at six phenological stages of growth

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Abstract. *Linum usitatissimum* L. is an annual dicotyledonous multipurpose crop grown either for fibre (fibre flax) or seed (linseed, oil seed flax or flax seed). Linseed straw can be exploited as source of roughage and although at the late stages is very fibrous, at the early stages, can be very nutritious, comparable to canola hay. Linseed and grass are the most important n-3 fatty acid sources for ruminants. Linoleic acid (LA, 18:2n-6) and α -linolenic acid (ALA, 18:2n-3) are both available in linseed. Plant secondary metabolites have attracted interest as potential antioxidants for both inhibiting deterioration of foodstuffs and for providing beneficial metabolic effects in animals. Evaluation of quality of linseed biomass, as an important source of precursors of fatty acids, and its chemical composition (total polyphenols, flavonoids and condensed tannins) at different morphological stages, was the first aim of this work.

Keywords. Fatty acid – Bioactive compound – Phenological stages.

Composition chimique, teneur en acides gras et composés phénoliques (bioactifs) du lin (*Linum usitatissimum* L.) récolté à six stades phénologiques de croissance

Résumé. *Linum usitatissimum* L. est une plante annuelle polyvalente de la famille des dicotylédones, produite soit pour les fibres (lin textile) ou les graines (lin oléagineux). La paille de lin peut être exploitée en tant que source de fourrage grossier et bien que très fibreuse lors des derniers stades, elle peut être très nutritive aux premiers stades phénologiques, comparable au foin de canola. Les graines et l'herbe de lin sont les sources les plus importantes d'acides gras n-3 pour les ruminants. L'acide linoléique (LA, 18:2n-6) et l'acide α -linoléique (ALA, 18:2n-3) sont tous deux disponibles dans les graines de lin. Les métabolites secondaires végétaux ont suscité de l'intérêt comme antioxydants potentiels à la fois pour l'inhibition de la détérioration des denrées alimentaires et pour fournir des effets métaboliques bénéfiques chez les animaux. L'évaluation de la qualité de la biomasse de lin, comme importante source de précurseurs d'acides gras, et de sa composition chimique (polyphénols totaux, flavonoïdes et tanins condensés) à différents stades morphologiques, était le premier objectif de ce travail.

Mots-clés. Acide gras – Composé bioactif – Stade phénologique.

I – Introduction

Linseed (*Linum usitatissimum* L.) is an herbaceous annual dual-purpose crop plant grown worldwide, with wide range of industrial uses due to its main products, fibre and seed (Jankauskienė and Grudevienė, 2015). Belonging to Linaceae family, it is native to West Asia and has been cultivated since 5,000 BC. Moreover, linseed is an important nutraceutical crop as a rich source of omega-3 fatty acid and antioxidant compounds. Polyphenols are among the most significant compounds related to the antioxidant properties of plant materials. Flavonoids have also been shown to act as scavengers of various oxidizing species. Linseed straw can be ammoniated and made adequate forage base for wintering beef cows (Mann *et al.*, 1988) and used safely as the only source of roughage for cattle (Peiretti and Meineri, 2008). The main

objective of this work was to assess the effects of the stage of maturity on total plant chemical composition for crude protein, fibrous fractions, fatty acids and phenolic compounds.

II – Materials and methods

The experiment was conducted during 2014 at the experimental station of Leccari, Sassari (40°45'12" N, 8°25'17" E; 27 m a.s.l.), in Sardinia (Italy). The climate of the area is typically Mediterranean with mild winter, characterized by a long-term average annual rainfall of 554 mm, prevalently distributed in autumn and winter months, and a mean annual air temperature of 16.2 °C. Soil has been classified as *Eutric, Calcaric* and *Mollic Fluvisol* according to FAO (2006) and is sandy-clay-loam, alkaline with a scarce average nitrogen content (0.96‰) and adequate contents of phosphorous (20.33 ppm), organic matter (1.46%) and organic carbon (0.85%). The morphological stages of linseed were evaluated on a sample of 50 stems randomly clipped to ground level and classified according to a BBCH scale (Smith and Froment, 1998). The six morphological stages were: **Stage 3** – Stem extension; **Stage 5** – Inflorescence development and emergence; **Stage 6** – Flowering and capsule formation; **Stage 7** – Development of the seed and capsule; **Stage 8** – Capsule and seed ripening; and **Stage 9** – Stem senescence. Forage quality was evaluated by drying samples of biomass in oven at 80 °C for 48 h, then milling the samples for chemical traits determination. Total N was determined using Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral, acid detergent fibres and lignin (NDF, ADF and ADL), were determined according to Van Soest (1994) procedure. Secondary plant metabolites were evaluated in samples kept on ice during harvesting, freeze dried and ground to a fine powder for the chemical analysis. The powdered material was then used for extract preparations as reported by Piluzza *et al.*, (2014). Total phenolic content (TotP) of extracts was determined using the Folin–Ciocalteu reagent according to Singleton and Rossi (1965), with some modifications by Piluzza and Bullitta (2010). Results were expressed as g gallic acid equivalent kg⁻¹ dry weight of plant material (g GAE kg⁻¹ DW). The butanol assay of Porter *et al.* (1986) was adapted (Piluzza and Bullitta, 2010) for quantification of extractable condensed tannins content from our samples. The condensed tannins content was expressed as g delphinidin equivalent kg⁻¹ dry matter (g DE kg⁻¹ DM). Total flavonoids (TotF) were quantified by colorimetric assay with the AlCl₃ method (Kim *et al.*, 2003). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent kg⁻¹ dry weight of plant material (g CE kg⁻¹ DW). Fatty acids were determined in an external laboratory (Agriecobio, Pomezia, Rome). Chemical composition and secondary metabolites were correlated by regression with morphological stages.

III – Results and discussion

CP was negatively related to the phenological stages whereas NDF, ADF lignin contents, were positively related to the phenological stages, respectively (Fig. 1 a,b,c and d); R² ranged from 0.85 to 0.97. Concentration of CP ranged from 150 at early stages to 90 (g kg⁻¹ DM) at maturity, according to Peiretti and Meineri (2008). The highest protein level (about 150 g kg⁻¹ DM) was found at stem extension (stage 3). On later stages, seeds formation and changes in cell wall components occurred, resulting in NDF, ADF and lignin increases. The lowest NDF and ADF contents (Fig. 1 b and c) were found at the same stage (stage 3), but later for ADL (stage 5) (Fig.1 d). Levels of NDF, ADF and ADL were 480, 330 and 90 (g kg⁻¹ DM) at early stages, while they increased to 650, 460 and 170 (g kg⁻¹ DM) at seed ripening, confirming the fibrous characteristics of linseed.

Total phenolic and total flavonoid contents were negatively related with morphological stages (Fig. 2 a, b). Moreover, total phenolic showed the highest values (16.7 g GAE kg⁻¹ DW) at stage 2, whereas total flavonoid at stage 5 (16.5 CE kg⁻¹ DW).

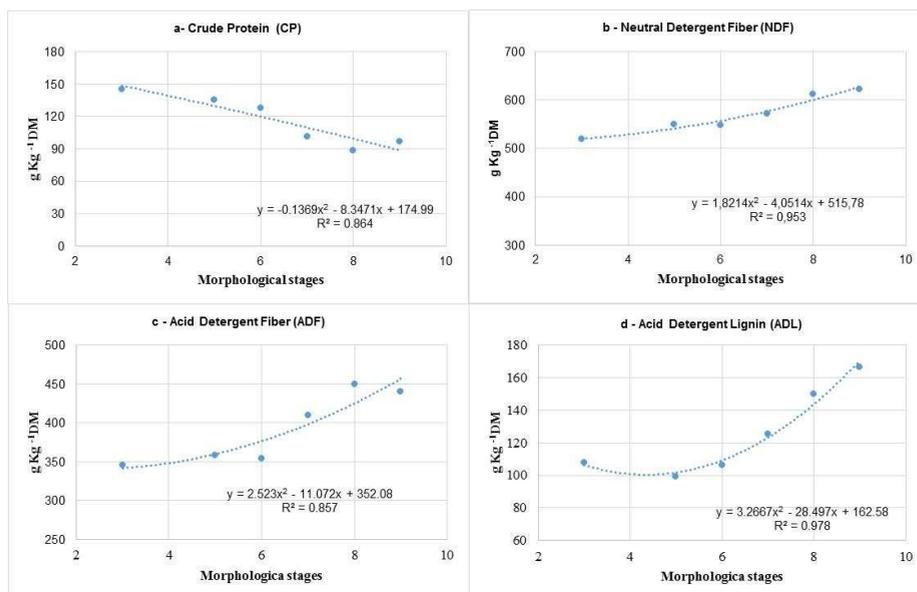


Fig. 1. Relationship between chemical composition (CP, NDF, ADF, ADL) and morphological stages in linseed biomass.

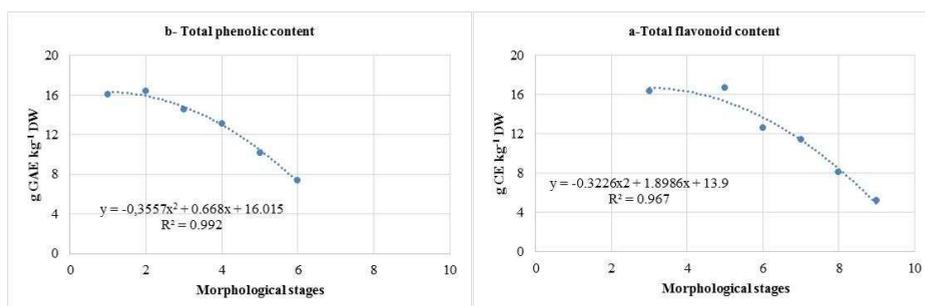


Fig. 2. Relationship between total phenolic (g GAE kg⁻¹ DW) and total flavonoid content (g CE kg⁻¹ DW) content in relation to linseed morphological stages.

The lowest values were reached at stage 9 (5.2 g GAE kg⁻¹ DW) and 7.4 (g CE kg⁻¹ DW), respectively. El-Lethy *et al.* (2010) found in linseed plant harvested at vegetative growth stage, a total phenolic content of 0.88% in the control and 1.81% and 1.64% in leaves treated using the antioxidants stigmasterol and putrescine, respectively. No condensed tannins were detected in linseed under study. Fatty acids (Fig. 3) were not affected by the different morphological stages, α -linolenic acid (ALA) ranging from 33.5 % to 40.6% and linoleic acid (LA) from 8.1% to 10.5%. El-Lethy *et al.* (2010), found similar levels of LA but lower levels of ALA, 5.5% and 12.2% respectively, in linseed grown in Egypt. In contrast with our study, Peiretti and Meineri (2008) found higher values for both fatty acid (LA and ALA) and evidenced that LA and ALA were affected by phenological stages of linseed. This was probably caused by the different environment and sowing date: late autumn in Sardinia (40°45'N. 8°25'E) and June in Piedmont (44°41'N. 7°11'E).

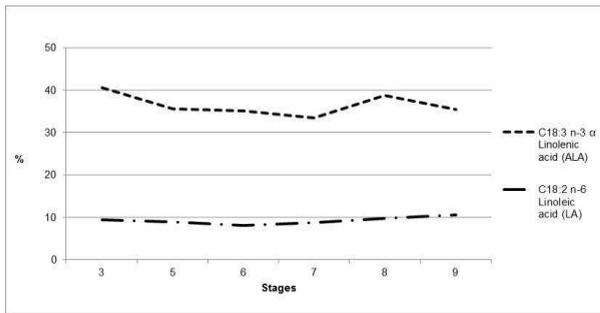


Fig. 3. Concentrations of Linoleic acid (LA) and α -Linolenic (ALA) acid at different morphological stages in linseed.

IV – Conclusions

As many studies were mainly referred to the seed chemical composition of *L. usitatissimum*, our research contribute to give new insights into the chemical composition of linseed biomass. In particular, our results highlight that the levels of NDF, ADF and ADL were positively related to the phenological stages, whereas CP level was negatively affected, as it was expected. A similar trend was recorded for the contents of phenolic and flavonoid. On the contrary, the levels of fatty acids proved to be not affected by the morphological stages. The obtained information is useful for a complete exploitation of linseed plant biomass as a forage source.

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