

# Fatty acid composition and antioxidant capacity in linseed grown as forage in Mediterranean environment

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## Abstract

This research was aimed at studying the bromatological traits, fatty acid profile, bioactive compounds, and antioxidant capacity in linseed (*Linum usitatissimum* L.) shoots harvested at six codified morphological stages. Quality traits were significantly related to cumulated growing degree days from seedling emergence to senescence. The crude protein and ash contents exhibited a gradual decrease and were negatively correlated with morphological stages, whereas cell wall components such as neutral, acid detergent fibers and lignin (NDF, ADF, and ADL) and ether extract (EE) showed a positive correlation. Both ABTS [(2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays indicated a reduction in antioxidant capacities from stem extension to senescence, from 16 to 7.1, and 19 to 7 mmol TEAC/100g DW, for ABTS and DPPH, respectively. Significant linear correlation among the antioxidant activity, phenolics, NDF, ADF, ADL, and EE were found usually. Total phenolic (9.6-26.4 g GAE kg<sup>-1</sup>) and total flavonoid (5.2-16.7 g CE kg<sup>-1</sup>) contents were negatively related with morphological stages. The morphological stage was significantly correlated with oil content, although individual fatty acid content did not. Research gives new insights into the evolution of chemical composition of linseed shoot. Remarkable varia-

tions in quality traits, fatty acid contents, bioactive compounds, and antioxidant capacity evidence the possibility to use green linseed in animals' diet, also suggesting the exploitation of linseed plant as forage source.

## Introduction

*Linum usitatissimum* L. (linseed, also called flax or flaxseed), an annual herbaceous species belonging to the Linaceae family, is originating from the Middle East and has been cultivated in Mesopotamia since ancient times. Its use has been documented as far back as 3000 BC with a long history of food, medicine, and textile fiber. Linseed was subsequently introduced to several regions including Europe (Zuk *et al.*, 2015).

Linseed is generally regarded as a dual-purpose crop due to its main products, namely fiber and seed (Jankauskienė *et al.*, 2015; Zuk *et al.*, 2015), and it is still widely grown for oil, fiber, and food. Recently, it is emerging as one of the key sources of phytochemicals in functional foods (Kajla *et al.*, 2015). In fact, linseed is rich in  $\alpha$ -linolenic acid and lignans, is an essential source of high-quality protein and soluble fiber and has also considerable potential as a source of phenolic compounds (Oomah, 2001). As a result of divergent plant selection (Diederichsen and Ulrich, 2009), linseed for oil grows up to 40-60 cm tall, whereas fiber linseed plant grows up to 80-120 cm and is less branched. Since many years ago, the fiber has been converted to yarn, which served as a major source to manufacture textiles for table or bed covering and clothing, whereas seeds have been pressed to extract edible oil (Zuk *et al.*, 2015).

Furthermore, the same authors reported that total fat content in linseed plant was 35-46%, total protein accounted for 18-25% and carbohydrate constituted 23-30%. Linoleic acid content was in the range 16 to 75% and  $\alpha$ -linolenic acid varied from 1.7 to 59%.

Cloutier *et al.* (2011) and Zuk *et al.* (2015) detailed that the average content of linseed oil is 35-50% of the seed weight. In linseed oil, the most frequently found fatty acids are palmitic (6%), stearic (2.5%), oleic (19%), linoleic (13%) and  $\alpha$ -linolenic acid (55%). Moreover, the oil content and fatty acid composition often change due to crop adaptation to local environmental conditions (Cloutier *et al.*, 2011). Duguid (2009) and Fofana *et al.* (2006) reported that the seed oil content varied up to 15%, and the percentage of  $\alpha$ -linolenic acid might increase (ca. 5%) in cooler environments, respectively. Linoleic acid (LA) and  $\alpha$ -linolenic acid (ALA) are the main fatty acids that are mostly modified by anaerobic bacteria in the rumen via biohydrogenation process aimed to produce saturated fatty acids (Chilliard *et al.*, 2000). LA, ALA, and vaccenic acid (VA) found in dairy products have been shown to possess peculiar nutritional properties as modifiers of cardiovascular risk (Pintus *et al.*, 2013). Moreover, pasture and lipid

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supplementation of the ruminant's diet have been reported to be reliable dietary strategies in ruminant nutrition, in order to increase milk content of n-3 fatty acids, conjugated linoleic acid (CLA) and VA (Nudda *et al.*, 2006; Mele 2009; Mele *et al.*, 2011; Pintus *et al.*, 2013). Conversely, Cabiddu *et al.* (2017) reported that supplementation of grazing ewes with oilseeds is proven effective to increase the level of CLA, polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) in milk, compared to unsupplemented grazing; they also evidenced that oilseed can increase putatively noxious trans fatty acid in respect to the unsupplemented herbage-fed treatment. According to Correddu *et al.* (2015), in the last few decades, a lot of attention has been directed to the content of healthy fatty acid (FA), especially polyunsaturated fatty acids belonging to the n-3 (PUFA n-3), such as LA and ALA in ruminant milk and dairy productions. Moreover, many studies have demonstrated that diet composition and rumen microbial biohydrogenation strongly influence the fatty acid profile of milk and dairy products (Lourenço *et al.*, 2010). Sejian *et al.* (2010) reported that adding linseed seed to high-quality forage in livestock diet could be an effective mean for reducing CH<sub>4</sub> gas emission.

Nowadays, a novel aspect concerning the comprehensive utilisation of linseed seeds to produce food additives or supplements with high nutritional value, attracts increasing interest (Wang *et al.*, 2017). Linseed meal and straw are feed to livestock, but while linseed meal is an excellent protein source (about 350 g kg<sup>-1</sup>) linseed straw, even if of good quality, has about the same feed value of oat (*Avena sativa* L.) or barley (*Hordeum vulgare* L.) straw (Peiretti and Meineri, 2008). Nevertheless, linseed straw can be used safely as the only source of roughage for cattle (Peiretti and Meineri, 2008). Green linseed is nutritionally comparable to canola (*Brassica napus* L.) hay (Feist *et al.*, 2007).

Health benefits of linseed can be credited mainly to its abundance in biologically active components (Russo and Reggiani, 2015). In particular, phenolic compounds (phenolic acid, flavonoids phenylpropanoids, and tannins) are excellent in preventing the excess of free radicals and avoiding their pathological effects (Kasote, 2013; Russo and Reggiani, 2015). Polyphenols are among the most significant compounds related to the antioxidant properties of plants materials. In plants, phenols play an important role in protection against photo-oxidation and disease resistance (Pandey and Rizvy 2009; Mojzer *et al.*, 2016). Flavonoids have also been shown to act as scavengers of various oxidising species [*i.e.* superoxide (O<sub>2</sub><sup>-</sup>), hydroxyl radicals, and peroxy radicals], and they may also act as quenchers of single oxygen (Harborne and Williams, 2000; Trem and Šmejkal, 2016). Recently, linseed have been incorporated as seed into food or feed with other nutraceuticals or ingredients to improve their quality (Nudda *et al.*, 2006; Mele *et al.*, 2011; Dong *et al.*, 2015; de Moura *et al.*, 2016; Wang *et al.*, 2017).

Several authors found that the content of phenolics varies with the growth stage of plants such as birdsfoot trefoil (*Lotus corniculatus* L.), chicory (*Chicorium intybus* L.) sulla (*Hedysarum coronarium* L.), sainfoin (*Onobrychis viciifolia* Scop.), milk thistle (*Silybum marianum* L.) Gaertn.), and garland (*Chrysanthemum coronarium* L.) (Håring *et al.*, 2007; Piluzza and Bullitta 2010; Re *et al.*, 2013; Piluzza *et al.*, 2014a; Sulas *et al.*, 2016; Sulas *et al.*, 2017).

Peiretti and Meineri (2008) investigated the effect of maturity stage on FA profile in linseed and found that it is closely connected to plant development and that quality of the crop decreased from the first to the last morphological stage. The FA profile trend in the plant during growth is generally different from that of the corresponding seed oil, as reported for some herbage as borage

(*Borrago officinalis* L.), galega (*Galega officinalis* L.), false flax (*Camelina sativa* L.), (Peiretti *et al.*, 2004; Peiretti and Gai, 2006; Peiretti and Meineri, 2006). Linseed supplementation (as oil or seed) is a reliable feeding strategy to enhance unsaturated fatty acid in milk fat from cattle, sheep and goat (Chilliard *et al.*, 2007; Mele *et al.*, 2011; Santurino *et al.*, 2017). Although several studies have reported its use as seed for feeding, the quality aspects of linseed shoots (aerial biomass) are little investigated so far. This research aimed at investigating the evolution of quality traits, fatty acid content, bioactive compounds (total polyphenolics, non-tannic phenolics, tannic phenolics, flavonoids, condensed tannins) and antioxidant capacity in linseed shoots harvested at six different codified morphological stages.

## Materials and methods

The research was carried out at the experimental field of Leccari, Sassari (40°45'12"N, 8°25'17"E; 27 m a.s.l.), in Sardinia (Italy), from 2013 to 2014. The climate of the area is typically Mediterranean with mild winter, characterised by a long-term average annual rainfall of 554 mm mainly distributed in autumn and winter, and a mean annual air temperature of 16.2°C. Daily maximum and minimum air temperatures and rainfall events were monitored by a weather station at the experimental site. From September 2013 to August 2014 total rainfall reached 718 mm, concentrated in winter and spring exceeding climate means by 29%. Mean temperatures never dropped below the average monthly value of 7°C (February 2013) and did not differ from long-term values. The soil at the experimental site has been classified as a Eutric, Calcaric and Mollic Fluvisol (FAO, 2006); it is sandy-clay-loam, alkaline with a scarce average nitrogen content (0.09%) and adequate contents of phosphorous (20.33 ppm), organic matter (1.46%) and organic carbon (0.85%).

In autumn 2013, plots (each sized about 25 m<sup>2</sup>) of the oilseed variety, *Natural*, were sown at a seeding rate of 50 kg ha<sup>-1</sup> corresponding to 450 plants per m<sup>-2</sup>, under a randomised block with 3 replicates. Fertilisation of 40 kg ha<sup>-1</sup> of N and 100 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, was applied at sowing. No irrigation or herbicides were applied.

The main morphological stages of linseed (Table 1), from stem extension (stage 3) to senescence (stage 9) were identified according to a BBCH scale (Smith and Froment, 1998) by adopting on a sample of 50 stems the average stage weight method (Borreani *et al.*, 1999). Accumulated growing degree days (GDD) were calculated by summing the daily degree values (°C) obtained subtracting the average temperature with the base temperature, which is 5°C for linseed (Fontana *et al.*, 1998; Mirshekari *et al.*, 2012).

At each morphological stage, representative aerial biomass (shoots) samples from 0.5 m<sup>2</sup> per plot were cut at 5 cm above ground level. Fresh shoots were weighted and then oven-dried at 60° C until constant weight, and dry matter (DM) content and yield were calculated. Dried sub-samples of shoots were ground finely enough to pass through a 1 mm mesh before chemical determinations. Total N was determined by using a Kjeldahl method (AOAC Official Method 991.20) and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral, acid detergent fibers and lignin (NDF, ADF, and ADL), were determined according to Van Soest (1994) procedure and ether extract using Soxhlet extraction (AOAC official method 942.05).

Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE<sub>l</sub>) were estimated according to the following equa-

tions adapted from Lithourgidis *et al.* (2006) and Sadeghpour *et al.* (2014):

$$\begin{aligned} \text{TDN} &= (-1.291 \times \text{ADF}) + 101.35 \\ \text{DDM} &= 88.9 - (0.779 \times \% \text{ADF, dry matter basis}) \\ \text{DMI} &= 120 / \% \text{NDF dry matter basis} \\ \text{RFV} &= \% \text{DDM} \times \% \text{DMI} \times 0.775 \\ \text{NE}_1 &= [1.044 - (0.0119 \times \% \text{ADF})] \times 2.205 \end{aligned}$$

Additional fresh shoot subsamples were kept on ice at harvest, freeze-dried and ground to a fine powder for the analysis of secondary metabolites. The powdered material was then used for extract preparations as reported by Piluzza *et al.* (2014b). Total phenolics (TotP), non tannic phenolics (NTP) and tannic phenolics (TP) were determined using the Folin-Ciocalteu reagent according to Singleton and Rossi (1965), with some modifications (Piluzza *et al.*, 2014b). Results were expressed as g gallic acid equivalent  $\text{kg}^{-1}$  dry weight of plant material (g GAE  $\text{kg}^{-1}$  DW). The butanol assay of Porter *et al.* (1986) was adapted (Piluzza and Bullitta, 2010) for quantification of extractable condensed tannin content from samples, expressed as g delphinidin equivalent  $\text{kg}^{-1}$  dry matter (g DE  $\text{kg}^{-1}$  DM). Total flavonoids (TotF) were quantified by colorimetric assay with the  $\text{AlCl}_3$  method (Kim *et al.*, 2003). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent  $\text{kg}^{-1}$  dry weight of plant material (g CE  $\text{kg}^{-1}$  DW).

Antioxidant capacity was determined by means of improved ABTS [(2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays with some modifications (Surveswaran *et al.*, 2007; Piluzza and Bullitta, 2011). Trolox, a water-soluble analogue of vitamin E was used as the reference standard. The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), as mmol Trolox equivalents per 100 g dry weight of plant material (mmol TEAC/100 g DW). Fatty acid compositions were determined in an external laboratory (AGRI-BIO-ECO, Pomezia, Rome, according to Pamioli 05 method, 2011).

### Statistical analysis

Data were analysed using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009). The regression analysis between polyphenols and antioxidant capacity, and between antioxidant capacity (ABTS, DPPH), total phenolics, non-tannic phenolics, tannic phenolics, flavonoids and quality values were processed using Microsoft Excel 2016. Homogeneity test of variance and arcsin transformation of percentages relative to data were performed.

## Results and discussion

About 1790 GDD  $^{\circ}\text{C}$  were required from seedling emergence to stem senescence of linseed and morphological stages were significantly related to GDD, ( $R^2=97\%$ ;  $P<0.0003$ ; Figure 1). Mirshekari *et al.* (2012), found a very close value of 1799 GDD, considering the same base temperature ( $5^{\circ}\text{C}$ ) in Iran. Zhang *et al.* (2014) and Miller *et al.* (2001) reported similar GDD  $^{\circ}\text{C}$  data (1603-1801) and 1702, in Canada and Montana, respectively, even if the base temperature was  $0^{\circ}\text{C}$ . Shoot dry matter yield (Figure 2) was controlled by GDD accumulation ranging from  $0.4 \text{ t ha}^{-1}$  at stem extension (stage 3) to  $6.8 \text{ t ha}^{-1}$  at stem senescence (Stage 9). Quality traits were significantly GDD related, in agreement with

Mirshekari *et al.* (2012) findings. According to Singh *et al.* (2013) and Soto-Cerda *et al.* (2014), genotype and environmental conditions could significantly affect linseed properties. The CP and ash content showed a gradual decrease whereas cell walls content (fiber fractions) and ether extract increased (Figure 3). For both quality parameter, correlation analysis exhibited a significant negatively association with morphological stages,  $R^2=0.88$  ( $P<0.007$  and  $P<0.002$ ). CP content decreased from 150 to 90 ( $\text{g kg}^{-1}$  DM) at stage 3 (stem extension) and stage 8 (ripening), respectively. In accordance with other authors (Bhatty and Cherdkiatgumchai, 1990; Saastamoinen *et al.*, 2013; Tavarini *et al.*, 2016), shoot CP content was negatively correlated ( $R^2=0.73$   $P\leq 0.02$ , data not shown) to its oil content, confirming that higher oil content corresponds to a lower CP concentration. Green linseed plant can potentially contain 80-180  $\text{g kg}^{-1}$  of crude protein and 400-650  $\text{g kg}^{-1}$  of total digestible nutrients and in the early stages of growth (prior capsule formation) and may be very nutritious, whereas in the later stages it may be very fibrous to be of practical use (Peiretti and Meineri, 2008). Cell wall components showed a positive correla-

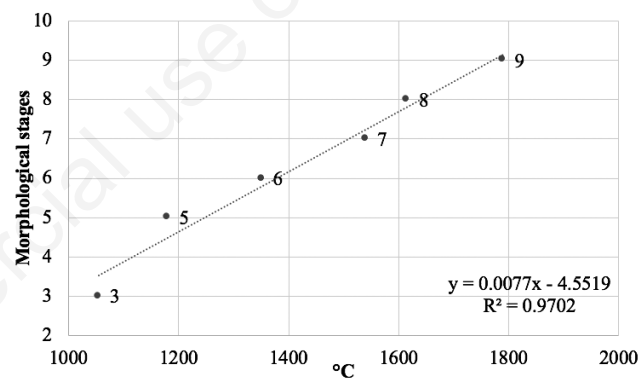


Figure 1. Linseed morphological stages in relation to growing degree days. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.

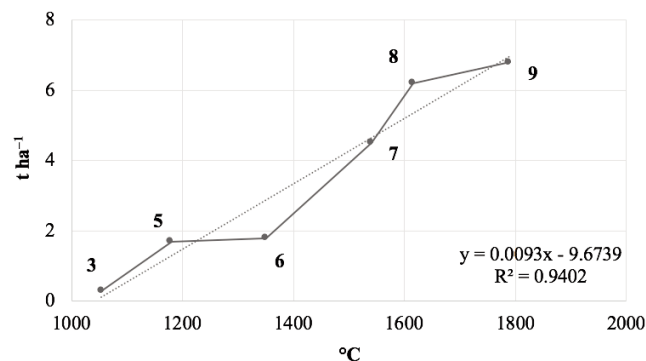


Figure 2. Shoot dry matter yield ( $\text{t ha}^{-1}$  DM) at six morphological stages across growing degree days. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.

tion with morphological stages;  $R^2$  were 0.90 ( $P < 0.003$ ), 0.84 ( $P < 0.015$ ) and 0.84 ( $P < 0.03$ ) for NDF, ADF and ADL, respectively. In addition, ether extract concentration was positively correlated with morphological stages ( $R^2 = 0.81$ ,  $P < 0.02$ ). On later stages, (seeds formation) changes in cell wall components and ether extract concentration occurred, resulting in increases of NDF, ADF, lignin, and ether extract. The lowest NDF, ADF and fat contents (Figure 3B, C, and F) were found at the same stage (stage 3 - stem extension), but later for ADL (stage 5 - inflorescence emergence (main stem) (Figure 3D). Levels of NDF, ADF and ADL were 480, 330 and 90 ( $\text{g kg}^{-1}$  DM) at early stages, while they increased to 650, 460 and 170 ( $\text{g kg}^{-1}$  DM) at seed ripening, confirming the fibrous characteristics of linseed shoot. Ash and ether extract ranged from 50.9 to 130  $\text{g kg}^{-1}$  DM and from 30 to 170  $\text{g kg}^{-1}$  DM, respectively. Peiretti and Meineri (2008) found a similar value and trend for quality traits in vegetative linseed sown at springtime in North Italy; only ash and ether extract were higher in the present research. According to them, the quality of the crop decreased from the first to the last morphological stage, mainly with a digestibility reduction across the growing season.

As a general trend, TDN, DMI, DDM, RFV and  $\text{NE}_i$  values, showed a significant linear correlation with morphological stages (Table 1). The TDN refers to the available nutrients related to the ADF concentration and, as expected, TDN declines when ADF concentration increases, which mean that forage reduces its quality. TDN varied from 593 to 417  $\text{g kg}^{-1}$  DM at stage 3 (stem extension) and stage 9 (stem senescence), respectively, when ADF was 44%. Usually, DMI is negatively correlated with NDF and this trend was also confirmed in linseed, DMI ranging from 18.4 to 25.2  $\text{g kg}^{-1}$  DM. RFV exceeded the 151 value, which is indicative of prime forage (Horrocks and Vallentine, 1999) only at stage 3.

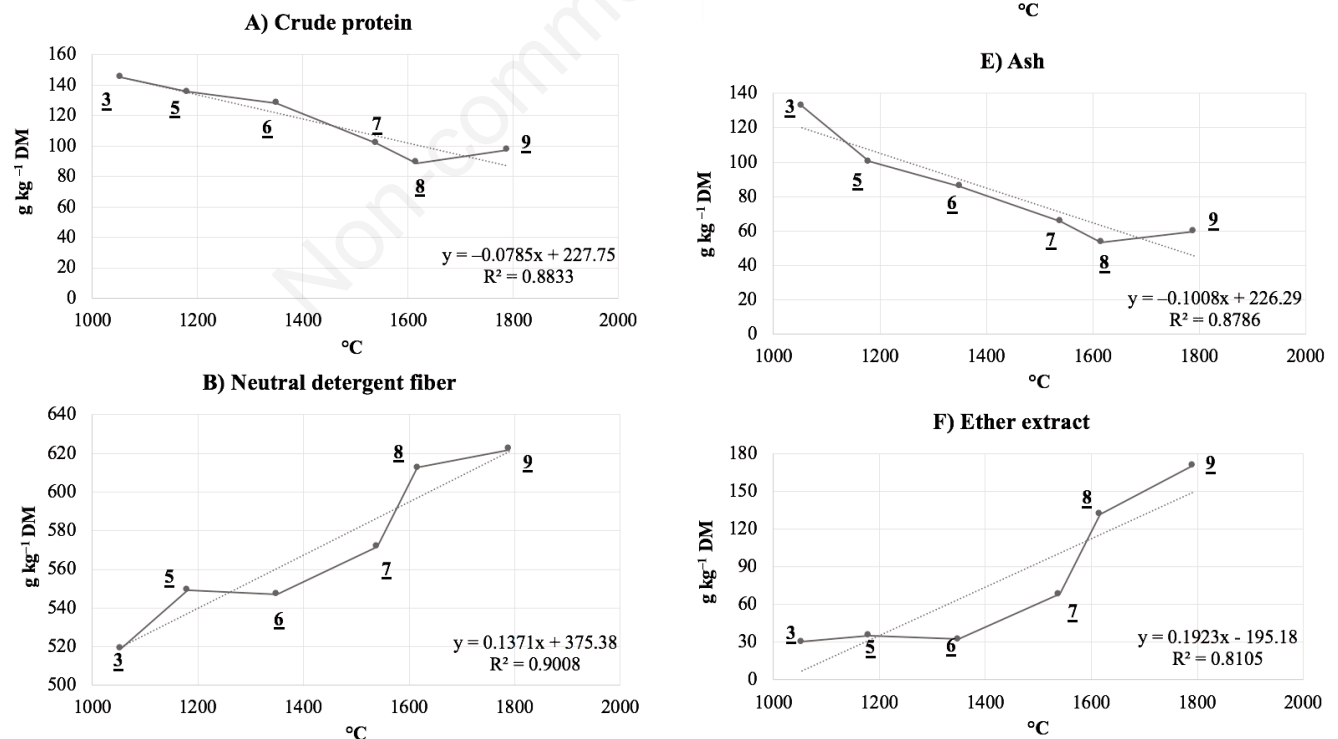


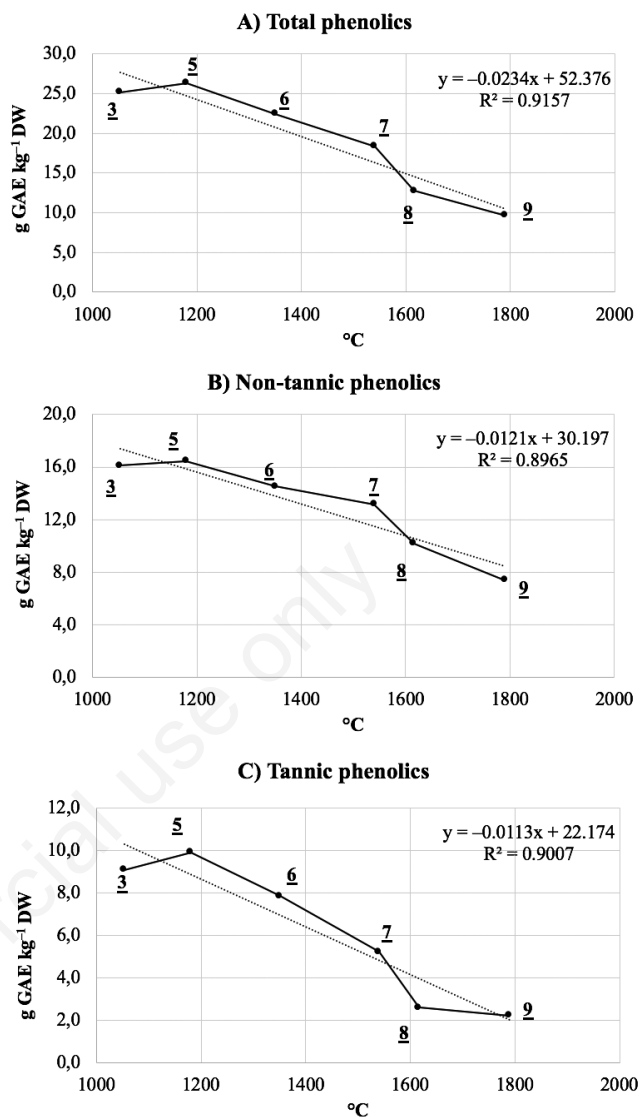
Figure 3. Bromatological composition of linseed shoot at six morphological stages: A) crude protein; B) neutral detergent fiber; C) acid detergent fiber; D) acid detergent lignin; E) ash; F) ether extract. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.

and fruits, regulating auxin transport, and protecting against pathogens, parasites, and predators (Winkel-Shirley, 2001).

Total phenolics, non-tannic phenolics, tannic phenolics (Figure 4) and total flavonoids (Figure 5) contents were negatively related with the morphological stages. Total phenolics, non-tannic phenolics, and tannic phenolics reached their highest values of 26.4, 16.4, 9.9 g GAE kg<sup>-1</sup> DW, respectively, at stage 5 (inflorescence emergence – main stem) corresponding to 1200 GDD and their lowest value of 9.6, 7.4, 2.2 g GAE kg<sup>-1</sup> DW, respectively, at stage 9 (stem senescence) after 1800 GDD. At the same GDD, total flavonoids decreased from 16.7 at stage 3 to 5.2 CE kg<sup>-1</sup> DW at stage 9. Re *et al.* (2013) reported a similar value (26.9 g GAE kg<sup>-1</sup>) of total phenolics for sainfoin (*Onobrychis vicifolia* Scop.) racemally, a study on garland (*Chrysanthemum coronarium* L.) shoot harvested at flowering showed values of total phenolics, non-tannic phenolics, and total flavonoids of 28.9, 14.0 g GAE kg<sup>-1</sup> and 21 g CE kg<sup>-1</sup> DW, respectively (Sulas *et al.*, 2017). With a different method for phytochemical extraction, Wang *et al.* (2017) reported for linseed sprouts values in total phenolics (an average of 2.05 g GAE<sup>-1</sup> kg DW) lower than our results, as well as, lower flavonoids contents (1.6 g CE kg<sup>-1</sup> DW). El-Lethy *et al.* (2010) found in linseed plant harvested at vegetative stage, a total phenolics content of 1.81% and 1.64% in leaves treated using the antioxidants stigmasterol and putrescine, respectively, and of 0.88% in the control. Russo and Reggiani (2015), for different linseed varieties, reported much lower values for phenolic contents in seeds than those found in the present study for shoots harvested at vegetative stage. No condensed tannins were detected in linseed shoot under study.

As shown in Figure 6A, ABTS assay exhibited a decreasing variation of antioxidant capacity from about 16 (stem extension) to 9.3 mmol TEAC/100 g DW (stem senescence). The total antioxidant capacity determined by the DPPH assay also decreased from 19 to 7 mmol TEAC/100 g DW at the same phenological stages, after 1050 and about 1800 GDD, respectively (Figure 6B). Our values of antioxidant activity were much higher than those obtained by Russo and Reggiani (2015), who performed the extraction using ethanol.

The correlations between the antioxidant activity revealed by the two assays (ABTS and DPPH), and TotP, NTP, TP, and TotF showed highly significant linear correlation (Table 2). Russo and Reggiani (2015) reported that the antioxidant capacity was significantly correlated with total polyphenolics in linseed varieties having different productive attitude (oil, fiber, intermediate). On the contrary, another study carried out by Souri *et al.* (2008), showed



**Figure 4.** Bioactive compounds in linseed six morphological stages: A) total phenolics; B) non-tannic phenolics; C) tannic phenolics. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.

**Table 1.** Total digestible nutrients, digestible dry matter, digestible dry matter intake, relative feed value and energy for lactation in relation to the morphological stages of linseed shoots.

Morphological stages	Stage code	TDN (g kg <sup>-1</sup> DM)	DDM (g kg <sup>-1</sup> DM)	DMI (g kg <sup>-1</sup> DM of body weight)	RFV (%)	NEI (Mcal kg <sup>-1</sup> )
Stem extension	3	593.0	886.5	25.2	173.2	1.45
Inflorescence emergence (main shoot)	5	567.6	886.3	22.2	152.5	1.40
Flowering and capsule formation (whole plant)	6	545.1	886.8	20.6	149.8	1.35
Development of seed and capsule (whole plant)	7	454.7	885.6	21.1	144.9	1.17
Capsule and seed ripening	8	433.5	885.5	19.6	134.5	1.12
Stem senescence	9	417.4	885.8	18.4	126.3	1.09
P≤0.05		***	***	***	***	***
R <sup>2</sup>		0.91	0.92	0.94	0.97	0.90

TDN, total digestible nutrients; DDM, digestible dry matter; DMI, digestible dry matter intake; RFV, relative feed value; NEI, energy for lactation; DM, dry matter.

no significant correlation between antioxidant activity and phenolics content of 24 medicinal plants, including seeds of linseed.

Highly significant linear correlations between the ABTS, DPPH and phenolics, as well as NDF, ADF, ADL, ash and ether extract were found (Table 3) except for ash and ABTS, NTP, TP. A highly significant correlation for NDF, ADF and antioxidant capacity and phenolics was reported by Sulas *et al.* (2016) in milk thistle (*Silybum marianum* (L.) Gaertn.). Conversely, Campion *et al.* (2013) found that cellulose accumulation is negatively correlated with total phenolic content in common bean (*Phaseolus vulgaris* L.) seeds.

The morphological stage was significantly correlated with shoot oil concentration ( $R^2=0.76$ ) although fatty acid content (unsature, mono, and polyunsature) showed an irregular trend and did not correlate with morphological stage (Table 4). On the contrary, Peiretti and Maineri (2008) reported that chemical composition and FA profile of linseed is closely connected to the development of plant and both nutritional quality and PUFA content of linseed decreases.

Oil content ranged from 10.1 to 17.9%,  $\alpha$ -linolenic acid from 33.5 to 40.6% and linoleic acid from 8.1 to 10.5%. Myristic acid (C14:0) was present in small amounts in all the phenological stages. Peiretti and Meineri (2008) in a study about linseed fatty acid content in different phenological stages, reported results in agreement to our values for myristic (C14:0), stearic (C18:0), and  $\alpha$ -linolenic acid (C18:3). However, another study on the fatty acid profile for seed oil of flax reported a lower content of myristic, palmitic (C16:0), and palmitoleic acid (C16:1) than the present research, whereas the content of oleic (C18:1), linoleic (C18:2), and  $\alpha$ -linolenic acid (C18:3) were higher (Gutiérrez *et al.*, 2010).

El-Lethy *et al.* (2010) found similar levels of LA but lower of ALA (5.5 and 12.2%, respectively) in linseed grown in Egypt. In contrast with this study, Peiretti and Meineri (2008) found higher

**Table 2. Correlation ( $R^2$  and equation) established between total phenolics, non-tannic phenolics, tannic phenolics, flavonoids and antioxidant capacity (ABTS, DPPH), in linseed shoots.**

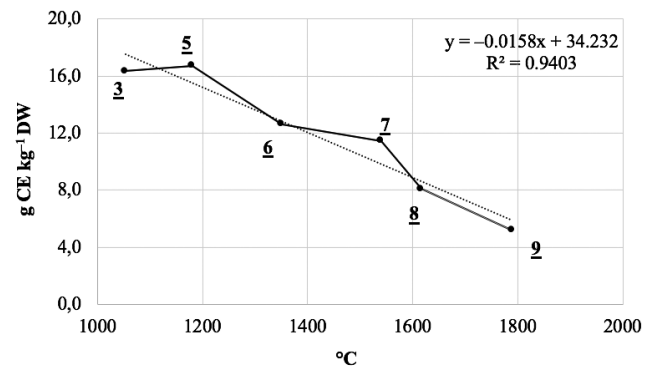
	ABTS		DPPH	
TotP	0.81*	$y=5.95+0.38x$	0.90**	$y=1.89+0.61x$
NTP	0.97**	$y=2.47+0.81x$	0.93**	$y=-2.62+1.22x$
TP	0.80*	$y=8.01+0.81x$	0.87**	$y=5.32+1.30x$
TotF	0.91*	$y=0.91+0.59x$	0.97**	$y=2.42+0.94x$

ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TotP, total phenolics; NTP, non-tannic phenolics; TP, tannic phenolics; TotF, flavonoids. \*Significance level at  $P \leq 0.05$ ; \*\*Significance level at  $P \leq 0.001$ .

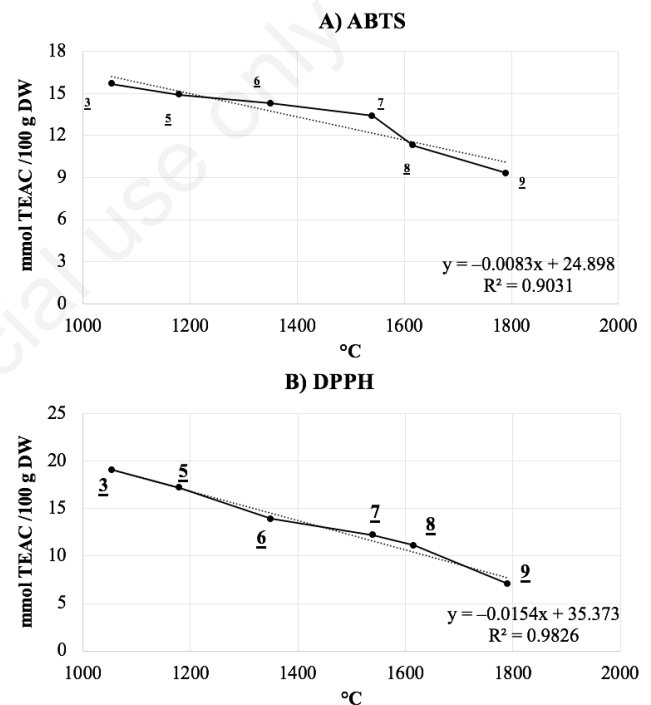
**Table 3. Correlation ( $R^2$  and equation) established between antioxidant capacity (ABTS, DPPH), total phenolics, non-tannic phenolics, tannic phenolics, flavonoids and quality features, in linseed shoots.**

	ABTS		DPPH		TotP		NTP		TP		TotF	
CP	0.67*	$y=0.41+0.11x$	0.86**	$y=-8.63+0.17x$	0.81*	$y=-14.0+0.28x$	0.72*	$y=-2.96+0.14x$	0.74*	$y=-8.55+0.12x$	0.78*	$y=-10.5+0.19x$
NDF	0.91**	$y=54.2-0.07x$	0.91**	$y=76.7-0.11x$	0.79*	$y=100.2-0.16x$	0.89**	$y=63.0-0.09x$	0.76*	$y=49.9-0.07x$	0.88**	$y=77.4-0.11x$
ADF	0.76*	$y=35.5-0.06x$	0.84*	$y=49.8-0.09x$	0.84*	$y=74.9-0.14x$	0.80*	$y=41.4-0.07x$	0.81*	$y=31.9-0.07x$	0.82*	$y=49.6-0.10x$
ADL	0.89**	$y=26.3-0.11x$	0.90**	$y=33.5-0.16x$	0.91**	$y=50.4-0.26x$	0.95**	$y=29.9-0.13x$	0.96**	$y=21.4-0.12x$	0.93**	$y=33.7-0.18x$
Ash	0.64 ns	$y=6.13+0.08x$	0.83*	$y=1.21-0.14x$	0.67*	$y=1.73-0.20x$	0.64 ns	$y=4.58-0.10x$	0.56 ns	$y=-1.02-0.08x$	0.70*	$y=-0.12-0.14x$
EE	0.94**	$y=16.8-0.05x$	0.85**	$y=18.8-0.07x$	0.84**	$y=27.0-0.11x$	0.95**	$y=17.7-0.06x$	0.88**	$y=10.2-0.05x$	0.90**	$y=17.6-0.08x$

ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TotP, total phenolics; NTP, non-tannic phenolics; TP, tannic phenolics; TotF, flavonoids; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; EE, ether extract. \*Significance level at  $P \leq 0.05$ ; \*\*Significance level at  $P \leq 0.001$ ; ns, not significant.



**Figure 5. Total flavonoids content in linseed shoots six morphological stages. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.**



**Figure 6. Antioxidant activity in linseed shoots six morphological stages: A) ABTS method; B) DPPH method. Stages: 3: stem extension; 5: inflorescence emergence (main shoot); 6: flowering and capsule formation (whole plant); 7: development of seed and capsule; 8: capsule and seed ripening; 9: stem senescence.**

**Table 4. Fatty acid composition (%) in relation to the morphological stages of linseed shoots.**

Morphological stages	Stage code	Myristic acid (C14:0) (%)	Palmitic acid (C16:0) (%)	Stearic acid (C18:0) (%)	Arachidic acid (C20:0) (%)	Palmitoleic acid (C16:1) (%)	Oleic acid (C18:1) (%)	Linoleic acid (C18:2) (%)	$\alpha$ -Linolenic acid (C18:3) (%)	Oil content (%)
Stem extension	3	<0.1	15.1	5.8	11.3	1.7	9.1	8.9	35.6	11.8
Inflorescence emergence (main shoot)	5	<0.1	15.1	10.6	10.3	2.4	6.2	9.4	40.6	10.1
Flowering and capsule formation (whole plant)	6	<0.1	12.9	5.5	12.5	3.1	9.6	8.1	33.5	13.7
Development of seed and capsule (whole plant)	7	<0.1	16.8	6.5	13.1	2.0	7.3	8.7	35.2	14.1
Capsule and seed ripening	8	<0.1	15.8	11.7	8.9	2.4	6.2	10.5	38.7	16.4
Stem senescence	9	<0.1	15.9	7.8	12.1	2.2	5.0	9.8	35.4	17.9
$P \leq 0.05$		ns	ns	ns	ns	ns	ns	ns	ns	***
$R^2$		-	0.27	0.25	$2.5 \times 10^{-4}$	0.06	0.30	0.43	0.35	0.76

values for both fatty acids and evidenced that LA and ALA were affected by phenological stages of linseed, presumably influenced by the different sowing time (late autumn in Sardinia and June in Piedmont, respectively) and environmental conditions. In galega (*Galega officinalis* L.) and quinoa (*Chenopodium quinoa* Willd.), Peiretti and Gai (2006) and Peiretti *et al.* (2013) found similar and higher concentration of linoleic acid compare to linseed, whereas  $\alpha$ -linolenic acid had an opposite trend.

## Conclusions

The quality traits of green linseed grown in a Mediterranean environment elucidated in the present study supply additional information regarding the entire range of utilisation of such crop, as linseed is usually considered as a dual purpose crop (fiber and seed). The evolution of bromatological composition, fatty acid content, bioactive compounds and antioxidant capacity of linseed shoots at six different codified morphological stages, was closely related to the plant development, indicating a decrease of shoot quality from the first to the last morphological stage.

Therefore, our results suggest harvesting/utilising linseed shoots before stage 6 (flowering and capsule formation). Moreover, our research evidenced that aerial biomass of linseed represent, (particularly at early stage) a valuable source of phenolic antioxidants, with potential benefits for animal welfare and as an alternative to synthetic antioxidants.

A further achievement is an increased awareness about the evolution of fatty acid content that resulted little influenced by the morphological stages, with implications for a whole exploitation of green linseed as a forage source. Future studies require elucidation of the chemical composition of phenolics in relation to the potential development of new natural antioxidant for natural health care of animals.

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