Effect of the Growth Stage of False Flax (Camelina sativa L.) on the Phenolic Compound Content and Antioxidant Potential of the Aerial Part of the Plant

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INTRODUCTION

False flax (Camelina sativa L.) is a member of the Brassicaceae family and is an ancient crop that is native to Europe in areas from the Mediterranean Sea to the Arctic Circle. The commercially utilized part of C. sativa is its seeds, due to their oil content, which accounts for up to 42% of the seed matter [Peiretti & Meineri, 2007; Belayneh et al., 2017]. False flax seed oil is appreciated as a food ingredient, and interest in it as a potential low input source of biofuel has recently been growing [Berti et al., 2016]. From a nutritional point of view, a high content of its polyunsaturated fatty acids (about 30% of the total fatty acids) and its favorable fatty acid profile (its α-linolenic acid content constitutes up to 40% of the total fatty acids) are important [Zubr & Matthäus, 2002; Kirkhus et al., 2013]. C. sativa oil is also a rich source of antioxidants, such as tocopherols, phytosterols and phenolic compounds, which are beneficial to human health and increase the oxidative stability of the oil [Zubr & Matthäus, 2002; Abramović et al., 2007; Kirkhus et al., 2013; Belayneh et al., 2017]. Among the phenolic compounds, sinapic, p-hydroxybenzoic, salicylic and gallic acids, as well as catechin and quercetin, have been identified in false flax seed oil [Terpinc et al., 2012a].

The main by-product of false flax seeds are cakes, obtained after seed oil pressing, which can be exploited as a fat- and protein-rich ingredient for animal nutrition [Berti et al., 2016]. However, false flax cake has also recently been considered as a valuable source of phenolic compounds for a potential use in selected foods or food formulations [Terpinc et al., 2012b; Rahman et al., 2018a, b]. C. sativa cake contains more phenolic compounds than its oil. Terpinc et al. [2012a] reported that the total phenolic content (TPC) in oil and cake was 9.1 and 1666 mg chlorogenic acid eq/100 g of dry matter (DM), respectively. The phenolic compounds identified in cake and defatted meal included mainly flavonoids and hydroxycinnamates [Terpinc et al., 2011; Rahman et al., 2018a]. Sinapine, the ester of sinapic acid and choline, has often been determined as the main phenolic compound in false flax cake or meal [Salminen et al., 2006; Terpinc et al., 2011]. Hydroxybenzoic acids have also been identified [Terpinc et al., 2012a; Rahman et al., 2018a], although Salminen et al. [2006] did not find them in false flax seed meal. Condensed tannins are another class of phenolic compounds that have been determined in false flax cake [Matthäus & Zubr, 2000]. Rahman et al. [2018a] identified several procyaminid dimers among these tannins.

The phenolic compound profile of false flax cake determines its significant antioxidant activity [Matthäus, 2002; Quezada & Cherian, 2012]. Rahman et al. [2018b] showed...
that C. sativa meals inhibited the in vitro oxidation of low density lipoprotein and DNA damage and effectively inhibited pancreatic lipase and α-glucosidase activities. The bioactivity of the phenolic compounds of false flax can be enhanced by the presence of other phytochemicals. Das et al. [2014] reported a synergy of NAD(P)H quinone oxidoreductase 1 (NQO1, phase II detoxification enzyme) induction for a combination of quercetin and some alkyl sulfanyl glucosinolates found in C. sativa seeds. False flax cake has been studied not only because of its potentially health-promoting food compounds, but also as a food ingredient to increase the shelf-life of products. It has been proved, for instance, that camelina meal effectively inhibits the oxidation of lipids and protein in cooked pork meat patties [Salminen et al., 2006].

As shown above, false flax seeds (both oil and defatted meal) have been studied extensively for their composition and the presence of bioactive substances. However, the composition of the other parts of the C. sativa is not so well known. Petretti & Meineri [2007] determined the nutritive value and fatty acid profile of the aerial part of the plant during growth, but did not analyze the phytochemicals. To the best of our knowledge, apart from the studies related to its seeds, only one study has been carried out on the phenolic compounds of C. sativa. Onyilagha et al. [2003] identified the presence of flavonoids in false flax leaves. It seems important to complete our knowledge about the phenolic compounds of the aerial part of C. sativa, especially because of the ever-growing interest in this plant [Faure & Tepfer, 2016]. The composition of the phenolic compounds changes during the growth cycle of plants [Gai et al., 2017; Karamać et al., 2019; Pavlović et al., 2019], therefore, the purpose of our research has been to determine the phenolic compound profile and antioxidant potential of the aerial part of false flax at several morphological stages, that is, from the vegetative to the ripe seed-pod stage, in order to select those with the highest parameters as a potential source of phenolic antioxidants.

MATERIAL AND METHODS

Plant material collection

The false flax seeds were obtained from Ornitalia Product Service s.a.s. (Colleredo di Monte Albano (UD), Italy). The plant was cultivated in the Western Po Valley near Cuneo, Italy. The stands were seeded in the spring (15 May), and no irrigations or fertilizers were applied after sowing. The aerial parts of the plants were collected, using edging shears (0.1 m cutting width), at five growth stages from the vegetative to the ripe seed-pod stage (Table 1), on subplots of 2 m² randomly located in 4 × 10 m² plots with three replicates cut to a 1 to 2 cm stubble height. The sampling time ranged from the end of June to the end of July. Sampling was not performed on rainy days and was carried out in the morning, only after the disappearance of dew. The collected plant material was frozen immediately and then freeze-dried (LIO-5P, 5 Pascal, Trezzano sul Naviglio, Italy). After grinding in a mortar (particles passing through a 1 mm screen), the samples were stored in tightly closed vessels at -20°C until analysis.

Standards and reagents

The Folin-Ciocalteu’s phenol reagent (FCR), gallic acid, 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2’-diphenyl-1-picrylhydrazyl (DPPH radical), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl-choran-2-carboxylic acid (Trolox), β-carotene, linoelic acid, Tween 40, butylhydroxyanisole (BHA), and high performance liquid chromatography (HPLC) standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). The remaining chemicals (analytical grade) and HPLC solvents (gradient grade) were acquired from Avantor Performance Materials (Gliwice, Poland).

Extract preparation

The phenolic compounds were extracted from freeze-dried false flax plants using 80% (v/v) methanol at 65°C and a 1:10 (v/w) material-to-solvent ratio, according to a procedure previously described by Karamać et al. [2018]. The crude extracts were dried by evaporating methanol under vacuum (Rotavapor R-200, Büchi Labortechnik, Flawil, Switzerland) and freeze drying the remaining aqueous residue (FreeZone Freeze Dry System, Labconco, Kansas City, MO, USA). A mass balance was carried out to evaluate the extraction yield.

Total phenolic content determination

The TPC of the false flax extracts and plants was determined using a colorimetric assay with FCR and gallic acid as the standard [Kosińska et al., 2011]. The results were expressed as mg of gallic acid equivalents (GAE) per g of extract or per g of plant fresh matter (FM).

Trolox equivalent antioxidant capacity determination

The Trolox equivalent antioxidant capacity (TEAC) was determined using the ABTS•+ decolorization assay [Re et al., 1999]. First, the ABTS radical cations were activated by mixing aqueous solutions of ABTS (7 mM) and sodium persulfate (2.45 mM), and then shaking the mixture overnight in the dark. The ABTS•+ stock solution was diluted with methanol to an absorbance of 0.720 at 734 nm (DU-7500 spectrophotometer, Beckman Instruments, Fullerton, CA, USA) immediately before conducting the colorimetric reaction. A 2 mL portion of this solution was vortexed with 20 μL of the extract solution in methanol (1.5 mg/mL) and the reaction was carried out at 37°C for 6 min. The absorbance was then measured at 734 nm. Trolox was used as the standard. The results were expressed as mmol Trolox equivalents (TE) per g of extract or μmol TE per g of plant FM.

Ferric-reducing antioxidant power determination

The ferric-reducing antioxidant power (FRAP) assay was performed as previously described by Benzie & Strain [1996]. In brief, the extracts were dissolved in water (1 mg/mL), and portions of a 75 μL solution were mixed with 2.25 mL of a FRAP reagent and 225 μL of water, both warmed to 37°C. The FRAP reagent consisted of 10 volumes of 300 mM acetate buffer, pH 3.6, 1 volume of 10 mM TPTZ in 40 mM HCl, and 1 volume of 20 mM FeCl3×6H2O. The absorbance of the reaction mixtures was
measured at 593 nm (DU-7500 spectrophotometer) after 30 min of incubation at 37°C. The FRAP values were calculated on the basis of the calibration curve prepared for ferrous sulfate. The results were expressed as mmol Fe²⁺ equivalents per g of extract or μmol Fe²⁺ equivalents per g of plant FM.

Scavenging the DPPH radicals

The DPPH⁺ scavenging activity of the false flax extracts was determined as described by Brand-Williams et al. [1995]. Methanolic solutions of the extract were prepared in the 1.2–6.0 mg/mL concentration range. A 0.1 mL aliquot of the extract solution was vortexed with 2 mL of methanol and 0.25 mL of methanolic solution of DPPH⁺ (1 mM). The reaction was conducted in the darkness at ambient temperature. After 20 min, the absorbance was measured at 517 nm. The curves of absorbance vs. extract concentration in the reaction mixture were plotted and the EC₅₀ values were calculated. EC₅₀ was defined as the concentration of extract needed to scavenge 50% of the initial DPPH⁺.

Oxidation of β-carotene-linoleic acid emulsion

The ability of the false flax extracts to inhibit the oxidation of the emulsion system with β-carotene and linoleic acid was determined according to Miller [1971], with some modifications, as described in a previous publication [Karamač et al., 2018]. A 20 μL aliquot of a methanolic solution of extracts (1 mg/mL) or BHA (0.5 mg/mL) was pipetted into plate wells. The emulsion (250 μL) was then added. Emulsion without an antioxidant was used as a control sample. The plate was immediately placed in an Infinite M1000 microplate reader (Tecan, Männedorf, Switzerland) heated to 42°C, and the absorbance was measured at 470 nm. The plate was left in the device for 180 min and absorbance was read every 15 min. The percentage of non-oxidized β-carotene was calculated for each measurement time.

HPLC analysis

The HPLC-DAD analysis of the phenolic compounds of the false flax extracts was performed using a Shimadzu system (Kyoto, Japan) consisting of two LC-10ADp pumps, an SCL-10Avp system controller and an SPD-M10Avp diode array detector (DAD). The pre-packed Luna C18 (4.6×250 mm, 5 μm, Phenomenex, Torrance, CA, USA) column was connected to an HPLC system. Methanolic solutions of the extracts (6.67 mg/mL) were injected (20 μL) into the column and the compounds were eluted for 30 min with a mobile phase in a linear gradient system consisting of 5–60% acetonitrile in water (v/v) with trifluoroacetic acid (0.1, v/v). The flow rate was 1 mL/min. The DAD scanned over a wavelength range of 200 to 400 nm. The eluted compounds were identified from a comparison of their retention times and UV spectra with the corresponding standards. The calibration curves of the standards were used to quantify the compounds. Chlorogenic acid was determined at 320 nm and flavonoids at 350 nm.

Statistical analysis

Three samples of the aerial parts of false flax were collected for each growth stage and duplicate extracts were prepared separately from each sample. The chemical determinations were carried out at least in triplicate. The results were reported as means with standard deviations. An analysis of variance (ANOVA) and Fisher’s LSD test were performed at a level of p<0.05 (GraphPad Prism; GraphPad Software, San Diego, CA, USA) to evaluate the significance of any differences among the mean values. A Principal Component Analysis (PCA) was performed to describe any variations among the growth stages. Statistica 13.1 software (StatSoft Corp., Kraków, Polska) was used for the PCA.

RESULTS AND DISCUSSION

False flax is a plant which has a short growth cycle and reaches maturity after an average cultivation of 92 days [Berti et al., 2016]. In our study, the aerial part of plants was harvested between 42 and 70 days after sowing, corresponding to vegetative and ripe seed-pods stages, respectively (Table 1). The five growth stages selected in our experiment corresponded to 4 (development of the vegetative parts), 5 (inflorescence emergence), 6 (flowering), 7 (development of the fruit), and 8 (ripening) phenological growth stage of false flax, respectively, according to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie scale [Martinelli & Galasso, 2011]. In line with the literature, the flowering plants collected on the 56th day of the growth cycle (Table 1), showed that 50% of the flowering of spring genotypes of false flax may be obtained within 36–59 days of planting [Berti et al., 2016].

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Days after sowing</th>
<th>Extraction yield (%)</th>
<th>TPC mg GAE/g extract</th>
<th>TPC mg GAE/g FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>42</td>
<td>24.0±0.5⁴</td>
<td>49.2±1.4</td>
<td>1.46±0.04</td>
</tr>
<tr>
<td>Bud</td>
<td>49</td>
<td>21.8±0.7⁶</td>
<td>56.9±2.1</td>
<td>2.16±0.21</td>
</tr>
<tr>
<td>Flowering</td>
<td>56</td>
<td>18.8±0.6³</td>
<td>59.1±6.0</td>
<td>2.31±0.17</td>
</tr>
<tr>
<td>Early seed-pod</td>
<td>64</td>
<td>18.2±0.6⁶</td>
<td>57.6±1.8</td>
<td>2.48±0.11</td>
</tr>
<tr>
<td>Ripe seed-pod</td>
<td>70</td>
<td>19.1±1.3³</td>
<td>55.4±3.8</td>
<td>3.10±0.43</td>
</tr>
</tbody>
</table>

GAE, gallic acid equivalents. Means with different letters in the column are significantly different (p<0.05).
Extraction yield and total phenolic content

Table 1 reports the extraction yield and TPC of the false flax at different plant growth stages. The highest yield was obtained from the extraction of false flax at the vegetative stage. The plants extracted at the subsequent growth states had lower yields, although the flowering, early and ripe seed-pod stage values did not differ significantly (p≥0.05). In a previous research, in which the aerial part of amaranth was extracted, we found that the budding stage of the plant also allowed a higher extraction yield to be obtained than at the early flowering or grain filling stages [Karamać et al., 2019].

The TPC of the false flax extracts ranged from 49.2 to 59.1 mg GAE/g and the differences in TPC were not statistically significant (p≥0.05) for several growth stages (Table 1). Considerable variations in TPC were observed when the values were expressed on a fresh plant matter basis. The highest TPC (3.10 mg GAE/g FM) was determined in false flax at the ripe seed-pod stage and the lowest value (1.46 mg GAE/g FM) was noted for the vegetative stage. This greater variability in TPC calculated for FM than for the extract may be due to the decreasing water content in false flax during growth, which has been noted in this study and in the previous ones [Peiretti & Meineri, 2007]. The TPCs of the plant extracts at all the growth stages (Table 1) were higher than the values reported by Matthäus [2002] for defatted seed extracts obtained using several types of solvents (3.2–21.8 mg GAE/g of extract). The TPC determined in the FM of the false flax at the ripe seed-pod stage (Table 1) was similar to those found in C. sativa seed meal (3940 μg/g) [Salminen & Heinonen, 2008] and in seeds and defatted meal (3248.3 and 4591.8 μg GAE/g, respectively) [Quezada & Cherian, 2012]. However, Terpinc et al. [2012a] reported about 3–5 times higher TPC of defatted cake and seeds, which is probably the result of a different way of expressing the results (using chlorogenic.

FIGURE 1. HPLC-DAD separation of the phenolic compounds of the false flax extract; 1 – chlorogenic acid; 2 – rutin; 3 – quercetin 3-O-glucoside; 4 – quercetin glycoside; Graphs 1–4, UV-DAD spectra of compounds 1–4, respectively.
TABLE 2. Phenolic compound content of the extracts (mg/g extract) of false flax at different growth stages.

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Chlorogenic acid</th>
<th>Rutin</th>
<th>Quercetin 3-O-glucoside</th>
<th>Quercetin glycoside†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>31.6±1.1a</td>
<td>13.3±0.7a</td>
<td>8.8±1.1a</td>
<td>21.5±1.2a</td>
</tr>
<tr>
<td>Bud</td>
<td>35.9±1.3a</td>
<td>15.1±0.8a</td>
<td>10.0±1.3a</td>
<td>24.4±1.4a</td>
</tr>
<tr>
<td>Flowering</td>
<td>31.7±1.8a</td>
<td>13.9±1.1a</td>
<td>11.0±0.4a</td>
<td>19.3±3.1a</td>
</tr>
<tr>
<td>Early seed-pod</td>
<td>23.4±2.4a</td>
<td>10.9±2.0a</td>
<td>10.4±0.4a</td>
<td>21.4±0.9a</td>
</tr>
<tr>
<td>Ripe seed-pod</td>
<td>19.0±0.6a</td>
<td>10.3±1.3a</td>
<td>10.3±1.0a</td>
<td>19.0±2.1b</td>
</tr>
</tbody>
</table>

†Expressed as quercetin equivalents. Means with different letters in the column are significantly different (p<0.05).

TABLE 3. Phenolic compound content of the fresh matter (mg/g FM) of false flax at different growth stages.

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Chlorogenic acid</th>
<th>Rutin</th>
<th>Quercetin 3-O-glucoside</th>
<th>Quercetin glycoside†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>0.94±0.03a</td>
<td>0.39±0.02a</td>
<td>0.26±0.03a</td>
<td>0.64±0.04a</td>
</tr>
<tr>
<td>Bud</td>
<td>1.36±0.05a</td>
<td>0.57±0.03a</td>
<td>0.38±0.05a</td>
<td>0.93±0.05a</td>
</tr>
<tr>
<td>Flowering</td>
<td>1.24±0.07a</td>
<td>0.54±0.04a</td>
<td>0.43±0.02a</td>
<td>0.76±0.12a</td>
</tr>
<tr>
<td>Early seed-pod</td>
<td>1.01±0.10a</td>
<td>0.47±0.09a</td>
<td>0.45±0.02a</td>
<td>0.92±0.04a</td>
</tr>
<tr>
<td>Ripe seed-pod</td>
<td>1.06±0.03a</td>
<td>0.57±0.07a</td>
<td>0.57±0.06a</td>
<td>1.06±0.12a</td>
</tr>
</tbody>
</table>

†Expressed as quercetin equivalents. Means with different letters in the column are significantly different (p<0.05).

Distribution of the individual phenolic compounds

The HPLC-DAD separation of the false flax extracts is shown in Figure 1. Four main peaks (1–4), with retention times in the 13.2–18.9 min range, are visible on the chromatogram. These peaks correspond to compounds with the maximum absorption of UV-Vis spectra at 325 nm (compound 1) or at 255 and 354 nm (compounds 2–4). They were identified as chlorogenic acid, rutin (quercetin 3-O-rutinoside), quercetin 3-O-glucoside, and quercetin glycoside, respectively. The presence of quercetin glycosides in the aerial part of false flax was consistent with a report of Onyilagha et al. [2003], who characterized flavonoids in the leaves of C. sativa and found that quercetin derivatives were the only type of flavonoid present. These authors identified four quercetin glycosides (quercetin heptose-pentose-deoxypentose derivative, quercetin 3-O-rutinoside, quercetin 3-O-glucoside and -O-galactoside, and quercetin 3-O-glucuronide). Quercetin glycosides were also identified in the seeds, cake and defatted seed meal of false flax, although they were accompanied by other flavonoids, such as catechin, epicatechin, dihydrodi-hydroxyisorhamnetin, daidzin, and myricetin [Terpinc et al., 2011; 2012a; Rahman et al., 2018a]. Chlorogenic acid was previously detected in defatted false flax seeds [Rahman et al., 2018a]. Surprisingly, sinapic acid and its choline ester (sinapine), which have frequently been determined in C. sativa seeds [Matthäus & Zubr, 2000; Salminen et al., 2006; Amyot et al., 2019], have not been instead identified in the aerial part of false flax at all investigated stages in our study, even at the ripe seed-pod stage.

The individual phenolic compound contents expressed in the extracts and FM of the false flax at different growth stages are reported in Tables 2 and 3, respectively. Chlorogenic acid was the most abundant phenolic constituent in the first four growth states (in both the extracts and FM), while its content was equal to the level of quercetin glycoside at the ripe seed-pod stage. The chlorogenic acid content increased from the vegetative to the bud stage, reaching 35.9 mg/g in the extract and 1.36 mg/g in the FM of the plant, and then gradually decreased. This trend has not been observed for individual flavonoids. The growth stages were divided into two groups (p<0.05) on the basis of the rutin level in the extracts; vegetative, bud and flowering stages with a high content, and early and ripe seed-pod stages with a low content. The rutin content values expressed in the fresh plant matter ranged from 0.39 to 0.57 mg/g FM, with the lowest level being found for the vegetative stage. No significant differences (p>0.05) were reported for the quercetin 3-O-glucoside content expressed as mg/g of extract, while a growth stage effect appeared when expressed on FM basis, with the lowest and the highest values (0.26 to 0.57 mg/g FM) being determined in the vegetative and ripe seed-pod stage, respectively. The quercetin glycoside content ranged from 19.0 to 24.4 mg/g of extract or from 0.64 to 1.06 mg/g of FM, and changed irregularly with the age of the false flax. Interestingly, the amount of flavonoids in the extract was the most abundant at the bud stage (49.5 mg/g of extract), as was the chlorogenic acid. In the case of fresh plant matter, the highest amounts of flavonoids were found in the bud and ripe seed-pod stages (1.88 and 2.20 mg/g of FM, respec-
The higher flavonoid content than that of hydroxycinnamic acids noted for all the growth stages was consistent with literature data regarding C. sativa seed meal [Salminen et al., 2006]. Onyilagha et al. [2003] determined the content of quercetin glycosides in false flax leaves and found a very low value (50 μg/g FM) compared to that reported in our study, even at the vegetative stage of the plant (1.29 mg/g FM), in which the proportion of leaves to other botanical parts was high. However, it should be taken into account that the aforementioned authors did not analyze the compounds directly in the crude extract, but re-extracted them in butanol and then used semi-preparative TPC, which could have caused a “loss” of a part of the flavonoids. However, the important contribution of chlorogenic acid to the phenolic compound pool determined in our study was not found for false flax seeds or for the by-products from oil pressing [Rahman et al., 2018a]. According to the authors, who analyzed free and esterified forms of extractable phenolics of defatted meal, the free chlorogenic acid content was only 17.02 μg/g and the content of caffeic acid liberated from esters was 16.36 μg/g [Rahman et al., 2018a]. In the same study, rutin was the major extractable flavonoid. Its defatted meal content (277.5 μg/g) was lower than the lowest content quantified in the present study at the vegetative growth stage (Table 3). These differences could be ascribed to the different distributions of phenolics in the examined plant part (seed vs. leaf), as reported by several authors for other plants belonging to the Brassicaceae family [Ferreres et al., 2005; 2006].

**Antioxidant potential**

The TEAC and FRAP of the false flax extracts and of the fresh plant matter are shown for different growth stages in Table 4. The results (both assays) obtained for the extracts were similar for the different growth states, although significant statistical differences (p<0.05) were found for some of them. These limited TEAC and FRAP variations between the growth stages were consistent with those found for TPC. Moreover, the trend of the TEAC changes in the fresh plant matter during the growth cycle was similar to that of TPC, with the highest value for the ripe seed-pod stage (16.5 mmol TE/g extract) and the lowest for the vegetative stage (8.4 mmol TE/g of extract). The FRAP determined for the FM of the plant at the vegetative stage was lower

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>TEAC mmol TE/g extract</th>
<th>TEAC μmol TE/g FM</th>
<th>FRAP mmol Fe2+/g extract</th>
<th>FRAP μmol Fe2+/g FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>0.28±0.00b</td>
<td>8.4±0.4c</td>
<td>0.93±0.04b</td>
<td>27.5±1.3b</td>
</tr>
<tr>
<td>Bud</td>
<td>0.32±0.01a</td>
<td>12.2±1.1b</td>
<td>1.05±0.04b</td>
<td>40.0±4.0a</td>
</tr>
<tr>
<td>Flowering</td>
<td>0.31±0.02a</td>
<td>12.3±0.6b</td>
<td>0.96±0.19b</td>
<td>37.7±7.1a</td>
</tr>
<tr>
<td>Early seed-pod</td>
<td>0.30±0.01b</td>
<td>13.0±0.4b</td>
<td>0.89±0.16b</td>
<td>38.4±6.9a</td>
</tr>
<tr>
<td>Ripe seed-pod</td>
<td>0.30±0.02b</td>
<td>16.5±2.4c</td>
<td>0.77±0.09b</td>
<td>42.9±2.9a</td>
</tr>
</tbody>
</table>

TE, Trolox equivalents. Means with different letters in the column are significantly different (p<0.05).
ported by and ing growth stages, respectively. On the as EC50, is respectively. The scavenging activity of flavonoids, in the lower EC50 value (113.7 µg/mL) was found for the ripe seed-pod extract stage. The DPPH· scavenging activity of the aerial part of false flax was lower than the antiradical activity against DPPH· of the seeds and cake [Terpinc et al., 2012a, b]. These differences (which were also mentioned earlier for TEAC) may be due to the different phenolic compound profiles of the aerial part and seeds of false flax. Mainly flavonoids, in the form of glycosides, which are known to have less ability to scavenge free radicals than their aglycons [Rice-Evans et al., 1996], were found in the aerial parts (Table 3). On the other hand, chlorogenic acid is a powerful antioxidant [Shahidi & Chandrasekara, 2010]. Its DPPH· scavenging activity was found to be slightly higher than that of sinapic acid and sinapine [Shahidi & Chandrasekara, 2010] – major phenolic compounds of C. sativa seeds belonging to phenolic acids and their derivatives [Terpinc et al., 2011; Rahman et al., 2018a].

The inhibition of the oxidation of β-carotene-linoleic acid emulsion by false flax extracts is reported in Figure 3. The antioxidant activity of the plant extracts at different growth stages decreases as follows: flowering > ripe seed-pod > early seed-pod > vegetative > bud. The low antioxidant activity of the plant at bud stage extracts and the high antioxidant activity of the ripe seed-pod extract were not compatible with the previously discussed results from other antioxidant assays. This is probably the result of different activities of the antioxidant compounds present in the extracts under polar conditions (ABTS, FRAP and DPPH assays) and in the lipid emulsion system. Although the β-carotene bleaching assay of false flax seeds has already been performed previously [Matthäus, 2002; Terpinc et al., 2012a, b; Rahman et al., 2018b], it was difficult to compare our results with literature data due to differences in the emulsion oxidation conditions or the way the results were expressed.

Principal component analysis

The PCA was carried out separately for two data sets; the first one was for the values obtained on an extract basis (Figures 4A and B) and second one was for the values expressed on plant fresh matter (Figures 4C and D), both considering five growth stages. The variables were: antioxidant assays, four phenolic compounds, and TPC. In the case of the data set related to the extracts, the first two principal components (PC1 and PCA) accounted for 88.79% of the total variance. The growth stages differed from each other, as can be observed in plot B (Figure 4). Most of the variables (FRAP, compounds 1, 2, and 4) described the bud stage. The flowering stage was associated with TEAC, TPC,

![Graph](image_url)  
**FIGURE 3.** Inhibition of the oxidation of β-carotene-linoleic acid emulsion by false flax extracts at different growth stages; BHA, butylhydroxyanisole.
Phenolic Content and Antioxidant Potential of False Flax

As expected, the EC_{50} values of the DPPH assay were negatively correlated with FRAP and TEAC. They influenced the distribution of the ripe seed-pod stage, due to its low antiradical activity against DPPH• with high EC_{50} value. The vegetative stage was not related to any variable.

The PCA of the second data set showed that the first two principal components were able to significantly explain up to 97.04% of the total variance. TEAC, FRAP, TPC and compounds 3 and 4 correlated with each other, as shown in plot C (Figure 4), and they also affected the ripe seed-pod stage. The bud and flowering stages were associated with compound 1. The vegetative stage was once again not associated with any variable.

**CONCLUSIONS**

Our research has shown that chlorogenic acid and quercetin glycosides are present in the aerial parts of *C. sativa* as the main phenolic compounds. Their contents changed in different ways during the growth cycle, although the chlorogenic acid content and the sum of the flavonoids reached the highest levels in the plant extracts at the bud stage. The high individual phenolic content in the plant extract at the bud and flowering stages determined their significant antioxidant activity under polar conditions (TEAC, FRAP and DPPH assay). As far as the antioxidant potential of fresh false flax matter is concerned, the best activity was observed for the plant at the ripe seed-pod stage. PCA indicated that quercetin 3-O-glucoside and quercetin glycoside were the main contributors to this potential. The extract and fresh matter of the youngest plant (vegetative stage) both showed a low antioxidant potential. The antioxidant activity of the extracts determined in the emulsion system was slightly different, the highest antioxidant activity was observed for the false flax extracts at the flowering and ripe seed-pod stages.

In short, the aerial part of *C. sativa*, which has a significant content of phenolic compounds, can be used as a source...
of protective antioxidant activity ingredients. However, in order to obtain the best properties, the plant should be harvested at the bud, flowering or ripe seed-pod stage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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