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**Short communication**

# Phenolics and antioxidant activity in flax varieties with different productive attitude

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**Abstract**

Eighteen varieties of flax (*Linum usitatissimum* L.) with different productive attitude (oil, fiber and intermediate) were analyzed for total phenolic content (TPC) and antioxidant activity. The TPC ranged from 4.64 to 9.40 mg caffeic acid equivalents (CAE) $g^{-1}$ dry weight of linseed. TPC were statistically different among productive attitude groups and increasing in this order: oil varieties → intermediate varieties → fiber varieties. The antioxidant activity ranged from 0.56 to 0.86 mmol trolox equivalents (TE) $g^{-1}$ dry weight of linseed. The antioxidant activity was statistically lower in oil varieties than the other two groups although within each group a greater variability respect to TPC was observed. The antioxidant activity was significantly correlated to TPC (r = 0.549). The data presented show that the oil flax varieties are poorer in antioxidants. Consequently, if there is an industrial interest on the flour for their TPC, intermediate varieties would be recommended.



## Introduction

Flax (*Linum usitatissimum* L.) is an annual plant and a member of the family Linaceae (Anwar and Przybylski, 2012). It can be grown in every country with moderate climate. Flax is grown both for fiber and for oil, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen and Richards 2003; Vaisey-Genser and Morris 2003). Linseed varieties contain usually about 40% of oil in the seeds. Flaxseed, besides its traditional oleochemical uses, is now gaining recognition as a functional food ingredient for human nutrition (Oomah, 2001; Lei *et al*., 2003). Seeds of flax are the richest source of alpha-linolenic acid, lignans and other nutritional components. In term of polyphenol content, flax seeds are at the top among plant species and phenolic compounds are excellent natural antioxidants (Perez-Jimenez *et al*., 2010; Kasote, 2013).

Consumption of flaxseed has been demonstrated to have multitude positive health benefits including decreasing rate of tumor growth, reducing serum cholesterol level and decreasing incidence of breast, prostrate, and colon cancers (Muir and Westcott, 2003; Hemmings *et al*., 2004; Hosseinian *et al*., 2006; Choo *et al.,* 2007). The health benefits of flaxseed can be credited mainly to its abundance to biologically active components (Kitts *et al*., 1999; Westcott and Paton, 2001; Tarpila *et al*., 2005; Hosseinian *et al*., 2006; Hussain *et al*., 2011). In particular, phenolic compounds (lignans, phenolic acid, flavonoids, phenylpropanoids and tannins) are excellent in preventing the excess of free radicals and avoiding their pathological effects (Kasote, 2013). Phenolic compounds exert their antioxidant capacity by acting as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Demiray *et al*., 2009). Therefore, there is a huge interest in assessing the variability in antioxidant activity in flax seeds as these are potential ingredients in foods, as well as in the pharmaceutical and cosmetic industries. In order to better define the nutraceutical attributes of flaxseed, there is great interest in the characterization of phytochemicals and antioxidant properties of this crop (Anwar and Przybylski, 2012). The main objectives of the present study was to evaluate the total polyphenol content (TPC) and antioxidant activity in eighteen flax varieties with different productive attitudes (oil, fiber and intermediate).

## Materials and Methods

### *Plant materials and sample extraction*

Seeds of different flax varieties were obtained from Semfor (Italy) or kindly provided by Centro di Ricerca per le Colture Industriali, CRA (Bologna, Italy). The eighteen varieties used in this study (six per group) were chosen according to the different productive attitude: Ita269, Ecotype R., Claudia, IT2421, Credo and Roma are oilseed varieties, Berber, Belinka, Blue di Riga, Datcha, Cruciata and

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Table 1. Total phenolic content (TPC) in different flax varieties and groups of different productive attitude

| Oil group | TPC[†] | Intermediate group | TPC[†] | Fiber group | TPC[†] |
|---|---|---|---|---|---|
| Ita269 | 5.02 ± 0.35 (gh) | Merlin | 7.21 ± 0.52 (c) | Berber | 8.92 ± 0.08 (a) |
| Ecotipo R. | 5.61 ± 0.06 (fg) | Solal | 6.40 ± 0.11 (def) | Belinka | 9.09 ± 0.38 (a) |
| Claudia | 5.88 ± 0.06 (ef) | Kaolin | 6.64 ± 0.32 (cde) | Blu di Riga | 7.40 ± 0.35 (bc) |
| IT2421 | 4.64 ± 0.18 (h) | Natural | 6.34 ± 0.32 (def) | Datcha | 8.11 ± 0.21 (b) |
| Credo | 5.81 ± 0.13 (f) | Linoal | 7.01 ± 0.17 (cd) | Cruciata | 8.95 ± 0.25( a) |
| Roma | 5.88 ± 0.11 (ef) | Festival | 7.23 ± 0.08 (c) | Ariane | 9.40 ± 0.23 (a) |
| Mean | 5.47 ± 0.13 | Mean | 6.80 ± 0.13 | Mean | 8.64 ± 0.19 |
| **P variety** | 32.8** | | | | |
| **P group** | 107.6** | | | | |

Notes: [†]Data are expressed as mg CAE $g^{-1}$ DW ± SEM; [**]Significant at ≤ 0.01; Means with different letters in parentheses within the same row differ significantly by Duncan's range test (P ≤ 0.05)

Ariane are fiber varieties, while Merlin, Solal, Kaolin, Natural, Linoal and Festival have intermediate traits. Whole flaxseeds were ground in a mortar and defatted by extracting with hexane (1:10, w/v, twice). Samples were prepared from defatted flours and extracted with 80% ethanol at 70˚C twice.

*Total phenolics and antioxidant activity analyses*

The total phenolic content (TPC) was determined using the Folin-Coicalteau method according to Velioglu *et al.* (2006). 100 µL of the ethanolic extract or caffeic acid standard was combined with 500 µL of Folin-Coicalteau reagent (which had previously been diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. Then, 400 µL of 60 g $L^{-1}$ sodium carbonate solution were added to the mix and the tubes were heated at 45°C for 15 min. The absorbance was measured at 765 nm after sitting for 30 min in the dark. Distilled water was used as a blank, and the caffeic acid standards (50, 100, 200, 400 µg) were prepared using 80% ethanol. The results were expressed as caffeic acid equivalents per g of dry weight (mg CAE $g^{-1}$ DW).

The antioxidant activity of the ethanolic extracts was determined by the Antioxidant Assay Kit (Sigma-Aldrich, Milan, Italy) according to the manufacturer's protocol. In the kit, trolox (a water-soluble vitamin E analog) was present as a standard for antioxidant control. Trolox has been broadly applied in assaying food samples (Re *et al.*, 1999; Huang *et al.*, 2005). The results were expressed as mmol trolox equivalents per g of dry weight (mmol TE $g^{-1}$ DW).

*Statistical analysis*

All analyses were carried out in triplicate. The results were subjected to One-Way ANOVA using SPSS version 16.0 software. Differences between mean values were tested for significance by Duncan's test (P ≤ 0.05). Pearson's correlations between total phenol content and antioxidant activity was also calculated.

## Results and Discussion

The TPC in flax varieties is shown in Table 1. The ANOVA showed that the differences in TPC among varieties and groups are significant at 0.01 level. The TPC was extremely different between the groups with different productive attitude (see lowercase letters in parenthesis by the Duncan's range test). According to Table 1, the TPC varied from 4.64 to 9.40 mg CAE $g^{-1}$ DW of defatted flour. Fiber varieties presented a average value of TPC significantly higher than the oil and intermediate varieties (+3.17 and +1.33 mg CAE $g^{-1}$ DW, respectively). Among the fiber varieties, Blu di Riga showed TPC value similar to the varieties of the intermediate group, while Ariane was the variety possessing the highest TPC (9.40 mg CAE $g^{-1}$ DW). A previous comparative study showed that flax ranks high for TPC among foods and first among plant seeds (Pérez-Jimenez *et al.*, 2010). The same study reported a value of TPC in flaxseed of 15.3 mg $g^{-1}$ DW slightly higher than those shown in Table 1 (in that paper however TPC was obtained by chromatography after hydrolysis of the glycosides and esters). Here, we emphasize that there is considerable variability in TPC among flax varieties. The breeding for a different productive attitude (oil or fiber) has led to different TPC levels.

In Table 2 are shown the antioxidant activities of extracts from defatted flours of eighteen flax varieties. Even here, the ANOVA showed that the differences in antioxidant activity among varieties and groups are significant at 0.01 level. As can be seen in Table 2, antioxidant activity ranged from

Table 2. Antioxidant activity in different flax varieties and groups of different productive attitude.

| Oil group | Antioxidant activity† | Intermediate group | Antioxidant activity† | Fiber group | Antioxidant activity† |
|---|---|---|---|---|---|
| Ita269 | 0.70 ± 0.05 (bc) | Merlin | 0.86 ± 0.02 (a) | Berber | 0.86 ± 0.02 (a) |
| Ecotipo R. | 0.58 ± 0.04 (de) | Solal | 0.70 ± 0.03 (bc) | Belinka | 0.72 ± 0.03 (b) |
| Claudia | 0.68 ± 0.05 (bc) | Kaolin | 0.70 ± 0.03 (bc) | Blu di Riga | 0.65 ± 0.02 (bcd) |
| IT2421 | 0.56 ± 0.03 (e) | Natural | 0.81 ± 0.01 (a) | Datcha | 0.68 ± 0.02 (b) |
| Credo | 0.66 ± 0.03 (bcd) | Linoal | 0.80 ± 0.02 (a) | Cruciata | 0.82 ± 0.02 (a) |
| Roma | 0.70 ± 0.02 (bc) | Festival | 0.63 ± 0.01 (cde) | Ariane | 0.84 ± 0.01 (a) |
| Mean | 0.65 ± 0.02 | Mean | 0.75 ± 0.02 | Mean | 0.76 ± 0.02 |
| **P variety** | 11.3** | | | | |
| **P group** | 10.0** | | | | |

Notes: †Data are expressed as mmol TE $g^{-1}$ DW ± SEM; **Significant at ≤ 0.01; Means with different letters in parentheses within the same row differ significantly by Duncan's range test (P ≤ 0.05)

0.56 to 0.86 mmol TE $g^{-1}$ DW of defatted flour. There was higher variability in antioxidant activity inside every group of productive attitude (evidenced also by the lowercase letters in parenthesis). Nevertheless, antioxidant activity in oilseed varieties resulted to be statistically lower than in fiber and intermediate groups (-0.11 and -0.10 mmol TE $g^{-1}$ DW, respectively). These value of antioxidant activity were much higher than those previously observed in defatted flaxseed extracts using trolox as a standard (Brodowska *et al*., 2014). The Pearson analysis showed that the antioxidant activity was positively correlated to TPC (r = 0.549) at the 0.01 level. A correlation between scavenging capacity and TPC in flaxseed was already put in evidence (Anwar and Przybylski, 2012), thus supporting the notion that phenolics are effective scavengers of free radicals.

Extensive studies has been undertaken to demonstrate antioxidant potential of flaxseed and their phenolics (Kasote, 2013). The novelty of this study lies in the fact that for the first time is shown as the breeding for fiber flax or linseed has altered the content in TPC and antioxidant activity. Our data indicate that flax varieties selected for oil are poorer in TPC compared to fiber varieties (Table 1). This means that if the oil and flour are both required products for industrial use and/or healthy, intermediate varieties, richer in phenolic compounds than linseed would be recommended.

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