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ORIGINAL PAPER

Changes in the Content of Phenolic Compounds in Flaxseed Oil During Development

Wahid Herchi · Faouzi Sakouhi · David Arráez-Román · Antonio Segura-Carretero · Sadok Boukhchina · Habib Kallel · Alberto Fernández-Gutierrez

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Abstract The phenolic fraction of flaxseed oil was quantified during the development of three varieties (H52, O116 and P129). Seed samples were collected at regular intervals from 7 to 56 days after flowering (DAF). During oilseed processing, less polar compounds are co-extracted with oil. The methanolic extracts were obtained by solid phase extraction. Separation of phenolic compounds was conducted by high-performance liquid chromatography-mass spectrometry. The main phenolic compounds detected during maturation were: diphyllin, pinoresinol, matairesinol, secoisolariciresinol, vanillic acid, ferulic acid and vanillin. The highest amount of lignans (6.74 mg of analyte/kg of flaxseed oil) was detected at 7 DAF in P129 variety. The maximum level of phenolic acids (2.57 mg of analyte/kg of flaxseed oil) was reached at 7 DAF in P129 which had also the highest content of simple phenols (1.37 mg of analyte/kg of flaxseed oil) at the same date after flowering. At full maturity, the content of phenolic compounds in three varieties ranged from 0.26 to 0.36 mg of analyte/kg of flaxseed oil. The highest content of total phenolic compounds using the Folin–Ciocalteu method was detected in P129 variety (196.42 mg CAEs/kg of flaxseed oil) at 7 DAF. Results of this study indicate that flaxseed oils contain different amounts of phenolic compounds using different methods.

Keywords Flaxseed oil · Folin–Ciocalteu · Development · Lignans · Phenolic acids · Simple phenols

W. Herchi (✉) · F. Sakouhi · S. Boukhchina · H. Kallel
Laboratoire de Biochimie des Lipides, Département de Biologie, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092 Tunis, Tunisia
e-mail: wahid1bio@yahoo.fr

D. Arráez-Román · A. Segura-Carretero · A. Fernández-Gutierrez
Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, 18071 Granada, Spain

Introduction

Phenolic compounds have a great influence on the stability, sensory and nutritional characteristics of oil products and may prevent deterioration through quenching of radical reactions responsible for lipid oxidation [1]. Many non-traditional vegetable oils have been introduced onto the market relatively recently, and therefore data on their phenolic compounds have not been reported. Such data are of importance for the evaluation of the nutritional and health impact of these oils [2]. Flaxseed oil (*Linum usitatissimum* L.) is an interesting raw material for food applications within the emerging concept of functional foods because it is a valuable source of omega-3 fatty acids and fiber with beneficial health effects [3]. There has been increasing interest in the phenolic compounds, particularly the lignan fraction [3]. Matairesinol is a lignan which is present in flaxseed oil in relatively low levels [4]. Secoisolariciresinol and matairesinol are believed to exert phytoestrogenic effects by acting as precursors of two metabolites: enterodiol and enterolactone [5]. Other lignans have been found in flaxseed oil: pinoresinol and isolariciresinol [6]. Besides lignans, Flaxseed oil was reported to contain some phenylpropanoids, e.g. *p*-coumaric, ferulic, *p*-hydroxybenzoic, and vanillic, in free and/or bound forms [7].

Although interest in phenolic compounds is related primarily to their antioxidant activity, they also show

important biological activity. It has been reported that the intake of several plant products containing phenolic compounds reduces the risk of cardiovascular disease, carcinogenesis, and inflammation [8]. All these studies provide the basis for the present rapidly increasing interest for the use of natural antioxidants as functional food ingredients and as food supplements. The quantification of these bioactive compounds was of importance for their physical properties and their nutritional value.

The phenolic compounds composition has been studied in flaxseed [9] and in flaxseed oil at complete maturity [2, 7]; however the content of the phenolic fraction of flaxseed oil during development has not previously been investigated. The aim of the present study was to carry out a quantitative characterization of the phenolic compounds fraction of three varieties of flaxseed (H52, O116, and P129) cultivated in Tunisia. Such information is valuable for many aspects of vegetable oil production, including the assessment of oil quality and product development.

Materials and Methods

Chemicals and Standards

All chemicals were of analytical reagent grade and used as received. The organic solvents, hexane, methanol, and ACN used in the extraction procedure and as the HPLC mobile phase were purchased from Lab-Scan (Dublin, Ireland). Acetic acid used in the HPLC phase A was purchased from Fluka (Switzerland). Deionized water was obtained from a water purifier system (Millipore, Bedford, MA). All the standard compounds (98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions containing these analytes were prepared in methanol/water (50/50, v/v). All the solvents used in the HPLC system were filtered through 0.20-μm Millipore (Bedford, MA, USA) membrane filters. Folin–Ciocalteu reagent and caffeic acid (90% purity) were purchased from Sigma (St. Louis, MO).

Samples

Three varieties of flaxseeds, H52, O116 and P129, were obtained from the Institut National Recherche Agronomie Tunis (INRAT), Tunisia. The varieties of flaxseed (*L. usitatissimum* L.) were grown in restricted plots (15 m × 3 m) on the Agronomy farm of the INRAT from the middle of November 2006 until the end of June 2007. Each sample was collected at intervals after the dates of flowering. The harvest period extended from 7 days after flowering (DAF) to 56 DAF, the time required for complete maturity.

Lipid Extraction

The total lipids were extracted by the method of Folch et al. [10] modified by Bligh et al. [11]. Seeds (2.5 g) were washed with boiling water for 5 min to denature the phospholipases [12] and then crushed in a mortar with a mixture of $CHCl_3$–MeOH (2:1, v/v). The water of fixation was added and the homogenate was centrifuged at 3,000*g* for 15 min. The lower chloroform phase containing the total lipids was kept and dried in a rotary evaporator at 40 °C.

Total Phenolic Content

The content of total phenols was determined by using the Folin–Ciocalteu colorimetric method, based on the reaction of the reagent with the functional hydroxyl groups of phenols. A one-gram oil sample was weighed, dissolved in 10 mL hexane and transferred to a separatory funnel. Then, 20 mL of a methanol–water mixture (80:10 v/v) was added. After 3 min of shaking the lower methanol–water layer was removed. The extraction was repeated twice and the methanol–water phases were combined. The methanol–water extract was driven to dryness in a rotary evaporator under a vacuum at 40 °C. The dry residue was then dissolved in 1 mL of methanol. The extraction procedure described above was performed three times [13]. An aliquot (0.2 mL) of the methanolic extract was placed in a volumetric flask (10 mL). Diluted Folin–Ciocalteu reagent (0.5 mL) was added. After 3 min, saturated sodium carbonate (1 mL) was added. The flask was filled with water up to 10 mL. After 1 h, the absorbance at 765 nm was measured using a UV–vis spectrophotometer (LKB—Spectronic 20D+) with a 1-cm cell. Total phenolic compounds were determined after preparation of a standard curve. Caffeic acid was used as a standard. Results are expressed as mg of caffeic acid equivalent per kg of oil.

Solid-Phase Extraction (SPE) of Phenolics

A 100-mg sample of DSC-Diol (Supelco, Bellefonte, PS, USA) as a powder was added to a 10-mL test tube and it was conditioned as follows: (1) 100 μl of methanol was added, shaken by vortex for 5 min, centrifuged at 4,500 rpm for 10 min and then the liquid part was discarded. (2) 100 μl of hexane was added, shaken by vortex for 5 min, centrifuged at 4,500 rpm for 10 min and then the liquid part was discarded. Flaxseed oil (1 g) was dissolved in 1.2 mL hexane in a test tube of 10 mL, shaken by vortex for 5 min and the solution was added into the test tube with the conditioned Diol. All was shaken by vortex for 5 min, centrifuged at 4,500 rpm for 10 min and the liquid part was discarded. Then, the DSC-Diol was washed

with 1.2 mL of hexane, shaken by vortex for 5 min, centrifuged at 1,000 rpm for 10 min and the hexane were then discarded in order to remove the non-polar fraction of the flaxseed oil. The sample was recovered by adding 1.2 mL of methanol; the solution was shaken by vortex for 5 min and centrifuged at 1,000 rpm for 10 min. Finally, the methanolic part was removed into an Eppendorf 2-mL tube and evaporated by a rotary evaporator (Concentrator plus, Eppendorf AG, Hamburg, Germany) under reduced pressure at 30 °C. The sample was redissolved in 20 µl of methanol and filtered through a 0.2-µm filter.

HPLC

The separation of the phenolic compounds from flaxseed oil was performed using an Agilent 1200 series Rapid Resolution LC (Agilent Technologies, Palo Alto, CA, USA) was equipped with a vacuum degasser, an autosampler, a diode-array detector (DAD), a binary pump, and a thermostated column department. The standards and samples were separated using a reversed-phase C_{18} analytical column (4.6 × 150 mm, 1.8 µm particle size, Agilent ZORBAX Eclipse plus). The mobile phase A and B consisted of water with 0.5% acetic acid, and ACN. The chromatographic method was as follows: gradient from 5 B to 30% B in 10 min; 30 B to 33% B in 2 min; 33 B to 38% B in 5 min; 38 B to 50% B in 3 min; 50 to 95% in 3 min. The initial conditions were re-established in 2 min and held for 10 min. The total run time, including the conditioning of the column to the initial conditions, was 35 min. The flow rate used was set at 0.80 mL/min throughout the gradient. The effluent from the HPLC column was split using a "T" before being introduced into the mass spectrometer (split ratio 1:3). Thus, the flow which arrived to the ESI-TOF–MS detector was 0.2 mL/min. The column temperature was maintained at 25 °C and the injection volume was 10 µL. The wavelength of the DAD detector was set at 240 and 280 nm and the on-line UV/vis spectra were recorded in the range 190–950 nm. This data are also recorded and available from the files although they have not been employed for the identification of compounds. The amount of phenolic compounds in the extract samples was calculated as mg of analyte per kg oil using external calibration curves [7].

ESI–TOF–MS

ESI–TOF–MS conditions were optimized in order to provide strong mass signals for all the studied phenolic compounds. The HPLC system was coupled to a TOF–MS instrument equipped with an ESI interface operating in negative ion mode. The optimum ESI parameters were as follows: nebulizing gas pressure, 2 bar; drying gas flow, 9 L/min; drying gas temperature, 190 °C. MS was performed using the microTOF (Bruker Daltonik, Bremen, Germany), an orthogonal-accelerated TOF mass spectrometer (oaTOF–MS). Transfer parameters were optimized by direct infusion experiments with Tuning Mix (Agilent Technologies) in the range of 50–800 *m/z* looking for the best conditions regarding sensitivity and resolution. Thus, the endplate offset was −500 V; capillary voltage 4,500 V, the trigger time was set to 50 µs, 49 µs for set transfer time and 1 µs pre-puls storage time, corresponding to a mass range of 50–800 *m/z*. Spectra were acquired by summarizing 20,000 single spectra, defining the spectral rate to 1 Hz. The accurate mass data of the molecular ions were processed through the software Data Analysis 3.4 (Bruker Daltonik), which provided a list of possible elemental formulas by using the Generate Molecular Formula™ Editor. The Generate Formula™ Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double bonds equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (sigma value) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note #008, Molecular formula determination under automation). The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 5 ppm [14]. We also have to say that even with very high mass accuracy (<1 ppm) many chemically possible formulas are obtained depending on the mass regions considered. So, high mass accuracy (<1 ppm) alone is not enough to exclude enough candidates with complex elemental compositions. The use of isotopic abundance patterns as a single further constraint removes >95% of false candidates. This orthogonal filter can condense several thousand candidates down to only a small number of molecular formulas. During the development of the HPLC method, external instrument calibration was performed using a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, Illinois, USA) directly connected to the interface, passing a solution of sodium formate cluster containing 5 mM sodium hydroxide in water/isopropanol 1/1 (v/v), with 0.2% (v/v) of formic acid at the end of each run. Using this method an exact calibration curve based on numerous cluster masses each differing by 68 Da ($NaCHO_2$) was obtained. Due to the compensation of temperature drift in the MicroTOF, this external calibration provided accurate mass values (better than 5 ppm) for a complete run without the need for a dual sprayer setup for internal mass calibration. These calibrations were performed in quadratic + high precision calibration (HPC) regression mode.

Statistics

Statistical analysis was performed by using the Proc ANOVA in SAS (software version 8). Results are

presented as the means ± from three triplicates of each experiment. The results are reported as mg analyte/kg of flaxseed oil.

Results and Discussion

Total Phenolic Content During Flaxseed Development

The total phenolic content of flaxseed oils during development is shown in Fig. 1. This result was determined using the Folin–Ciocalteu reagent method. During the early stages of flaxseed development, total phenolic content in flaxseed oil was higher: 140.71–107.14 mg CAEs/kg of flaxseed oil, and then decreased during maturation to achieve the harvest values: 14.23–16.64 mg CAEs/kg of flaxseed oil according to varieties. At 7 DAF, P129 had a significantly ($p \leq 0.05$) higher level of total phenolic content than H52 and O116. Flaxseed in first stages of development had the highest levels of phenolic compounds. Siger et al. [2] reported that phenolic compounds are very important for the oxidative stability of the polyunsaturated fatty acids of vegetables oils. The last two decades brought evidence that phenolic compounds can play an important role in the control of many processes including seed development, growth, flowering and may protect plants from different stresses [15]. Doherty et al. [16] reported that genetic and environmental factors affect the type and quantity of phenolic compounds in plant material. The content of phenolic compound in flaxseed oil investigated in this study was in accordance with previously reported results [2].

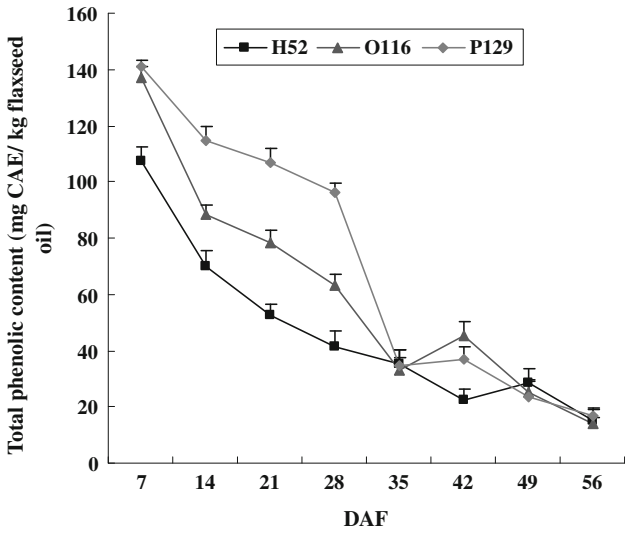

Fig. 1 Changes in total phenolic content in flaxseed oil during development determined by the Folin–Ciocalteu method

Changes in Phenolic Compounds Content during Flaxseed Development

The application of a diol SPE column allowed for the extraction of polar compounds from a non polar matrix, such as oil. The HPLC–TOF–MS chromatogram (base peak 50–800 *m*/*z*) for the analysis of the Diol-SPE extract of flaxseed oil is presented by Herchi et al. [7]. Figure 2 shows the chemical structures of the principal phenolic compounds. The content of phenolic compounds in the oil fraction, analyzed by high performance liquid chromatography-mass spectrometry, during flaxseed development is shown in Table 1. The highest level of phenolic compounds in flaxseed oil was determined during the early stages of development (10.68–3.84 mg of analyte/kg of flaxseed oil). The variety H52 had a significantly ($p \leq 0.05$) lower level of phenolic compound content than O116 and P129. At full maturity (56 DAF), the harvest values were found in the range of 0.26–0.36 mg of analyte/kg of flaxseed oil according to varieties. The most rapid change in the phenolic compounds content of flaxseed oil is complete at 7 DAF. This rapid decline of phenolic compounds in developing seed may be attributed to a change of the chemical composition of the lipid fraction affecting consequently solubility of these compounds [17]. Mhamdi et al. [18] claimed that the level of phenolic compounds using high-performance liquid chromatography decreased during the early stages of seed development. Lercker et al. [19] reported that plant oils contain small amounts of phenolic compounds. The amount of phenols determined with the Folin–Ciocalteu method was higher than those determined by LC–MS method. Probably the main cause of the difference obtained by the two methods is the fact that the Folin–Ciocalteu method does not provide a specific assay for phenolic compounds as it reacts positively with many easily oxidizable non-phenolic compounds [20]. Folin–Ciocalteu reagent measures the ability of any mixture to reduce phosphomolybdic and phosphotungstic acids to a blue complex. The presence of ascorbic acid or other very easily oxidized substances, not considered as phenolic compounds, may also result in the formation of a blue color with Folin–Ciocalteu reagent, causing an overestimation of total phenolic content [21]. It has been argued that Folin reagents may be inappropriate for plant extracts with high levels of other easily oxidizable substances [22].

Changes in Lignan Content During Flaxseed Maturation

The lignans present in the three varieties were: diphyllin, secoisolariciresinol, matairesinol, and pinoresinol. Table 2 represents the content of the lignan fraction in flaxseed oils at different stages of maturity. It can be seen that during

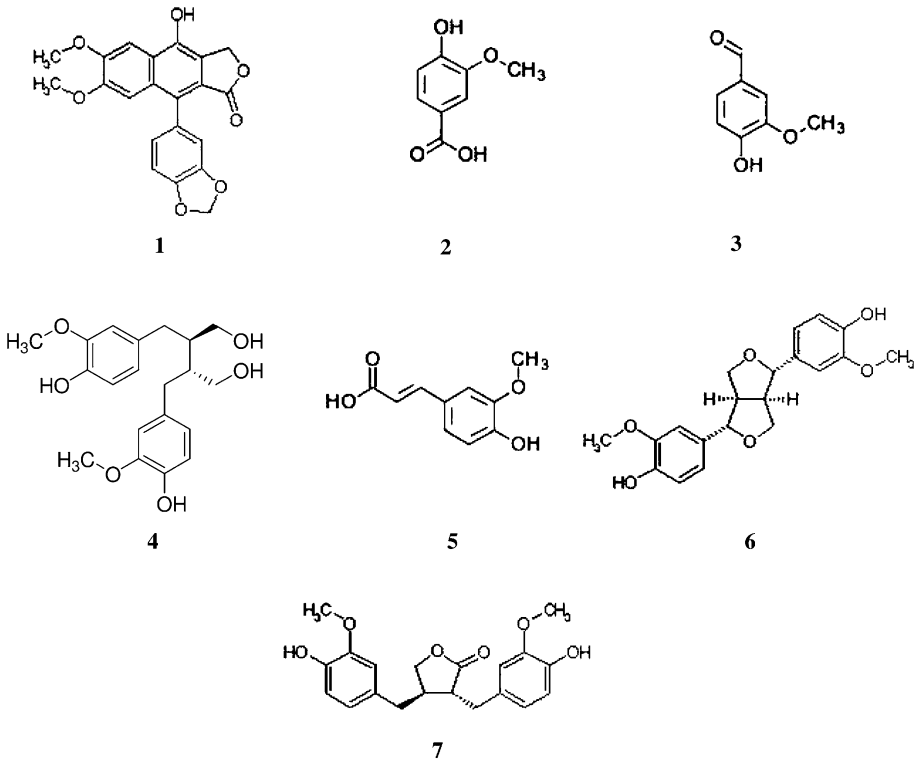

Fig. 2 Structures of the characterized compounds in the phenolic fraction of flaxseed oil: (1) diphyllin, (2) vanillic acid, (3) vanillin, (4) secoisolariciresinol, (5) ferulic acid, (6) pinoresinol, (7) matairesinol

Table 1 Phenolic compounds content (mg of analyte/kg of flaxseed oil) during development determined by an HPLC–MS method

DAF	H52	O116	P129
7	3.84 ± 0.22[a]	5.68 ± 0.19[b]	10.68 ± 0.39[c]
14	0.60 ± 0.07[a]	1.73 ± 0.10[b]	1.82 ± 0.11[b]
21	0.14 ± 0.06[a]	0.65 ± 0.08[b]	1.50 ± 0.09[c]
28	0.16 ± 0.07[a]	0.35 ± 0.07[b]	0.47 ± 0.07[c]
35	0.17 ± 0.05[a]	0.27 ± 0.07[b]	0.23 ± 0.06[b]
42	0.23 ± 0.06[a]	0.19 ± 0.05[b]	0.28 ± 0.05[b]
49	0.20 ± 0.07[a]	0.24 ± 0.06[a]	0.14 ± 0.05[b]
56	0.30 ± 0.07[a]	0.36 ± 0.05[a]	0.26 ± 0.05[b]

Each value is the mean ± standard deviation (SD) of triplicate analyses. Means with different letters (a–c) within a row are significantly different at $p \leq 0.05$

flaxseed development, the content of lignan decreased more progressively from 2.51, 4.04 and 6.74 mg of analyte/kg of flaxseed oil to 0.11, 0.11 and 0.10 mg of analyte/kg of flaxseed oil in H52, O116 and P129 respectively. A comparison between the three varieties showed that there was a significant difference ($p \leq 0.05$) in their lignan contents. In flaxseed oil, pinoresinol was detected in higher level while diphyllin, matairesinol and secoisolariciresinol were found in lower levels. The maximum level of pinoresinol (6.18 mg of analyte/kg of flaxseed oil), matairesinol (0.23 mg of analyte/kg of flaxseed oil), secoisolariciresinol (0.23 mg of analyte/kg of flaxseed oil) and diphyllin (0.22 mg of analyte/kg of flaxseed oil) were detected at 7 DAF in P129 and O116 varieties. All these lignans have the same accumulation profile. The content of pinoresinol decreased during flaxseed development to a constant value of 0.01 mg of analyte/kg of flaxseed oil. Considering the fact that "Pinoresinol Synthase" (PS) catalyzes the coupling of two molecules of *E*-coniferyl alcohol with the help of an auxiliary dirigent protein to give pinoresinol [23], we suggested that this enzyme was low and inactive during flaxseed development. Qualitative composition of lignan fraction in flaxseed oil was similar to those reported in the literature [4].

Changes in Phenolic Acid Content During Flaxseed Maturation

Phenolic acids content in flaxseed oil decreased during development (Table 3). The maximum level of phenolic acids was detected in P129 variety at 7 DAF (2.57 mg of analyte/kg of flaxseed oil). At full maturity: H52, O116 and P129 have a phenolic acid content of 0.04, 0.04 and 0.03 mg of analyte/kg of flaxseed oil, respectively. Manach et al. [24] reported that phenolic acids generally decrease during the course of maturation. Indeed, phenolic acids are involved in the response of plants to different types of stress [25]. Ferulic acid and vanillic acid were the main phenolic acids identified in flaxseed oil. Figure 3 shows TOF–MS spectra of ferulic acid identified in flaxseed oil.

Table 2 Lignans content (mg of analyte/kg of flaxseed oil) during development determined by HPLC–MS method

DAF	Diphyllin	Secoisolariciresinol	Pinoresinol	Matairesinol	Σ Lignans	Oil content (%)[a]
H52						
7	0.08 ± 0.01[b]	0.09 ± 0.01[b]	2.24 ± 0.08[b]	0.10 ± 0.01[b]	2.51 ± 0.11[b]	9.35 ± 0.72
14	0.02 ± 0.01[b]	0.03 ± 0.01[b]	0.29 ± 0.01[b]	0.03 ± 0.01[b]	0.37 ± 0.04[b]	22.02 ± 0.55
21	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.03 ± 0.01[b]	0.02 ± 0.01[b]	0.09 ± 0.04[b]	30.18 ± 0.88
28	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.01 ± 0.002[b]	0.02 ± 0.01[b]	0.07 ± 0.03[b]	34.48 ± 0.73
35	0.03 ± 0.01[b]	0.02 ± 0.01[b]	0.01 ± 0.001[b]	0.02 ± 0.01[b]	0.08 ± 0.03[b]	38.38 ± 0.84
42	0.04 ± 0.01[b]	0.03 ± 0.01[b]	0.02 ± 0.01[b]	0.03 ± 0.01[b]	0.12 ± 0.04[b]	42.65 ± 1.75
49	0.04 ± 0.01[b]	0.03 ± 0.01[b]	0.01 ± 0.002[b]	0.03 ± 0.01[b]	0.11 ± 0.05[b]	37.14 ± 0.67
56	0.04 ± 0.01[b]	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.03 ± 0.01[b]	0.11 ± 0.04[b]	35.03 ± 1.11
O116						
7	0.22 ± 0.01[c]	0.23 ± 0.02[c]	3.36 ± 0.05[c]	0.23 ± 0.01[c]	4.04 ± 0.09[c]	8.04 ± 0.49
14	0.03 ± 0.01[b]	0.04 ± 0.01[c]	0.77 ± 0.03[c]	0.04 ± 0.01[c]	0.88 ± 0.05[c]	16.63 ± 0.33
21	0.03 ± 0.01[c]	0.03 ± 0.01[c]	0.32 ± 0.02[c]	0.03 ± 0.01[b]	0.41 ± 0.05[c]	21.87 ± 0.91
28	0.03 ± 0.01[c]	0.03 ± 0.01[c]	0.12 ± 0.01[c]	0.03 ± 0.01[b]	0.21 ± 0.04[c]	31.42 ± 0.81
35	0.03 ± 0.01[c]	0.04 ± 0.01[c]	0.02 ± 0.01[c]	0.03 ± 0.01[c]	0.12 ± 0.04[c]	34.42 ± 0.92
42	0.03 ± 0.01[c]	0.03 ± 0.01[b]	0.01 ± 0.001[c]	0.03 ± 0.01[b]	0.10 ± 0.03[c]	38.75 ± 1.33
49	0.03 ± 0.01[c]	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.03 ± 0.01[b]	0.10 ± 0.03[b]	43.70 ± 1.24
56	0.04 ± 0.01[b]	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.03 ± 0.01[b]	0.11 ± 0.03[b]	36.61 ± 1.14
P129						
7	0.16 ± 0.01[d]	0.17 ± 0.01[d]	6.18 ± 0.12[d]	0.23 ± 0.02[c]	6.74 ± 0.16[d]	8.10 ± 0.48
14	0.06 ± 0.01[c]	0.06 ± 0.01[c]	1.10 ± 0.04[d]	0.07 ± 0.01[d]	1.29 ± 0.07[d]	20.71 ± 1.02
21	0.04 ± 0.01[d]	0.04 ± 0.01[d]	1.04 ± 0.03[d]	0.07 ± 0.01[c]	1.19 ± 0.06[d]	26.08 ± 0.52
28	0.03 ± 0.01[c]	0.04 ± 0.01[d]	0.12 ± 0.01[c]	0.05 ± 0.01[c]	0.24 ± 0.04[d]	31.48 ± 0.69
35	0.03 ± 0.01[c]	0.03 ± 0.01[b]	0.01 ± 0.002[c]	0.03 ± 0.01[c]	0.10 ± 0.03[c]	38.44 ± 1.02
42	0.05 ± 0.01[b]	0.04 ± 0.01[c]	0.01 ± 0.001[c]	0.04 ± 0.01[c]	0.14 ± 0.03[b]	46.58 ± 1.56
49	0.02 ± 0.01[d]	0.02 ± 0.01[c]	0.01 ± 0.001[b]	0.02 ± 0.01[c]	0.07 ± 0.03[c]	36.79 ± 0.93
56	0.03 ± 0.01[c]	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.03 ± 0.01[b]	0.10 ± 0.03[b]	34.80 ± 0.98

[a] according to Herchi et al. [17]

Each value is the mean (standard deviation (SD) of triplicate analyses. Means with different letters (b–d) within a row are significantly different at $p \leq 0.05$

The highest level of ferulic acid was determined in P129 at 7 DAF (2.15 mg of analyte/kg of flaxseed oil). The three varieties were characterized by a lower level of vanillic acid. It was in the range of 0.42–0.02 mg of analyte/kg of flaxseed oil during development. This result is in agreement with those obtained by Siger et al. [2]. These two phenolic acids have the same pathway. The decrease of ferulic acid and vanillic acid during development may be explained by a low activity of O-Methyl transferase [26]. Siger et al. [2] reported that the highest level of phenolic acids was determined in rapeseed oil (0.26 mg/kg oil) in comparison with flaxseed oil which had 0.005 mg/kg oil.

Changes in Simple Phenols Content During Flaxseed Maturation

Simple phenols were found to comprise less than 15% for the phenolic compounds content in flaxseed oil at the early stages of development. Vanillin is the main simple phenol detected in flaxseed oil. The accumulation of simple phenols followed a similar trend in all varieties and the highest level (0.46, 0.57 and 1.37 mg of analyte/kg of flaxseed oil) was observed at 7 DAF in H52, O116 and P129 respectively (Table 4). At complete maturity, vanillin represents 0.15, 0.21 and 0.13 mg of analyte/kg of flaxseed oil for H52, O116 and P129, respectively. In the three varieties, the content of vanillin decreased during seed development. Actually, this result may be explained by a higher activity of P_{450} Monooxygenase [27] which leads to a decrease in the formation of simple phenols.

Conclusion

The results of our investigation provide useful information on the content of phenolic compounds in flaxseed oil

Table 3 Phenolic acid content (mg of analyte/kg of flaxseed oil) during development determined by HPLC–MS method

DAF	Vanillic acid	Ferulic acid	Σ phenolic acid
H52			
7	0.14 ± 0.02[a]	0.73 ± 0.05[a]	0.87 ± 0.07[a]
14	0.02 ± 0.01[a]	0.17 ± 0.01[a]	0.19 ± 0.02[a]
21	0.01 ± 0.001[a]	0.01 ± 0.001[a]	0.02 ± 0.01[a]
28	0.02 ± 0.001[a]	0.01 ± 0.001[a]	0.03 ± 0.01[a]
35	0.03 ± 0.01[a]	0.01 ± 0.002[a]	0.04 ± 0.01[a]
42	0.02 ± 0.01[a]	0.02 ± 0.004[a]	0.04 ± 0.01[a]
49	0.02 ± 0.01[a]	0.01 ± 0.002[a]	0.03 ± 0.01[a]
56	0.03 ± 0.01[a]	0.01 ± 0.004[a]	0.04 ± 0.01[a]
O116			
7	0.16 ± 0.01[a]	0.91 ± 0.05[b]	1.07 ± 0.06[b]
14	0.04 ± 0.01[b]	0.66 ± 0.03[b]	0.70 ± 0.04[b]
21	0.03 ± 0.01[b]	0.14 ± 0.01[b]	0.17 ± 0.02[b]
28	0.04 ± 0.01[b]	0.02 ± 0.01[a]	0.06 ± 0.02[b]
35	0.03 ± 0.01[a]	0.04 ± 0.01[b]	0.07 ± 0.02[b]
42	0.02 ± 0.01[a]	0.01 ± 0.002[b]	0.03 ± 0.01[a]
49	0.03 ± 0.01[a]	0.02 ± 0.01[b]	0.05 ± 0.02[b]
56	0.03 ± 0.01[b]	0.01 ± 0.001[b]	0.04 ± 0.01[a]
P129			
7	0.42 ± 0.04[b]	2.15 ± 0.10[c]	2.57 ± 0.14[c]
14	0.07 ± 0.01[b]	0.16 ± 0.01[a]	0.23 ± 0.02[a]
21	0.03 ± 0.01[b]	0.06 ± 0.01[c]	0.09 ± 0.02[c]
28	0.03 ± 0.01[b]	0.08 ± 0.01[b]	0.11 ± 0.02[c]
35	0.02 ± 0.01[a]	0.02 ± 0.01[c]	0.04 ± 0.02[a]
42	0.03 ± 0.01[a]	0.01 ± 0.002[b]	0.04 ± 0.01[a]
49	0.02 ± 0.01[a]	0.01 ± 0.001[c]	0.03 ± 0.01[c]
56	0.02 ± 0.01[a]	0.01 ± 0.001[b]	0.03 ± 0.01[b]

Each value is the mean ± standard deviation (SD) of triplicate analyses. Means with different letters (a–c) within a row are significantly different at $p \leq 0.05$

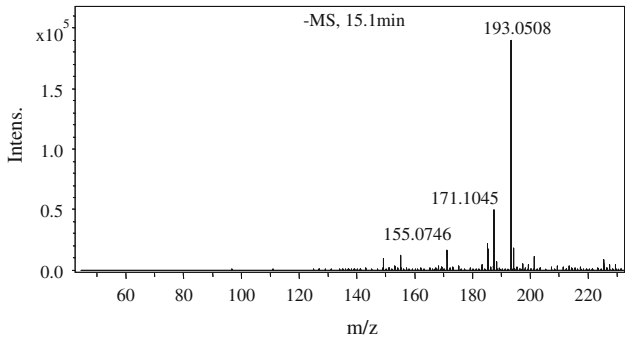

Fig. 3 ToF–MS (50–800 *m/z*) spectra of ferulic acid identified in flaxseed oil

during development. The contents of phenols determined with the Folin–Ciocalteu method were higher than those determined by the LC–MS method. The lignan fraction was

Table 4 Simple phenols content (mg of analyte/kg of flaxseed oil) during development determined by HPLC–MS method

DAF	H52	O116	P129
7	0.46 ± 0.04[a]	0.57 ± 0.04[a]	1.37 ± 0.09[b]
14	0.04 ± 0.01[a]	0.15 ± 0.01[b]	0.30 ± 0.02[c]
21	0.03 ± 0.01[a]	0.07 ± 0.01[a]	0.22 ± 0.01[b]
28	0.06 ± 0.01[a]	0.08 ± 0.01[b]	0.12 ± 0.01[c]
35	0.05 ± 0.01[a]	0.08 ± 0.01[b]	0.09 ± 0.01[b]
42	0.07 ± 0.01[a]	0.06 ± 0.01[a]	0.10 ± 0.01[b]
49	0.06 ± 0.01[a]	0.09 ± 0.01[a]	0.04 ± 0.01[b]
56	0.15 ± 0.02[a]	0.21 ± 0.01[b]	0.13 ± 0.01[a]

Each value is the mean ± standard deviation (SD) of triplicate analyses. Means with different letters (a–c) within a row are significantly different at $p \leq 0.05$

the major phenolic compounds fraction in flaxseed oil during development. Phenolic acids and simple phenols were found in small concentrations. The most rapid change in phenolic compounds of flaxseed oil is complete at 7 DAF. The content of phenolic compounds was influenced by the ripening stage of seed.

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