Chemical characterisation of old cabbage (*Brassica oleracea* L. var. *acephala*) seed oil by liquid chromatography and different spectroscopic detection systems

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**ABSTRACT**

We report an extensive chemical characterisation of fatty acids, triacylglycerols, tocopherols, carotenoids and polyphenols contained in the oil extracted from old cabbage (*Brassica oleracea* L. var. *acephala*) by cold-pressing of the seeds. Analyses were performed by GC-FID combined with mass spectrometry, HPLC with photodiode array, fluorescence and mass spectrometry detection. The 94% of the total fatty acids were unsaturated, represented by erucic acid (more than 50%) followed by linoleic, linolenic and oleic acids accounting for approximately 10% each. The most abundant triacylglycerols (>13%) were represented by erucic–gadolenic–linoleic, erucic–eruci–linoleic and erucic–erucic–oleic. Among tocopherols, γ-tocopherol accounted for over 70% of the total content. Thirteen carotenoids and 11 polyphenols were identified and measured. In particular, the total content in carotenoids was 10.9 ppm and all-E-lutein was the main component (7.7 ppm); among polyphenols, six hydroxycinnamic acids and five flavonoids, were identified by combining information from retention times, PDA and MS data.

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**KEYWORDS**

*Brassica oleracea*; fatty acids; triacylglycerols; carotenoids; tocopherols; polyphenols; HPLC-PDA-ESI-MS

**ARTICLE HISTORY**

Received 15 June 2015
Accepted 14 November 2015

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Supplemental data for this article can be accessed at [http://dx.doi.org/10.1080/14786419.2015.1131982](http://dx.doi.org/10.1080/14786419.2015.1131982).

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1. Introduction

Recently, there has been a worldwide interest in the characterisation of yet underexploited high-quality oils. The remarkably high content of these oils in nutritionally, medicinally or industrially desirable fatty acids (FAs) make them highly valuable for various purposes (Tranchida et al. 2007; Dugo et al. 2011; Fanali et al. 2011; Mondello, Beccaria, et al. 2011; Ragonese et al. 2014; Dugo et al. 2015).

Despite the wide range of vegetable oils sources, the world consumption is dominated by palm, soybean, rapeseed and sunflower oils. Vegetable oils with a high relative amount of minor lipid components are of great importance for human health (Nasri et al. 2012) and their composition is important from the nutritional point of view. In particular, ω-3 FAs play a fundamental role in physiology, especially during foetal and infant growth and they are also important for the prevention of cardiovascular diseases as they are antithrombotic, anti-inflammatory, antiarrythmic and promote plaque stabilisation (Galli & Marangoni 2006).

Cabbage (Brassica oleracea L.) is one of the most consumed fresh vegetables all over the world. Cabbage belongs to the Cruciferae family, which includes cauliflower, kale, broccoli and brussels sprouts. It originates from Western Europe and its different varieties are characterised by variable sizes, shapes and colours of both leaves and heads (Nieuwhof 1969). Cabbage was and is still currently used in the treatment of different diseases such as headaches, gout, diarrhoea and peptic ulcers. Several epidemiological studies indicated an inverse association between consumption of vegetables from B. oleracea and a reduced risk of cancer and cardiovascular diseases. The biological action is assumed to be provided by its content in compounds such as carotenes, tocopherols and glucosinolates (Brooks et al. 2001; Liang et al. 2013). It was also demonstrated that the most bioactive compounds in cabbage are phenolic compounds such as flavonoids, isoflavone, flavones, anthocyanin and catechins (McDougall et al. 2007).

A particular variety of cabbage, cultivated in the Sicilian village of Rosolini (Italy), is known as ‘old cabbage’, a particular variety named after its long life span up to 7 years mainly due to the fact that it is cultivated on the border of the stocking place of organic manure (Figure S1). The old cabbage belongs to the ‘acephala’ variety and is able to survive also in dry soils. It is cultivated starting from self-production seeds and can cover up to one square metre of surface. To date, a detailed chemical characterisation of the oil extracted from the seeds of B. oleracea var. acephala grown in Rosolini is not available. In this study, we evaluate the triacylglycerol (TAG), carotenoid, tocopherol and polyphenol contents of this oil. FAs were analysed as methyl ester derivatives (FAMES) by gas chromatography (GC) combined with flame ionisation detection (FID) and mass spectrometry (MS). TAGs were analysed by non-aqueous reversed-phase high-performance liquid chromatography (NARP-HPLC) combined with atmospheric pressure chemical ionisation mass spectrometry (APCI-MS). Tocopherols were separated by normal-phase liquid chromatography (NP-HPLC) coupled to a fluorescence detection (RF). Finally, the polyphenolic fingerprint of the major polyphenols was achieved by RP-HPLC with photodiode array (PDA) and electrospray (ESI) MS detection.

2. Materials and methods

2.1. Chemicals and reagents

Reagent grade N,N-dimethyl-formamide (DMF), n-hexane (Hex), acetone, ethyl acetate, ethyl ether and LC-MS grade methanol (MeOH), water (H₂O), ethanol (EtOH), methyl tert-butyl
ether (MTBE), acetonitrile (ACN), isopropanol (IPA) were all obtained from Sigma-Aldrich/Supelco (Milan, Italy).

α-tocopherol, γ-tocopherol and δ-tocopherol were provided by Sigma-Aldrich/Supelco (Milan, Italy). Carotenoid standards for HPLC analysis (lutein, β-carotene) were purchased from extrasynthese (Genay, France).

### 2.2. Seed material

Mature pods of *B. oleracea* L. var. *acephala* species were collected in January 2015 from Rosolini located in the South-eastern part of the Sicilian region. The seeds were collected and then hand-picked to eliminate damaged ones. The selected seeds were sun-dried for three days, carefully cleaned, weighed (5 g) and ground to powder. Press-extraction was carried out using screwless cold presses; the oil thus obtained (450 mg) was subsequently treated according to the class of analytes to investigate.

#### 2.2.1. Analysis of the fatty acid content

The seed oil was dissolved in 1 mL of Hex and 1 mL of a 2 N solution of NaOH in MeOH was added, shaken for 15 s and left to stratify (about 5 min). The supernatant representing the hexane layer was then analysed by GC-MS and GC-FID system. (See supplementary materials).

#### 2.2.2. Analysis of the triacylglycerol content

11.4 mg of the seed oil was weighed and dissolved in 1 mL of acetone; afterwards the sample was filtered through a 0.45-μm Acrodisc nylon membrane filter (Pall Life Sciences, Ann Arbor, MI, USA) prior to LC-MS analyses. (See supplementary materials).

#### 2.2.3. Analysis of the tocopherol content

10 mg of the seed oil sample was carefully weighed and dissolved in 1 mL of Hex. (See supplementary materials).

#### 2.2.4. Analysis of the carotenoid content

See supplementary materials

#### 2.2.5. Analysis of the polyphenolic content

See supplementary materials

### 3. Results and discussion

#### 3.1. FAMEs composition by GC-FID and GC-MS

The fatty acid profile of the old cabbage oil was determined by GC-FID and GC-MS analysis after preparation of FAMEs as previously described (Ragonese, Tranchida, Dugo, et al. 2009; Ragonese, Tranchida, Sciarrone, et al. 2009; Fanali et al. 2011; Tuttolomondo et al. 2015). FAMEs were identified by comparing their mass spectra and their retention indices with those listed in a dedicated database (Mondello 2011). Individual FA quantities were determined by GC-FID analysis of FAMEs using theoretical relative response factors (TRF) and expressed as mass fraction of the total FAME content (%) applying the formula:
where \( FA_{x(TRF)} \) refers to the peak area of the FAME considered and \( FA_{TOT(TRF)} \) refers to the total peak area of the FAMEs contained in the sample, both corrected by using TRF. Figure 1 shows the GC-FID chromatogram of the FAMEs identified whose names along with the relative percentage and standard deviation are reported in Table S1. These results show that approximately 94.4% of total FAs consisted of unsaturated FAs (UFA), approximately three quarters of which were monounsaturated (MUFA), while the rest was represented by polyunsaturated FAs (PUFA). The PUFA fraction consisted primarily of two essential FAs, linoleic (L) and linolenic (Ln) acid, accounting for 11.4% and 10.2% of total FA contents. Among MUFAs, erucic acid (Er) accounted for being the about 50% of the whole FA content, and this finding is in agreement with previous reports (Mahler and Auld 1989; Mackenzie et al. 1997).

3.2. Triacylglycerol analysis by NARP-HPLC-APCI-MS

TAGs were identified by positive-ion APCI-MS: protonated molecular ions \([M+H]^+\) were used for molecular weight assignments and fragment ions \([M+H-RiCOOH]^+\) for the identification of the FAs on the glycerol chain (Holcapek et al. 2005; Fanali et al. 2011; Dugo et al. 2012; Beccaria et al. 2014). The NARP-HPLC-APCI-MS total ion current (TIC) chromatogram of the oil is shown in Figure 2, whereas Table S2 reports the retention times, corrected average Area %, SD and CV% (three replicates) of the identified TAGs. Since NARP-HPLC is not capable of resolving TAGs according to the regioisomeric position, the conventional notation of TAGs used refers to the initial of FA trivial names arranged according to their decreasing molecular weights (Dugo et al. 2012; Beccaria et al. 2014). Figure 2 and Table S2 show that some TAGs partially or completely co-eluted the incomplete separation of (e.g. PN = 46, GPLn + SOLn; PN = 52, ErGL + NrGLn). These results may be explained considering that TAG retention

![Figure 1. 40–70 min enlargement of the GC-FID chromatogram of the FAMEs identified. The experimental conditions are reported in the supplementary material.](image-url)
times in NARP-HPLC increase with increasing partition number (PN) defined as the total carbon number (CN) of all acyl chains minus two times the number of double bonds (DBs), PN = CN−2DB. As a consequence, TAGs with the same PN are, usually, very difficult to resolve. In addition, the retention behaviour of TAGs with the same CN is strongly influenced by the FA composition of the individual TAG, mainly by the unsaturation degree and acyl chain length. The triacylglycerol profile of the old cabbage oil sample determined by NARP-HPLC is well-correlated with the FA composition measured by GC-FID and GC-MS. NARP-HPLC identified TAGs containing 12 different FAs (P: Palmitic acid (C16:0); S: Stearic acid (C18:0); O: Oleic acid (C18:1); L: Linoleic acid (C18:2); Ln: Linolenic acid (C18:3); A: Arachidic acid (C20:0); G: Gadoleic acid (C20:1); B: Behenic acid (C22:0); Es: Eicosadienoic acid (C20:2); Er: Erucic acid (C22:1); Li: Lignoceric acid (C24:0); Nr: Nervonic acid (C24:1)). The predominant components accounting for >40% of total composition were erucyl–gadoleil linolein (ErGL), dierucyl linolein (ErErL) and dierucyl olein (ErErO). Most of the TAGs in the old cabbage oil (about 50%) contained at least one residue of erucic acid. Such a high content in erucic acid raises serious concerns on the use of this variety to produce edible oil. TAG % contents were calculated as the ratio of the TIC area of the TAG and the sum of TIC areas of all identified TAGs, multiplied by 100. The NARP-HPLC-APCI-MS TAG areas were corrected by applying the relative response factors published by Holcapek and co-workers (2005).
3.3. Tocopherol analysis by NP-HPLC-RF

Tocopherols are a class of lipid-soluble compounds known as vitamin E which are essential to human health, are also strong antioxidants that protect oils from oxidation. Among the four homologues of tocopherols, α-tocopherol is considered the strongest antioxidant whereas γ-tocopherol is the strongest inflammatory agent (Sen et al. 2007; Zingg, 2007). α-tocopherol is the most abundant tocopherol in olive oil, whereas γ- and δ-tocopherols are found mainly in seed oils like soybean and sunflower oil (Fanali et al. 2011). In this contribution, the tocopherol content of the old cabbage sample was determined by NP-LC coupled to fluorimetric detection. Tocopherols were identified by comparing their retention times with the ones of reference materials. LOD and LOQ values were as follows: α-tocopherol, 0.8 and 1.4 mg/kg; γ-tocopherol, 0.1 mg/kg both; δ-tocopherol, 0.4 and 0.6 mg/kg. γ-tocopherol accounted for more than 66% of the entire tocopherol content in the extracted oil, followed by α-tocopherol (roughly 30%) and δ-tocopherol (Table 1). The total and individual tocopherol contents differed from those previously reported for common vegetable seed oils, e.g. soybean and sunflower characterised by 79% of δ-tocopherol and 84% of α-tocopherol, respectively (Grilo et al., 2014). The variability in the concentrations of α- and γ-tocopherol in vegetable oils can be related to the conditions of cultivation, storage and processing of the seeds or grains which must be properly and constantly evaluated and monitored for the preservation of the organoleptic and nutritional quality of the final product.

3.4. Carotenoid analysis by RP-HPLC-PDA/APCI-MS and UV–vis Spectrophotometry

The present report describes for the first time the carotenoid composition of old cabbage seed oil. In total, 13 different carotenoids were differentiated by utilising their retention time values, UV–vis and MS spectral data, and comparison with available reference materials. The UV–vis and APCI-MS identification parameters, along with quantitative data of the carotenoid content in the extracted oil, are reported in Table 2. The total content in carotenoids was 10.9 ppm and all-E-lutein was the main component (7.7 ppm). Various cis isomers of lutein were also detected. Taking into consideration the reference values suggested by Britton and Khachik (2009), that classified the quantity of a carotenoid as very high when above 20 μg g−1 and high when between 5 and 20 μg g−1, the all-E-lutein content measured in this work

| No | Compound          | UV–vis spectrum | [M + H]+ APCI (+) | Fragments APCI (+) | μg/kg       |
|----|------------------|----------------|-------------------|--------------------|------------|
| 1  | Violaxanthin     | 417, 439, 470  | 601               | 583, 565           | 351.5 ± 2.6|
| 2  | Apocarotenoid of lutein | 421 | n.d.         | 583, 565           | 72.3 ± 0.5 |
| 3  | 8-R-neochrome    | 400, 422, 449  | 601               | 583, 565           | 215.2 ± 0.9|
| 4  | Lutein-5,6-epoxide| 416, 438, 469  | 585               | 587, 549           | 116.5 ± 1.1|
| 5  | Mutatoxanthin    | 405, 429, 454  | 585               | 587, 549           | 74.2 ± 2.3 |
| 6  | 15-Z-lutein      | 332, 416, 440, 466 | 569     | 551, 533, 476     | 637.6 ± 3.9|
| 7  | 13/13’Z-lutein   | 332, 417, 439, 466 | 569     | 551, 533, 476     | 774.3 ± 62 |
| 8  | E-lutein         | 420, 445, 473  | 569               | 551, 533, 476     | 98.7 ± 0.8 |
| 9  | Zeaxanthin       | 426, 451, 476  | 569               | 551, 533, 476     | 526.9 ± 3.4|
| 10 | 9/9’-Z-lutein    | 330, 416, 440, 468 | 569     | 551, 533, 476     | 346.0 ± 2.8|
| 11 | 9/9’-Z-lutein    | 331, 417, 441, 468 | 569     | 551, 533, 476     | 346.0 ± 2.8|

Notes:

n.d.: not detected.

All compounds expressed quantitatively as All-E-lutein.
can be defined as relatively high. Interestingly, neither chlorophylls/chlorophyll derivatives nor xanthophyll esters were detected in the studied samples. An apo-carotenoid probably formed from an oxidative degradation of lutein was detected as a minor component, and due to its low concentration all spectra recorded were not clear, thus it was not fully characterised. The provitamin A, all-\(E\)-\(\beta\)-carotene and its 9-\(Z\)-isomer were detected and quantified by both HPLC and direct UV–vis spectrophotometry, although they were present in very low amounts (10.6 ppb and 4.2 ppb, respectively). Moreover, the values of the total carotenoids content determined by HPLC and by the photometric determination were comparable.

### 3.5. Polyphenol analysis by RP-HPLC-PDA/ESI-MS

The polyphenol compounds were characterised by RP-HPLC-PDA/ESI-MS and their separation is shown in Figure 3. The partially porous C18 column, operated under gradient conditions, allowed baseline separation for all identified compounds within a total run time of roughly 40 min. A total of 11 different polyphenol compounds, 6 hydroxycinnamic acids and
5 flavonoids, were identified by combining information from retention times, PDA and MS data (Table 3). In particular, the early eluting compounds were represented by chlorogenic acid and hydrocinnamic acid derivatives, followed by acylated derivatives of kaempferol and quercetin glycosides whereas the last eluted ones were represented by hydrocinnamic acids glycosides. These results are consistent with a recent study on the polyphenol contents of the leaves of *B. oleracea* L. Convar. *acephala* (Olsen et al. 2009). Since in previous works only the leaves samples were taken under consideration, this is the first report on the polyphenol composition of old cabbage seed oil.

**4. Conclusions**

This study investigates in great details the composition of the oil extracted from the seeds of *B. oleracea*, variety *acephala* (old cabbage). The contents in FAs, triacylglycerols, tocopherols, carotenoids and polyphenols were determined by GC-FID, GC-MS, HPLC-MS and HPLC-FL. The fatty acid profile showed that erucic acid is the most abundant component of this oil accounting for more than 50% of its composition, followed by the essential FAs, linoleic and linolenic acid. γ-tocopherol was the most abundant tocopherol (>50% of total tocopherol composition). The carotenoid and polyphenol analysis led to the identification of 13 carotenoids and 15 polyphenols. This study shows that the old cabbage seed oil contains more than 30% in health promoting unsaturated FAs, although the high percentage of erucic acid could raise serious concern on human health; therefore, the utilisation for human consumption of this oil rich in many healthy phytochemicals should be discouraged unless its erucic acid content is removed or reduced. Alternatively, this oil characterised by high content in erucic acid could be a valuable and renewable raw material for the manufacturing of a wide array of industrial products.

**Acknowledgements**

The authors gratefully acknowledge Shimadzu and Sigma-Aldrich/Supelco Corporations for their continuous support.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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