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Insights into the phytochemical composition of selected genotypes of organic kale (Brassica oleracea, L. var. acephala)

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Abstract:	Seven genotypes of kale (B. oleracea L. var. acephala), selected from a collection set up in the framework of the BRESOV H2020 Project, aimed at breeding under organic conditions, were analysed for the content in the characteristic phytochemicals of Brassica spp. The presence of prebiotic oligosaccharides was an important characteristic of this crop, with values ranging from 4.2 to 10.8 g/100 g d.w. The other detected soluble sugars were sucrose, glucose, and fructose, with glucose predominating (3.9-9.0 g/100 g d.w.). Sulphur compounds, such as sulphoxides, were detected in the form of pyruvic acid, their catabolic product (36-154 mg/100 g d.w.). In addition, the levels of main breakdown products of glucosinolates, such as sulforaphane and indole-3-carbinol, were found to be in average contents of 6.7 and 3.9 mol/g d.w., respectively. Finally, the concentrations of major phytochemicals in kale, such as polyphenols and carotenoids, reported considerable concentrations (around 1400 mg/100 g d.w. and 19 mg/100 g d.w, respectively), typical for this Brassica crop.
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Insights into the phytochemical composition of selected genotypes of organic kale (*Brassica oleracea* L. var. *acephala*)

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Dear Editor,
I and all co-Authors of the proposed work completely agree with your final comment. We understand the reasons on which the JFCA policy regarding the definition of antioxidant in food and its consequent potential bioactivity is based.
Consequently, we carefully changed the manuscript according to your indications, changing the term "antioxidant" to the concept of "compositional traits", better agreeing with the Policy of the Journal. This remembering, in agreement with the JFCA policy, that our concept of "antioxidant", when expressed, was related to the potential antioxidant content of the food matter, which obviously should be validated by further *in vivo* assays targeted to the antioxidant action in living cells or bodies when counteracting the oxidative stress by ROS and RNS.
Hoping that the changes made will meet the Editor's recommendations, we send to you the revised manuscript.

Roberto Lo Scalzo
Corresponding Author
on behalf of all co-Authors

Reply to the Reviewers

We are very grateful for the Comments of the Reviewers, which surely improve the scientific quality of the proposed work. To our knowledge, we have not further replies to the comments, so the reply has been included in the Cover Letter, being the comments made by the Editor.

Highlights

- The breeding hystory of germplasm strongly determines the kale biochemical profile
- Sulphur defence compounds appeared equally distributed in the studied kale genotypes
- Sucrose and total tocopherols were positively correlated with total carotenoids
- Organic kale crops showed adequate to elevated levels of bioactive phytochemicals

1 **Insights into the phytochemical composition of selected genotypes of organic kale**
2 **(*Brassica oleracea* L. var. *acephala*)**

3

4 **Abstract**

5 Seven genotypes of kale (*B. oleracea* L. var. *acephala*), selected from a collection set up in the
6 framework of the BRESOV H2020 Project, aimed at breeding under organic conditions, were
7 analysed for the content in the characteristic phytochemicals of *Brassica* spp. The presence of
8 prebiotic oligosaccharides was an important characteristic of this crop, with values ranging from 4.2
9 to 10.8 g/100 g d.w. The other detected soluble sugars were sucrose, glucose, and fructose, with
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13 were found to be in average contents of 6.7 and 3.9 µmol/g d.w., respectively. Finally, the
14 concentrations of major phytochemicals in kale, such as polyphenols and carotenoids, reported
15 considerable concentrations (around 1400 mg/100 g d.w. and 19 mg/100 g d.w, respectively), typical
16 for this *Brassica* crop.

17 ***Keywords***: *Brassicaceae*, biodiversity, organic cropping, phytochemical quality, sulphur compounds,
18 carotenoids, phenolic compounds, prebiotic oligosaccharides

19 ***Abbreviations:*** HAD, hydroxycinnamoyl derivatives; QD, quercetin derivatives; KD, kaempferol derivatives; HCG,
20 hydroxycinnamoyl gentiobiosides; GSH, glutathione; SRA, sulforaphane; I3C, indole-3-carbinol

21

22 1. **Introduction**

23 The *Brassicaceae* family has a complex taxonomy and systematics: the number of genera and species
24 are often reviewed, due to the discovery of new species or to the application of novel genetic
25 techniques, which allow the distinction of different groups (Šamec et al., 2019; Maggioni et al., 2015;
26 Treccarichi et al., 2023). More specifically, the term kale refers to a group of plants belonging to the
27 species *Brassica oleracea* var. *acephala*, which comprises plants whose leaves do not form a head
28 (Arena et al., 2022). The *acephala* group includes the following morphotypes: kale (*B. oleracea* L.
29 var. *acephala* DC.), scotch kale (*B. oleracea* L. convar. *acephala* DC. Alef. var. *sabellica* L.), collard
30 (*B. oleracea* L. var. *viridis* L.), palm kale (*B. oleracea* L. convar. *acephala* DC. Alef. var. *palmifolia*
31 L.), marrow stem kale (*B. oleracea* L. convar. *acephala* DC. Alef. var. *medullosa* L.), thousand-head
32 kale (*B. oleracea* L. var. *ramosa* DC.) and Portuguese Tronchuda cabbage (*B. oleracea* L. var. *costata*
33 DC.) (Diederichsen 2001). In some cases, the *acephala* varieties include landraces with distinct
34 properties, such as the Laciniato kale, known as “Cavolo Nero Toscano”, an appreciated Italian palm
35 kale.

36 Kale has a wide variety of culinary uses; it can be eaten raw in salads or cooked in soups or side
37 dishes. Many recipes belong to local traditions and others arise from the renewed attention which is
38 paid to this *Brassica* species. In recent years, indeed, there has been a growing interest in the
39 nutritional properties of kale plants, due to their complex profile of bioactive compounds (Di Bella et
40 al., 2021; Tribulato et al., 2020). Previous studies report the correlation between *Brassicaceae*
41 consumption and the protection against cancer and cardiovascular diseases (Sarıkamış et al, 2008;
42 Manchali et al., 2012). In fact, these vegetables are a rich source of ascorbic acid, carotenoids, sulphur
43 organic compounds and phenolics. Kale contains high level of ascorbic acid, in the range between
44 680 and 1120 mg/100 g dry weight (Korus, 2011; Vargas et al., 2022). In addition to ascorbic acid,
45 thiol-type compounds constitute a class of organic sulphur derivatives with sulfhydryl functional
46 groups (−SH), which play a crucial role as ROS (Reactive Oxygen Species) scavengers. Among them,

47 glutathione (GSH) is the prevalent metabolite, which is involved in the regeneration of reduced
48 ascorbic acid via the ascorbate-glutathione cycle. Being a strong ROS scavenger, glutathione
49 represents a phytochemical that is worth considering both for plant and human health. Compared to
50 other phytochemicals, glutathione and total thiols are often less studied and quantified in plants, and
51 little literature is available regarding their content in kale. In the work by Łata (2014), concerning the
52 variability in bioactive compounds in red and green-leafy kale in relation to soil type and N-level, the
53 author showed that GSH was the main thiol compound in kale (around 6 mg/100 g fresh weight), and
54 the share of GSH in the global GSH pool amounted to approximately 80%. In addition to GSH, L-
55 cysteine was the other more abundant thiol compound (around 0.6 mg/100 fresh weight), followed
56 by γ-glutamylcysteine.

57 In *Brassica oleracea*, sulphur content is high, ranging from 0.6 to 1.0% of dry matter (Rosa and
58 Heaney, 1996). Sulphur is present as inorganic sulphate and involved in the formation of proteins, S-
59 methyl-L-cysteine sulphoxide (SMCO) and glucosinolates (GSLs), a group of phytochemicals
60 characteristics of cruciferous plants. As a response to abiotic stress or biotic attacks, myrosinase
61 enzyme catalyzes the hydrolysis of GLSs, producing glucose and other breakdown products,
62 including isothiocyanates, thiocyanates and nitriles. These specialized metabolites are known for their
63 anti-inflammatory, anti-bacterial, and phase II detoxification effects (Steinbrecher et al., 2010).
64 Specifically, two of these metabolites, such as the sulforaphane (SRA), which derives from the
65 hydrolysis of glucoraphanin, and the indole-3-carbinol (I3C), which derives from the hydrolysis of
66 glucobrassicin, are widely recognized to have relevant healthy properties (Singh et al., 2021;
67 Sivakumar et al., 2007).

68 Among other leafy vegetables, kale stands out for the high content of carotenoids, such as lutein,
69 violaxanthin and β-carotene.

70 Phenolic compounds are important specialized metabolites which contribute to kale health-promoting
71 properties. They include glycosides of quercetin and kaempferol and derivatives of *p*-coumaric,

72 ferulic, sinapic and caffeic acid (Olsen et al., 2009), with caffeic and ferulic acid as predominant
73 compounds (Ayaz et al., 2008).

74 The increasing concern about environmental issues and the demand for sustainable products with
75 high nutritional and sensory quality has led to the diffusion of organic cropping systems: specifically,
76 previous research on *Brassica* crops indicate the positive influence of organic fertilizers on the
77 flavonoids and carotenoids content (Dos Reis et al., 2015). Moreover, some experimental results
78 indicate that the effect of the cropping system is cultivar dependent (Picchi et al., 2012; Naguib et al.,
79 2012). The choice of plant material specifically developed for organic agriculture, which can
80 guarantee a good yield and high quality in diverse climate and stress conditions, is therefore a key
81 requirement to meet the demands of expanding organic farming systems.

82 In the framework of the BRESOV H2020 project "Shaping the future of organic breeding and
83 farming" (https://bresov.eu) a breeding program was established with the aim of developing new
84 high-quality *Brassica* spp. genotypes suitable for organic farming, through the exploitation of genetic
85 biodiversity. The present study has been focused on 7 selected kale genotypes belonging to the
86 collections of the University of Liverpool (UL) and the University of Catania (UNICT) that were
87 grown together under organic conditions in a controlled environment. The selection was based on the
88 genotypes showing the most relevant content in phytochemicals. Until now, few studies have reported
89 the concentration of other kale micronutrients, such as sulforaphane (SRA) and indole-3-carbinol
90 (I3C), organic volatiles and tocopherols (Sasaki et al., 2012; Wibowo et al., 2019; Korus 2020).

91 Resuming, the aim of the present study was a thorough characterization of phytochemical
92 composition of these selected genotypes of kale specifically adapted for organic cultivation. The
93 levels of soluble solids content, single sugars content, organic acids, ascorbic acid, thiols,
94 glucosinolate breakdown products (I3C and SRA), volatile compounds, polyphenols, chlorophylls,
95 tocopherols, and total carotenoids, were determined. Moreover, variability within each genotype was
96 analysed, as well as the correlation among the different measured phytochemicals.

97

98 ## 2. **Materials and methods**

99 *2.1 Plant material*

100 The assayed plant materials were selected among a germplasm collection grown in the experimental
101 field crops of University of Catania Di3A during season 2018-2019 in the framework of the BRESOV
102 H2020 Project (www.bresov.eu). The BRESOV collection is represented by hundreds of *Brassica*
103 genotypes, provided by several genebanks of different countries, such as Italy, United Kingdom,
104 Portugal, Czech Republic, People's Republic of China. The organic growing system was carried out
105 according to the EC Rule 834/2007 and the Italian Ministerial Decree 220/1995. The experiment
106 focused on seven *Brassica oleracea* var. *acephala* genotypes, selected with a screening approach
107 based on the relevant presence of phytochemicals by the total reducing power measured through the
108 Folin-Ciocalteu reaction and the DPPH scavenging capacity, carried out on the entire available
109 BRESOV collection of *Brassica* genotypes. These measurements, even if aspecific, were considered
110 a useful tool to distinguish, among a broad number of genotypes, the ones with higher potential
111 phytochemical content. In fact, a number of studies indicate high positive correlations between DPPH
112 scavenging capacity and ascorbic acid (Picchi et al., 2012 and 2020; Heimler et al., 2006) or phenolic
113 content (Jin et al., 2012). The genotypes (Table 1) were provided by the University of Liverpool (UK)
114 and the University of Catania, Di3A (Italy) and the seeds were sown in August 2018 cellular trays
115 using organic substrate (Terri® Bio) placed in a cold greenhouse in Catania. The plants were
116 transplanted in September 2018 in a potted experiment field at the University of Catania (37°31'10''
117 N 15°04'18''E) under natural light (4.6 to 9.2 MJ m^{-2} d^{-1}), and the mean temperature registered
118 during the growing year was 19.1°C ± 8.6°C. The plants were growing according to organic
119 agriculture methods utilizing amino acids and microorganisms for the nutritional support of the
120 plants, and *Bacillus thuringiensis* and pyrethrin based products for pest plant protection. Plant samples
121 were collected after the first year of growth from three different plants for each genotype. Single plant

122 samples were quickly flash-frozen at -50°C, freeze-dried, homogenized (22-74 mesh powder) and
123 stored at -20°C until analyses. The analyses have been performed in triplicate for each sample.

124 *2.2 Soluble solids content and sugars composition*

125 The soluble solid content (SSC) was measured following the rationale by Lamb (1972), with few
126 modifications, on an aqueous extract obtained by adding 0.5 g of freeze-dried kale powder to 5 mL
127 of distilled water, after 1,000g centrifugation. The SSC was expressed as °Bx on a dry weight (d.w.)
128 basis, using a digital bench refractometer (Bellingham-Stanley, mod. RFM 91). The same extract,
129 after centrifuging at 10,000g, was used for the determination of single soluble sugars, such as high
130 DP sugars (retention time 6.1 to 7.5 min), sucrose (8.5 min), glucose (10.4 min), and fructose (12.4
131 min). Soluble sugars have been analyzed following the validated guidelines by Li et al (2002), slightly
132 modified: they were analysed by HPLC with a refractive index as detector, using a Benson BP-100
133 300×7.8 carbohydrate column and quantification was made by comparison with external standards at
134 known concentrations. The mobile phase was H_2O at 80 °C, at a flow rate of 0.6 mL/min, and the
135 injection volume was 30 µL. The calibration was made by solutions of raffinose for high DP sugars,
136 sucrose, glucose, and fructose ranging from 0.05 to 0.33 mg/mL concentrations. The resulting average
137 conversion factors (concentration *vs* peak area) were 0.0021, 0.0023, 0.0025 and 0.0024 for raffinose,
138 sucrose, glucose, and fructose, respectively.

139 *2.3 Analysis of organic acids*

140 For organic acids determination, an HPLC method was developed following the validated guidelines
141 by Jayaprakasha et al. (2003), with some modifications. Briefly, 3 mL of distilled water was added
142 to 100 mg of freeze-dried powder. The mixture was vortexed at 1000 rpm for 5 minutes at room
143 temperature, centrifuged (10000*g*, 5 min at 4 °C) and the supernatant was filtrated on a 0.45 µm nylon
144 filter.

145 The main identified organic acids (citric, malic, pyruvic and fumaric) were analysed by HPLC using
146 a Repromer H+ column, 300×8 mm, 9 µm (Dr. Maisch, Ammerbuch-Entringen, Germany) at 50 °C,
147 33 mM H_2SO_4 as mobile phase at a flow of 0.5 mL/min, with UV detection at 214 nm. Solutions of
148 commercial standards at known concentrations were used for calibration in concentration ranges of
149 0.05-1.50 mg/mL for citric, malic, and pyruvic and a range of 0.002-0.02 mg/mL for fumaric acid.
150 The resulting response factors were of 2277, 2452, 4118 and 156461, respectively. In these conditions
151 the retention times were: 10.9 min citric, 12.8 min malic,14.6 min pyruvic, and 20.6 min fumaric.
152 Data were given as mg/100 g d.w.

153 *2.4 Ascorbic acid (AsA)*

154 For this analysis, a previously validated method on *Brassica* samples was followed (Picchi et al.,
155 2012): 50 mg of lyophilized powder was homogenized in the dark with 1.5 mL of 6% metaphosphoric
156 acid and centrifuged at 10000*g* for 5 min at 4 °C. The supernatant was filtered on 0.45 mm nylon
157 filters and immediately analysed. L-ascorbic acid (AsA) content was determined by HPLC using a
158 Supelco Ascentis Phenyl column (250 × 4 mm i.d.) at 44 °C, and 0.02 M orthophosphoric acid as
159 mobile phase. The flow rate was 0.6 mL/min and 20 µL samples were injected and monitored at 254
160 nm, and the retention time of AsA was observed at 5.9 minutes. The concentration was calculated by
161 interpolation of samples area data with a calibration curve made using solutions at known
162 concentrations of an AsA standard (purity HPLC ≥ 98%, Sigma-Aldrich) ($y = 36447x$, $r^2 = 0.9967$,
163 range 2-100 µg/ml), and was expressed as milligrams per 100 g/d.w.

164 *2.5 Total thiols*

165 The analysis of free soluble −SH groups was performed on 50 mg of lyophilised powder extracted
166 with 1 mL of a 6% metaphosphoric acid solution, according to the already validated method used by
167 Riener et al (2002), with few modifications. Briefly, an aliquot of 0.25 mL was treated with 0.5 mL
168 of a solution composed of 160 mM 5-sulphosalicylic acid, 15 mM Na ascorbate and 2 mM Na_2–
169 EDTA. Then, 1 mL of 0.8 M, pH 8.0 mono- and di-Na phosphate buffer and 0.25 mL of 10 mM

170 DTNB (dithionitrobenzoic acid) were added. The absorbance of the resulting solution was read at
171 415 nm after 30 seconds of reaction in a 1 cm-path cuvette. The level of total thiols was calculated
172 from a standard curve made with glutathione (GSH) ($y = 11.681x$, $r^2 = 0.9998$, range 10-50 µg/ml),
173 and data were given as mg/100 g of glutathione (GSH) equivalents per 100 g of d.w.

174 *2.6 HPLC analysis of sulforaphane (SRA) and indole-3-carbinol (I3C) contents*

175 These two catabolites of glucoraphanin and glucobrassicin, respectively, were separated, identified,
176 and quantified by an SPE followed by an HPLC-DAD method, following the procedures proposed
177 by Pilipczuk et al., 2015 and 2017, with slight modifications: a kale powder aliquot of 50 mg was
178 incubated with 1 mL distilled water at room temperature for 30 minutes. Then, 1 mL of HPLC grade
179 EtOH 80% was added to the mixture, vortexed and centrifuged at 10000g. The clear supernatant was
180 placed on a C_{18} SPE cartridge (Alltech, MaxiClean, 300 mg), previously conditioned with 1×2 mL of
181 EtOH and 1×2 mL of water. After washing with 1×2 mL of water, the elution was performed with
182 3×0.5 mL of EtOH 96%, the eluate was filtered on 0.45 mm nylon filters and divided into two aliquots
183 for subsequent analyses of I3C and SRA, following previously validated methods: I3C has been
184 detected by fluorescence (Pilipczuk et al., 2015) and SRA was derivatized with N-acetyl cysteine in
185 sodium bicarbonate and detected at 272 nm (Pilipczuk et al., 2017). The HPLC separation was carried
186 out with a Pinnacle C_{18} column (250 mm × 4.6 mm i.d., 5 µm) under gradient elution of solvent A
187 (HCOOH 0.5%, MeOH 10% in water) and solvent B (CH_3CN/MeOH 9:1 HCOOH 0.5%) as follows:
188 from 90% (5 min isocratic condition) to 50% of solvent A in 20 min, then to 50% of solvent A in 25
189 min and to 90% of solvent A in 10 min. Compounds were eluted at 0.60 mL/min at 43°C and UV
190 detected at 272 for SRA and 280/360 nm ex./em. for I3C, with retention times at 8.3 and 11.4 minutes,
191 respectively. Peak identification was based on the comparison of retention times with those of pure
192 reference compounds treated the same as the samples. Quantitation of the detected compounds was
193 performed by an external standard method using available commercial standards (Sigma, purity

194 HPLC ≥98%) of SRA with a concentration range of 0.1-0.8 µmol/mL and of I3C with a concentration
195 range of 0.2-1.8 µmol/mL. The given concentration units were µmol/g d.w.

196 *2.7 Volatile compounds profile*

197 The volatile analysis from kale samples was performed by using a headspace solid-phase micro
198 extraction (HS-SPME) coupled with GC-MS and GC-FID analyses. Each sample was composed by
199 200 mg freeze-dried *Brassica* kale sample added with 10 mL deionized water in a 20 mL glass vial,
200 kept for 5 h at 60°C. Each vial was then closed with an aluminium/silicone-PTFE septum after the
201 addition of 2 g of NaCl. Volatiles were identified using GC-MS and quantified using GC-FID.

202 Samples extraction was performed using a DVB-CAR-PDMS fiber, at 80°C for 20 min. Volatiles
203 desorption was obtained exposing the fiber in the GC injector at 200°C for 5 min.

204 GC-MS analyses were performed using a previously validated method (Picchi et al., 2012), with
205 modifications; they were carried out with an Agilent 6890 N GC connected to an Agilent 5973 mass
206 spectrometer and equipped with a DB-1 column (60 m × 0.25 mm I.D., film thickness 0.25 µm), in
207 splitless mode, using He as carrier gas (flow 1 mL/min). The column temperature program was: 40
208 °C for 5 min, 3°C/min to 180 °C, 8 °C/min to 220 °C for 5 min (duration: 61,7 min). Injector and
209 detector temperatures were 200 and 230 °C, respectively; interconnecting line temperature, 200 °C.
210 The MS settings were as follows: filament voltage, 70 eV; scan range, 39-450 amu; scan speed, 1.4
211 scan/s. The compounds were identified comparing their mass spectra with those stored in Wiley 7n
212 library and analysing authentic standards.

213 The quantification GC-FID system comprised extraction, injection, and desorption from SPME fibers
214 performed using a HT2800T autosampler (HTA S.r.l., Brescia, Italy) connected to the GC-FID
215 system. The GC-FID analysis followed a procedure previously validated on other vegetables (Lo
216 Scalzo et al., 2020) and was performed on 16 selected compounds using the same column and
217 chromatographic conditions as GC-MS. Quantification was performed by the interpolation of

218 calibration curves made with known concentrations of hexanal (slope equation y=0.0052 x, r^2=0.90,
219 LOQ 0.4 ng/g, LOD 0.1 ng/g, average ranges 1-100 ng/g, used for aldehydes and ketones) and allyl
220 isothiocyanate (slope equation y=0.0055x, r^2=0.98, LOQ 0.3 ng/g, LOD 0.1 ng/g, average ranges
221 0.5-100 ng/g, used for other compounds) and the values were expressed as ng/g d.w.

222 *2.8 Chlorophylls and total carotenoids*

223 For the analysis of chlorophylls and total carotenoids, a spectrophotometric method was used,
224 following the instructions and equations previously performed and validated by Lichtenthaler and
225 Wellburn (1983). An aliquot of around 50 mg sample exactly weighted was extracted with 1.5 mL of
226 an EtOH/acetone 1:1 solution added with 0.1% BHT, to avoid autoxidation. After vortexing,
227 sonication and centrifugation, the clear supernatant was diluted with acetone and analysed by
228 spectrophotometer, set at the wavelengths of 470, 646 and 663 nm, requested by the equations from
229 Lichtenthaler and Wellburn (1983). Results were given in mg/100 g d.w.

230 *2.9 Tocopherols*

231 The evaluation of tocopherols was performed by HPLC, with a fluorescence detection, following the
232 rationale by Meckelmann et al. (2015).

233 The chromatographic system used for the separation, identification and quantification of kale
234 tocopherols comprised a column C_{30} YMC Europe S-3 μm, 4.6 mm × 250 mm, flow 0.4 mL min^{-1} at
235 40 °C, eluent A CH_3CN/MeOH 9:1, eluent B MTBE (methyl *tert*-butyl ether), with a gradient elution
236 from 95% to 85% solvent A in ten minutes, then 85% solvent A for 10 minutes and return to the
237 starting conditions in ten minutes, for a total run length of 30 minutes. The detection was
238 spectrofluorimetric, setting the detector at 295 nm excitation and 325 nm emission.

239 In these conditions, the retention times were 13.4 min for δ-tocopherol, 14.7 min for γ-tocopherol and
240 16.4 min for α-tocopherol. The calibration was performed by standard solutions of δ-, γ- and
241 α–tocopherols at known concentrations in an average range from 10 to 100 mg/mL, with resulting

242 equations for sample data interpolation having slopes of 2×10^7, 2×10^7, 4×10^7, respectively, and an
243 average experimental point fitting of $r^2= 0.999$.

244 *2.10 Phenols analysis*

245 Phenols were extracted starting from powdered plant material (20 mg), treated with 1 mL of
246 EtOH/0.06 N HCl (1:1) and shaken at room temperature overnight. The samples were then
247 centrifuged at 25,000g for 20 minutes, the supernatant was filtered on 0.2 mm nylon filters and stored
248 at -80 °C until analyses.

249 The phenolic composition was evaluated by a double chromatographic approach, including the extract
250 analysis by UPLC-ESI-MS and the subsequent compound quantification in an HPLC-DAD system.

251 For ESI/MS-MS, a Jasco UPLC system equipped with a binary pump system, photo diode array
252 detector (Jasco Chemstation ChromNAV) and coupled to a Thermo LTQ (linear ion trap mass
253 detector) with an electrospray ionization (ESI) source was used. All data were acquired and processed
254 using Thermo Xcalibur Qual Browser software. Chromatographic runs were carried out with an
255 Acquity UPLC BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm particles, 13 nm pore size) (Waters)
256 under a linear gradient of solvent A (H_2O/0.1% HCOOH) and solvent B (CH_3CN/0.1% HCOOH) as
257 follows: 0.0–5.0 min (5% B), 30.0 min (25% B), 40.0 min (70% B), 50.0 min (100% B). The flow
258 rate was 0.3 mL/min, the column temperature was set at 30 °C, injection volume 5.0 μL. The eluates
259 were spectrophotometrically checked at 9 different wavelengths (from 250 nm to 340 nm). For MS
260 detection, negative ESI was used as ionization mode. Capillary voltage, 3100 V; sheath gas (He), aux
261 gas (He), sweep gas (He) heated at 275 °C and introduced with a source heater temperature of 80 °C.
262 Full scan spectra were acquired over the range of 100–2000 m/z. Automated MS/MS was performed
263 by isolating the base peaks (molecular ions) using an isolation width of 2.0 m/z, normalized collision
264 energy of 25 V, threshold set at 500 and ion charge control on, with max acquire time set at 300 ms.
265 All the compounds were tentatively identified by a comparison of their retention times and mass
266 spectral data obtained in positive and negative ion mode with those of standard compounds or with

267 compounds previously reported in literature for *Brassica* spp. (Francisco et al., 2009; Lin et al., 2011;
268 Velasco et al., 2011; Yang et al., 2015; Picchi et al., 2020).

269 The phenolic composition of all extracts was also evaluated by HPLC-DAD using a PerkinElmer
270 (Norwalk, CT, USA) chromatograph equipped with a LC250 binary pump and DAD 235 detector.
271 Chromatographic runs were carried out with a Discovery® HS C18 column (250 mm × 4.6 mm i.d.,
272 5 µm) (Supelco) under gradient elution of solvent A (CH_3CN/0.05% CF_3COOH) and solvent B
273 (H_2O/1%MeOH/0.05%CF_3COOH) as follows: from 2% (10 min isocratic condition) to 15% of
274 solvent A in 30 min, then to 50% of solvent A in 50 min and to 90% of solvent A in 10 min. Twenty
275 µL of all extracts were injected. Compounds were eluted at 1.0 mL/min and detected by UV
276 monitoring at 330 nm, as illustrated in the example chromatogram (Figure 1). Peak identification was
277 based on LC-MS data and UV spectra were also recorded. Quantitation of the detected compounds
278 was performed at 330 nm by an external standard method using available commercial standards
279 (Sigma, purity HPLC ≥98%) of chlorogenic acid ($y = 63581x - 358152$, $r^2 = 0.9982$) for compound
280 1; kaempferol-3-O-glucoside ($y = 10701x + 78553$, $r^2 = 0.9946$) and quercetin-3-O-glucoside ($y =$
281 $42804 x + 5375$, $r^2 = 0.9995$) for flavonol derivatives (peaks 2-36) and sinapoyl-4-O-glucoside ($y =$
282 $43694x - 156367$, $r^2 = 0.9935$) for hydroxycinnamoyl gentiobiosides (peaks 37-42). For calibration
283 curves, all standards were injected in triplicate in the range from 6 to 350 µg/ml and a linear response
284 was observed. The given units for phenol quantification were mg/100 g d.w. As for the data analysis,
285 the phenol composition was grouped into four classes of compounds, comprising the phenols with
286 similar structures, such as hydroxycinnamic esters, quercetin derivatives, kaempferol derivatives and
287 hydroxycinnamoyl gentiobiosides.

288 *2.11 Data analysis*

289 The present results are the mean ± standard deviation (SD) of three plants per genotype. Each plant
290 was analysed in triplicate. The percentage coefficient of variation (CV) among plants of the same
291 genotype was evaluated as (SD/mean) *100.

292 For each parameter, mean values for each genotype were compared using Tukey's test and significant
293 differences were accepted at $P \leq 0.05$. One-way ANOVA was carried out using Statgraphics v. 5.1
294 (Manugistics, US). Correlations among variables and principal component analysis (PCA) were
295 computed with PAST v. 4.03 (Hammer et al., 2001).

296 3. Results and Discussion

297 *3.1 SSC and single sugars content*

298 The SSC is related to total soluble sugars content, and therefore represents an important sensory
299 quality index. SSC significantly differed among genotypes and ranged from 26.2 ± 10.3 °Bx/d.w. (in
300 UNICT 4959) to 41.1 ± 3.2 °Bx/d.w. (in UL 5010) (Table 2). Previous studies have measured SSC
301 on fresh Galega kale plants, obtaining results of 11.2 and 13.2 °Bx referred to fresh weight (Armesto
302 et al., 2015; Martìnez et al., 2010). Moreover, Akdaş and Bakkalbaşı (2017) indicate a value of 9.4
303 °Bx for kale grown in Turkey. In the work by Lefsrud et al (2008), the dry weight of kale widely
304 ranged from 15.0 to 21.4 % with high and low nitrogen input, respectively. Therefore, the here
305 presented data are within the ranges given by existing literature.

306 The presence of high degree of polymerization (DP) sugars (Table 2) followed that of SSC, with the
307 higher amount observed in UL genotypes. In fact, SSC was strongly correlated with high DP sugars
308 (r= 0.77, P= 0.000), and with fructose (r=0.49, P= 0.023). In the analytical conditions used in the
309 present work, this fraction includes oligosaccharides composed by three or more sugar units. The
310 presence of these high potential prebiotic sugars was confirmed in previous studies (Jovanovic-
311 Malinovska et al., 2014). In more recent studies on kale (Thavarajah et al., 2021) the characterization
312 of sugar profile highlighted the presence of the prebiotic oligosaccharides stachyose, raffinose,
313 verbascose, nystose and kestose in commercial kale genotypes adapted for organic cropping, ranging
314 from 5.7 to 8.7 g/100g of total prebiotic carbohydrates, in a similar range to the levels reported in the
315 present study (Table 2).

316 The sucrose content was generally lower compared to glucose and fructose, with slightly higher
317 amounts in UNICT genotypes, namely 375, 4946 and 4959 (0.5 g/100 g d.w.). Among single
318 monomeric sugars (Table 2), the prevalent compound was glucose, which ranged from 3.8 (in UL
319 5010) to 9.0 g/100 g d.w. (in UL 2066), without significant differences among the genotypes. The
320 highest (4.5 ± 0.6 g/100 g d.w.) and the lowest (1.8 ± 0.4 g/100 g d.w.) fructose levels were observed
321 in UL 2066 and UNICT 3332, respectively. Fructose and glucose amounts are overall higher than
322 those observed by Ayaz et al. (2006), which indicates fructose as the predominant sugar (2 g/100 g
323 d.w.) followed by glucose (1 g/100 g d.w.) and sucrose (0.9 g/100 g d.w.). By contrast, the present
324 kale genotypes seem to have slightly lower sugar content than those reported by Megìaz-Pérez et al.
325 (2020), and Jovanovic-Malinovska et al. (2014), with the predominance of glucose, in accordance
326 with here presented data.

327 Plant development stage and environmental conditions affect both sugar profile and amount. Megias-
328 Pérez et al. (2020) studied the relation of sugar composition to cold acclimation. Using HILIC-ESI-
329 MS and GC-MS techniques, they observed that sucrose decreased significantly during kale plant
330 development; however, at low temperature, fructose increased in kales grown at 2 °C, evidently
331 increasing the potential sweetness.

332 *3.2 Organic acids*

333 Citric and malic acids were the two main organic acids detected in the seven kale genotypes (Table
334 3). They were found in amounts that are overall in accordance with previous studies (Ayaz et al.,
335 2006; Nemzer at al., 2021). Citric acid concentrations (Table 3) ranged from 1962 to 3743 mg/100 g
336 d.w. in UL 2066 and UL 2075 respectively, without significant differences. Malic acid, instead, varied
337 from 454 mg/100 g d.w. in UNICT 4946 to 3175 mg/100 g d.w. in UL 5010 (Table 3). Interestingly,
338 the UL genotypes showed almost equal contents of these acids, while in UNICT genotypes citric acid
339 was largely prevalent, reflecting the acid pattern observed by Ayaz et al. (2006) in kales grown in
340 Turkey. Pyruvic acid content (Table 3) was similarly higher in UL genotypes and in UNICT 3332

341 (range 148-154 mg/100 g d.w.) than in the other UNICT genotypes (range 36-81 mg/100 g d.w.).
342 Pyruvic acid, together with ammonia and methanesulphenic acid, is a catabolic product of S-
343 methylcysteine sulfoxide (MCSO), produced by cysteine sulphoxide lyases released after cellular
344 disruption (Edmands et al., 2013). In the past, the main use of kale was as forage, however breeding
345 activity has led to the decrease of this compound in modern kale varieties, due to the antinutrient
346 effects of MCSO, which is normally present in relevant concentrations (1-2 % on d.w.) and it is the
347 cause of hemolytic anemia of cattle (Edmands et al., 2013). This is particularly evident in UNICT
348 genotypes (Table 3), where pyruvic acid has an average content of 82 mg/100 g d.w., with respect to
349 UL genotypes (150 mg/100 g d.w.). The content in MCSO of these kale genotypes (average 13.2
350 μmol/g d.w.) is similar to that found by Marks et al. (1992) using other *Brassica* species, and lower
351 than that found in broccoli (around 50 μmol/g d.w.) (Traka et al., 2013). Interestingly, the average
352 MCSO concentration is similar to the sum of the breakdown forms of glucosinolates, since SRA and
353 I3C averaged 6.7 and 3.9 μmol/g d.w., respectively, and it was higher than the levels of reducing
354 thiols, averaging at 3.3 μmol/g d.w; this may indicate that, in the studied genotypes, S-containing
355 defence compounds are almost equally distributed.

356 *3.3 Ascorbic acid and total thiols*

357 Ascorbic acid concentrations (Table 3) ranged from 209.4 (in UNICT 4959) to 243.8 mg/100 g d.w.
358 (in UL 2075). The values were overall slightly higher in UL genotypes, even though differences
359 among means were not significant. These amounts are in accordance with those reported by Hagen
360 et al. (2009) and Korus (2011) for dried kale samples. Total thiols content represents an important
361 part of plant sulphur metabolism, including total glutathione and non-protein –SH groups (Hawrylak
362 and Szymanka, 2004). In the present samples, thiol content differed significantly among genotypes
363 (Table 3). UNICT 3332 showed the highest level (119.9 mg/100 g d.w.) while the lowest (77.7
364 mg/100 g d.w.) was found in UL 2075. In her work, Łata (2014) found an average of 6 mg/100g fresh
365 weight of GSH in kale, while much higher concentrations of GSH, 6.9 μmol/g d.w. (211 mg/100 g

366 d.w.), were found in broccoli samples by Traka et al. (2013), and the present have shown intermediate
367 levels between the referenced ones. Ascorbic acid and glutathione are two bioactive compounds that
368 scavenge the ROS (mainly hydrogen peroxide) produced during the physiological processes of the
369 cells or in response to stress conditions. AsA and GSH cooperate in the AsA/GSH cycle to detoxify
370 the hydrogen peroxide (Noctor and Foyer, 1998). The total thiols were at some extent higher in
371 UNICT genotypes. The AsA/thiols ratio (Table 3) differed among genotypes, being significantly
372 higher in UL 2075 than other genotypes. AsA and thiols are both important parameters in determining
373 plant resistance to oxidative stress, such as environmental stress (Picchi et al., 2017). High GSH
374 regeneration is essential for AsA formation and AsA to thiols ratio is positively related to oxidative
375 stress tolerance (Fatima et al., 2019). In the present study, plants were not exposed to oxidative stress,
376 but the positive values of AsA to thiols ratio indicated a constitutive plant capacity to cope with
377 possible oxidative stress.

378 *3.4 Sulforaphane and indole-3-carbinol*

379 The content of glucosinolates and their breakdown products in *Brassica* spp. is dependent on several
380 environmental and developmental factors (Velasco et al., 2007). The I3C and SRA HPLC data (Figure
381 2) indicate that the kale genotypes used in this work are a potential good source of glucoraphanin,
382 representing the native glucosinolate precursor of the sulphoraphane, well noted for its health
383 promoting properties (Matusheski et al., 2004).

384 The average content of the breakdown product of glucoraphanin, such as sulforaphane (6.7 μmol/g
385 d.w.) almost double than the average content of indole-3-carbinol (I3C), the glucobrassicin catabolite
386 (3.9 μmol/g d.w.). These data are in a full accordance with the findings of Sasaki et al. (2012), where
387 the concentration of glucosinolates precursors was considered. Moreover, similar amounts of
388 glucobrassicins were found in kale by Son et al. (2021), while West et al. (2004) found relevant
389 concentrations of glucoraphanin and glucobrassicin in kale seeds. Sulforaphane content of the kale
390 genotypes used in this study ranged from 3.4 μmol/g d.w.in UL 5010 to 12.4 μmol/g d.w. in UNICT

391 375 (Figure 2). Overall, UL genotypes had lower sulforaphane levels and a higher variability among
392 the plants of the same genotype. In UL 5010 the CV was 78.9%. Unfortunately, relatively few
393 published data with respect to raw glucosinolates is available to compare the presence of sulforaphane
394 in kale samples. An amount ranging from 2 to 8 μmol/g d.w was reported in previous studies (Li et
395 al., 2016; Sivakumar et al., 2007). Interestingly, kale can be a good source of sulforaphane, since
396 some samples have concentrations at around 12 μmol/g d.w, similar to the commercialized
397 "Benefortè ™" broccoli (Traka et al., 2013).

398 The distribution of I3C concentration did not show clear differences between UL and UNICT
399 genotypes (Figure 2). The higher average levels, found in UL 2075 and UNICT 4959 were 5.35 and
400 5.34 μmol/ g d.w., respectively, and the variability among plants was highest in UNICT 375, with a
401 CV of 115.1%. Due to this high variability, no significant differences in the I3C content were found.
402 Overall, the I3C concentrations were in full accordance with published data on commercial kale
403 samples (Yu et al., 2018), while much lower concentrations were found in other works (Kapusta-
404 Duch et al., 2016; Pilipczuk et al., 2015).

405 *3.5 Volatile compounds profile*

406 The 16 selected compounds identified and quantified in the SPME headspace of all genotypes are
407 listed in Table 4, with the data regarding the tentative identification listed in Table S2. Among them,
408 six compounds were aldehydes, with hexanal and (*E*)-2 hexenal as the prevalent compounds. These
409 aldehydes are the main products of the lipoxygenases (LOX) catabolism of linoleic and linolenic
410 acids, respectively (Cao et al., 2014). Kale is a good source of these essential fatty acids (Ayaz et al.,
411 2006). (*E*)-2-hexenal concentration was higher in UL 5010 (100 ng/g d.w.), and in almost all other
412 genotypes was around 60 ng/g d.w., with the exceptions of UNICT 3332 and UNICT 375 which had
413 lower levels (about 10 ng/g d.w.). The CV% among plants of the same genotype was higher in UNICT
414 3332 (34.7%). Hexanal content ranged from 1.0 in UNICT 3332 to 17.1 ng/g d.w. in UNICT 4946,
415 with a maximum CV related to the different plants in UNICT 375 (37.8%).

416 As regards sulphur volatile compounds, two main isothiocyanates were identified and quantified,
417 allyl- and 3-butenyl isothiocyanate (respectively a sinigrin and a gluconapin breakdown product).
418 Overall, higher concentrations of these compounds were found in UL compared to UNICT genotypes;
419 however, due to the large variability among plants, the differences among genotypes were not
420 significant. The 3-butenyl-isothiocyanate, characteristic of kale (Zeng et al, 2021; Oh and Cho, 2021)
421 was prevalent in HS-SPME, with the higher concentration in UL 2075 (539 ng/g d.w.) and lowest in
422 UNICT 3332 (0.4 ng/g d.w.). Allyl isothiocyanate was similarly higher in UL 2075 (141.3 ng/g d.w.)
423 and was present in concentrations around 1-2 ng/g in UNICT genotypes. For allyl isothiocyanate CV
424 ranged from 19.4% (BH 96) to 87.3% (UL 2075), while for 3-butenyl isothiocyanate it was not lower
425 than 52.6% (UL 2066), ranging in other genotypes from 55.8 to 80.3%.

426 Sulphides represent another class of sulphur compounds present in *Brassicaceae,* deriving from the
427 MCSO catabolism (Edmands et al., 2013). The main compound of this class found in the studied kale
428 accessions was dimethyl trisulfide, whose concentration varied from 23.9 ng/g d.w. in UL 5010 to
429 0.5 ng/g d.w. in UNICT 375, even though the CV% widely varied (0.21-62.8 in UNICT 375 and UL
430 2075, respectively).

431 Kales are naturally rich in carotenoids (Perry et al., 2009; Ashenafi et al., 2022). Apocarotenoids,
432 their respective catabolites, are another important class of volatile compounds, to which 6-methyl-5-
433 hepten-2-one, geranyl acetone and β-ionone belong. 6-Methyl-5-hepten-2-one and geranyl acetone
434 are products of lycopene cleavage: the 6-methyl-5-hepten-2-one concentration varied from 0.6 in UL
435 2075 to 6.8 ng/g d.w. in UNICT 3332, and geranyl acetone amounts showed a similar distribution,
436 ranging from 0.1 ng/g d.w. in UNICT 3332 to 1.9 in UL 2075 (Table 4). UNICT 3332 and UNICT
437 375 had the lowest β–ionone amounts (0.3 ng/g d.w.), while UL 2075 had the highest (4.1 ng/g d.w.)
438 (Table 4).

439 Few literature data are available on kale volatile profiles, especially from a quantitative point of view.
440 Previous studies deal with changes in volatile profiles induced by processing (Wibowo et al., 2019;

441 Oh and Cho, 2021) or pest attack (Fernandes et al., 2010). Among the compounds found in this work,
442 (*E*)-2-hexenal, allyl isothiocyanate, dimethyl trisulphide and phenylacetaldehyde were identified by
443 Wibowo et al. (2019) and Oh and Cho (2021) as odour-active compounds in processed kale, while
444 some compounds found by Fernandes et al. (2010), such as (*E*)-2-hexenal, allyl isothiocyanate,
445 dimethyl trisulphide, and 6-methyl-5-hepten-2-one, were consistent with the volatile profile here
446 studied.

447 *3.6 Chlorophylls (Chl) and total carotenoids*

448 As reported in Table 5, the genotype UL 5010 distinguished by the highest concentration in
449 chlorophylls, both Chl a and Chl b at 199.8 and 71.1 mg/100 g d.w. respectively, followed by UL
450 2066 with 167.4 and 59.2 mg/100 g d.w. UNICT 3332 and UNICT 4946 showed lower amounts,
451 around 85 mg/100 g d.w. for Chl a and 25 mg/100 g d.w. for Chl b (Table 5). The differences in Chl
452 a and Chl b amounts observed among genotypes were significant. The detected levels are lower than
453 those reported in other studies (Kopsell et al., 2007; Akdaş and Bakkalbasi, 2017; Ljubej et al., 2021).
454 Previous research by Kopsell et al. (2007) indicate genotype as an important variation factor for
455 carotenoids concentration in kale; however, across the board, kale has been recognised as an
456 outstanding source for these secondary metabolites (Perry et al., 2009). In the analysed genotypes
457 some significant differences were found in total carotenoid content, which showed the same
458 distribution as chlorophylls, as evidenced by the positive correlation observed between both Chl a
459 ($r=0.95$, $P=0.000$) and Chl b ($r=0.88$, $P=0.000$). The carotenoid levels (Table 5), ranging from 11.9
460 in UNICT 3332 to 27.9 in UL 5010, were higher than those measured in other studies (Kopsell et al.,
461 2007; Akdaş and Bakkalbasi, 2017). Ferioli et al. (2013) carried out a cross-country study on
462 phytochemical levels in local kale populations; compared to that study, the genotypes analysed in this
463 work ranked lower for chlorophylls and higher for carotenoids.

464 *3.7 Tocopherols*

465 Kale is considered a rich source of essential vitamins. The present work included an analysis of
466 tocopherols (Table 6). In all genotypes, α-tocopherol was the prevalent form. Total average
467 tocopherols (α+δ+γ) ranged from 10.5 (in UNICT 3332) to 22.8 mg/100 g d.w (in UNICT 375).
468 Except for UNICT 3332, which possessed the lowest amount of each tocopherol form, overall higher
469 levels of α- and total tocopherols were found in UNICT genotypes, while γ- and δ- amounts were
470 higher in UL genotypes. UNICT 375 was higher in α-tocopherol (22.0 mg/100 g d.w.), while the
471 higher amounts of γ- and δ- were found in UL 2075, 1.7 and 0.9 mg/100 g d.w. respectively. High
472 variability among plants of the same genotype, especially in genotypes UL 5010 and UL 2066 was
473 observed, as shown by high values of standard deviations (Table 6). The observed distribution and
474 amounts are in accordance with those reported by Korus (2020, 2022). Total tocopherols content
475 significantly correlated with sucrose (r = 0.45, P = 0.039) and total carotenoids (r = 0.47, P = 0.033).

476 *3.8 Phenolic compounds*

477 The UPLC-ESI-MS analysis led to the identification of 43 phenolic compounds (Table 7), which
478 have been grouped into four main classes (Table S1): hydroxycinnamic acid derivatives (HAD)
479 flavonols, distinguished in quercetin (QD) and kaempferol derivatives (KD), and, finally,
480 hydroxycinnamoyl gentiobiosides (HCG), as shown in Figure 3, excluding the isorhamnetins
481 derivatives, present in trace amounts (Table S1). The composition of the phenolic profile did not show
482 great differences among the samples, and flavonols were the prevalent compounds in all genotypes.
483 The total phenols content, instead, significantly differed among genotypes, except for the kaempferol
484 derivatives group (Figure 3). However, wide variability among plants was observed, especially in the
485 UL genotypes (Table S1). Overall, UNICT genotypes had higher phenol levels. Total phenols content
486 ranged from 763.4 mg/100 g d.w. in UL 2075 to 1920.2 mg/100 g d.w. in UNICT 3332. As regards
487 the single compounds, a higher content of neo-chlorogenic acid was found in UNICT 3332 (130.3
488 mg/100 g d.w.). K-3-O-caffeoyldiglucoside-7-O-glucoside (higher content in UNICT 4946, 132.0-
489 166.5 mg/100 g d.w.), K-3-O-sinapoyldiglucoside-7-O-glucoside (higher content in UNICT 375,

490 113.3-153.0 mg/100 g d.w.) and K-3-O-sinapoyldiglucoside-7-O-diglucoside (higher content in
491 UNICT 4959, 63.8- 189.2 mg/100 g) were in most genotypes the prevalent phenols. The quercetin
492 sinapoyl glucosides (compounds nr. 32 and 33, Figure 1, Table 7) were found in high amounts (82.0-
493 197.1 mg/100 g d.w.) in UNICT 4946. Conversely, the lowest amounts of 32 and 33 were in UL2075
494 (19.2-126.9 mg/100 g d.w.) UL 2075 (Table S1). Among gentiobiosides, the most abundant
495 compounds were compounds nr. 39 and 42: UNICT 4959 was characterized by a higher amount of
496 39 (55.3-110.4 mg/100 g d.w.), while UNICT 3332 possessed a notable content of 42 (75.2-137.4
497 mg/100 g d.w).

498 Phenolic compound profiles agreed with previously reported data (Olsen et al., 2009; Hagen et al.,
499 2009; Ferioli et al., 2013); the sum of all quercetin and kaempferol derivatives indicated a very high
500 flavonol content (above 1000 mg/100 g. d.w., except for UL 2075 and UL 2066), higher than those
501 reported by Hagen and colleagues (2009). Moreover, total phenolic content was in accordance with
502 the ranges found by Ferioli et al (2013). Interestingly, the quali-quantitative comparison with data
503 reported by Soengas et al. (2012) reported a perfect match both with regard to the presence of different
504 phenol classes and to their concentration. It could be resumed that kale is an interesting source of
505 soluble phenolic phytochemicals, mainly ascribed to the class of flavonols. The literature data
506 describe higher amounts of total flavonoids in collard kale than broccoli, 20-50 mg/100g fw
507 (Radošević et al., 2017): these values resulted much higher in the present study, ranging from around
508 700 to 1500 mg/100g dw for the sum of kaempferol and quercetin derivatives (Figure 3), meaning
509 104-224 mg/100g fw, if assumed a presence of dry matter of 15 % (Lefsrud et al., 2008).

510 *3.9 Principal Component Analysis (PCA)*

511 To summarize and highlight the main differences among genotypes, PCA was performed, including
512 all genotypes and the average values of each significantly different biochemical parameter. Five PCs
513 were extracted, together accounting for 98.5% of the variance. Figure 4 shows a biplot of the first two
514 components which explain 74.5% of the variance. As regards the scores, PC1 divides UL genotypes,

515 with positive values, from UNICT genotypes, with negative values. Loadings for volatile
516 apocarotenoids, isothiocyanates, hexanal and delta-tocopherols have negative PC1 and PC2 values
517 and are associated with UL 2075, while other UL genotypes have positive PC2 values and are
518 associated with organic acids, SSC, fructose, high DP sugars, dimethyl trisulfide, hexanal and
519 chlorophylls. UNICT 3332 has a positive PC2 value and is associated with phenol compounds and
520 total thiols, while other UNICT genotypes have negative PC2 values and are associated with SRA
521 and I3C.

522

523 **4. Conclusions**

524 The genotypes analysed in this work are part of the BRESOV *Brassica* core collection; however, they
525 originate from two collections selected in different environments (Liverpool, UL, and Catania,
526 UNICT). The selected genotypes were cultivated under organic conditions to highlight differences in
527 both morphotype and genetic profile. As part of this, the aim of the present work was to select suitable
528 accessions that can be used directly for organic growing, and to contribute towards organic breeding
529 programmes for enhanced nutritional content. Interestingly, it has been determined that the two
530 groups of genotypes have different biochemical characteristics which are maintained when grown in
531 the same controlled environment. This was surprising since the major phytochemicals studied are
532 secondary metabolites, which can be largely influenced by environmental conditions. This study
533 shows that the breeding history of germplasm can strongly determine their biochemical profile.
534 Moreover, this work also highlights that kale crops with adequate to elevated levels of phytochemicals
535 potentially relevant for human health can be produced under organic growing conditions.

536 The presence of considerable concentrations of potential biologically active sulphur compounds,
537 mainly in the forms of sulphoxides, reduced thiols and isothiocyanates, as well as the relevant
538 concentration of ascorbic acid and phenols, makes these genotypes of kale very interesting material
539 with appropriate nutritional characteristics related to *Brassica* spp.

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Figure

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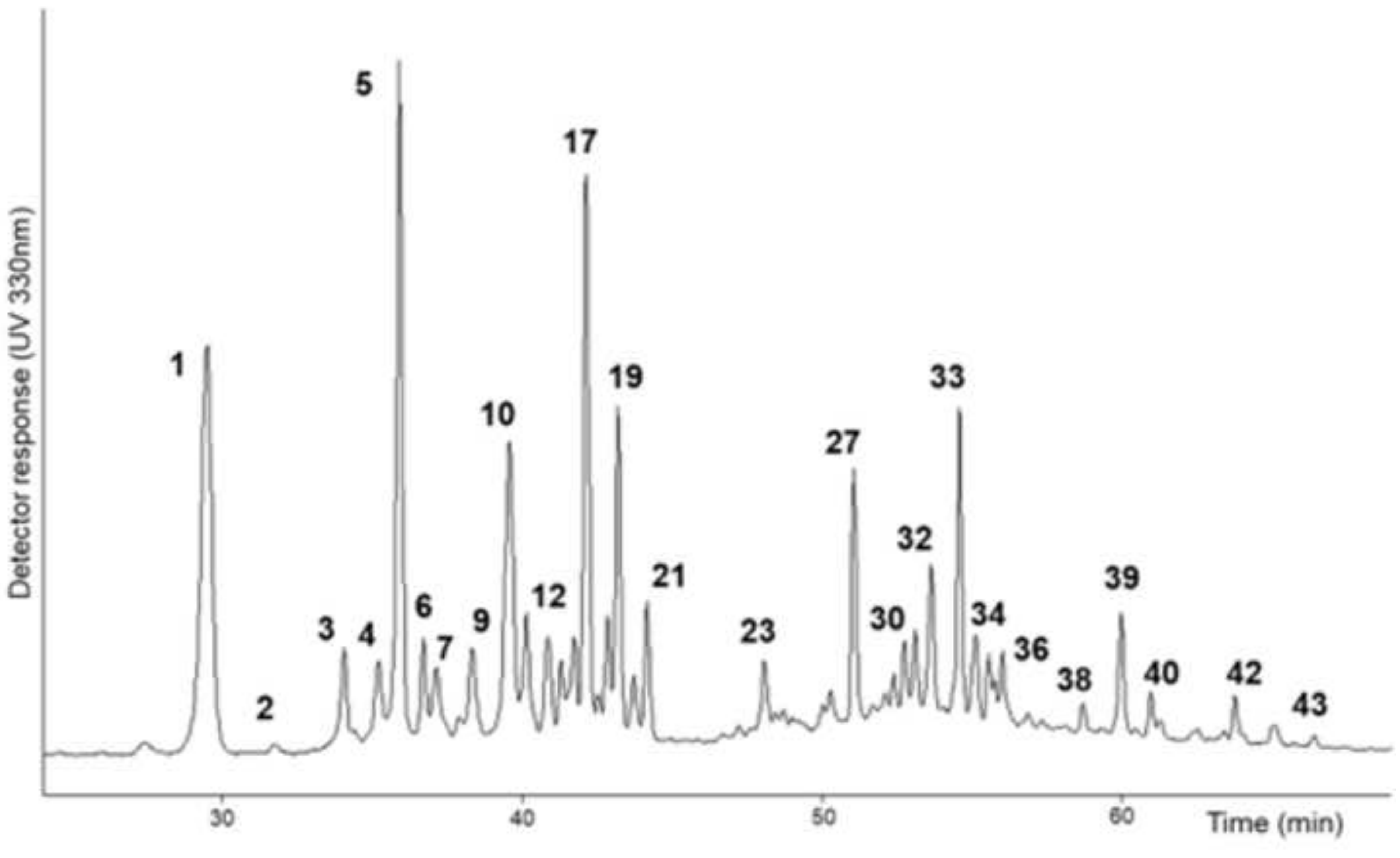

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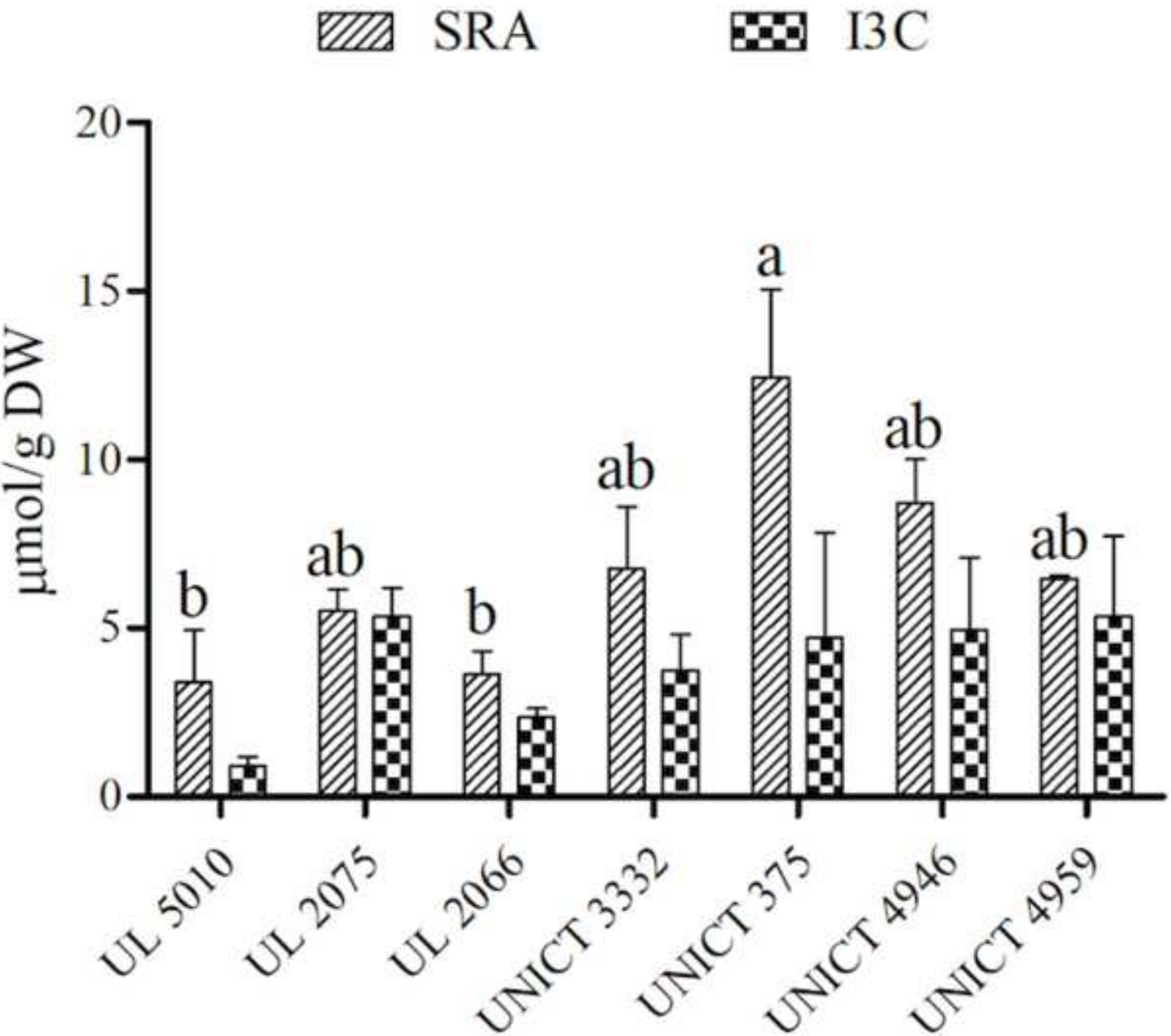

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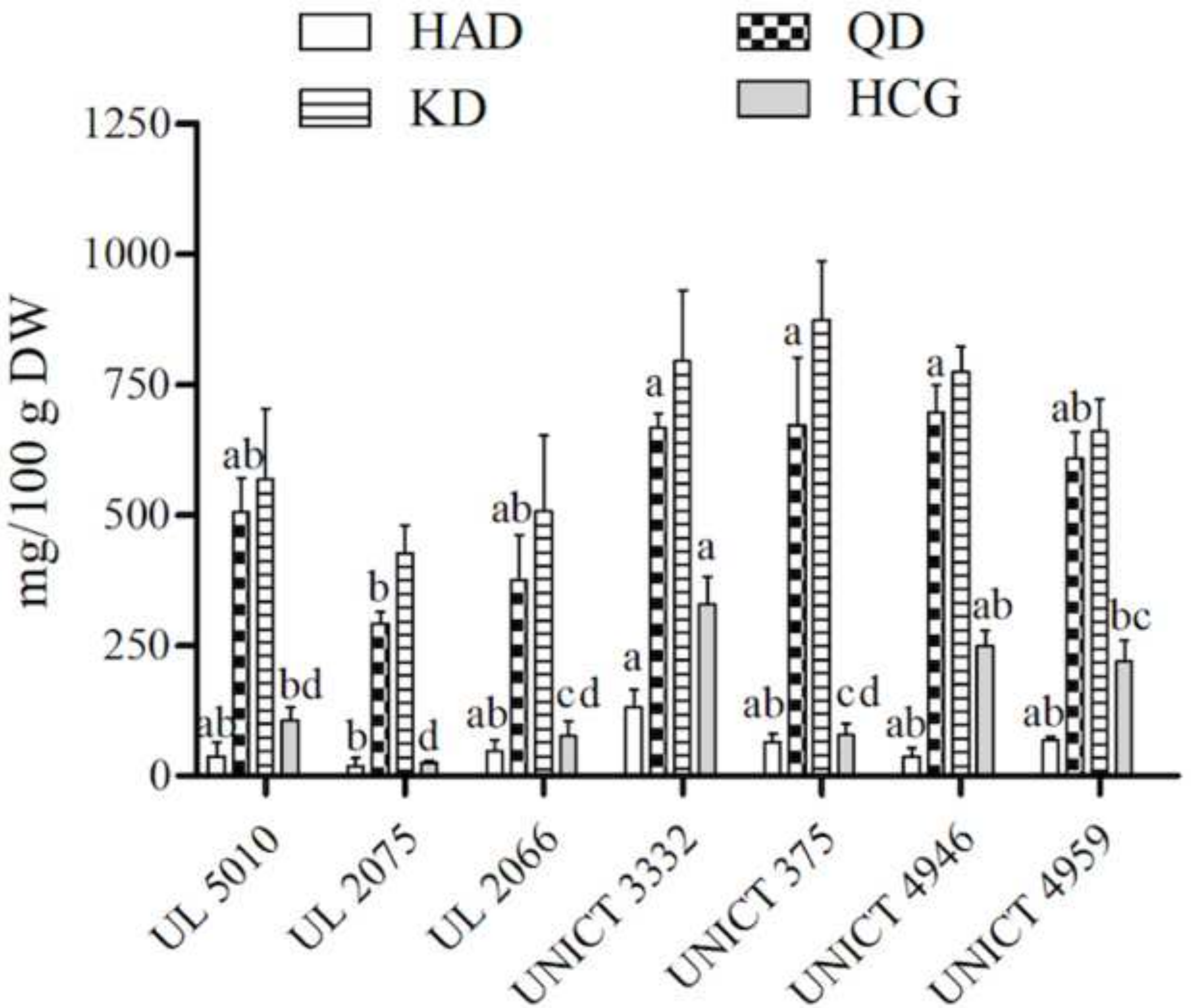

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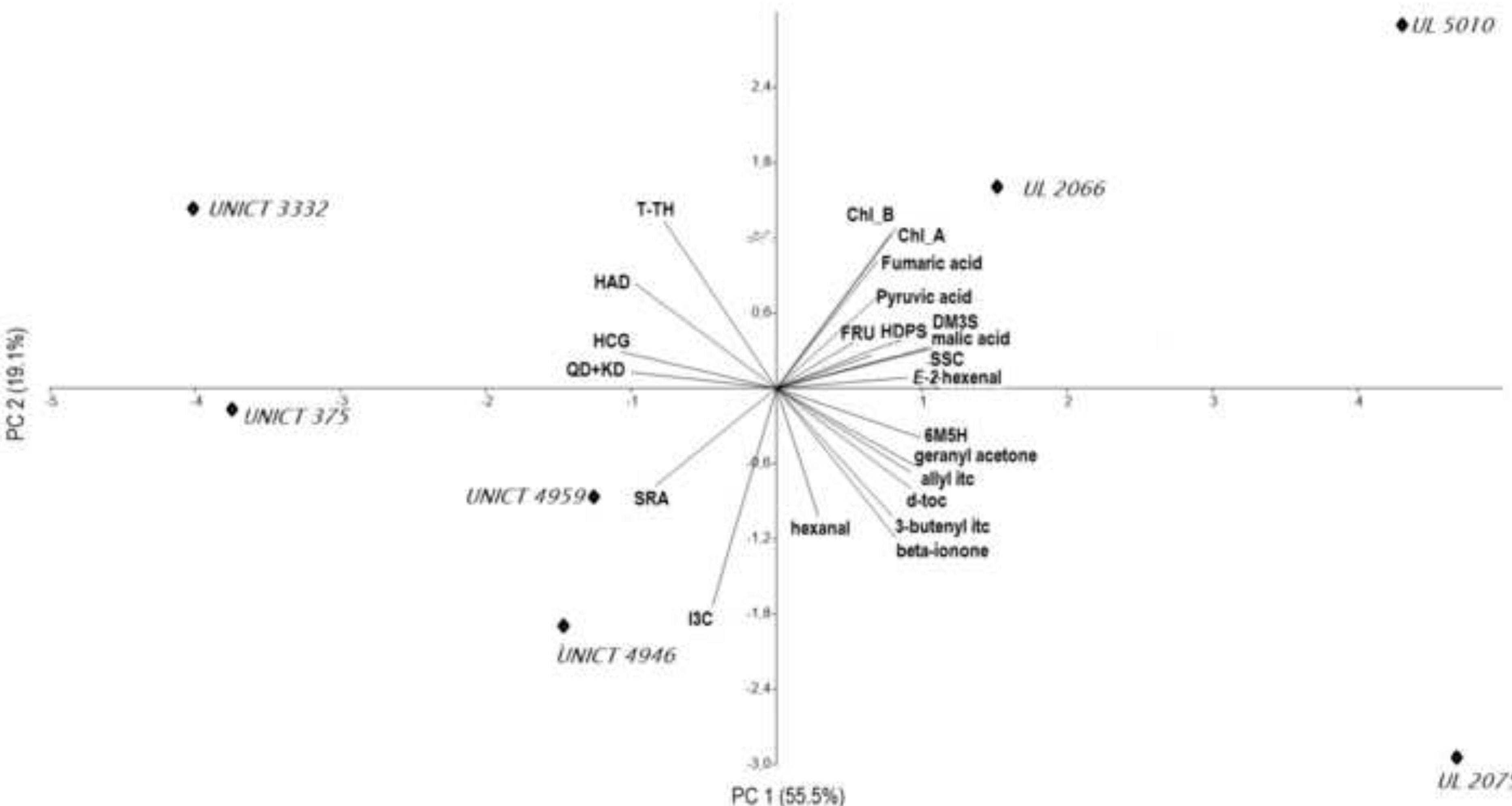

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Figure 1. HPLC chromatogram (UV 330 nm) of raw extract of the *Brassica oleracea* var. *acephala* sample. For compound identification see Table S1.

Figure 2. Content (μmol/g d.w.) of sulforaphane (SRA) and indole-3-carbinol (I3C) in the seven genotypes of kale. Data are represented as means and error bars refer to the standard deviation. Different letters above each data series indicate significant differences among genotypes according to the Tukey's HSD test.

Figure 3. Content (mg/100 g d.w.) of total hydroxycinnaic acid derivatives (HAD), flavonol derivatives (quercetin, QD, and kaempferol, KD, derivatives) and hydroxycinnamoyl gentiobiosides (HCG) in the seven genotypes of kale. Data are represented as mean and bars refer to the standard deviation. Different letters above each data series indicate significant differences among genotypes according to the Tukey's HSD test.

Figure 4. PCA of average values of main parameters: biplot of the first two components.

(Footnote): T-TH, total thiols; HDPS, high degree polymerization sugars; d-toc, delta-tocopherol; FRU, fructose; itc, isothiocyanate; DM3S: dimethyl trisulphide; 6M5H, 6-methyl-5-hepten-2-one

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Table 1. List of the seven selected genotypes of *B. oleracea* cv *acephala* analysed in the present work.

Species	***Trivial name***	***Genebank code***	***BRESOV id code****	***Plant codes***	***Seeds production***
B. oleracea cv *acephala*	kale	UL 5010	B1800343	93-1, 93-3, 93-5	University of Liverpool, UK
B. oleracea cv *acephala*	kale	UL 2075	B1800315	55-1, 55-3, 55-5	University of Liverpool, UK
B. oleracea cv *acephala*	kale	UL 2066	B1800309	100-1, 100-2, 100-4	University of Liverpool, UK
B. oleracea cv *acephala*	kale	UNICT 3332	B1800091	145-1, 145-4, 145-7	University of Catania, Di3A
B. oleracea cv *acephala*	kale	UNICT 375	B1800087	215-4, 215-6, 215-7	University of Catania, Di3A
B. oleracea cv *acephala*	kale	UNICT 4946	NA	218-1, 218-2, 218-6	University of Catania, Di3A
B. oleracea cv *acephala*	kale	UNICT 4959	B1800903	219-1, 219-7, 219-8	University of Catania, Di3A

*BRESOV id code lists all the accessions studied in the frame of the H2020 BRESOV, NA means not available.

Table 2. Soluble solids content (SSC, °Bx on d.w.), high DP sugars and single sugars content (g/100g d.w.) of *B. oleracea* cv *acephala*

Genotype	SSC			High DP sugars			Sucrose			Glucose			Fructose		
UL 5010	41.1	±	3.2 a	10.8	±	3.4a	0.1	±	0.0	3.8	±	2.7	2.8	±	0.8ab
UL 2075	40.5	±	2.3 ab	9.3	±	3.1ab	0.1	±	0.1	5.3	±	0.6	2.8	±	0.2ab
UL 2066	37.6	±	2.6 ab	6.0	±	0.5ab	0.1	±	0.0	9.0	±	1.9	4.5	±	0.6a
UNICT 3332	26.8	±	3.3 ab	5.4	±	1.4ab	0.0	±	0.0	3.9	±	2.1	1.8	±	0.4b
UNICT 375	27.4	±	3.5 ab	4.8	±	0.9ab	0.6	±	0.5	5.4	±	3.9	2.6	±	0.7b
UNICT 4946	29.3	±	6.9 ab	4.8	±	2.3ab	0.5	±	0.1	4.6	±	1.3	2.5	±	0.5b
UNICT 4959	26.2	±	10.3 b	4.2	±	2.6b	0.5	±	0.4	4.7	±	1.5	2.7	±	0.9b
P	**			*			ns			ns			**		

Results are reported as means ±standard deviation. Different letters on the same column indicate significant differences among genotypes according to the Tukey's HSD test. *P* significance: ns >0.05, * < 0.05, ** <0.01, *** <0.001

Table 3. Organic acids, ascorbic acid (AsA) and total thiols content (mg/100 g d.w.) of *B. oleracea* cv *acephala*

Genotype	Citric acid	Malic acid	Pyruvic acid	Fumaric acid	AsA	Total thiols	AsA/total thiols
UL 5010	2347 ± 286	3175 ± 210a	148 ± 54a	199 ± 13a	222.5 ± 25.5	106.6 ± 9.8 ab	2.1 ± 0.2 b
UL 2075	3743 ± 984	3072 ± 750a	154 ± 49a	63 ± 25b	243.8 ± 28.1	77.7 b ± 13.4 b	3.2 ± 0.7 a
UL 2066	1962 ± 351	1896 ± 376b	149 ± 43a	28 ± 7b	235.3 ± 34.3	110.7 ± 12.0 ab	2.1 ± 0.3 b
UNICT 3332	2693 ± 726	664 ± 232c	151 ± 43a	51 ± 46b	212.7 ± 28.0	119.9 a ± 19.6 a	1.8 ± 0.5 b
UNICT 375	3186 ± 1137	736 ± 427c	36 ± 7b	24 ± 2b	219.3 ± 8.6	112.9 ± 9.2 ab	2.0 ± 0.2 b
UNICT 4946	3109 ± 1555	454 ± 175c	60 ± 23ab	36 ± 22b	215.6 ± 1.9	105.8 ± 9.1 ab	2.0 ± 0.2 b
UNICT 4959	2800 ± 1026	601 ± 216c	81 ± 14ab	40 ± 13b	209.4 ± 10.0	100.7 ± 16.4 ab	2.1 ± 0.2 b
P	ns	***	**	***	ns	*	**

Results are reported as means ±standard deviation. Different letters on the same column indicate significant differences among genotypes according to the Tukey's HSD test. *P* significance: ns >0.05, * < 0.05, ** <0.01, *** <0.001

Table 4. Volatile compounds amount (ng/g d.w.) in *B. oleracea* cv *acephala*

Compound	UL 2075	UL 5010	UL 2066	UNICT 3332	UNICT 375	UNICT 4946	UNICT 4959	*P*
3-Methyl butanal	19.8 ± 9.0a	1.5 ± 0.6b	10.0 ± 4.2ab	0.7 ± 0.2b	0.1 ± 0.0b	0.8 ± 0.1b	0.3 ± 0.1b	*
2-Methyl butanal	5.8 ± 0.3a	5.3 ± 2.3ab	2.1 ± 0.3b	0.8 ± 0.3 b	0.5 ± 0.2 b	6.0 ± 0.9 a	5.1 ± 1.3ab	**
Hexanal	8.3 ± 0.8b	6.5 ± 0.7bc	6.2 ± 0.3bc	1.0 ± 0.1c	2.0 ± 0.7c	17.1 ± 1.9a	8.4 ± 2.4b	***
(E)-2-Hexenal	63.1 ± 3.7ab	100.0 ± 27.9a	56.2 ± 5.0ab	10.4 ± 3.6b	10.5 ± 2.4b	68.1 ± 10.1a	65.6 ± 4.3a	***
Allyl isothiocyanate	141.3 ± 123.3	62.8 ± 51.5	4.5 ± 1.8	1.5 ± 0.8	0.7 ± 0.2	1.0 ± 0.2	2.2 ± 0.6	ns
Dimethyl trisulfide	15.1 ± 9.5ab	23.9 ± 7.8a	10.6 ± 1.3ab	1.5 ± 0.5ab	0.5 ± 0.0b	7.7 ± 0.9ab	7.4 ± 1.4ab	*
3-Butenyl isothiocyanate	539.0 ± 381.0	104.4 ± 58.2	15.4 ± 8.1	1.7 ± 1.2	0.4 ± 0.3	14.8 ± 11.0	7.1 ± 5.7	ns
6-Methyl-5-hepten-2-one	6.4 ± 1.1ab	6.8 ± 0.3a	3.8 ± 0.7b	0.6 ± 0.2c	0.7 ± 0.1c	5.3 ± 0.4ab	4.3 ± 0.5ab	***
Phenylacetaldehyde	3.1 ± 0.1ab	4.1 ± 0.4a	5.9 ± 1.2a	0.5 ± 0.1b	0.6 ± 0.0b	4.5 ± 0.2a	5.3 ± 0.9a	***
Phenylethanone	3.5 ± 0.9ab	3.9 ± 0.5a	1.9 ± 0.3abc	0.2 ± 0.0c	0.3 ± 0.1c	2.4 ± 0.4abc	1.6 ± 0.1bc	***
3,5-Octadien-2-one	3.6 ± 2.1	1.2 ± 0.6	0.8 ± 0.3	0.1 ± 0.0	0.3 ± 0.1	3.7 ± 0.6	2.2 ± 0.9	ns
4,5-epithiovaleronitrile	23.8 ± 18.0	3.2 ± 1.4	7.0 ± 2.0	0.5 ± 0.0	0.3 ± 0.0	6.2 ± 1.6	4.2 ± 2.8	ns
Nonanal	6.2 ± 2.6a	1.3 ± 0.5ab	1.1 ± 0.3ab	0.2 ± 0.1b	0.2 ± 0.0b	1.7 ± 0.4ab	1.0 ± 0.2b	*
2,6-Dimethylcyclohexanol	3.3 ± 0.4abc	4.7 ± 1.0a	4.3 ± 1.9ab	0.6 ± 0.2bc	0.3 ± 0.0c	2.0 ± 0.1abc	2.2 ± 0.2abc	**
Geranyl acetone	1.9 ± 0.3a	1.5 ± 0.2a	1.2 ± 0.2ab	0.1 ± 0.0c	0.3 ± 0.1bc	1.3 ± 0.3a	0.9 ± 0.1abc	***
β-Ionone	4.1 ± 1.2a	2.0 ± 0.4ab	1.8 ± 0.4ab	0.3 ± 0.1b	0.3 ± 0.0b	2.6 ± 0.2ab	2.7 ± 0.4ab	**

Results are reported as means ±standard deviation. Different letters on the same row indicate significant differences among genotypes according to the Tukey's HSD test. *P* significance: ns >0.05, * < 0.05, ** <0.01, *** <0.001. Compound identification is described in Table S3.

Table 5. Chlorophyll a, chlorophyll b and total carotenoid contents (mg/100 g d.w.) in *B. oleracea* cv *acephala*

Genotype	Chl a			Chl b			Total carotenoids		
UL 5010	199.8	±	78.3a	71.1	±	22.1 a	27.9	±	14.0
UL 2075	114.9	±	4.8 ab	39.9	±	0.9 abc	16.7	±	1.9
UL 2066	167.4	±	25.5ab	59.2	±	6.3 ab	25.3	±	5.4
UNICT 3332	85.4	±	22.2b	29.8	±	9.1 bc	11.9	±	1.0
UNICT 375	106.8	±	16.6ab	31.6	±	17.9 bc	17,9	±	3.2
UNICT 4946	86.5	±	28.0b	25.0	±	8.7 c	14.2	±	3.8
UNICT 4959	125.1	±	40.4ab	39,6	±	18.6 abc	21.3	±	6.5
P		*			**			ns	

Results are reported as means ±standard deviation. Different letters on the same column indicate significant differences among genotypes according to the Tukey's HSD test. P significance: ns >0.05, * < 0.05, ** <0.01, *** <0.001

Table 6. Tocopherols content in *B. oleracea* cv *acephala* (mg/100 g d.w.)

Genotype	α-tocopherol			δ-tocopherol			γ-tocopherol			Total tocopherols		
UL 5010	15.0	±	10.1	0.5	±	0.4	1.5	±	1.2	16.9	±	11.7
UL 2075	11.1	±	3.2	0.9	±	0.4	1.7	±	0.98	13.7	±	4.2
UL 2066	14.8	±	9.3	0.5	±	0.2	1.2	±	0.4	16.5	±	11.7
UNICT 3332	10.0	±	4.9	0.2	±	0.2	0.3	±	0.2	10.5	±	5.0
UNICT 375	22.0	±	5.3	0.3	±	0.2	0.6	±	0.2	22.8	±	5.5
UNICT 4946	20.4	±	5.7	0.3	±	0.2	0.7	±	0.4	21.3	±	6.0
UNICT 4959	21.2	±	8.4	0.4	±	0.3	0.6	±	0.1	22.2	±	8.5
P	ns			ns			ns			ns		

Results are reported as means ±standard deviation. Different letters on the same column indicate significant differences among genotypes according to the Tukey's HSD test. P significance: ns >0.05, * < 0.05, ** <0.01, *** <0.001

Table 7. Chemical characteristics of the tentatively identified phenolic components detected in the *Brassica oleracea* cv *acephala* samples.

#	UV λ_{max} (nm)	$[M+H]^+/[M-H]^-$	Diagnostic MS^2 negative ions, m/z (%)	Formula	MW	Compound[a]
1	240, 298, 327	-/353	191(100); 179(43); 135(10)	$C_{16}H_{18}O_9$	354	Caffeoylquinic acid
2	226, 310	-/337	191(9); 163(100); 119(5)	$C_{16}H_{18}O_8$	338	*p*-Coumaroylquinic acid
3	254, 266sh, 352	789/787	625(100); 463(21); 301(79)	$C_{30}H_{40}O_{22}$	788	Q-3-*O*-diglucoside-7-*O*-glucoside
4	256, 266, 334	981/979	817(100); 787(85); 625(51); 301(3)	$C_{43}H_{48}O_{26}$	980	Q-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside
5	265, 316sh, 346	773/771	609(100); 447(35); 285(38)	$C_{33}H_{40}O_{21}$	772	K-3-*O*-diglucoside-7-*O*-glucoside
6	252, 266sh, 352	951/949	787(100); 625(95); 301(7)	$C_{39}H_{50}O_{27}$	950	Q-3-*O*-diglucoside-7-*O*-diglucoside
7	256, 266, 332	951/949	787(100); 625(61); 463(3); 301(3)	$C_{42}H_{46}O_{25}$	950	Q-3-*O*-caffeoyldiglucoside-7-*O*-glucoside
8	256, 266, 332	1143/1141	949(100); 817(18); 625(33); 301(1)	$C_{49}H_{58}O_{31}$	1142	Q-3-*O*-hydroxyferuloyldiglucoside-7-*O*-diglucoside
9	255, 266, 334	1113/1111	949(100); 787(52); 301(2)	$C_{48}H_{56}O_{30}$	1112	Q-3-*O*-caffeoyltriglucoside-7-*O*-glucoside
10	266, 332	965/963	801(100); 609(4); 285(2)	$C_{43}H_{48}O_{25}$	964	K-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside
11	266, 332	935/933	771(100); 609(5); 285(2)	$C_{42}H_{46}O_{24}$	934	K-3-*O*-caffeoyldiglucoside-7-*O*-glucoside
12	256, 266, 334	995/993	831(100); 787(88); 625(54); 301(3)	$C_{44}H_{50}O_{26}$	994	Q-3-*O*-sinapoyldiglucoside-7-*O*-glucoside
13	256, 266, 336	965/963	801(100); 787(45);625(28); 301(2)	$C_{43}H_{48}O_{25}$	964	Q-3-*O*-feruloyldiglucoside-7-*O*-glucoside
14	255, 266, 334	1157/1155	993(10); 949(100); 831(20); 625(38)	$C_{50}H_{60}O_{31}$	1156	Q-3-*O*-sinapoyltriglucoside-7-*O*-glucoside
15	266, 332	1097/1095	933(5); 771(100); 609(21); 285(1)	$C_{48}H_{56}O_{29}$	1096	K-3-*O*-caffeoyldiglucoside-7-*O*-diglucoside
16	256, 265, 334	1127/1125	963(5); 949(100); 801(48); 625(35)	$C_{49}H_{58}O_{30}$	1126	Q-3-*O*-feruloyltriglucoside-7-*O*-glucoside
17	266, 332	979/977	815(100); 609(5); 285(2)	$C_{44}H_{50}O_{25}$	978	K-3-*O*-sinapoyldiglucoside-7-*O*-glucoside
18	266, 332	1141/1139	977(10); 815(100);609(25); 285(1)	$C_{50}H_{60}O_{30}$	1140	K-3-*O*-sinapoyldiglucoside-7-*O*-diglucoside
19	266, 332	949/947	785(100); 609(7); 285(2)	$C_{43}H_{48}O_{24}$	948	K-3-*O*-feruloyldiglucoside-7-*O*-glucoside (25 isomer)
20	266, 332	1111/1109	947(2); 785(100); 609(26); 285(1)	$C_{49}H_{58}O_{29}$	1110	K-3-*O*-feruloyldiglucoside-7-*O*-diglucoside
21	266, 319	919/917	755(100); 609(3); 285(2)	$C_{42}H_{46}O_{23}$	918	K-3-*O*-coumaroyldiglucoside-7-*O*-glucoside
22	266, 319	1081/1079	755(100); 609(16); 285(1)	$C_{42}H_{46}O_{23}$	1080	K-3-*O*-coumaroyldiglucoside-7-*O*-diglucoside
23	254, 266, 335	819/817	625(100); 463(3); 301(2)	$C_{37}H_{38}O_{21}$	818	Q-3-*O*-hydroxyferuloyldiglucoside
24	256, 265sh, 353	627/625	463(72); 301(100)	$C_{27}H_{30}O_{17}$	626	Q-3-*O*-diglucoside
25	256, 266, 335	789/787	625(100); 463(1); 301(2)	$C_{36}H_{36}O_{20}$	788	Q-3-*O*-caffeoylglucoside-7-*O*-glucoside
26	266, 332	949/947	785(100); 609(3); 285(3)	$C_{43}H_{48}O_{24}$	948	K-3-*O*-feruloyldiglucoside-7-*O*-glucoside (18 isomer)
27	265, 295sh, 348	611/609	429(91);285(100)	$C_{27}H_{30}O_{16}$	610	K-3-*O*-diglucoside

28	266, 331	773/771	609(100); 285(5)	$C_{36}H_{36}O_{19}$	772	K-3-*O*-caffeoyldiglucoside
29	266, 331	817/815	609(100); 285(7)	$C_{38}H_{40}O_{20}$	816	K-3-*O*-sinapoyldiglucoside
30	266, 317	757/755	609(100); 285(11)	$C_{36}H_{36}O_{18}$	756	K-3-*O*-coumaroyldiglucoside
31	265, 331	787/785	609(100); 285(4)	$C_{37}H_{38}O_{19}$	786	K-3-*O*-feruloyldiglucoside
32	256, 266, 334	1363/1361	1199(100); 1155(45); 993(62); 787(6)	$C_{61}H_{70}O_{35}$	1362	Q-3-*O*-disinapoyltriglucoside-7-*O*-glucoside
33	256, 266, 332	1333/1331	1169(100); 1155(28); 1125(11); 930(35); 963(11)	$C_{60}H_{68}O_{34}$	1332	Q-3-*O*-sinapoylferuloyltriglucoside-7-*O*-glucoside
34	266, 348	449/447	285(100)	$C_{21}H_{20}O_{11}$	448	K-3-*O*-glucoside
35	256, 354	479/477	285(100)	$C_{22}H_{22}O_{12}$	478	I-3-*O*-glucoside
36	265, 365	449/447	285(100)	$C_{21}H_{20}O_{11}$	448	K-7-*O*-glucoside
37	256, 266sh, 368	479/477	285(100)	$C_{22}H_{22}O_{12}$	478	I-7-*O*-glucoside
38	240, 332	777[b]/753	529(100)	$C_{34}H_{42}O_{19}$	754	1,2-disinapoyl gentiobioside
39	240, 332	747[b]/723	529(18); 499(100)	$C_{33}H_{40}O_{18}$	724	1-sinapoyl-2-feruloyl gentiobioside
40	240, 331	717[b]/693	499(100)	$C_{32}H_{48}O_{17}$	694	1,2-diferuloyl gentiobioside
41	239, 332	983[b]/959	735(100); 511(18)	$C_{45}H_{52}O_{23}$	960	1,2,2'-trisinapoyl gentiobioside
42	239, 331	953[b]/929	735(4); 705(100); 511(7)	$C_{44}H_{50}O_{22}$	930	1,2'-disinapoyl-2-feruloyl gentiobioside
43	240, 332	923[b]/899	705(100); 675(38); 511(15)	$C_{43}H_{48}O_{21}$	900	1-sinapoyl-2,2'-diferuloyl gentiobioside

[a]K: kaempferol; Q: quercetin; I: isorhamnetin; [b][M+Na]+ for gentiobiosides

Table S2. Identification of volatile compounds listed in Table 2.

Compound	KI calc	KI tab (NIST)	MW	MS spectrum*
3-Methyl butanal	625	634	86	44(100), 58(50), 57(40), 41(40), 86(15)
2-Methyl butanal	645	639	86	44(100), 41(95), 43(90), 58(70), 86(10)
Dimethyl disulphide	717	718	94	94(100), 79(50), 45(40), 46(30), 61(30)
Hexanal	774	777	100	44(100), 56(100), 41(75), 57(70), 43(70)
(*E*)-2-Hexenal	826	830	98	41(100), 55(90), 69(90), 83(80), 39(60)
Allyl isothiocyanate	849	846	99	99(100), 41(50), 39(50), 72(30)
Dimethyl trisulphide	944	944	126	126(100), 45(40), 79(30), 111(20)
3-Butenyl isothiocyanate	947	951	113	72(100), 113(50), 55(30), 41(30), 39(20)
6-Methyl-5-hepten-2-one	964	966	126	43(100), 108(60), 41(50), 69(50), 126(10)
Phenylacetaldehyde	1006	1007	120	91(100), 92(40), 120(30), 85(20), 51(10)
Phenylethanone	1035	1044	120	105(100), 77(60), 43(50), 120(40), 51(30)
3,5-Octadien-2-one	1044	1046	124	95(100), 43(75), 81(50), 53(30), 124(20)
4,5-Epithiovaleronitrile	1062	1075	113	113(100), 45(50), 73(40), 60(30), 67(20)
Nonanal	1063	1078	142	57(100), 41(90), 43(90), 56(80), 124(10)
2,6-Dimethylcyclohexanol	1087	1098	128	71(100), 68(30), 95(25), 110(20), 128(10)
Geranyl acetone	1430	1428	194	43(100), 69(60), 41(40), 125(20), 136(20)
β-Ionone	1464	1466	192	177(100), 149(50), 43(50)

* main product ion fragments and relative percentage amounts; m/z range 39-400

Table S1. Quantitative evaluation (mg/100g DW) of the tentatively identified polyphenols detected in the *Brassica oleracea cv acephala* samples. See Table 7 for compounds identification. HAD: hydroxycinnamic derivatives, QD: quercetin derivatives, KD: kaempferol derivatives, HCG: hydroxycinnamic gentiobiosides.

		UL 5010, kale			**UL 2075, kale**			**UL 2066, kale**			***B. oleracea* acephala Isnello BH50**			***B. oleracea* acephala BH25 (Portogallo)**			***B. oleracea* acephala BH96**			***B. oleracea* acephala BH100 (Galati Mamertino)**		
		93_5	93_3	93_1	55_5	55_3	55_1	100_1	100_2	100_4	145_1	145_4	145_7	215_7	215_6	215_4	218_1	218_6	218_2	219_1	219_7	219_8
#	**HAD**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Caffeoylquinic acid	90.26	9.61	11.48	49.73	4.50	1.16	9.03	44.51	85.36	187.86	134.98	67.94	60.30	41.61	93.82	62.13	5.50	44.24	61.72	59.35	83.35
2	*p*-Coumaroylquinic acid	0.31	1.57	0.92	0.53	0.39	0.08	0.24	0.77	1.52	0.23	0.08	2.50	0.12	0.08	0.02	0.31	0.27	0.69	0.28	0.24	0.04
	QD																					
3	Q-3-*O*-diglucoside-7-*O*-glucoside	23.33	12.09	46.17	19.58	37.12	42.56	13.38	24.49	41.53	10.08	7.66	12.45	25.94	99.19	96.29	29.75	12.66	1.95	5.52	7.61	9.97
4	Q-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside	22.96	56.06	129.15	25.92	91.64	145.66	56.67	24.95	61.09	13.72	38.77	39.07	15.82	91.90	54.42	14.22	49.27	52.56	56.00	47.65	42.11
6	Q-3-*O*-diglucoside-7-*O*-diglucoside	27.66	5.81		16.80	10.19	14.09	4.51	23.83	33.53	46.39	45.56	14.62	20.58	91.97	38.11	83.37	67.22	107.89	58.20	54.25	36.52
7	Q-3-*O*-caffeoyldiglucoside-7-*O*-glucoside	30.87	39.63	80.53	17.12	6.03		15.03	9.44	29.01	5.26	33.05	13.51	11.17	23.85	32.20	22.15	67.66	142.38	24.80	82.00	86.35
8	Q-3-*O*-hydroxyferuloyldiglucoside-7-*O*-diglucoside		19.42		11.14		6.43	4.46			54.32	118.75	94.44	6.58	32.58		137.83	29.72		57.59	0.00	65.16
9	Q-3-*O*-caffeoyltriglucoside-7-*O*-glucoside	7.21	8.24	11.82	6.09	7.17	3.71	8.22	1.99	22.02	9.53	22.91	30.50	2.86	5.24	13.05	15.43	33.73	57.00	13.91	28.27	23.55
12	Q-3-*O*-sinapoyldiglucoside-7-*O*-glucoside	48.37	86.70	94.81	44.53	6.20	4.74	41.70	26.05	45.60	58.82	80.27	107.28	48.22	98.06	103.37	46.48	5.46		74.13	17.69	87.16
13	Q-3-*O*-feruloyldiglucoside-7-*O*-glucoside	33.37	80.22	89.21	44.78	26.97	12.03	48.20	42.51	45.74	93.09	145.82	146.68	41.69	98.01	99.83	106.37	58.24	33.94	54.96	45.34	123.19
14	Q-3-*O*-sinapoyltriglucoside-7-*O*-glucoside	3.51	3.50	2.85	1.41	3.52	2.00	0.60	0.86	1.42	1.16	0.29	0.87	1.47	5.69	1.44	4.36	4.26	11.45	4.40	7.35	1.03
16	Q-3-*O*-feruloyltriglucoside-7-*O*-glucoside	20.38	43.08	31.09	18.40	9.39	27.35	19.16	7.89	32.76	46.44	112.81	58.82	21.52	113.59	50.21	87.44	36.75	30.12	85.09	71.82	102.23
23	Q-3-*O*-hydroxyferuloyldiglucoside	2.82	4.59	7.40	1.00	1.45		5.50	3.73	3.85	35.77	8.12	21.18	4.43	5.00	7.41	18.97	4.55	8.18	2.57	5.84	7.87
24	Q-3-*O*-diglucoside	13.37	8.35	2.61	12.73	9.34	11.90	7.45	9.35	9.04	30.40	12.90	36.69	58.09	32.05	19.71	12.84	10.10	10.84	14.66	16.85	15.67
25	Q-3-*O*-caffeoylglucoside-7-*O*-glucoside	2.58	5.78	8.38	2.31	2.17	8.43	3.57	2.94	1.66	71.98	8.96	13.57	12.96	3.59	6.92	12.93	5.97	6.82	4.45	7.33	19.26
32	Q-3-*O*-disinapoyltriglucoside-7-*O*-glucoside	61.46	56.11	81.85	28.07	21.20	20.61	42.30	43.95	85.03	137.95	54.67	27.11	139.10	96.45	110.26	82.01	115.99	197.10	71.12	99.07	55.96
33	Q-3-*O*-sinapoylferuloyltriglucoside-7-*O*-glucoside	115.43	24.36	42.16	48.81	14.25	22.80	19.19	63.66	125.85	24.25	21.88	7.18	11.90	50.10	100.69	32.23	72.70	90.30	24.81	63.98	28.58
	KD																					
5	K-3-*O*-diglucoside-7-*O*-glucoside	167.15	25.18	36.15	89.41	51.12	70.61	16.90	90.05	130.30	44.61	18.73	25.38	86.19	157.93	271.00	77.73	35.19	12.22	8.66	24.29	7.80
10	K-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside	35.76	15.63	20.58	23.62	9.24	4.77	12.22	17.27	27.56	95.14	74.13	26.48	6.03	49.65	61.22	44.85	48.64	11.93	36.09	54.03	55.77
11	K-3-*O*-caffeoyldiglucoside-7-*O*-glucoside	145.30	82.44	129.42	83.12	17.84	18.47	51.71	62.83	145.87	43.45	130.76	120.19	61.46	155.20	189.20	166.49	202.81	213.02	92.45	167.88	188.32
15	K-3-*O*-caffeoyldiglucoside-7-*O*-diglucoside	12.96	9.32	54.19	10.10	19.17	9.40	32.90	11.94	36.66	68.99		2.45	8.71	8.09	39.65	5.51	17.20	19.10	15.76	2.79	18.12
17	K-3-*O*-sinapoyldiglucoside-7-*O*-glucoside	160.67	64.22	78.41	102.89	38.51	49.46	34.80	92.83	154.48	85.41	54.48	103.49	113.25	77.53	152.95	65.00	47.93	36.00	22.33	55.80	59.48
18	K-3-*O*-sinapoyldiglucoside-7-*O*-diglucoside	32.65	35.62	35.93	34.40	29.55	20.63	31.38	35.39	40.14	55.41	55.99	51.60	33.15	119.56	60.27	123.85	83.38	49.19	171.63	63.76	189.15
19	K-3-*O*-feruloyldiglucoside-7-*O*-glucoside (25 isomer)	92.29	68.29	73.49	85.03	70.28	61.69	48.75	88.55	86.31	64.43	78.67	147.74	73.60	82.78	146.29	108.23	89.91	49.54	11.91	62.90	85.62
20	K-3-*O*-feruloyldiglucoside-7-*O*-diglucoside	19.59	0.28	7.07	9.75	2.82	2.75	3.47	4.26	18.85	21.66	33.90	22.60	2.46	14.95	2.83	19.52	9.33	12.93	15.41	7.72	18.58
21	K-3-*O*-coumaroyldiglucoside-7-*O*-glucoside	37.48	3.15	21.57	19.32	24.17	3.83	3.32	14.27	34.31	27.29	6.43	17.50		8.25	16.67	6.57	10.40	3.97	3.04	4.83	5.00
22	K-3-*O*-coumaroyldiglucoside-7-*O*-diglucoside	0.27	6.81	9.77	2.63	0.00	36.93	6.71	0.27	0.26	0.27	0.03	13.61				0.27			0.27	0.27	6.03
26	K-3-*O*-feruloyldiglucoside-7-*O*-glucoside (18 isomer)	12.47	4.76	1.36	4.84	5.44	2.00		5.01	7.76			12.04	24.12			0.00	3.64	3.24	0.00	4.52	12.25
27	K-3-*O*-diglucoside	43.66	11.24	6.46	27.88	50.15	81.11	7.98	25.71	32.18	88.13	14.23	24.74	140.40	50.83	71.45	30.19	41.75	20.66	2.08	28.38	16.57
28	K-3-*O*-caffeoyldiglucoside	2.65	3.29	36.34	8.01	4.25	1.94	1.84	4.11	5.67	131.15	35.70	10.51	79.51	4.65	24.79	20.13	85.68	109.78	12.68	79.81	48.34
29	K-3-*O*-sinapoyldiglucoside	7.24	1.80		2.92	4.16	2.13		5.58	4.41	195.36		17.35	71.86	2.29	13.66	5.79	29.39	33.25	81.61	26.47	17.72
30	K-3-*O*-coumaroyldiglucoside	10.48	6.73	11.01	3.05		1.06		6.73	18.23	97.92	91.86	24.67	40.58	23.71	13.16	104.02	37.80	79.43	64.81	32.49	7.57
31	K-3-*O*-feruloyldiglucoside	13.95	5.39		8.88	7.55	12.22	5.50	9.80	13.73	47.94	68.89	23.84	5.25	2.67	11.00	66.66	7.47	11.84	7.34	13.83	15.13
34	K-3-*O*-glucoside	13.23	10.10	10.13	12.00	16.29	18.11	6.78	13.61	12.08		5.26	1.31	10.71	9.38	18.67	11.93	25.55	25.11	11.31	20.47	21.64
36	K-7-*O*-glucoside	10.91	0.94				4.03						0.47			4.26						
	ISORHAMNETINS (FLAVONOLS)																					
35	I-3-*O*-glucoside	8.29	4.27	3.57	3.81		1.07	1.86	2.48	8.04		4.70	3.85		4.23	5.13	4.72	12.74	12.38		9.13	2.62
37	I-7-*O*-glucoside	2.03		1.51	1.60	3.76		1.19		0.73	7.12	4.95	2.72		1.74	2.08	1.44	11.01	16.40		2.98	1.45
	HCG																					
38	1,2-disinapoyl gentiobioside	6.96	13.57	20.00	3.17	3.63	4.10	12.25	4.49	17.86	34.38	23.98	14.49	19.03	6.87	18.09	16.30	14.09	18.66	20.71	8.98	13.31
39	1-sinapoyl-2-feruloyl gentiobioside	32.47	40.42	67.85	17.19	11.90	14.81	21.16	20.35	48.32	87.57	47.78	43.77	3.28	27.42	51.56	82.68	84.38	80.77	103.34	55.31	110.35
40	1,2-diferuloyl gentiobioside	11.17	7.45	12.71	5.46	1.91	4.01	2.33	12.44	10.43	37.38	27.70	16.05	6.66	5.48	5.35	47.55	50.26	53.56	55.23	29.19	88.13
41	1,2,2'-trisinapoyl gentiobioside	6.96	6.38	9.17							49.18	109.43	34.43	8.40	4.19	8.97	8.16	5.01	20.50	10.12	5.92	11.50
42	1,2'-disinapoyl-2-feruloyl gentiobioside	11.87	13.76	41.97	2.11	0.84	4.10	4.88	9.63	42.34	98.15	137.95	75.20	9.36	16.58	32.26	41.08	45.70	95.18	20.74	32.74	38.81
43	1-sinapoyl-2,2'-diferuloyl gentiobioside	9.62	4.20	3.96	3.29			5.35	5.33	13.31	40.55	62.38	45.58	1.71	7.21	4.52	19.53	21.44	39.77	12.65	14.38	25.68
Total		**1411.94**	**910.35**	**1333.07**	**913.42**	**624.12**	**752.80**	**612.52**	**873.87**	**1534.84**	**2248.74**	**1935.42**	**1576.50**	**1288.45**	**1730.15**	**2052.77**	**1847.04**	**1600.75**	**1823.87**	**1394.39**	**1423.49**	**1852.93**

The corresponding authors, on behalf of all other co-Authors, regarding the paper entitled "Insights into the phytochemical composition of selected genotypes of organic kale (*Brassica oleracea* L. var. *acephala*), submitted to "Journal of Food Composition and Analysis" declares that the data presented in the manuscript are all authentic and that they are all available, upon a reasonable request.

Sincerely,

Roberto Lo Scalzo (corresponding Author)

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