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Comparison of five agro-industrial waste-based composts as growing media for lettuce: Effect on yield, phenolic compounds and vitamin C

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ABSTRACT

Overall phenolic content in plants is on average higher in organic farming, including when renewable resources such as composts are used as soil amendments. In most cases, however, the composting process needs to be optimized to reach the desired outcome. Using composts obtained from chestnut, red and white grapes, olive and broccoli wastes, the relative antioxidative abilities of lettuces cultivated in greenhouse were examined. Results clearly coupled high phenolic levels with high yield in lettuce grown on the chestnut-based compost. A huge accumulation of phenolics was observed with the white grape-based compost, but this coincided with low yield. Three compounds were identified as discriminating factors between treated samples, namely quercetin 3-*O*-glucoside, luteolin 7-*O*-glucoside, and cyanidin 3-*O*-(6″-malonyl)-*β*-D-glucoside; these are also some of the compounds receiving health claims on lettuce consumption. On a negative note, all composts led to decreased vitamin C levels. Collectively, the data suggest that compost amendments can help add value to lettuce by increasing its antioxidant activity as compared to other organic resources.

1. Introduction

Lettuce (*Lactuca sativa* L.) farming practices have become much larger in scale in terms of growth facilities, growth seasons, and inputs. Nowadays, lettuce is one of the main crops grown in greenhouses worldwide; open-air-grown lettuce, however, still holds the highest portion of the market (Becker, Klaering, Kroh, & Krumbein, 2014; Durazzo et al., 2014; Li, Zhao, Sandhu, & Gu, 2010). Several varieties have been bred for different climates (Baslam, Morales, Garmendia, & Goicoechea, 2013; Nicolle et al., 2004), making lettuce available over the whole year. In terms of input source, conventionally – as opposed to organically-grown lettuce is still dominant (Heimler, Vignolini, Arfaioli, Isolani, & Romani, 2012; Liu et al., 2007). However, the share of organic land in lettuce production worldwide has steadily increased during the last decades.

The concept of organic agriculture was popularized because of the need to avoid synthetic chemical residues in foods (Smith-Spangler et al., 2012). There have been two other major arguments put forward for promoting organic agriculture i.e., more nutrients in the food produced and lower negative environmental impacts. There is robust evidence supporting the perception that the risk for contamination with pesticide residues is lower among organic than conventional produces. However, that appears to be nutritionally irrelevant since the levels found do not exceed maximum limits set by environmental protection agencies (Nicoletto, Santagata, Zanin, & Sambo, 2014). The long-held belief that organic foods are significantly more nutritious than conventional foods has been challenged by recent meta-analyses. Besides phenolics which were significantly higher in organic produces, no major differences were found in the nutrients contents of organic and conventional plant foods (Smith-Spangler et al., 2012). As for the third reason, it seems that organic agriculture is more environmental-friendly than its conventional counterpart; the concept of friendliness concerning two main aspects, which are soil management and greenhouse gases emissions (Pereira & Trindade, 2015).

As a consequence of the recognition of organic agriculture as an instrument of environmental policy, application of organic supplements is now widely promoted. A number of potential organic fertilizers have been identified in efforts to achieve more sustainable agriculture, and composts have proved to be solid alternatives to

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synthetic chemicals (Montemurro et al., 2015). With the increasing popularity of organic foods, strategies to improve the organic cultivation system using advanced technologies have also been developed. In the case of lettuce, growing evidence suggests that it is now possible under optimized conditions to achieve yields close to average conventional agriculture, as observed with olive mill waste – (Kelepesi & Tzortzakis, 2009), fruits/dregs distillery residues – (Nicoletto et al., 2014), olive pomace – (Montemurro et al., 2015), and posidonia-based composts (Grassi et al., 2015).

In addition, the quality of lettuce in response to compost amendments has been reported, with substantial proportions of studies showing either lower or higher nutrients contents compared with the conventional field. For example, both protein decreases (Montemurro et al., 2015) and increases (Nicoletto et al., 2014) have been observed. Lettuce cultivated with composted spent-coffee grounds showed enhanced carotenoids, but decreased vitamin E (Cruz et al., 2014). In the study by Nicoletto et al. (2014), the vitamin C content of lettuce was higher for all compost treatments.

While yield and nutrients still dominate lettuce improvement efforts in the composting industry, selection for antioxidant properties is slowly emerging. In the few studies published on the subject, garden-based composts were shown to increase phenolics in lettuce (Heimler et al., 2012), while the response varied depending on the compost concentration for fruits/dregs distillery residues – (Nicoletto et al., 2014), and spent-coffee grounds-based composts (Cruz et al., 2014). While the existing scientific evidence regarding a primary role for phenolics in imparting health-benefits on lettuce consumption is still unclear, scattered studies on dietary supplementation with phenolic-rich extracts from lettuce have demonstrated improvements in lipid and antioxidant profiles in neuronal PC-12 cells (Im et al., 2010), Caco-2 cells (Durazzo et al., 2014), J774A.1 monocyte/macrophage cells (Pepe et al., 2015), rodents (Cheng et al., 2014) and healthy humans (Serafini et al., 2002). It has been hypothesized that as the health effects of lettuce phenolics are better understood and established, contents of these compounds may become part of the requirements for organic products (Cheng et al., 2014).

Although lettuce reportedly provides relatively low levels of antioxidative phytochemicals (Caldwell, 2003), its high per capita consumption makes it a considerable contributor to the amount of antioxidants in the diet. On the other hand, studies have highlighted the importance of variation in factors such as cultivar, agronomic practices, climatic conditions, and storage conditions, as a key tool to obtain healthful and more nutritious food crops (Buer, Imin, & Djordjevic, 2010; Queiroz, Morais, & Nascimento, 2002). In that regard, composting could become a major component of strategies aiming at maximizing the levels of bioactive molecules in lettuce.

In this study, it was hypothesized that the contents of phenolics in the compost may have roles in the transport and synthesis of antioxidative compounds in lettuce. There is precedence for considering that possibility, as it has been recently demonstrated that flavonoids are selectively taken up from the roots and are capable of long-distance movement within the plant (Buer et al., 2010). To test that hypothesis, five plant food-based wastes coming from the olive, chestnut, grape, and broccoli industries were selected for composting. In Mediterranean countries, these industries generate large amounts of wastes, which are routinely dumped in landfill sites or incinerated, causing serious environmental concerns due to their high organic load (Kelepesi & Tzortzakis, 2009). It follows that a parallel environmental issue is the disposal of these agricultural wastes; and their use in compositing clearly represents a profitable recycling approach.

The objectives of this study were to (i) evaluate the five plant food-based composts as medium amendments in greenhouse organic lettuce production, and as alternatives to non-renewable organic substrates such as peat, and (ii) assess the impact of compost polyphenols on the phenolic composition, vitamin C and carotenoids contents of lettuce.

2. Materials and methods

2.1. Agro-industrial plant food-based wastes and composting set-up

In order to provide contrasting growing conditions for lettuce, five raw materials were used for composting. Each initial batch was assembled with wastes collected from privately owned industries located in the Vila Real area in Portugal: (i) broccoli stems and florets were collected because of their allegedly bio-fumigant properties; (ii) white grape rachis were obtained after separation of berries of *Vitis vinifera* L. var. Moscatel for fermentation; as was (iii) red grape rachis from *Vitis vinifera* L. var. Alfrocheiro; (iv) olive leaves, and (v) chestnut shells and peels were discarded from a 3-phase centrifugation mill, and an agro-food company, respectively. Wheat straw was provided by a neighbouring farm and was used as bulking agent during composting. After manually removing the non-biodegradable coarse part of the materials, the remaining matrix was crushed using a shredder (Yike 9FQ-360 straw hammer mill, Zhengzhou, China), and passed through a 40 mm sieve.

Composting was done at a specialised pilot composting plant built at UTAD. The composting process was optimized to have a final product containing close to 20 g/kg nitrogen. A windrow of 20 kg was prepared by piling the wastes with wheat straw in a 135-L reactor in the proportions 30:70 for broccoli and 40:60 for the other materials (dry matter – DM basis). A treatment was also prepared with only wheat straw. Mechanical aeration was done by air injection through a pump. Moisture was controlled weekly, and was maintained around 45–60% by adding water. The windrow was turned mechanically once a week during the most active biooxidative phase, and then every 15 days throughout the maturation period. The composting process was held until complete temperature stabilization ca. 5.5 months. One week after the end of the process, the compost was removed from the reactor and stored at 4 °C until used. In all cases, the final product showed a high degree of humification, and no phytotoxic effect on seed germination. The nitrogen contents measured were 23.48, 22.59, 23.77, 20.10, and 20.73 g/kg for broccoli, white grape, olive, red grape, and chestnut, respectively (data not shown), with no statistical differences, except between olive and red grape ($P < 0.05$). Portions of raw materials and composts were homogenized in a Tecator Cyclotec 1093 Mill (Foss, Hoganas, Sweden) to a 0.2 mm particle size, freeze-dried and submitted to phenolic measurements.

2.2. Design of the experiment and growth conditions

The composts were carefully mixed with sand in a proportion allowing an amendment equivalent to 15 t DM/ha in 1 L plastic pots. Potted organic lettuce is widely grown in substrates that consist of peat and inorganic materials such as sand, perlite or vermiculite; therefore, the treatment with peat and sand was used as control. The experiment was conducted during the winter of 2014 in a greenhouse in Vila Real (N 41°17′7.28″; W 7°44′36.83″), first because of the expanding growth of winter greenhouse-grown lettuce in the region, second because cool-cultivated lettuce reportedly contain higher levels of phenolics than warm-cultivated ones (Becker et al., 2014).

Two lettuces were used for nursery tests. Maravilha Inverno, extensively cultivated in greenhouses during winter, is a bright green lettuce. Quatro Estações has a red pigmentation, especially in the borders of the most ruffled leaves, and is adapted to be grown during all year. Both lettuces develop a round, dense head, with very broad leaves and a consistent crisp texture. The two varieties have undergone repeated field selections for pests and diseases and shown polygenic resistance to several diseases such as bottom rot and downy mildew, justifying their use in organic farming. The seeds were purchased from a local shop, and sown on November 28th in pots filled with the composts (three seeds per pot and 4 pots per treatment); the treatments are herein referred to as CONTROL, BROCCOLI, WHITEGRAPE, OLIVE, REDGRAPE, and CHESTNUT. When seedlings had emerged, they were thinned to 1 plant per pot. The experiment was carried out in a completely randomized design. Weather conditions were recorded in the greenhouse as follow: 12.9/5.8 °C day/night temperatures, 68.6/87.4% day/night relative humidity and photosynthetic photon flux of 300–400 μmol/m^2/s during a 10 h photoperiod. The plants were manually irrigated according to plant needs based on the Reference Evapotranspiration value of lettuce, using the same amount of water in all pots. Weeding was done by hand when needed.

2.3. Harvest and measurement of morphological traits

When an acceptable leaf size and growing shape was attained, plants were harvested (90 days after sowing) by uprooting and cutting the above-ground parts; the roots were washed with water, blotted between two layers of paper towel, and all materials were transported to the laboratory within 5 min. The following morphological parameters were determined: fresh weight of leaves and roots (by weighing), plant height (with a ruler), number of leaves (by counting), root length (with a rhizometer), and stem diameter (with a caliper). Since no injured leaves were found, the whole leaves and roots were frozen with liquid nitrogen, freeze-dried, and the moisture losses recorded. Freeze-dried samples were blended into powder and used for analytical determinations.

2.4. Extraction and chromatographic separation of phenolic compounds

Samples (40 mg) were added with 1 mL of 70% methanol (Panreac Quimica; Barcelona, Spain), incubated at room temperature for 30 min with vortexing every 5 min, and then centrifuged (13,000, 15 min, 25 °C). The supernatant was collected and passed through a 0.2 μm filter (Spartan 13/0.2 RC; Whatman, Dassel, Germany) using a syringe. Ultra pure water was used throughout the study.

Individual phenolics were determined by high-performance liquid chromatography (HPLC) using a Gilson system (Villers-le-bel, France) equipped with a Finnigan Surveyor photo diode array detector (DAD 81401; Thermo Electron, San Jose, USA), and the software Xcalibur 2.0 (Thermo Fisher Scientific, Waltham, USA), which generated a 3-dimensional data set (absorbance, retention time, and wavelength). Phenolics in the injected extracts (10 μL) were separated using a C18 column (5 μm, 250 × 4.5 mm; Sigma/Aldrich, Steinheim, Germany) enclosed in an oven maintained at 25 °C. The mobile phase consisted of (A) 0.1% trifluoroacetic acid in water and (B) 0.1% trifluoroacetic acid in acetonitrile (all from Sigma/Aldrich, Steinheim, Germany), using a linear gradient starting with 100% A for 5 min, decreasing to 80% A at 15 min, 50% A at 30 min, 0% A at 45 min, and then reverting to 100% A at 55 till 60 min. The flow rate was constant at 1 mL/min. DAD data acquisition was set in the range of 200–400 nm, and peak areas were registered at the maximum absorbance of the compounds of interest i.e., 320 nm for phenolic acids, 370 nm for flavonoids and 520 nm for anthocyanins (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008; Ribas-Agustí, Gratacós-Cubarsí, Sárraga, García-Regueiro, & Castellari, 2011).

The chromatographic peaks obtained were identified by matching samples with standards on the basis of their retention times, UV spectra, and co-chromatography. Seven compounds were identified with some certainty, including caftaric, chlorogenic, and chicoric acids, quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside, quercetin 3-*O*-glucoside, luteolin 7-*O*-glucoside, and cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside. The quantitation of these compounds was done using a six-point regression curve ($r \geqslant 0.989$) for each standard. The four first standards + caffeic acid, gallic acid and trolox were from Sigma/Aldrich (Steinheim, Germany), and the two others + cyanidin 3-*O*-glucoside and quercetin 3-*O*-rutinoside from Extrasynthese (Lyon, France). Several peaks did not agree with any of the standards available in the laboratory; in those cases, bibliographic data was used for their identification. Tentatively identified compounds were quantified as equivalents of the most similar compound i.e., caffeic acid for caffeoylmalic acid and caffeic acid derivatives, cyanidin 3-*O*-glucoside for cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside, and quercetin 3-*O*-rutinoside for the unidentified flavonoid. Since lettuce is consumed fresh, the results are expressed in mg/g fresh weight (FW) after normalization with the moisture content.

2.5. Other analytical methods

2.5.1. HPLC determination of vitamin C in lettuce

For the extraction of ascorbic acid, 0.2 g sample in a dark tube was homogenized with an Ultra-Turrax T25 (IKA, Staufen, Germany) for 30 s on an ice bath with 5 mL of extraction medium (3% metaphosphoric acid +8% acetic acid added with 1 mM tert-butylhydroquinone) (Llorach et al., 2008; Nicolle et al., 2004). All chemicals were from Panreac Quimica (Barcelona, Spain). The homogenate was centrifuged (4000 rpm, 2 min, 4 °C) and the supernatant was recovered, filtered through a 0.45 μm Spartan filter, and immediately injected into the HPLC equipment, column and DAD described above for phenolics. The mobile phase was 0.2% orthophosphoric acid with an isocratic elution. The injection volume and the flow rate were 20 μL, and 1.2 mL/min, respectively. Vitamin C was quantified on the basis of the chromatograms obtained at 440 nm, using standard ascorbic acid (Sigma-Aldrich, Buchs, Switzerland).

2.5.2. Spectrophotometric determination of other phytochemicals in lettuce

The total phenolics content (TPC) was measured in the extract obtained in 2.4. by a modified Folin-Ciocalteu method (Cheng et al., 2014) and results expressed as mg gallic acid equivalent (GAE)/g FW. A microplate reader method based on the conventional method described by Lakhdar et al. (2011) was used for the total flavonoids content (TFC) and results expressed as mg catechin equivalent (CAE)/g FW. The ability of the extracts to scavenge DPPH was measured and expressed in mg trolox equivalent (TE)/g FW as described by Li, Zhao, Sandhu, and Gu (2010). The total monomeric and anthocyanins (TAC) contents were measured by the pH differential assay (Baslam, Morales, Garmendia, & Goicoechea, 2013), calculated with the equations reported by Li et al. (2010) and expressed as cyanidin 3-*O*-glucoside equivalent (mg CGE/g FW). Chlorophylls and carotenoids were extracted and calculated (mg/g FW) using the equations established by Hartmut Lichtenthaler and reported by Li et al. (2010).

2.5.3. Determination of phenolics in the raw materials and composts

Total phenolics and simple phenolics were extracted (0.2 g sample; 10 mL of 70% methanol) as described by Queiroz et al. (2002), and expressed in GAE mass basis. Gallic acid was quantified as in 2.4.

2.6. Statistical analyses

The results are presented as mean ± standard deviation (SD, n = 4). All analyses were done with compost type, lettuce variety, and measured parameters as class variables in the statistical models. Dependent variables were first studied with ANOVA and the Tukey's honestly significant difference (HSD) procedure applied for means comparison at a 5% significance level. Pearson's correlation tests were then established between all morphological traits; between TPC, TFC and TAC determined by chromatography and spectrophotometry; between compost phenolics and lettuce phenolics; and between yield and phytochemicals. Principal components analysis (PCA) was finally applied to single out composts which performed best in increasing the phenolic content of lettuce, and to identify phenolics which adequately summarised the effects observed. All analyses were performed using SPSS 15.0 (Chicago, USA).

3. Results and discussion

In this study, the possibility of using composts as organic mineral fertilizer in lettuce production was investigated, with an emphasis on the contents of antioxidant compounds.

3.1. Effect of composts on growth parameters

As compared to CONTROL, two of the composts were clearly beneficial to lettuce growth, namely CHESTNUT and OLIVE (Fig. 1). CHESTNUT increased the yield of Quatro Estações by 31% ($P < 0.05$). The second highest yield means were with CONTROL and OLIVE. With Maravilha Inverno, CHESTNUT also resulted in the highest yield mean, although it was statistically similar with the yield obtained with CONTROL and OLIVE, a pattern also seen with root yield (Supporting Information 1). WHITEGRAPE, REDGRAPE, and BROCCOLI showed 1.5–2.4 times lower yields than CONTROL, with BROCCOLI at the lower end of the range (Fig. 1). Several studies have emphasized the importance of plant food-based composts as sources of minerals for vegetable production (Grassi et al., 2015; Lakhdar et al., 2011). Given that all the composts had the same nitrogen content, the intrinsic nature of some of the composts predictably retarded mineral absorption, thus ensuring a lower crop production. For example, although having much more nutrients compared to peats, composts often compact a phenomenon which impedes the movement of nutrients. Furthermore, the availability of minerals in the composts depends on its interaction with the media microorganisms and weeds, whereas peat is usually sterile with no weed seeds. The current observation of lower yield with composts is not isolated. In fact, Montemurro et al. (2015) found that depending on the stage of maturity, olive pomace-based composts could lead to lower lettuce yield as compared with the unfertilized control.

A negative correlation was found between lettuce yield and moisture ($r = -0.76$). Fig. 1 indicated that the tissues of lettuce grown on WHITEGRAPE and BROCCOLI were more hydrated than those of lettuces grown on the other media, despite all plants grew under optimal water regime. Other morphological traits affected included alterations in the number of leaves, plant height, stem diameter and root length. However, the differences in yields seem to be mostly related to differences in the number of leaves produced ($r = 0.92$). Lettuce grown on CHESTNUT developed 1–2 more leaves per plant than the other lettuces (Fig. 1). Although the same trends were observed with the other parameters, no significant differences were found among treatments, other than BROCCOLI with plant height (Fig. 1), and WHITEGRAPE with root length in Maravilha Inverno (Supporting Information 1).

3.2. Identification of phenolic compounds in lettuce

The HPLC method used in this study allowed the separation of 15 peaks. Interferences of other matrix components were minimised and besides peaks 9 and 10 which co-eluted in the conditions selected, a satisfactory resolution between the target phenolics was observed (Fig. 2). UV–vis spectra showed three

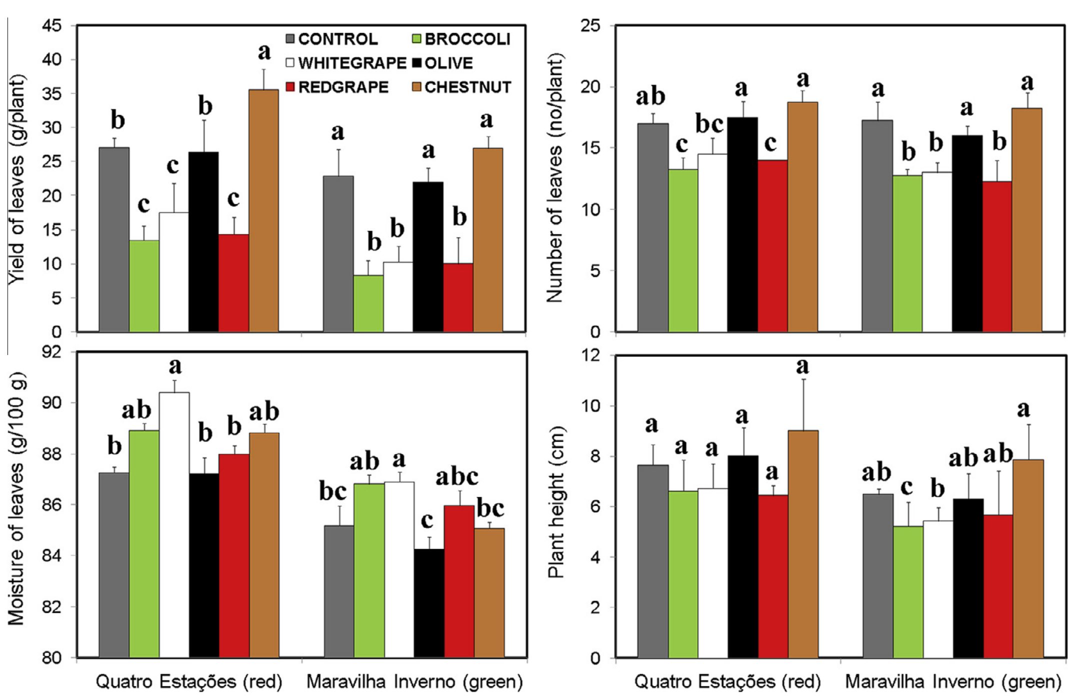

Fig. 1. Changes in aboveground traits of lettuce grown in media with composts; Means and SD as error bars with the same letter do not differ (Turkey's HSD test; $P < 0.05$).

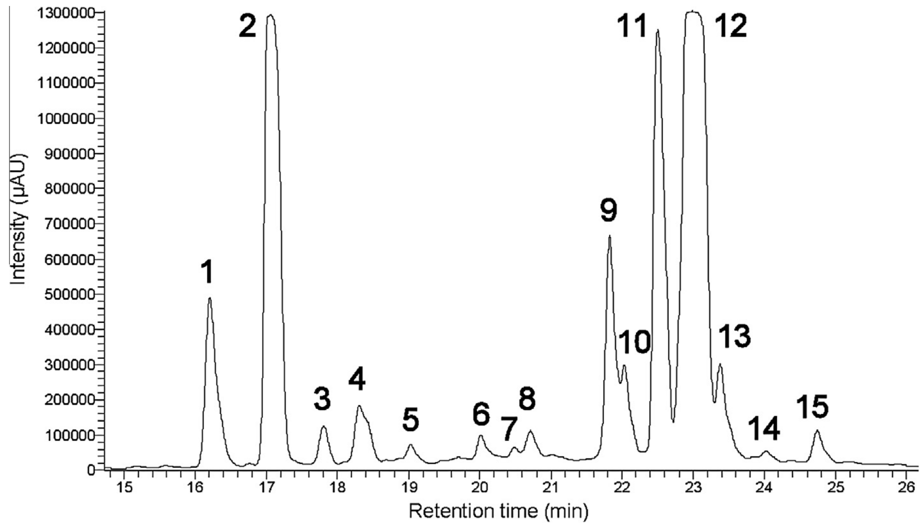

Fig. 2. Typical HPLC-DAD chromatogram of lettuce extract recorded at λ320 nm. Compounds correspond to those in Table 1 and Supporting Information 2. (1) Caftaric acid, (2) Chlorogenic acid, (3) Unidentified flavonol, (4) Caffeoylmalic acid, (5) Caffeic acid derivative 1, (6) Caffeic acid derivative 2, (7) Caffeic acid derivative 3, (8) Cyanidin 3-O-(6″-malonyl)-β-D-glucoside, (9) Luteolin 7-O-glucoside, (10) Quercetin 3-O-glucoside, (11) Quercetin 3-O-(6″-O-malonyl)-β-D-glucoside, (12) Chicoric acid, (13) Caffeic acid derivative 4, (14) Caffeic acid derivative 5, (15) Caffeic acid derivative 6.

spectrum typologies, corresponding to: (i) 3 known, 1 tentatively identified (caffeoylmalic acid) and 6 unidentified phenolic acids (caffeic acid derivatives 1–6) with maximum bands at λ311–330 nm; (ii) 3 known and 1 unidentified flavonoid with maximum bands at λ352–367 nm; and (iii) 1 tentatively identified anthocyanin (cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside) with a maximum band at λ516 nm.

The two lettuces showed both quantitative and qualitative differences regarding their composition of phenolics. As expected (Li et al., 2010), cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside was identified in only the red Quatro Estações (Table 1). Quatro Estações was also unusual in that it contained no detectable amount of caffeic acid derivative 3 (Supporting Information 2). When separated by colour, the red lettuce possessed higher phenolic acids and flavonoids than the green one, reflecting a 1.14, and 1.23-fold difference, respectively.

Using commercial standards for quantification, phenolic contents in lettuce (mg/g FW) were observed in the following order: chicoric acid (2.48) > chlorogenic acid (2.15) > quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside (0.92) > luteolin 7-*O*-glucoside (0.51) > quercetin 3-*O*-glucoside (0.27) > caftaric acid (0.19). Next was cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside which was quantified as CGE (Table 1). These major phenolic constituents were similar to those reported in other studies, notably showing chicoric acid and quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside as the main phenolic acid and flavonoid in lettuce, respectively (Becker et al.,

Table 1
Effect of composts on lettuce phenolics (mean ± SD; mg/g FW). Caffeoylmalic acid and cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside are tentatively identified and expressed as caffeic acid, and cyanidin 3-*O*-glucoside equivalent, respectively.

No[a]	Varieties/compounds	CONTROL	BROCCOLI	WHITEGRAPE	OLIVE	REDGRAPE	CHESTNUT
Quatro Estações (red)							
1	Caftaric acid	0.28 ± 0.06a	0.23 ± 0.04a	0.04 ± 0.03b	0.19 ± 0.04ab	0.31 ± 0.08a	0.29 ± 0.04a
2	Chlorogenic acid	1.68 ± 0.19b	2.43 ± 0.21b	2.31 ± 0.34b	1.98 ± 0.22b	2.00 ± 0.49b	2.64 ± 0.34a
4	Caffeoylmalic acid	0.03 ± 0.00ab	0.03 ± 0.0ab	0.03 ± 0.01ab	0.02 ± 0.01b	0.02 ± 0.01b	0.05 ± 0.01a
12	Chicoric acid	2.43 ± 0.46a	2.73 ± 0.24a	1.14 ± 0.10b	2.83 ± 0.37a	3.01 ± 0.41a	3.35 ± 0.48a
8	Cyanidin 3-O-(6″-malonyl) -β-D-glucoside	0.14 ± 0.05b	0.15 ± 0.04b	0.25 ± 0.07ab	0.15 ± 0.02b	0.15 ± 0.09b	0.30 ± 0.03a
9	Luteolin 7-*O*-glucoside	0.37 ± 0.05ab	0.26 ± 0.03b	0.62 ± 0.15a	0.33 ± 0.07ab	0.44 ± 0.20ab	0.67 ± 0.09a
10	Quercetin 3-*O*-glucoside	0.17 ± 0.05b	0.14 ± 0.02b	0.44 ± 0.23a	0.18 ± 0.04ab	0.29 ± 0.06ab	0.39 ± 0.04a
11	Quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside	1.05 ± 0.13ab	1.14 ± 0.11ab	0.87 ± 0.19b	1.20 ± 0.12ab	1.25 ± 0.28ab	1.59 ± 0.33a
	Total flavonoids (TFC)	1.66 ± 0.21b	1.61 ± 0.16b	2.03 ± 0.36ab	1.78 ± 0.17b	2.05 ± 0.49ab	2.76 ± 0.33a
	Total phenolics (TPC)	6.37 ± 0.88b	7.32 ± 0.64b	6.00 ± 0.72b	7.07 ± 0.50b	7.83 ± 1.68ab	9.58 ± 0.61a
Maravilha Inverno (green)							
1	Caftaric acid	0.18 ± 0.03a	0.11 ± 0.04a	0.16 ± 0.01a	0.18 ± 0.02a	0.16 ± 0.02a	0.12 ± 0.01a
2	Chlorogenic acid	1.65 ± 0.05 cd	1.60 ± 0.29d	2.97 ± 0.11a	2.60 ± 0.26ab	2.43 ± 0.32abc	1.63 ± 0.53 cd
4	Caffeoylmalic acid	0.01 ± 0.00a	0.01 ± 0.00a	0.03 ± 0.01a	0.04 ± 0.02a	0.02 ± 0.00a	0.02 ± 0.00a
12	Chicoric acid	2.51 ± 0.20a	1.90 ± 0.23a	2.37 ± 1.04a	2.70 ± 0.22a	2.82 ± 0.79a	2.16 ± 0.15a
8	Cyanidin 3-O-(6″-malonyl) -β-D-glucoside	ND	ND	ND	ND	ND	ND
9	Luteolin 7-*O*-glucoside	0.50 ± 0.07bc	0.26 ± 0.06d	0.94 ± 0.06a	0.62 ± 0.03bc	0.57 ± 0.10bc	0.68 ± 0.07b
10	Quercetin 3-*O*-glucoside	0.19 ± 0.03b	0.18 ± 0.09b	0.66 ± 0.12a	0.24 ± 0.01b	0.26 ± 0.07b	0.35 ± 0.05b
11	Quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside	0.67 ± 0.15a	0.40 ± 0.08b	0.62 ± 0.20a	0.68 ± 0.07a	0.72 ± 0.13a	0.62 ± 0.07a
	Total flavonoids (TFC)	1.44 ± 0.23b	0.88 ± 0.19c	2.33 ± 0.16a	1.62 ± 0.09b	1.63 ± 0.23b	1.77 ± 0.20b
	Total phenolics (TPC)	5.95 ± 0.44bc	4.61 ± 0.32c	8.04 ± 1.12a	7.37 ± 0.54ab	7.24 ± 1.27ab	5.85 ± 0.87bc

Row values with no letter in common differ at $P < 0.05$ (Tukey's HSD test).
ND = non detected.
[a] Letters refers to compounds in Fig. 2.

2014; Llorach et al., 2008;). The high content of chlorogenic acid found in this study was in agreement with Li et al. (2010), and Ribas-Agustí et al. (2011), but differed from Caldwell (2003), and Pepe et al. (2015), which observed the compound in low amounts. Several studies (Liu et al., 2007; Llorach et al., 2008) have pointed out discrepancies on the occurrence and relative abundance of several phenolics in lettuce, which is likely due to variety, but also to extraction and quantification methods.

3.3. Effect of composts on lettuce phenolics

The response of phenolics to composts was strongly genotype-specific; therefore data from the two lettuces were separately submitted to PCA to discriminate between the composts and better decipher the compounds accounting for the effects observed.

For Quatro Estações, higher levels of phenolic acids, flavonoids and anthocyanins in CHESTNUT-grown lettuce versus CONTROL were found (Fig. 3A). Only CHESTNUT and WHITEGRAPE were effective in increasing the cyanidin 3-*O*-(6″-malonyl)-*β*-D-glucoside content. For flavonoids, moderate performances were observed with WHITEGRAPE and REDGRAPE (Table 1). The impact of individual phenolics to the overall variance in lettuce was visualized on the loading plot of PCA (Fig. 3B); although all compounds had higher mean values with CHESTNUT, only few were truly responsible for the effect observed. The contents of all flavonoids and cyanidin 3-*O*-(6″-malonyl)-*β*-D-glucoside were highly sensitive to the growing media; in the case of phenolic acids, the differences induced by CHESTNUT were most clearly seen with caffeic acid derivative 1, caffeoylmalic acid, and chlorogenic acid (Fig. 3B). All these caffeic acid derivatives contain one or two caffeoyl moieties, which are important structures involved in antiperoxidative and radical scavenging activities (Caldwell, 2003; Nicolle et al., 2004); and therefore changes in their contents could have health consequences. Isochlorogenic acid is interesting in this regard as it has been identified as the main phenolic involved in the neuroprotective effect of lettuce extracts (Im et al., 2010). WHITEGRAPE significantly ($P < 0.05$) induced the accumulation of caffeic acid derivative 1, unidentified flavonol, quercetin 3-*O*-glucoside, and luteolin 7-*O*-glucoside, but that was not sufficient to produce a major effect as with CHESTNUT, because of decreases in chicoric acid, caftaric acid, and quercetin 3-*O*-(6″-*O*-malonyl)-*β*-D-glucoside contents (Table 1; Supporting Information 2).

On the other hand, Maravilha Inverno grown on WHITEGRAPE contained significantly ($P < 0.05$) higher levels of flavonoids and phenolic acids. This was followed by CHESTNUT although the effect observed was not as pronounced as with Quatro Estações (Fig. 3C). In fact, only WHITEGRAPE let to values higher than CONTROL with respect to flavonoids (Table 1). In all cases, BROCCOLI induced significantly ($P < 0.05$) lower accumulation of phenolics (Fig. 3C). Four phenolics formed a cluster on the PCA loading plot and their accumulation in Maravilha Inverno seems to be responsible for the effect observed with WHITEGRAPE: quercetin 3-*O*-glucoside, luteolin 7-*O*-glucoside, unidentified flavonol and caffeic acid derivative 6 (Fig. 3D). Levels of most other compounds were not affected by any of the growing media (Table 1; Supporting Information 2). Consistent with what was observed with CHESTNUT in Quatro

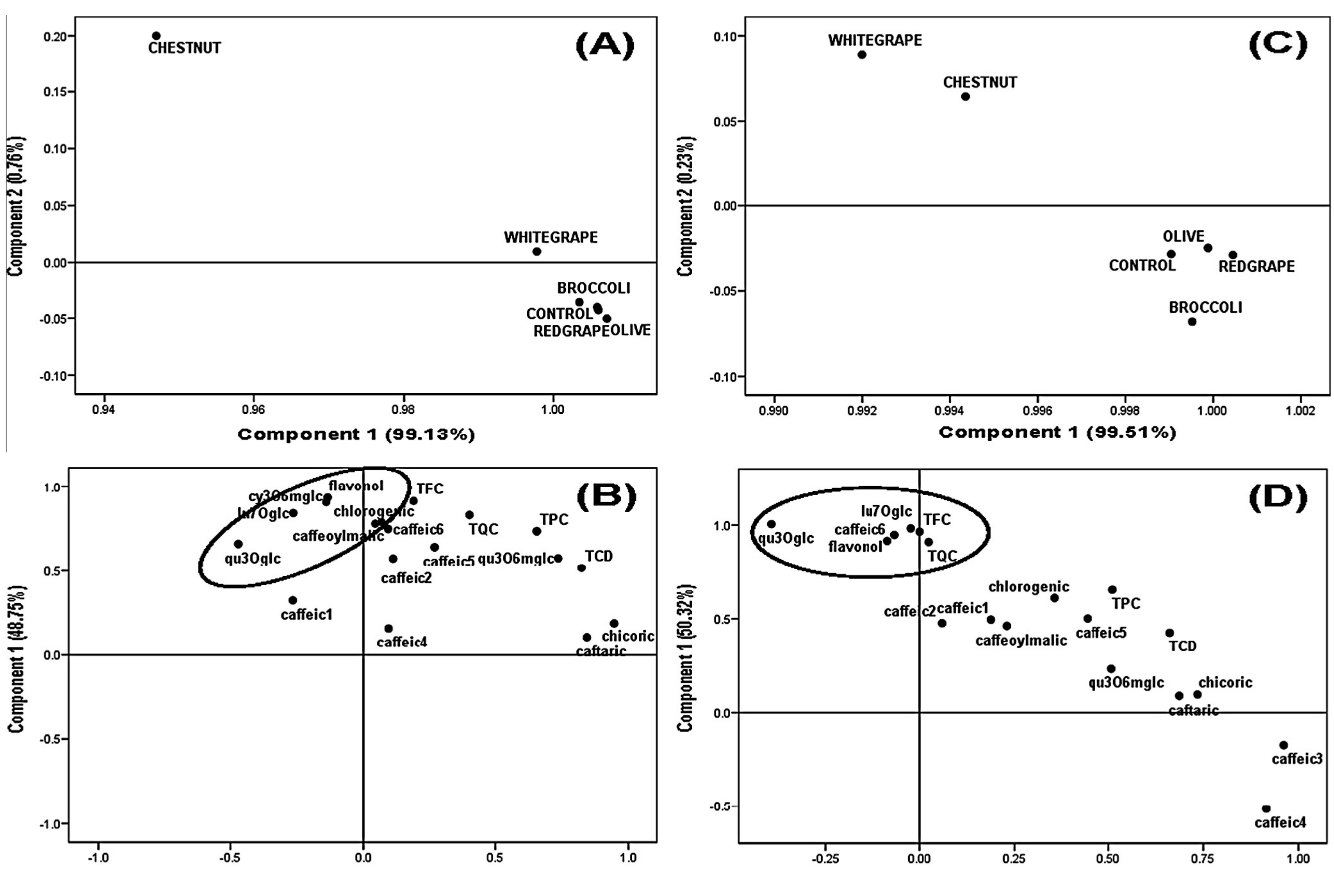

Fig. 3. PCA biplots showing the influence of composts on phenolics: (A) separation of composts in red Quatro Estações, (B) distribution of phenolics in relation to the treatments in Quatro Estações (C) separation of composts in green Maravilha Inverno, and (D) distribution of phenolics in relation to the treatments in Maravilha Inverno. For abbreviations, see Table 1 and Supporting Information 2.

Estações, flavonoids contributed the most to the improvements observed with WHITEGRAPE in Maravilha Inverno. In the study by Caldwell (2003), the decreasing order of peroxyl radical scavenging activities for flavonoids was cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside > quercetin 3-*O*-glucoside > quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside. That ranking was used by the authors to suggest certain health consequences of increased consumption of lettuce. Some of the best supporting evidence for the role of lettuce flavonoids in diabetes prevention have recently been reported, and shown cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside and quercetin 3-*O*-(6″-*O*-malonyl)-β-D-glucoside to be associated with the reduction of hyperglycemia in mice (Cheng et al., 2014).

Although this study was not specifically designed to compare organically and conventionally-grown lettuces, it is important to state that polyphenol contents are significantly lower under conventional farming (Grassi et al., 2015; Heimler et al., 2012; Lakhdar et al., 2011); and even though it is not a universal phenomenon (Durazzo et al., 2014), that support the prediction that when cultivated in the presence of CHESTNUT and WHITEGRAPE applied under appropriate conditions versus chemical fertilizers, the lettuce plant could be enriched in bioactive compounds.

3.4. Effect of composts on DPPH, TPC, TFC, and TAC

The DPPH assay was selected to investigate if changes in lettuce phenolics with compost treatments could translate into improved antioxidant capacities. DPPH had a significant ($P < 0.05$) positive correlation with total phenolics/flavonoids in Quatro Estações ($r = 0.73$) and in Maravilha Inverno ($r = 0.86$); and high phenolic/flavonoid contents in lettuces from CHESTNUT and WHITEGRAPE were associated with high reducing capacities in Quatro Estações and in Maravilha Inverno, respectively (Fig. 4; Table 1). OLIVE and REDGRAPE in Maravilha Inverno also led to high DPPH values. Determination of the actual relevance of these changes will require consideration of cell or in vivo studies.

Marked differences existed between TPC and TFC measured spectrophotometrically, and those obtained by HPLC in Quatro Estações: the spectrophotometric TPC was not affected ($P < 0.05$) by any of the treatments, while highest HPLC TPC were obtained with CHESTNUT AND REDGRAPE; the highest spectrophotometric TFC and the lowest HPLC TFC were both obtained with BROCOLI (Fig. 4; Table 1). When compared in a linear correlation model, there was only a loose correlation ($r = 0.58$ for TFC; $r = 0.61$ for TPC) between the two methods in Maravilha Inverno. Although the Folin-Ciocalteu and aluminum chloride assays are regularly used to indirectly estimate total polyphenols contents in plants, these methods are not completely specific for the target substances, and not all phenolics exhibit the same level of activity in the assays (Liu et al., 2007); therefore depending on the vegetable matrix, their use should be carefully reconsidered.

Spectrophotometric measurements found TAC of 0.30 and 0.08 mg CGE/g FW in the red and green lettuces, respectively (Fig 4), although cyanidin 3-*O*-(6″-malonyl)-β-D-glucoside was only detected in the red lettuce. In most cases, lettuce is stripped of its outer wrapped leaves before analyses, which was not the case in this study. A darker coloration was found toward the extremities of the outer leaves of Maravilha Inverno; since this was clearly not related to leaves senescence, it could explain the level of anthocyanins measured. In fact, it was demonstrated that anthocyanin and vitamin C contents of lettuce are 10 times as high in the green outer leaves as in the inner light-coloured ones (Baslam et al., 2013). In contrast with the TPC and TFC, spectrophotometric TAC results were comparable to HPLC ones ($r = 0.70$), notably showing CHESTNUT and WHITEGRAPE as effective in increasing anthocyanin levels.

3.5. Effect of composts on vitamin C and carotenoids

Compost as amendments significantly decreased the content of vitamin C in the lettuces. The CONTROL on average had 1.29 mg/g FW vitamin C; the next highest value was with Quatro Estações grown on REDGRAPE, followed by Maravilha Inverno grown on WHITEGRAPE (Fig. 4). Although no strong correlation was observed between DPPH and vitamin C (Supporting Information 3), recent studies have reaffirmed a positive link between vitamin C and antioxidant capacities in lettuce (Llorach et al., 2008; Nicolle et al., 2004; Serafini et al., 2002).

The total chlorophylls and carotenoids contents of lettuces grown with the composts were mostly statistically similar to CONTROL (Supporting Information 4). However, the total carotenoids content is not the main factor to take into account as specific carotenoids may be affected to varying degrees. Lettuce carotenoids have been identified as lutein, β-carotene, neoxanthin, violaxanthin and lactucaxanthin (Baslam et al., 2013; Durazzo et al., 2014; Nicolle et al., 2004); and at least one study has shown increments in their levels in lettuce grown on compost (Cruz et al., 2014).

3.6. Correlation between compost and lettuce phenolics

The second objective of this study was to investigate if changes in lettuce antioxidant profiles with compost treatments fit the picture of phenolics uptake from roots.

A major challenge faced by the composting industry is to alleviate toxicity concerns associated with the use of raw wastes. Agro-industrial-based wastes are known to be rich in high MW phenolics, with the potential for production of phytotoxic leachates as they decompose (Kelepesi & Tzortzakis, 2009; Lakhdar et al., 2011). On the other hand, these chemicals at lower concentrations may elicit stimulatory effects on different plant organs. Therefore, their contents need to be manipulated to counter the polluting effect and ensure efficient plant growth. Composting the five wastes with wheat straw vastly reduced total (94%) and simple phenolics (85%) contents, but increased gallic acid content by 187% on average (Table 2). At the end of the composting process, the compost stability was closely related to polyphenol contents, denoting a high degree of maturity (data not shown).

From Tables 1 and 2, it seems that simple phenolics in the compost – not total phenolics – determined the contents of phenolics in lettuce. This tendency was verified by Pearson's correlation analysis; a positive correlation between the compost gallic acid content and Maravilha Inverno phenolics was established ($r = 0.48–0.62$), but this was less pronounced than the highly linear relationship ($r = 0.57–0.67$) observed between all simple phenolics in the compost, and the TPC, TFC, and DPPH in Quatro Estações (Supporting Information 3). Although correlation does not imply causation, the fact that high levels of simple phenolics and gallic acid in CHESTNUT and WHITEGRAPE, respectively, were associated with high levels of polyphenols in lettuce grown on these media, suggests a cause-effect relationship. It is possible that specific non-planar flavonoids produced during compositing may have acted as signal factors, prompting root-to-shoot phenolic uptake by protein transporters as recently observed in *Arabidopsis* (Buer et al., 2010), resulting in an enhanced accumulation and synthesis of these compounds in lettuce.

4. Conclusions

On the whole, type of compost has a marked influence on both yield and phenolics in lettuce; and genetics seems to play a large role in the ability of lettuce to respond to the growing media. By

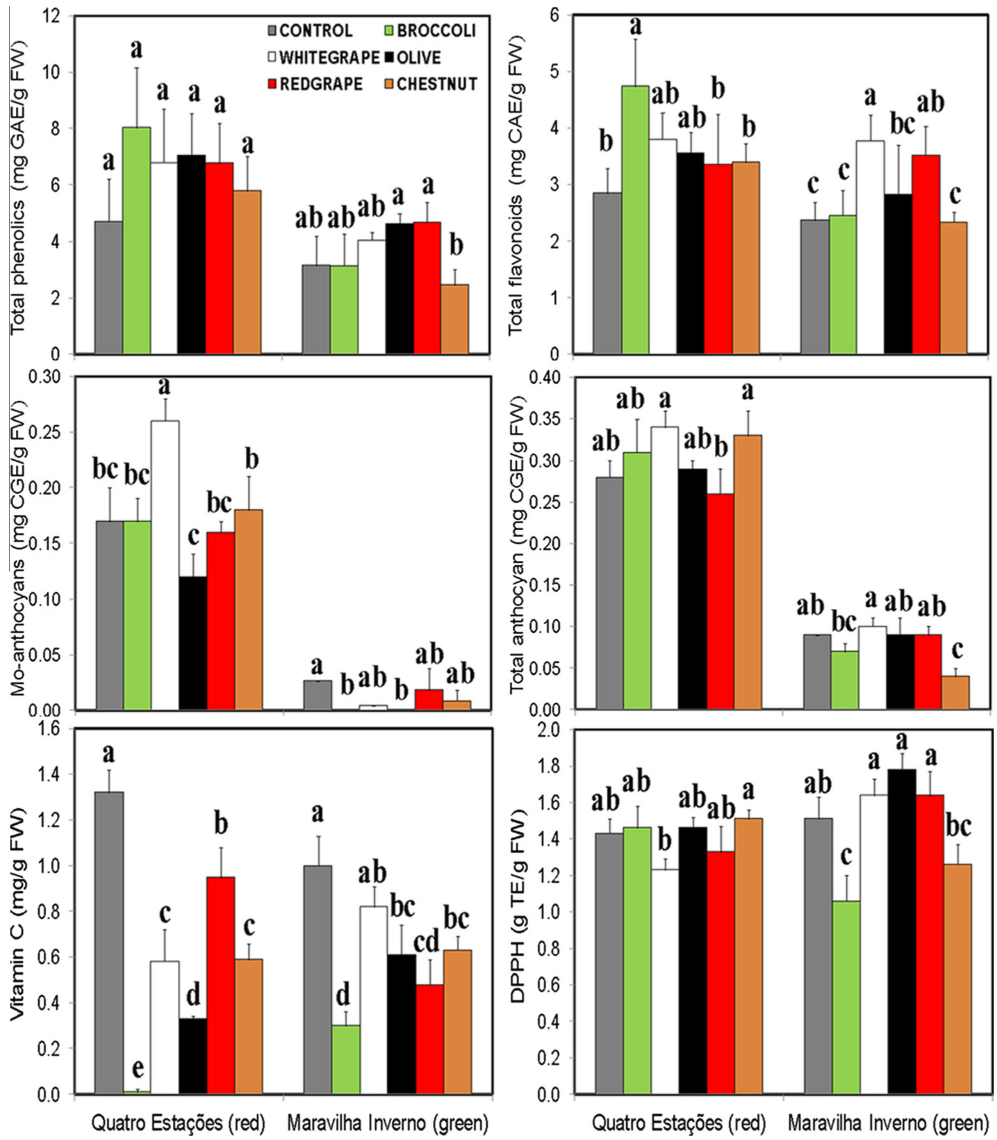

Fig. 4. Total flavonoids, phenolics, and anthocyanins, vitamin C and DPPH radical scavenging activity of lettuce cultivated with composts. Means and SD as error bars with the same letter do not differ (Turkey's HSD test; $P < 0.05$).

Table 2
Phenolic composition (mean ± SD; DW) of the raw material and composts used in the study.

	Total phenolics content (g GAE/kg)	Simple phenolics content (mg GAE/kg)	Galic acid (μg/kg)
Raw material			
Wheat straw[a]	3.83 ± 0.79e	2.29 ± 0.05f	27.71 ± 2.37a
BROCCOLI	6.65 ± 0.05d	7.08 ± 0.05e	4.17 ± 0.07d
WHITEGRAPE	29.57 ± 1.88a	25.04 ± 1.14a	12.60 ± 3.43c
OLIVE	25.52 ± 1.06b	20.93 ± 0.31b	13.41 ± 4.20c
REDGRAPE	25.31 ± 1.25b	18.68 ± 1.37c	13.17 ± 2.96c
CHESTNUT	15.22 ± 0.53c	11.17 ± 0.41d	17.95 ± 1.52b
Compost			
Wheat straw	1.14 ± 0.11a	1.05 ± 0.01d	22.89 ± 0.91b
BROCCOLI	1.08 ± 0.32a	1.15 ± 0.12d	23.79 ± 0.95b
WHITEGRAPE	1.15 ± 0.38a	1.18 ± 0.16d	34.37 ± 9.57a
OLIVE	0.16 ± 0.01c	2.72 ± 0.13b	28.18 ± 8.48ab
REDGRAPE	0.70 ± 0.09b	2.30 ± 0.23c	30.54 ± 4.09ab
CHESTNUT	1.10 ± 0.09a	3.46 ± 0.15a	26.53 ± 1.91ab

Different letters in a column show significant mean differences ($P < 0.05$; Turkey's HSD test).
[a] Wheat straw was used as co-substrate during composting.

developing a parallelism between yield and antioxidants, five main lessons could be learned: (i) Quatro Estações grown on CHESTNUT exhibited increased yield and antioxidant capacity; yield and polyphenol pools were positively correlated on average ($r = 0.60$), suggesting that factors that promoted growth might have also enhanced phenolic synthesis. Lettuce is mostly consumed fresh; thus these modifications can have significant direct nutritional impacts; (ii) Maravilha Inverno grown on WHITEGRAPE exhibited increased production of phenolics; however, that seems to act in direct competition for assimilated carbon to the plants' growth processes, given the low yield recorded. Provided growers decide to mainly select for increased antioxidant activity, there is scope to consider WHITEGRAPE for that variety; (iii) CHESTNUT also increased the yield of Maravilha Inverno without adversely affecting its phenolic content. A cooperative action between CHESTNUT and WHITEGRAPE could be beneficial in enhancing the properties of the latter, and this may be the focus of a future research direction; (iv) Lettuces grown on BROCCOLI exhibited the worst yields and phenolic levels. Therefore, it seems better to avoid their application in organic horticulture, unless adequate composting strategies are implemented to improve their fertilizing efficiency;

(v) With respect to vitamin C, all the composts led to decreased contents in lettuces, which is undesirable from a nutritional point of view, but does not necessarily denote an overall negative outcome. It is obvious that composting not only provides an adequate disposal approach for plant food-based wastes, but composts can be used as substitutes for non-renewable resources such as peat in organic lettuce production. The data show that if lettuces with high yield and polyphenol contents are desired, the chestnut-made compost developed in the course of this study and available at UTAD can be used for their cultivation.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.04.087.

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