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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