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# The Sustainable Use of Cotton, Hazelnut and Ground Peanut Waste in Vegetable Crop Production

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Received: 29 September 2020; Accepted: 9 October 2020; Published: 15 October 2020



**Abstract:** The environmental burden from crop production byproducts is gradually increasing and necessitates the sustainable management of waste towards a circular economy approach. In the present study, three byproducts (cotton ginning waste (CGW), ground hazelnut husks (GHH) and ground peanut husks (GPH)) were evaluated in lettuce cultivation. For this purpose, the tested materials were incorporated in soil at two different rates (25% and 50% of total substrate volume) while a control treatment (no addition of byproducts) was also considered. Fresh weight per plant and total yield was the highest for the GHH50% treatment. The highest fat, protein, carbohydrates and energy content were observed for the CGW25% treatment. Chemical composition also differed among the tested byproducts where CGW25% treatment had the highest total tocopherols, sugars (sucrose, fructose, trehalose and total sugars) and organic acids content. The most abundant fatty acids were  $\alpha$ -linolenic, linoleic and palmitic acid in all the tested treatments, while the highest antioxidant activity was observed for the GHH50% treatment. Regarding polyphenols, phenolic acids content was the highest in the GHH treatments, whereas flavonoids were the highest for the CGW25% treatment. No cytotoxicity against the PLP2 non-tumor cell line was observed, whereas only the GPH50% treatment showed moderate efficacy against HeLa, HepG2 and MCF-7 cell lines. The tested extracts also showed moderate antibacterial activities and only the extracts from the CGW50% treatment were more effective than the positive control against *Trichoderma viride*. In conclusion, the present results showed the great potential of using the tested byproducts as soil amendments for vegetable crops production, since they may improve the nutritional parameters, the chemical profile and the bioactivities of the final product. The suggested alternative use of the tested byproducts not only will increase the added value of crops but will also alleviate the environmental burden from bulky agroindustry byproducts.

**Keywords:** agroindustry byproducts; antimicrobial activities; cotton ginning waste; ground peanut husks; hazelnut husks; *Lactuca sativa* L.; phenolic compounds; tocopherols

## 1. Introduction

The environmental burden of agro-industry byproducts with bulky nature necessitates the finding of alternative/complementary applications that will enhance the added value of crops and the farmers' income, while further establishing the concept of circular economy and sustainable production in the agricultural sector [1,2]. Field and tree crops such as cotton, hazel and ground peanut are widely cultivated throughout the world and generate high amounts of waste. Cotton ginning waste, also known as cotton gin thrash or gin thrash, constitutes a great amount (15–42%) of the overall global yield (approximately 25 million tons) [3], which makes the handling of this material a nuisance for the processing sector. Ground peanut or groundnut (*Arachis hypogaea* L., Fabaceae) is widely used for seed oil production or in crop rotation programs due to nitrogen fixing properties [4]. A total amount of approximately 46 million tons of ground peanut are produced annually out of which 25% of total yield is discarded as waste in the form of hulls [5]. Moreover, hazelnut cultivation is mostly located in the eastern Mediterranean where Turkey is the leading world producer (approximately 70% of world production) [6], while hazelnut shells (or husks) constitute 20% of total yield [7].

So far, cotton ginning byproducts and hazelnut and ground peanut husks are usually discarded or burnt in the field, thus increasing the greenhouse gas emissions and nutrients loss [8,9], while the energy production from the obtained biomass is also evaluated [10–13]. An alternative approach is to use husks of hazelnuts as mulching material for the sustainable management of weeds [6], or the incorporation of peanut hulls compost in soil as natural biofertilizer [5].

Several studies suggested the use of various agro-industry wastes as growing substrates in soilless or pot cultivation of ornamental and horticultural crops aiming to substitute peat which is the main substrate currently used [1,14–18]. For example, the use of cotton ginning waste had positive effects on lettuce crop [19] and potted chrysanthemum performance [15], while the same material and cardoon byproducts showed promising results for the pot cultivation of *Cichorium spinosum* L. [1]. Moreover, decomposed hazelnut husks showed promising results as growing media in soilless systems due to their physicochemical properties (pH, EC, nutrients content and C/N) [14]. The use of hazelnut husks compost was proposed for the greenhouse cultivation of tomato and the production of tomato seedlings [7,20], as well as for the production of kiwifruit cuttings [21]. On the other hand, peanut hulls have been suggested for mushroom substrate supplementation where the biological efficiency (fresh weight of mushrooms divided by the dry weight of substrate) increased by 61% compared to the control treatment [4]. Other studies suggested the use of such materials for soil amelioration purposes through the improvement of physicochemical properties and organic matter and nutrients replenishment [19,22,23], or as bulking agents in composting of sewage sludge [24] and biosorbents [25].

Considering the pollution of the environment that improper management of crop production waste may cause, the first aim of this study was to examine the use of byproducts obtained from cotton industry (cotton ginning waste) and the production of hazelnuts and ground peanuts as soil amendments for the production of lettuce in order to suggest alternative uses of bulky byproducts. Lettuce was selected since it is one of the main vegetable crops being cultivated in 1.27 million hectares worldwide and producing approximately 27 million tons annually [3]. The wide distribution and the added value of this crop ensures the adequate assimilation of bulky agroindustry byproducts such as those tested in the present study. Moreover, the second aim of the study was to evaluate the effect of the tested byproducts on the nutritional characteristics, the chemical profile and the bioactive parameters of lettuce leaves in order to identify those materials that may benefit both the plant growth and the quality of the final produce.

## 2. Materials and Methods

### 2.1. Plant Material and Growing Conditions

The experiment was performed during November 2017–February 2018 in a commercial plastic greenhouse in the region of Trikala, Greece. Lettuce seedlings (*Lactuca sativa* L. cv. Starfighter; Batavia

type) were transplanted to soil on 25 November 2017 when they formed 3–4 true leaves in plots of  $2 \times 2$  m. The experimental treatments included the incorporation of three agro-industry by-products, namely cotton ginning waste (CGW), ground hazelnut husks (GHH) and ground peanut husks (GPH) in two amounts (25% and 50%; *v/v*), while a control treatment (C) with no addition of by-products was also included. The amount of by-products used in each treatment was calculated assuming that incorporation took place at the upper 30 cm of soil (total volume of each plot =  $1.2 \text{ m}^3$ ) and after determining the dry bulk density of each by-product ( $0.152 \text{ kg/m}^3$ ,  $0.474 \text{ kg/m}^3$  and  $0.156 \text{ kg/m}^3$  for CGW, GHH and GPH, respectively). The calculated amount of each material was incorporated at the depth of 30 cm via a rotary tiller. Table 1 presents physicochemical properties and minerals' content in soil and by-products. The soil was sandy clay loam (47% sand, 31% clay and 22% loam) with  $\text{pH} = 7.6$ , electrical conductivity (EC) =  $1731 \text{ }\mu\text{S/cm}$ , organic matter = 3.4%, total  $\text{CaCO}_3 = 11.0\%$  and total dissolved solids (TDS) =  $969 \text{ mg/L}$ . Each treatment was replicated three times while plants were arranged in three double rows with distances of  $0.25 \times 0.25$  m between plants and corridors of 0.50 m between each pair of rows (48 plants in each plot). Standard cultivation practices for pest and pathogens control were applied, whereas weed control was carried out manually. Irrigation took place at regular intervals and according to environmental conditions (once or twice a week). Fertilizers were applied with basal dressing by adding 67 kg of granular complex fertilizer 20-10-10 (N-P-K) +  $15 \text{ SO}_3$ .

**Table 1.** Mineral composition of the soil of the experimental field and the tested by-products.

By-Product	Bulk Density (g/cm)	WHC (%)	OM (%)	pH	EC (dS/cm)	N (%)	K (cmol/kg)
Soil	1.07	45.5	3.4	7.6	1.73	0.13	0.91
CGW *	0.30	139.3	82.9	6.8	5.42	0.19	0.87
GHH	0.54	78.9	110.7	5.8	1.93	0.85	2.18
GPH	0.17	262.2	59.1	5.9	1.36	1.1	3.05

\* CGW: cotton ginning waste; GHS: ground hazelnut husks; GPH: ground peanut husks.

Harvest took place on 5 February 2018 by cutting the aerial part of plants at the base of stem with a sharp knife. Yield was calculated based on the fresh weight of individual plants and assuming a plant density of 120,000 plants/ha without including the outer plants (12 plants) of each plot. Dry matter content was estimated after forced-air drying fresh samples at  $72 \text{ }^\circ\text{C}$  for at least 48 h and until constant weight. For chemical analyses, fresh samples of fully grown leaves from each treatment were used to prepare batch samples that were put in deep freezing conditions, then freeze-dried and pulverized (pestle and mortar) and finally kept at  $-80 \text{ }^\circ\text{C}$  until analysis.

## 2.2. Nutritional Value and Hydrophilic Compounds

### 2.2.1. Macronutrients and Energetic Value

According to the AOAC methods [26], the proximate composition was determined in the lyophilized samples and expressed in g per 100 g of fresh weight (fw). Total carbohydrates were determined by difference, and total energy was determined using the following equation: Energy (kcal/100 g fresh weight (fw)) =  $4 \times (\text{g protein} + \text{g carbohydrates}) + 9 \times (\text{g fat})$  [26].

### 2.2.2. Free Sugars

Free sugars content was estimated according to the procedure described in detail by the authors [27] using an HPLC equipment coupled with a refraction index detector (RI). The identification of compounds was performed via comparison with commercial standards, while the detected compounds were quantified using the internal standard method (IS; melezitose; Sigma, St. Louis, MO, USA).

### 2.2.3. Organic Acids

Organic acids were determined according to the protocol of Pereira et al. [28]. The analysis was performed using a Shimadzu 20A series UFLC with a diode array detector (DAD). The organic

acids were quantified by comparing the peak area with calibration curves obtained from commercial standards (oxalic acid, malic acid and fumaric acid acquired from Sigma-Aldrich, St. Louis, MO, USA) of the detected compounds.

### 2.3. Lipophilic Compounds

#### 2.3.1. Fatty Acids

Fatty acids were estimated following the protocol of Silva et al. [27] using a GC-FID equipment. The identification of fatty acids was performed via comparison of the relative retention times of peaks of the detected fatty acids methyl ester (FAME) with commercial standards (mixture 37 (standard 47885-U), Sigma-Aldrich, St. Louis, MO, USA).

#### 2.3.2. Tocopherols

The extraction of tocopherols from the lyophilized samples was carried out following the procedure described in detail by Silva et al. [27]. The identification of compounds was performed by comparisons of the detected peaks with authentic standards. Tocopherol isoforms were quantified according to their fluorescence signal response (IS method; tocol, Matreya, Pleasant Gap, PA, USA).

### 2.4. Phenolic Compounds Characterization

#### 2.4.1. Extracts Preparation

To prepare the hydroethanolic extracts, the powder obtained from lyophilized leaves was extracted after stirring for 1 h with 30 mL of ethanol/water (80:20, *v/v*) following filtering with Whatman No. 4 paper. The obtained residue was extracted for 1 h for one more time using 30 mL of ethanol/water. The hydroethanolic extracts obtained from two extractions were combined and evaporated until dryness. The phenolic compounds characterization and the bioactive assays were performed in the dried residues after redissolution in ethanol/water [29].

#### 2.4.2. Phenolic Compounds

The hydroethanolic extracts prepared above, were redissolved in ethanol/water (80:20, *v/v*), to a final concentration of 10 mg/mL for the phenolic compounds characterization [30]. The analysis was performed in a HPLC system coupled with a diode-array detector (DAD) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS) equipped with an electrospray ionization (ESI) source. Separation was made in a Waters Spherisorb S3 ODS-2 C18 column. The operating conditions and the procedure for the identification and quantification of the compounds were previously described in detail by Bessada et al. [30].

### 2.5. Selected Bioactivities

#### 2.5.1. Antioxidant Activity

Antioxidant activity was determined by applying two cell-based assays: the thiobarbituric acid reactive substances (TBARS) formation inhibition assays and the oxidative hemolysis (OxHLIA) previously described in detail by Spréa et al. [29] using the above-prepared hydroethanolic extracts. The TBARS assay was determined by the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant and the results were given as EC<sub>50</sub> values ( $\mu\text{g/mL}$ ) [29]. The antihemolytic activity was determined by the oxidative hemolysis inhibition assay (OxHLIA) and the results were presented as IC<sub>50</sub> values [29]. In both assays, trolox was used as positive control.

### 2.5.2. Cytotoxicity Assays

Cytotoxicity was evaluated using two assays according to the procedure described by the authors [31]. For non-tumor cell lines, the cytotoxicity of the extracts was determined using the sulforhodamine B assay against primary cell cultures (PLP2). For tumor cell lines cytotoxicity, the same method was implemented using four human tumor cell lines (HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) and NCI-H460 (non-small cell lung cancer)). Ellipticine was the positive control for both assays, and the results were expressed as GI<sub>50</sub> values (μg/mL).

### 2.5.3. Antimicrobial Properties

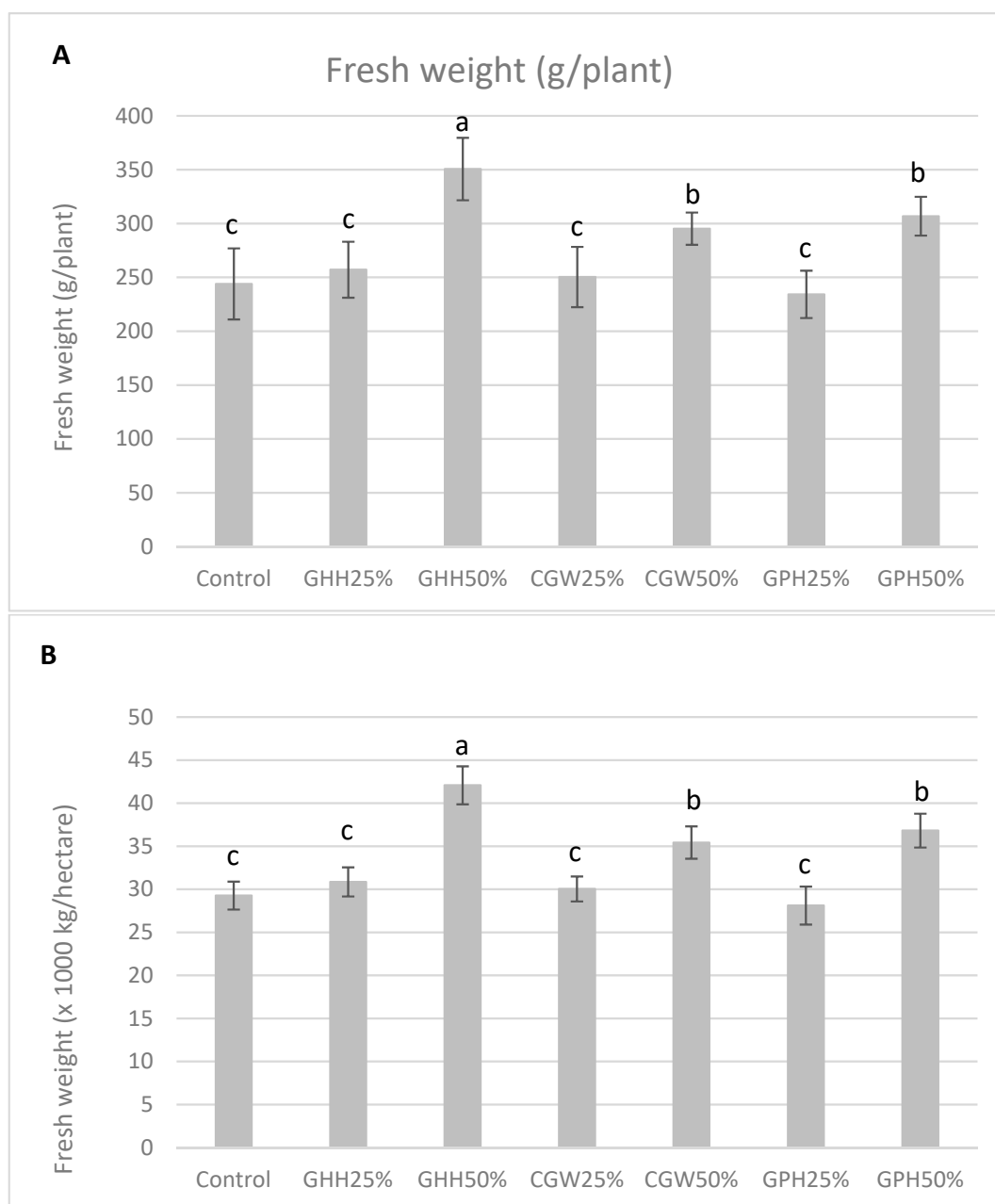
The hydroethanolic extracts prepared above were used for the determination of the antibacterial and antifungal properties according to the method of Soković et al. [32]. The results were expressed as the concentrations that resulted to the complete inhibition of the bacterial growth (MIC, minimal inhibition concentration), MBC (minimal bactericidal concentration) and MFC (minimal fungicidal concentration) values. Streptomycin, ampicillin and ketoconazole were positive controls, whereas 5% DMSO was the negative control.

### 2.6. Statistical Analysis

The experiment was performed according to the randomized complete block design (RCB) (n = 3). All chemical analyses were performed in triplicate (n = 3). The analysis of data was accomplished with the use of Statgraphics 5.1.plus (Statpoint Technologies, Inc., Warrenton, VA, USA) and the one-way ANOVA, while means were compared with the Tukey's HSD test ( $p = 0.05$ ) and Student's *t* test ( $p = 0.05$ ) when significant differences were detected.

## 3. Results and Discussion

Results of crop performance are presented in Figure 1. Fresh weight (g) per plant and total yield (kg/h) were the highest for the GHH50% treatment in both cases (350.6 g/plant and 42,072 kg/h, respectively), followed by the treatments of GPH50% and CGW50%. The lowest fresh weight per plant and total yield were observed in the GPH25%; however, there were no significant differences among the rest of the treatments (Control, CGW25% and GHH25%). These results suggest that the incorporation of the highest amount (50%) of each byproduct could result in a significant increase in the crop fresh weight and total yield compared to the control treatment and the treatment when 25% equivalents are applied. According to the physicochemical properties of the tested material (Table 1), it could be assumed that the recorded yields are mostly associated with the improvement in water holding capacity and the soil content in organic matter, as well as with the addition of macronutrients (N and K). Khah et al. [19] who evaluated cotton ginning byproducts as growth media of vegetable crops suggested the increase of plant growth parameters (plant height, leaf number, dry and fresh weight of leaves, chlorophyll content) of radish, spinach and lettuce. Moreover, Riley et al. [33] suggested that the incorporation of cotton gin thrash resulted in lower air space and similar water holding capacity to cotton stalks, although the amounts of unavailable water were higher for the cotton gin thrash treatment. This finding suggests that the application of high amounts of cotton gin waste may affect the water status of soil with further implications on plant growth. Positive effects on soil properties caused by hazelnut husks were also reported by Ozdemir et al. [34] where the authors suggested that hazelnut husks could be used in composted mixes with wastewater biosolids for the production of ornamental plants. Moreover, Aşkın and Aygün [35] highlighted the beneficial impact of hazelnut husk compost on soil organic matter content and water holding capacity, while Gülser et al. [36] and Gülser and Candemir [37] indicated the slow mineralization rate of hazelnut husks and the improvements they induce in soil hydraulic properties.



**Figure 1.** Yield in relation to growth medium expressed as fresh weight (g) per plant ( $n = 32$ ) (A) and fresh per hectare (B) ( $n = 3$ ). Different letters above the bars point out significant differences between the means based on Tukey's HSD test ( $p = 0.05$ ).

Regarding the nutritional parameters, the highest moisture content was recorded in the CHH 50% treatment, whereas the highest ash, protein, carbohydrates and energy content were recorded in the CGW25% treatment (Table 2). Finally, the highest fat content was measured in the GPH treatment regardless of the amount of byproduct incorporated in the soil. Similarly, the control treatment had the lowest amounts of protein and ash, whereas the incorporation of high amounts (50%) of cotton and hazelnut husks resulted in the lowest amounts of fat in the first case and carbohydrates and energy in the second one. The recorded values were within the same range of other reports indicating that lettuce is a leafy vegetable with high moisture content and low amounts of protein, fat, ash and carbohydrates [38]. However, these results are not comparable with our study since no identical growing media were implemented [39], while significant differences among the various lettuce



genotypes have been also reported [40]. The effect of substrates containing agroindustry byproducts on the nutritional parameters of vegetables has been highlighted in several studies. For example, by using biochar as hydroponic growth medium, an improved nutritional composition of various leafy vegetables was observed [41], while other materials such as oak sawdust, cotton seed hulls and olive press cake affected ash and protein content of *Hericium erinaceus* isolates [42]. Moreover, the substrate type may affect the nitrogen and nitrates content in spinach [43] and the nutritional composition of lettuce [44].

**Table 2.** Nutritional value (g/100 g fw), energy (kcal/100 g fw), free sugars (g/100 g fw) and organic acids (mg/100 g fw) of lettuce leaves in relation to the growth medium (mean  $\pm$  SD;  $n = 3$ ).

	Control	GHH25% *	GHH50%	CGW25%	CGW50%	GPH25%	GPH50%
<b>Nutritional Value</b>							
Moisture	95.9 $\pm$ 0.7 b	95.8 $\pm$ 0.6 b	96.2 $\pm$ 0.3 a	95.0 $\pm$ 0.5 c	95.9 $\pm$ 0.1 b	95.2 $\pm$ 0.1 c	95.8 $\pm$ 0.6 b
Fat	0.142 $\pm$ 0.002 e	0.15 $\pm$ 0.01 c	0.148 $\pm$ 0.003 c	0.157 $\pm$ 0.003 b	0.133 $\pm$ 0.009 e	0.162 $\pm$ 0.003 a	0.161 $\pm$ 0.003 a
Proteins	0.753 $\pm$ 0.005 f	0.889 $\pm$ 0.001 d	0.812 $\pm$ 0.004 e	1.15 $\pm$ 0.01 a	0.977 $\pm$ 0.003 c	1.02 $\pm$ 0.01 b	0.882 $\pm$ 0.001 e
Ash	0.56 $\pm$ 0.01 d	0.60 $\pm$ 0.01 c	0.60 $\pm$ 0.03 c	0.86 $\pm$ 0.02 a	0.73 $\pm$ 0.01 b	0.84 $\pm$ 0.01 a	0.74 $\pm$ 0.01 b
Carbohydrates	2.61 $\pm$ 0.01 c	2.58 $\pm$ 0.02 c	2.19 $\pm$ 0.02 f	2.81 $\pm$ 0.01 a	2.30 $\pm$ 0.01 e	2.74 $\pm$ 0.01 b	2.46 $\pm$ 0.01 d
Energy	14.73 $\pm$ 0.03 d	15.23 $\pm$ 0.01 c	13.35 $\pm$ 0.08 f	17.28 $\pm$ 0.05 a	14.31 $\pm$ 0.03 e	16.51 $\pm$ 0.04 b	14.83 $\pm$ 0.01 d
<b>Free Sugars</b>							
Fructose	0.47 $\pm$ 0.03 c	0.43 $\pm$ 0.03 d	0.43 $\pm$ 0.01 d	0.59 $\pm$ 0.01 a	0.43 $\pm$ 0.04 d	0.46 $\pm$ 0.02 c	0.49 $\pm$ 0.01 b
Glucose	0.267 $\pm$ 0.009 a	0.244 $\pm$ 0.005 b	0.22 $\pm$ 0.03 c	0.22 $\pm$ 0.02 c	0.18 $\pm$ 0.01 d	0.26 $\pm$ 0.03 a	0.223 $\pm$ 0.001 c
Sucrose	0.123 $\pm$ 0.003 a	0.088 $\pm$ 0.005 d	0.104 $\pm$ 0.001 b	0.125 $\pm$ 0.002 a	0.094 $\pm$ 0.002 c	0.087 $\pm$ 0.001 d	0.105 $\pm$ 0.001 b
Trehalose	0.017 $\pm$ 0.002 b	0.017 $\pm$ 0.002 b	0.013 $\pm$ 0.001 e	0.023 $\pm$ 0.001 a	0.015 $\pm$ 0.003 d	0.017 $\pm$ 0.001 b	0.016 $\pm$ 0.001 c
Sum	0.88 $\pm$ 0.02 b	0.77 $\pm$ 0.05 d	0.77 $\pm$ 0.02 d	0.96 $\pm$ 0.03 a	0.72 $\pm$ 0.05 e	0.83 $\pm$ 0.01 c	0.83 $\pm$ 0.01 c
<b>Organic Acids</b>							
Oxalic acid	286 $\pm$ 1 f	315 $\pm$ 3 e	278 $\pm$ 1 g	413 $\pm$ 8 a	339 $\pm$ 4 c	358 $\pm$ 1 b	330 $\pm$ 2 d
Malic acid	319 $\pm$ 7 e	389 $\pm$ 1 b	318 $\pm$ 6 e	401 $\pm$ 7 a	369 $\pm$ 5 c	405 $\pm$ 2 a	335 $\pm$ 6 d
Fumaric acid	tr	tr	tr	tr	tr	tr	tr
Sum	605 $\pm$ 9 e	704 $\pm$ 4 c	596 $\pm$ 7 f	814 $\pm$ 15 a	708 $\pm$ 1 c	763 $\pm$ 2 b	666 $\pm$ 8 d

\* CGW: cotton ginning waste; GHH: ground hazelnut husks; GPH: ground peanut husks; tr—traces. Different letters in the same row point out significant differences between the means based on Tukey's HSD test ( $p = 0.05$ ).

Table 2 presents the composition of free sugars. Fructose was the major compound, followed by glucose and sucrose, whereas trehalose was detected in lesser amounts. As reported in our study, Barickman et al. [45] suggested fructose to be the main detected sugar in lettuce leaves, whereas Fallovo et al. [46] recorded two to three times higher sucrose content than fructose and glucose. These contradictory results could be mainly associated with differences in the extraction protocols and determination assays used (liquid chromatography vs. spectrophotometric assays used by Barickman et al. [45] and Fallovo et al. [46], respectively). Regarding the effect of soil amendments, the application of cotton gin waste in low amounts (CGW25%) led to the highest amounts of fructose, sucrose, trehalose and total free sugars, while the highest glucose content was found in the control and the GPH25% treatments. Although the existing results regarding the impact of growing substrates on sugars content in lettuce are not comparable [47], according to the literature, growing conditions are strongly involved in sugars biosynthesis, especially the light quality [48–50]. Therefore, considering that the plants in our study were grown under identical light conditions it could be assumed that the observed differences could be assigned to different water and nutrient status in soil induced by the incorporation of different waste materials at different rates, since Fallovo et al. [39] already reported the effect of nutrients availability on sugars composition in lettuce. Moreover, sugars are the main substrate for flavonoids biosynthesis through the production of phenylalanine which is the precursor of flavonoid glycosides via the shikimic pathway [51]. Therefore, the low total sugars content observed for the CGW50% treatment could be associated with the increased flavonoids' content observed for the same treatment (see below the corresponding results).

The composition of organic acids is presented in Table 2. Only two organic acids were detected in traceable amounts, whereas traces of fumaric acid were also identified. The same compounds were identified through a metabolomics analysis in different lettuce varieties by Yang et al. [52]. The highest amounts of total and oxalic acid were observed in the CGW25% treatment, while malic acid content

was similarly high in CGW25% and GPH25% treatments. In contrast, the GHH50% treatment resulted in the lowest oxalic acid content which is an important quality feature of leafy vegetables [53,54]. Although lettuce is not a rich source of oxalic acid, the findings of the present study could be tested with vegetables that are oxalate accumulators, such as spinach [55]. As already mentioned in the case of free sugars composition, the recorded differences could be allocated to differences in water and nutrients availability in soil due to the incorporation of the tested byproducts at different rates.

Fatty acids composition is presented in Table 3. The major compound was  $\alpha$ -linolenic acid, followed by linoleic and palmitic acid (saturated fatty acid; SFA). Consequently, polyunsaturated fatty acids (PUFA) was the most abundant class (68.5% to 74.0%) followed by the saturated (SFA; 22.4–27.7%) and monounsaturated fatty acids (MUFA; 3.5–4.3%). Similarly to the present study, Kim et al. [56] and Ko et al. [57] reported that fatty acids in lettuce consist mostly of PUFA ( $\alpha$ -linolenic and linoleic acids) and despite its low lipid content, the high consumption of lettuce throughout the world may significantly contribute to the improvement of blood lipid profile and the fortification of human body against chronic diseases. Moreover, the same study as well as the study of Yang et al. [52] highlighted the differences in fatty acids profile that exist among the various types (leafy and head types) and varieties of lettuce, which has a great importance considering the established consumer preferences in specific markets. A variable response was observed to the tested materials and although most of the fatty acids had the highest content in the control treatment,  $\alpha$ -linolenic was the richest in the GHH50% treatment. This resulted in similar trends for the SFA and PUFA, while MUFA were the highest in the CGW25% and GPH25% treatments.

**Table 3.** Fatty acids (relative %) and tocopherols (mg/100 g fw) composition of lettuce leaves in relation to the growth medium (mean  $\pm$  SD;  $n = 3$ ).

Fatty Acids	Control	GHH25% *	GHH50%	CGW25%	CGW50%	GPH25%	GPH50%
C12:0	0.106 $\pm$ 0.004 a	0.089 $\pm$ 0.005 b	0.086 $\pm$ 0.006 b	0.066 $\pm$ 0.001 c	0.059 $\pm$ 0.002 d	0.069 $\pm$ 0.002 c	0.045 $\pm$ 0.001 e
C13:0	0.125 $\pm$ 0.001 a	0.034 $\pm$ 0.001 d	0.065 $\pm$ 0.004 b	0.025 $\pm$ 0.001 e	0.022 $\pm$ 0.002 f	0.051 $\pm$ 0.004 c	0.026 $\pm$ 0.002 e
C14:0	1.86 $\pm$ 0.02 a	1.5 $\pm$ 0.1 c	1.5 $\pm$ 0.1 c	1.46 $\pm$ 0.02 d	1.7 $\pm$ 0.1 b	1.03 $\pm$ 0.01 f	1.06 $\pm$ 0.01 e
C14:1	0.069 $\pm$ 0.001 c	0.047 $\pm$ 0.002 d	0.035 $\pm$ 0.002 e	0.012 $\pm$ 0.001 f	0.084 $\pm$ 0.002 b	0.38 $\pm$ 0.01 a	0.049 $\pm$ 0.001 d
C15:0	0.252 $\pm$ 0.007 a	0.25 $\pm$ 0.01 a	0.22 $\pm$ 0.01 c	0.230 $\pm$ 0.003 b	0.215 $\pm$ 0.004 d	0.153 $\pm$ 0.008 e	0.23 $\pm$ 0.01 b
C16:0	19.2 $\pm$ 0.2 a	16.93 $\pm$ 0.03 d	15.7 $\pm$ 0.8 f	17.41 $\pm$ 0.05 c	17.0 $\pm$ 0.5 d	17.7 $\pm$ 0.2 b	16.7 $\pm$ 0.4 e
C16:1	2.0 $\pm$ 0.1 d	1.9 $\pm$ 0.1 e	1.98 $\pm$ 0.05 d	1.95 $\pm$ 0.02 d	2.2 $\pm$ 0.1 b	2.3 $\pm$ 0.1 a	2.12 $\pm$ 0.06 c
C17:0	0.222 $\pm$ 0.001 a	0.20 $\pm$ 0.02 b	0.19 $\pm$ 0.01 c	0.202 $\pm$ 0.002 b	0.203 $\pm$ 0.004 b	0.18 $\pm$ 0.01 d	0.20 $\pm$ 0.01 b
C18:0	1.67 $\pm$ 0.07 b	1.56 $\pm$ 0.04 d	1.57 $\pm$ 0.06 c	1.73 $\pm$ 0.04 a	1.67 $\pm$ 0.01 b	1.67 $\pm$ 0.03 b	1.52 $\pm$ 0.01 e
C18:1n9 c	1.77 $\pm$ 0.01 c	1.75 $\pm$ 0.07 c	1.47 $\pm$ 0.04 e	2.4 $\pm$ 0.2 a	1.82 $\pm$ 0.07 b	1.61 $\pm$ 0.06 d	1.65 $\pm$ 0.02 d
C18:2n6 c	25.7 $\pm$ 0.3 a	24.6 $\pm$ 0.4 c	23.6 $\pm$ 0.3 d	24.4 $\pm$ 0.1 c	22.4 $\pm$ 0.3 e	24.3 $\pm$ 0.2 c	25.01 $\pm$ 0.03 b
C18:3n3	42.1 $\pm$ 0.1 e	47.1 $\pm$ 0.3 c	50.2 $\pm$ 0.7 a	46.0 $\pm$ 0.2 d	49.3 $\pm$ 0.2 b	45.6 $\pm$ 0.4 d	47.1 $\pm$ 0.3 c
C20:0	0.48 $\pm$ 0.01 c	0.47 $\pm$ 0.01 c	0.43 $\pm$ 0.02 d	0.52 $\pm$ 0.02 b	0.53 $\pm$ 0.01 b	0.62 $\pm$ 0.02 a	0.46 $\pm$ 0.01 c
C20:2	0.65 $\pm$ 0.02 a	0.313 $\pm$ 0.001 c	0.31 $\pm$ 0.01 c	0.259 $\pm$ 0.008 e	0.256 $\pm$ 0.004 e	0.362 $\pm$ 0.004 b	0.265 $\pm$ 0.001 d
C22:0	1.27 $\pm$ 0.02 c	1.17 $\pm$ 0.02 d	1.06 $\pm$ 0.01 e	1.30 $\pm$ 0.02 b	1.31 $\pm$ 0.04 b	1.48 $\pm$ 0.01 a	1.31 $\pm$ 0.05 b
C23:0	0.191 $\pm$ 0.001 d	0.215 $\pm$ 0.001 b	0.20 $\pm$ 0.01 c	0.17 $\pm$ 0.01 f	0.178 $\pm$ 0.002 e	0.366 $\pm$ 0.009 a	0.214 $\pm$ 0.001 b
C24:0	2.29 $\pm$ 0.07 a	1.83 $\pm$ 0.01 e	1.34 $\pm$ 0.03 f	1.91 $\pm$ 0.01 d	1.0 $\pm$ 0.3 g	2.14 $\pm$ 0.01 b	2.08 $\pm$ 0.16 c
SFA	27.7 $\pm$ 0.2 a	24.24 $\pm$ 0.07 d	22.4 $\pm$ 0.9 f	25.03 $\pm$ 0.07 v	23.9 $\pm$ 0.3 e	25.5 $\pm$ 0.2 b	23.8 $\pm$ 0.3 e
MUFA	3.8 $\pm$ 0.1 c	3.68 $\pm$ 0.03 d	3.49 $\pm$ 0.09 e	4.3 $\pm$ 0.2 a	4.1 $\pm$ 0.2 b	4.28 $\pm$ 0.06 a	3.82 $\pm$ 0.04 c
PUFA	68.5 $\pm$ 0.4 e	72.08 $\pm$ 0.04 c	74 $\pm$ 1 a	70.7 $\pm$ 0.3 d	72.0 $\pm$ 0.5 c	70.3 $\pm$ 0.2 d	72.3 $\pm$ 0.3 b
<b>Tocopherols</b>							
$\alpha$ -Tocopherol	0.054 $\pm$ 0.002 f	0.101 $\pm$ 0.002 c	0.141 $\pm$ 0.001 a	0.115 $\pm$ 0.001 b	0.091 $\pm$ 0.002 d	0.089 $\pm$ 0.001 e	0.089 $\pm$ 0.001 e
$\gamma$ -Tocopherol	0.293 $\pm$ 0.002 g	0.405 $\pm$ 0.001 e	0.420 $\pm$ 0.001 d	0.509 $\pm$ 0.002 b	0.459 $\pm$ 0.007 c	0.517 $\pm$ 0.006 a	0.348 $\pm$ 0.008 f
$\delta$ -Tocopherol	0.011 $\pm$ 0.001 e	0.016 $\pm$ 0.001 b	0.016 $\pm$ 0.001 b	0.014 $\pm$ 0.001 c	0.013 $\pm$ 0.001 d	0.018 $\pm$ 0.001 a	0.014 $\pm$ 0.001 c
Sum	0.360 $\pm$ 0.001 g	0.520 $\pm$ 0.001 e	0.580 $\pm$ 0.001 d	0.640 $\pm$ 0.001 a	0.570 $\pm$ 0.007 c	0.630 $\pm$ 0.007 b	0.460 $\pm$ 0.007 f

\* CGW: cotton ginning waste; GHH: ground hazelnut husks; GPH: ground peanut husks. Different letters in the same row point out significant differences between the means based on Tukey's HSD test ( $p = 0.05$ ).

Tocopherols composition is presented in Table 3.  $\gamma$ -tocopherol was the most abundant vitamin E isoform, followed by  $\alpha$ - and  $\delta$ -tocopherols, a result which agrees with the findings of Mou [40] who reported a similar profile of tocopherols for various lettuce types (except for the crisphead lettuce) although  $\delta$ -tocopherol was not present. Similarly, Samuolienė et al. [58] suggested the content of the same two vitamin E isoforms ( $\alpha$ - and  $\gamma$ -tocopherol) to be affected by light quality, whereas in another study all four tocopherols were detected [59]. Moreover, the CGW25% treatment increased significantly the overall tocopherols content, while in regard to individual tocopherols the GHH50%



treatment increased  $\alpha$ -tocopherol and that of GPH25% increased  $\gamma$ - and  $\delta$ -tocopherol. In any case, the content of individual and total tocopherols was the lowest in the control treatment indicating the positive effects of soil amendment with the tested materials on lettuce quality. According to the literature, the growing conditions and the genotype [60,61] or the harvesting stage and the plant part [62,63] are key factors for tocopherols composition in leafy vegetables, while in the case of fruit vegetables harvesting stage, fertilization regime and water availability may also have an effect on this parameter [64–66]. Other researchers have also mentioned the importance of cultivation management and growing system on phytochemicals composition via the induction of main genes involved in the biosynthetic pathways [67,68]. This finding is very important, since apart from the genotypic effect on tocopherols composition in lettuce, simple and cost-effective cultivation practices, such as the soil amendment with agroindustry byproducts tested in our study, could enhance the quality of the final produce.

The data regarding phenolic compounds identification and quantification are presented in Tables 4 and 5, respectively. Thirteen compounds were tentatively identified, namely ten phenolic acids (caffeic and *p*-coumaric acid derivatives) and three *O*-glycosylated flavonoids (quercetin and kaempferol derivatives) (Table 4). The phenolic profile of *L. sativa* leaves has been extensively described in the literature [69,70], also using HPLC methodologies coupled to mass spectrometry, such as in the variety *longifolia* by Ribas-Agustí et al. [71] or in six different varieties by Alarcón-Flores et al. [72], in red oak leaf (“Krysthine RZ”) and green oak leaf (“Versai RZ”) by Viacava et al. [73] and cv. *Omega* by Materska et al. [74]. As such the tentative identification was performed using the previously described profiles. Despite that the plant varieties studied by other authors were not herein present, the phenolic profile in our study was very similar, having been all the compounds found in the existing bibliography also present in the tested variety. Peaks 4 ([M-H]<sup>−</sup> at *m/z* 353), 11 ([M-H]<sup>−</sup> at *m/z* 477) and 12 ([M-H]<sup>−</sup> at *m/z* 461) were positively identified as 5-*O*-caffeoylquinic acid, quercetin-3-*O*-glucuronide and kaempferol-3-*O*-glucuronide, respectively, in comparison with available standard compounds.

**Table 4.** Retention time (Rt), wavelengths of maximum absorption in the visible region ( $\lambda_{\max}$ ), mass spectral data and tentative identification of the phenolic compounds present in the studied lettuce leaves.

Peak	Rt (min)	$\lambda_{\max}$ (nm)	[M-H] <sup>−</sup> ( <i>m/z</i> )	MS <sup>2</sup> ( <i>m/z</i> )	Tentative Identification
1	5.21	323	341	179 (100)	Caffeic acid hexoside isomer I
2	5.73	323	341	179 (100)	Caffeic acid hexoside isomer II
3	6.44	323	341	179 (100)	Caffeic acid hexoside isomer III
4	7.1	324	353	191 (100), 179 (11), 173 (3)	5- <i>O</i> -Caffeoylquinic acid
5	9.6	326	295	179 (100), 133 (33)	Caffeoylmalic acid isomer I
6	9.89	326	295	179 (100), 133 (42)	Caffeoylmalic acid isomer II
7	11.42	315	337	191 (100), 173 (3), 163 (17)	<i>p</i> -Coumaroylquinic acid
8	12.8	326	473	311 (100), 293 (92), 179 (5), 149 (3)	di- <i>O</i> -Caffeoyltartaric acid isomer I
9	13.25	329	473	311 (100), 293 (98), 179 (6), 149(4)	di- <i>O</i> -Caffeoyltartaric acid isomer II
10	13.72	328	473	311 (100), 293 (90), 179 (5), 149 (3)	di- <i>O</i> -Caffeoyltartaric acid isomer III
11	18.08	352	477	301 (100)	Quercetin-3- <i>O</i> -glucuronide
12	18.57	348	461	285 (100)	Kaempferol-3- <i>O</i> -glucuronide
13	20.12	354	549	505 (52), 463 (33), 301 (100)	Quercetin- <i>O</i> -malonylhexoside

The quantification of individual compounds revealed a variable composition among the tested byproducts (Table 5). In all the samples, phenolic acids were recorded in higher contents compared to flavonoids in amounts that ranged between 53.4  $\mu\text{g}/100\text{ g fw}$  to 89.0  $\mu\text{g}/100\text{ g fw}$  and 22.46  $\mu\text{g}/100\text{ g fw}$  to 29.49  $\mu\text{g}/100\text{ g fw}$ , respectively. Di-*O*-Caffeoyltartaric acid (isomer I) was the major compound followed by its isomers II and III and 5-*O*-Caffeoylquinic acid with the highest contents being observed in plants grown in soil where ground hazelnut husks were incorporated (Table 5). The same trend was observed for total phenolic acids and total phenolic compounds concentrations. On the contrary, the content of the detected flavonoids was the highest for the CGW25% treatment which was also reflected to the

total flavonoids content. Similarly to our study, the use of alternative growth substrates resulted in significant alterations in total phenols and total flavonoids content of two culinary herbs (parsley and dill) [75], whereas Chrysargyris et al. [76] suggested a variable response of three ornamental plants (marigold, petunia and matthiola) to substrates with different composition in terms of paper waste rates. A varied response of the total phenols content to the use of olive-stone waste as growing substrate was also reported in the seedlings of three vegetable species (cauliflower, broccoli and cabbage) [77], whereas Kim et al. [56] suggested significant differences between various types and varieties of lettuce in terms of total phenols content. In addition, Petropoulos et al. [1] reported significantly altered composition of phenolic compounds in pot-grown spiny chicory plants depending on the growth substrate composition, a finding which agrees with the results of this study.

**Table 5.** Phenolic compounds quantification ( $\mu\text{g}/100\text{ g fw}$ ) of lettuce leaves extracts in relation to the growth medium (mean  $\pm$  SD;  $n = 3$ ).

Peak	Compound	Control	GHH25% *	GHH50%	CGW25%	CGW50%	GPH25%	GPH50%
1	Caffeic acid hexoside isomer I	tr	0.36 $\pm$ 0.02 b	0.35 $\pm$ 0.02 c	0.38 $\pm$ 0.02 a	tr	0.36 $\pm$ 0.02 b	tr
2	Caffeic acid hexoside isomer II	tr	0.39 $\pm$ 0.02 b	0.35 $\pm$ 0.02 c	0.68 $\pm$ 0.04 a	0.13 $\pm$ 0.01 d	0.38 $\pm$ 0.01 b	tr
3	Caffeic acid hexoside isomer III	tr	0.75 $\pm$ 0.03 a	0.56 $\pm$ 0.01 b	0.10 $\pm$ 0.01 e	0.14 $\pm$ 0.01 d	0.25 $\pm$ 0.01 c	tr
4	5-O-Caffeoylquinic acid	10.2 $\pm$ 0.1 e	16.4 $\pm$ 0.7 b	18.7 $\pm$ 0.1 a	15.7 $\pm$ 0.4 c	5.60 $\pm$ 0.06 g	10.9 $\pm$ 0.1 d	9.80 $\pm$ 0.06 f
5	Caffeoylmalic acid isomer I	5.63 $\pm$ 0.04 e	9.75 $\pm$ 0.02 a	9.39 $\pm$ 0.02 b	8.20 $\pm$ 0.05 c	3.38 $\pm$ 0.05 g	3.54 $\pm$ 0.02 f	6.81 $\pm$ 0.02 d
6	Caffeoylmalic acid isomer II	7.8 $\pm$ 0.2 c	10.8 $\pm$ 0.1 a	10.1 $\pm$ 0.2 b	7.67 $\pm$ 0.07 dd	5.7 $\pm$ 0.1 f	6.72 $\pm$ 0.08 e	7.60 $\pm$ 0.03
7	<i>p</i> -Coumaroylquinic acid	1.49 $\pm$ 0.05 d	1.96 $\pm$ 0.05 b	2.02 $\pm$ 0.04 b	1.42 $\pm$ 0.02 e	2.96 $\pm$ 0.08 a	2.9 $\pm$ 0.1 a	1.86 $\pm$ 0.05 c
8	di-O-Caffeoyltartaric acid isomer I	12.8 $\pm$ 0.5 e	17.97 $\pm$ 0.06 a	18 $\pm$ 1 a	15.4 $\pm$ 0.6 d	17 $\pm$ 1 c	17.8 $\pm$ 0.4 b	10.1 $\pm$ 0.2 f
9	di-O-Caffeoyltartaric acid isomer II	11.8 $\pm$ 0.2 f	16.7 $\pm$ 0.1 a	15.9 $\pm$ 0.1 b	13.3 $\pm$ 0.4 e	15.6 $\pm$ 0.2 c	15.0 $\pm$ 0.3 d	9.71 $\pm$ 0.08 g
10	di-O-Caffeoyltartaric acid isomer III	8.5 $\pm$ 0.1 e	13.9 $\pm$ 0.2 a	12.3 $\pm$ 0.4 b	10.3 $\pm$ 0.1 d	12 $\pm$ 1 c	12.4 $\pm$ 0.7 b	7.57 $\pm$ 0.08 f
11	Quercetin-3-O-glucuronide	7.28 $\pm$ 0.01 f	8.05 $\pm$ 0.07 d	8.35 $\pm$ 0.01 c	9.28 $\pm$ 0.03 a	8.09 $\pm$ 0.02 d	8.92 $\pm$ 0.01 b	7.71 $\pm$ 0.01 e
12	Kaempferol-3-O-glucuronide	7.48 $\pm$ 0.02 f	8.3 $\pm$ 0.2 e	8.47 $\pm$ 0.05 d	10.09 $\pm$ 0.06 a	8.82 $\pm$ 0.03 c	9.56 $\pm$ 0.02 b	8.29 $\pm$ 0.05 e
13	Quercetin-O-malonylhexoside	7.70 $\pm$ 0.01 f	8.52 $\pm$ 0.01 d	9.31 $\pm$ 0.07 c	10.12 $\pm$ 0.04 a	8.41 $\pm$ 0.03 e	9.47 $\pm$ 0.02 b	8.5 $\pm$ 0.1 d
	Total Phenolic Acids	58 $\pm$ 1 e	89 $\pm$ 1 a	88 $\pm$ 1 a	73.2 $\pm$ 0.2 b	62.4 $\pm$ 0.1 d	70 $\pm$ 1 c	53.4 $\pm$ 0.3 f
	Total Flavonoids	22.46 $\pm$ 0.02 f	24.89 $\pm$ 0.08 d	26.1 $\pm$ 0.1 c	29.49 $\pm$ 0.08 a	25.3 $\pm$ 0.1 d	28.0 $\pm$ 0.1 b	24.5 $\pm$ 0.1 e
	Total Phenolic Compounds	81 $\pm$ 1 e	114 $\pm$ 1 a	114 $\pm$ 1 a	102.7 $\pm$ 0.1 b	87.7 $\pm$ 0.7 d	98 $\pm$ 1 c	77.9 $\pm$ 0.5 f

\* CGW: cotton ginning waste; GHH: ground hazelnut husks; GPH: ground peanut husks; tr—traces. Different letters in the same row point out significant differences between the means based on Tukey's HSD test ( $p = 0.05$ ).

The antioxidant activity of extracts was tested with two different assays (TBARS and OxHLIA) (Table 6). The highest antioxidant capacity was observed for the GHH 0% treatment, which is partly justified by the highest  $\alpha$ -tocopherol and PUFAs content (see Table 3) for the same treatment, especially in the case of TBARS assay which measures the peroxidation of lipids [78]. Although antioxidant activity of lettuce is strongly associated with total phenolic compounds content [56], the implemented assay [79] and the genotype [80,81] may also have a significant effect resulting in variable results. The substrate type may affect the antioxidant activity of leafy greens [1,77,82], culinary herbs [75] and ornamental plants [76], whereas Petropoulos et al. [1] suggested that *Cichorium spinosum* plants grown in soil exhibited higher antioxidant activity than plants grown in substrates containing agroindustry byproducts due to severe stress conditions which increased phenolic compounds content. However, this trend was not confirmed in our study since high phenolic compounds content was not followed by similarly high antioxidant activity and other compounds such as  $\alpha$ -tocopherol should be implicated in the antioxidant mechanism of lettuce plants [60,78].

Table 6 presents the cytotoxicity results, where none of the tested extracts exhibited in vitro toxicity to non-tumor (PLP2 cell line) or against non-small cell lung cancer cell lines (NCI-H460). Moreover, extracts obtained from leaves of GPH50% treatment grown in soil where 50% of ground peanut hulls were incorporated exhibited slight in vitro toxicity against the rest of the tested cell lines (HeLa, HepG2 and MCF-7), as well the treatment of GHH 50% (only against MCF-7 cell line). According to the literature, flavonoids present in lettuce extracts could exhibit in vitro toxic effects against human hepatoma (HepG2) cells [83], however the main compound responsible for these

effects was luteolin-7-*O*-glucoside which was not detected in our study. Moreover, extracts from iodine-biofortified lettuce were effective against Caco-2 cancer cell line [84], while Durazzo et al. [85] suggested significant effects of cultivation practices on cytotoxicity of lettuce extracts against the same cell line. Similarly to our study, Karkanis et al. [82] reported a significant impact of growth substrate on the cytotoxic effects of *Sanguisorba minor* leaf and root extracts, which indicates that differences in the physicochemical properties of the growing medium may affect the bioactivities of the final produce.

**Table 6.** Antioxidant activity (EC<sub>50</sub>, µg/mL) and cytotoxicity (GI<sub>50</sub>, values µg/mL) of lettuce leaves extracts in relation to the growth medium (mean ± SD; n = 3).

	Control	GHH25% *	GHH50%	CGW25%	CGW50%	GPH25%	GPH50%	Positive Control
Antioxidant activity								Trolox
TBARS	169 ± 8 a	50 ± 2 e	27 ± 1 f	96 ± 5 c	76 ± 1 d	74 ± 5 d	114 ± 6 b	23 ± 0.1
OxHLIA Δt = 60 min	383 ± 16 e	553 ± 32 b	186 ± 11 f	550 ± 28 b	500 ± 15 c	590 ± 73 a	451 ± 17 d	19.6 ± 0.7
Cytotoxicity to non-tumor cell lines								Ellipticine
PLP2	>400	>400	>400	>400	>400	>400	>400	2.3 ± 0.1
Cytotoxicity to tumor cell lines								
HeLa	>400	>400	>400	>400	>400	>400	258 ± 14	0.91 ± 0.1
HepG2	>400	>400	>400	>400	>400	>400	269 ± 20	1.10 ± 0.09
MCF-7	>400	>400	329 ± 17 a	>400	>400	>400	307 ± 6 b	1.21 ± 0.02
NCI-H460	>400	>400	>400	>400	>400	>400	>400	1.03 ± 0.09

\* CGW: cotton ginning waste; GHH: ground hazelnut husks; GPH: ground peanut husks; tr—traces. Different Latin letters in the same row indicate significant differences between the means according to Tukey's HSD test or Student's *t* test (*p* = 0.05).

The antimicrobial properties of lettuce leaves in response to the tested byproducts are presented in Table 7. None of the extracts showed better antibacterial activity than the used positive controls against the six evaluated bacteria. However, specific extracts were more efficient, such as the treatments of GCW (25% and 50%) and GPH25% against *Staphylococcus aureus* or the treatments of GHH50% and CGW25% against *Bacillus aureus*. For the rest of the tested bacteria, no significant differences between the tested extracts were observed, except for the case of GHH25% treatment which routinely showed the lowest efficacy. Similar results were suggested by Noumedem et al. [86] who also recorded a moderate efficacy of lettuce leaves' extracts against various bacteria strains without however being more efficient than the tested positive control. Moreover, according to the studies of Karkanis et al. [82] and Petropoulos et al. [1], growth substrate may have an effect on the antimicrobial properties of the final produce through the changes in the chemical profile of phytochemicals which are responsible for such properties.

**Table 7.** Antibacterial activity (MIC and MBC, mg/mL) and antifungal activity (MIC and MFC, mg/mL) of lettuce leaves extracts in relation to the growth medium (mean ± SD; n = 3).

	Control	GHH25% †	GHH50%	CGW 5%	CGW50%	GPH25%	GPH50%	Positive Controls	
Antibacterial Activity								Streptomycin	Ampicillin
<i>S. aureus</i> (ATCC 11632)	MIC *	1.75	1.75	1.75	0.89	0.89	0.89	1.75	0.012
	MBC	3.50	3.50	3.50	1.75	1.75	1.75	3.50	0.025
<i>B. cereus</i> (food isolate)	MIC	0.89	0.89	0.44	0.44	0.89	0.89	0.89	0.10
	MBC	1.75	1.75	0.89	0.89	1.75	1.75	1.75	0.20
<i>L. monocytogenes</i> (NCTC 7973)	MIC	1.75	3.50	1.75	1.75	1.75	1.75	1.75	0.20
	MBC	3.50	7.00	3.50	3.50	3.50	3.50	3.50	0.50
<i>S. typhimurium</i> (ATCC 13311)	MIC	1.75	3.50	1.75	1.75	1.75	1.75	1.75	0.20
	MBC	3.50	7.00	3.50	3.50	3.50	3.50	3.50	1.20
<i>E. cloacae</i> (ATCC 35030)	MIC	1.75	3.50	1.75	1.75	1.75	1.75	1.75	0.003
	MBC	3.50	7.00	3.50	3.50	3.50	3.50	3.50	0.012
<i>E. coli</i> (ATCC 25922)	MIC	0.89	3.50	0.89	0.89	0.89	0.89	0.89	0.20
	MBC	1.75	7.00	1.75	1.75	1.75	1.75	1.75	0.50
Antifungal Activity								Ketoconazole	
<i>A. fumigatus</i> (ATCC 9197)	MIC	0.88	0.88	0.44	0.44	0.44	0.88	0.22	0.20
	MFC	1.75	1.75	0.88	0.88	0.88	1.75	0.44	0.50

Table 7. Cont.

		Control	GHH25% ‡	GHH50%	CGW 5%	CGW50%	GPH25%	GPH50%	Positive Controls
<i>A. versicolor</i>	MIC	0.88	0.88	0.44	0.44	0.44	0.88	0.44	0.20
(ATCC 11730)	MFC	1.75	1.75	0.88	0.88	0.88	1.75	0.88	0.47
<i>A. niger</i>	MIC	0.88	0.88	0.44	0.44	0.44	0.44	0.44	0.20
(ATCC 6275)	MFC	1.75	1.75	0.88	0.88	0.88	0.88	0.88	0.50
<i>P. funiculosus</i>	MIC	0.44	0.44	0.44	0.44	0.22	0.44	0.44	0.20
(ATCC 36839)	MFC	0.88	0.88	0.88	0.88	0.44	0.88	0.88	0.50
<i>P. v. var.</i>	MIC	1.75	0.44	0.88	0.44	0.44	0.44	0.44	0.20
<i>cyclopium</i>	MFC	3.20	0.88	1.75	0.88	0.88	0.88	0.88	0.30
(food isolate)									
<i>T. viride</i>	MIC	0.44	0.22	0.44	0.22	0.11	0.22	0.22	0.20
(IAM 5061)	MFC	0.88	0.44	0.88	0.44	0.22	0.44	0.44	0.30

\* MIC: minimum inhibitory activity; MBC: minimum bactericidal activity; MFC: minimum fungicidal activity.

‡ CGW: cotton ginning waste; GHH: ground hazelnut husks; GPH: ground peanut husks.

Regarding the antifungal activities of the evaluated extracts, the positive controls were more efficient than the leaf extracts in most of the cases, except for *Aspergillus fumigatus* where the extracts of the GPH50% treatment had the lowest MFC values, as well as in the case of *Trichoderma viride* where the extracts of the CGW50% treatments were more efficient than the positive control (Table 7). Similar findings were observed by Karkanis et al. [82] who evaluated the effect of growth medium on the antifungal activities of *Sanguisorba minor* root and leaf extracts and reported a varied response to the tested growing medium, whereas Petropoulos et al. [1] did not observe any significant fungicidal effects for the extracts of spiny chicory leaves grown in different growth substrates.

#### 4. Conclusions

The findings of this study were promising and suggested the alternative use of organic waste from cotton, ground peanut and hazelnut as soil amendments, aiming to reduce the environmental pollution and the pressure to agro-ecosystems that the improper disposal of agroindustry waste may cause. The most beneficial effect on crop performance was observed for the ground peanut husks when applied in high amounts (GHH 50%) in the soil, followed by the other two tested materials (cotton ginning waste and ground hazelnut husks) at the same amounts. Considering that most of the studies related with organic waste utilization focus on the impact on soil characteristics and crop growth parameters, limited literature exists for the effect of these byproducts on the quality and the chemical profile of the final produce. As such, the findings of this study increase the knowledge towards the sustainable production of high-quality vegetables and indicate cost effective means that could allow the improvement of the quality of the final product. Therefore, the incorporation of crop byproducts in soil for lettuce cultivation may have a direct effect on improving soil physicochemical characteristics, as well as an indirect one through the increase in lettuce crop performance and the improvement of the quality of the final produce. However, prior to suggesting the extended use of these materials, further studies are needed with different soil types and different crops to identify the amounts of organic waste that will be beneficial for the physicochemical properties of soil and crop performance and quality as well.

**Author Contributions:** Conceptualization, S.A.P.; methodology, Â.F., S.P., M.I.D., C.P., R.C., J.P. and A.C.; software, M.I.D., C.P., R.C., A.C. and Â.F.; validation, M.I.D., C.P., R.C. and Â.F.; investigation, M.I.D., Â.F., L.B., C.P., A.C., J.P. and R.C.; data curation, S.A.P., M.I.D., C.P., R.C., S.P., J.P. and Â.F.; writing—original draft preparation, S.A.P., Â.F. and L.B.; writing—review and editing, S.A.P., L.B., N.T., M.D.S. and I.C.F.R.F.; visualization, S.A.P.; supervision, S.A.P., M.D.S. and I.C.F.R.F.; project administration, S.A.P., N.T., M.D.S. and I.C.F.R.F.; funding acquisition, I.C.F.R.F., Â.F., M.I.D., R.C., C.P. and L.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020) and national funding by FCT, P.I., through the institutional scientific employment program-contract for L. Barros, A. Fernandes, M. I. Dias and R. Calhelha. C. Pereira through the celebration of program-contract foreseen in No. 4, 5 and 6 of article 23 of Decree-Law No. 57/2016, of 29 August, mended by Law No. 57/2017, of 19 July. The authors are grateful to the FEDER-Interreg

España-Portugal programme for financial support through the project 0377\_Iberphenol\_6\_E; and to the Ministry of Education, Science and Technological Development of Republic of Serbia (451-03-68/2020-14/200007).

**Acknowledgments:** The authors are grateful to Kagiantzas Dimitrios and Sofia Simopoulou for their assistance during the experiment.

**Conflicts of Interest:** The authors declare no conflict of interest.

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