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Effects of brewer's spent grain biochar on the growth and quality of leaf lettuce (*Lactuca sativa* L. var. *crispa*.)

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Abstract

The need for organic soil amendments is increasing in the Republic of Korea against the backdrop of increased soil acidification and nutrient losses. The pyrolysis of biomass produces biochar which not only increases soil productivity but also provides environmental benefits through carbon sequestration. The portion of the brewer's spent grain (BSG) recycled is by far less than the amount generated, but pyrolysis can help to reverse this trend by turning BSG waste into a valuable soil amendment. The current study, therefore, evaluated the effects of brewer's spent grain biochar (BB_{xxx}) produced at three different temperatures of 300 °C, 500 °C and 700 °C on the yield and quality characteristics of the leaf lettuce as well as the effects on soil chemical properties through a pot experiment. Each of the BB_{xxx} and BSG were added to the soil at two rates of 2% and 5% by weight. The pH and carbon content of the BB_{xxx} increased with increasing pyrolysis temperatures and the trend was replicated in the soil upon biochar application i.e. the soil pH and carbon content increased alongside temperatures at which biochar was pyrolyzed. On the other hand, however, the soil electrical conductivity (EC) diminished with the increasing pyrolysis temperatures of the biochar applied. With regards to crop growth, the BB₅₀₀ 5% amendment produced the highest marketable yield of the leaf lettuce and while the lettuce grown on the control produced leaf lettuce with the lowest content of nitrate nitrogen, BB₅₀₀ 5% amendment generally produced the highest quality lettuce. The results indicate that BB₅₀₀ performed agronomically better than the rest of the amendments and is thus recommended as an effective BSG recycling measure.

Keywords: Biochar, Brewer's spent grain, Leaf lettuce, Pyrolysis, Soil amendment

Introduction

The granite derived soil predominant in the Republic of Korea has high silicic acid and low cation contents which render it impervious and thus susceptible to erosion from the surfeit of rainfall received which by far exceeds the evaporation rates, culminating into nutrient losses and soil acidification [1]. Soil acidification is further exacerbated directly or indirectly by excessive reactive nitrogen

in the Republic of Korea atmosphere ensuing from automobiles, power plants, chemical fertilizers, etc. the concentration of which is the highest amongst the OECD countries (1.3 times more than the OECD average) [1, 2]. There is thus an increasing need to improve the physical, chemical and biological properties of the soil, and biochar is touted as the right organic soil amendment for this job because of its wide-ranging benefits and is receiving great attention.

Biochar is a solid, carbon-rich product which results from heating biomass under conditions of no or limited supply of oxygen in a process called pyrolysis [3]. A lot of research has been done regarding the importance of biochar as a soil amendment with a majority of them

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reporting improved agricultural productivity attributable to improved soil properties including increasing pH, water retention, ion exchange capacity, and improving the microbial environment [4]. Biochar as a sustainable waste management technique provides environmental benefits because it reduces the volume of organic waste resources such as agricultural by-products [5]. It is also an efficient pollutant adsorbent [6–8] as well as a good greenhouse gas emission countermeasure [9–12]. The latter is largely because biochar applied to the soil semi-permanently sequesters carbon [12, 13]. This is possible due to the stability of biochar, which can exist in the soil for an extended period of time owing to its strong resistance to microbial decomposition [4].

Beer is one of the most popular alcoholic beverages in the world and it ranks first in terms of preference for alcoholic beverages in the Republic of Korea [14]. In the manufacture of beer, malt is removed before the fermentation process, in which case BSG, rich in several useful nutrients and functional substances, such as protein, fat, and fiber is generated. BSG is the most abundant brewing by-product, accounting for about 85% of the total detritus generated which represents approximately 20 kg per 100 L of beer produced [15]. BSG is mainly used as fodder for livestock due to its abundant nutrients, but it's not utilized at the same pace as its generated. In fact, about 40% of the BSG is dumped into the ocean presaging sea contamination [16]. Therefore, further recycling measures of BSG are required, especially because the London Dumping Convention, which South Korea joined completely banned the ocean dumping of organic wastes since 2012 [17].

Leaf lettuce is an annual herbaceous plant of the family Compositae, mostly used as ssam or salad in Korea [18]. Leaf lettuce accounts for a large proportion of leafy vegetables produced in the greenhouses. Domestic production of leaf lettuce is estimated at 224.6 billion won, which is the second largest of all leafy vegetables grown [19]. Leaf lettuce contains functional substances such as carotenoid, and anthocyanin in red lettuce, vitamins A, B, C, E, and a large amount of iron and fiber, which are known for physiological activities such as anti-inflammatory, antioxidant, and calming effect [20]. Previous studies by Jung [21] and Oh et al. [5] have reported increased EC upon biochar addition to the soil, and since leaf lettuce is one of the crops relatively sensitive to salt abundance [22], it is the suitable crop to assess whether biochar induced EC increments cause salt stress to plants.

Therefore, this study was conducted to examine pyrolysis as a way of recycling BSG, delineating the effect of pyrolysis temperatures on the physicochemical properties of the biochar produced. The study also aimed to

evaluate the effects of BB_{XXX} on the growth and quality of leaf lettuce, and chemical properties of the soil.

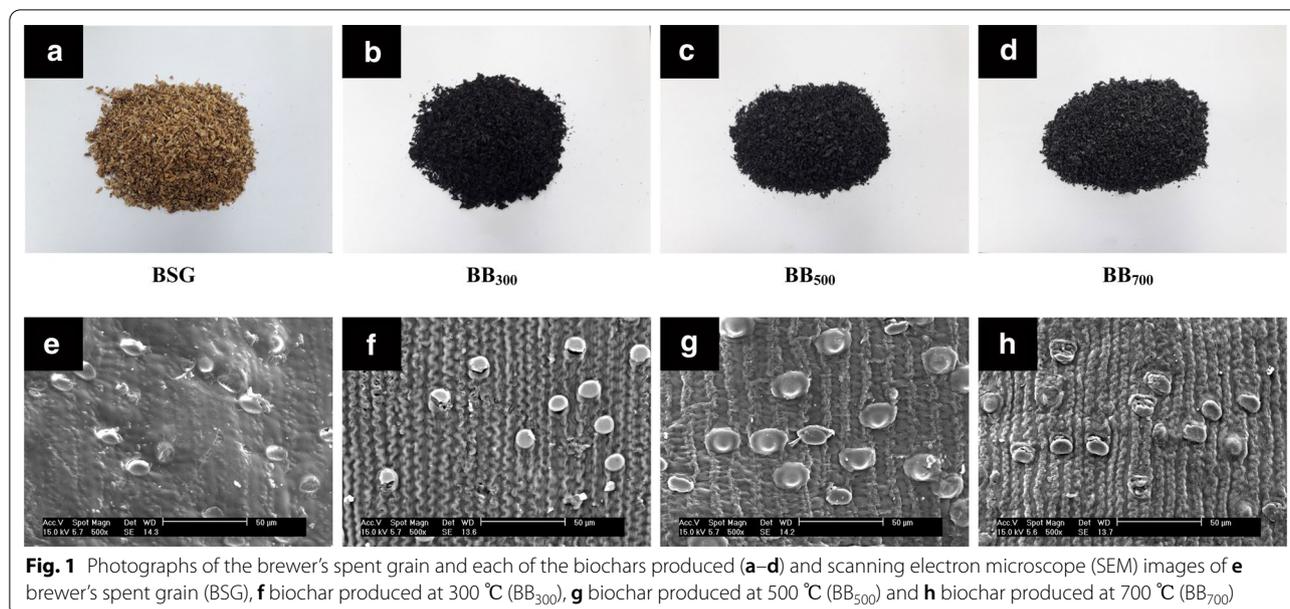
Materials and methods

Soil preparation and analysis

The soil used in the experiment was dried at 105 °C for 24 h and then strained through a 2 mm sieve. Soil texture was classified following the U.S. Department of Agriculture (USDA) soil taxonomy after analysis with the hydrometer. Soil pH and electrical conductivity (EC) were measured by an Electrochemical meter (Orion Versa Star Pro, Thermo Fisher Scientific, USA) after extraction of 1 part of soil with 5 parts of ultrapure water (w/v) and shaking for 30 min at 160 rpm on the Orbital Shaker (SH30 Orbital Shaker, Fine PCR, Korea). The contents of carbon (C) and nitrogen (N) were measured by Automatic Elemental Analyzers (Flash 1112 series EA, Thermo Fisher Scientific, USA) while the available phosphorus (Av. P₂O₅) was extracted with Mehlich No. 3 solution and determined colorimetrically with UV-Vis Spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, Thermo Fisher Scientific, USA) at 880 nm. Exchangeable cations were assessed with an Inductively Coupled Plasma (iCAP 7000 Series ICP-OES, Thermo Fisher Scientific, USA) after extraction with a 1N neutral ammonium acetate solution.

Biochar production and analysis

BSG was obtained from a local brewing company situated in Jeongnim-dong, Daejeon called The Ranch Brewing co. and was dried for 24 h in Forced Convection Oven (ON-12Gw L080125, Jeio Tech, Korea) at 85 °C. Dried BSG was then pyrolyzed for 2 h in a Box furnace (1100 °C Box Furnace BF51800 Series, Thermo Fisher Scientific, USA) with the internal temperatures set at 300 °C, 500 °C and 700 °C. Photographs of BSG and each of the biochar and their scanning electron microscopy (SEM) images are shown in Fig. 1, biochar is symbolized as BB_{XXX} with subscripts referring to corresponding pyrolysis temperatures. The pH, EC, C and N of BB_{XXX} were measured in the same way as the soil samples but in the case of pH and EC, the mixing ratio of biochar and ultrapure water was 1:10 (w/w). The available P₂O₅ was determined colorimetrically at 470 nm after extraction with 2% formic acid. Inorganic contents were measured using ICP-OES (iCAP 7000 Series ICP-OES, Thermo Fisher Scientific, USA) after ashing with the nitric, perchloric and sulfuric acids mixed in ratios of 10:4:1 on a large digital hotplate (HPLP-C-P, Daihan Scientific, Korea) set at 200 °C followed by dilution (100,000 times) with ultrapure water.



Growth conditions of leaf lettuce

To evaluate the impact of BB_{XXX} pyrolyzed at varying temperatures on the growth and quality of leaf lettuce, a pot experiment lasting for 35 days was conducted from March 27, 2019 to May 02, 2019 in a greenhouse at Chungnam National University, College of Agriculture and Life Sciences using a 1/5000 (a) Wagner pot. The *Lactuca sativa* L. var. *crispa* (cv. 'Jeockchima', Danong Co., Korea) was used in the pot experiment. Seeds were planted in a plug tray, transplanted to the Wagner pots 30 days later and growing them for another 35 days. All biochars and BSG were added to the soil at rates of 2% and 5% (w/w) while the control received neither of the two amendments. The essential elements of N, P and K were supplied at rates recommended by the Rural Development Administration of the republic of Korea. After transplanting, each pot was watered with 200 ml once a day and each of the treatment was replicated thrice.

Assessment of growth and quality of leaf lettuce

The yield and quality parameters of the leaf lettuce were assessed by paying strict adherence to the Standards for Research and Analysis of Agricultural Science and Technology of the Rural Development Administration [24]. The yield parameters assessed included both the fresh and dry weights of the shoot and the root, the number of leaves per plant, leaf length and width, and the average weight of the leaves while the quality assessments involved measuring sweetness, nitrate-nitrogen (NO₃-N), chlorophyll, and anthocyanin. All the assessments were conducted immediately after harvesting for the optimized accuracy of

the measurements. Fresh weight was measured after removing moisture remaining on the leaf lettuce, and dry weight was measured after drying the lettuce at 60 °C for 24 h in a Forced Convection Oven (ON-12Gw L080125, Jiio Tech, Korea). The average weight of the leaves was computed as the average value of the weight of five heaviest leaves on the plant and the number of leaves index included only leaves that had lengths of 5 cm and above. Sweetness and NO₃-N were measured with Digital Saccharimeter (HI 96801, Hanna Instruments Inc, USA) and NO₃⁻-N meter (S040, HORIBA Ltd., Japan), respectively after juice extraction out of the leaves. Chlorophyll content was measured from the mid leaves using chlorophyll meter (SPAD-502, Konica Minolta, Japan) between 10 and 11 a.m. on the harvesting day. The anthocyanin content was measured by taking a 2 g piece of the leaf lettuce from the second leaf node crushing it in a mortar containing 2 ml of extracting solution made by mixing 95% ethanol and 1.5 N HCl in a ratio of 85:15 (v/v). 1 ml of the supernatant was siphoned off in a micro test tube and stored in a refrigerator set at 4 °C for 24 h. The supernatant obtained was centrifuged for 20 min at 13,000 rpm and later diluted with the extracting solution. The anthocyanin content was then analyzed with UV–Vis Spectrophotometer (Genesys 10 s UV–Vis, Thermo Fisher Scientific, USA) set at 535 nm (Fuleki and Francis 1968).

Statistical analysis

Statistical analysis of the data obtained was conducted by a one-way analysis of variance (ANOVA) of the SPSS

Table 1 Physicochemical properties of soil before the pot experiment

Sample	Texture (%)			pH (1:5 H ₂ O)	EC (dS m ⁻¹)	Av. P ₂ O ₅ (mg kg ⁻¹)	Elemental content (%)		Exchangeable cations (cmol _c kg ⁻¹)			
	Sand	Silt	Clay				C	N	OM (%)	K ⁺	Ca ²⁺	Mg ²⁺
Soil	74.72 ± 3.00	10.67 ± 1.15	14.61 ± 1.15	6.16 ± 0.03	0.18 ± 0.01	498.81 ± 13.43	0.07 ± 0.00	0.00 ± 0.00	0.12 ± 0.00	0.23 ± 0.01	9.64 ± 0.30	6.06 ± 0.17

EC electrical conductivity, Av. P₂O₅ available P₂O₅, C carbon, N nitrogen, OM organic matter

(IBM SPSS Statistics version 24.0) for significance and post-testing was performed through Duncan method, variability in the data was expressed as the standard deviation, and a value of $P < 0.05$ was considered to be statistically significant.

Results

Physicochemical properties of soil, BSG, and BB_{XXX}

The results of the physicochemical properties of the soil before leaf lettuce cultivation are shown in Table 1. The ratio of sand, silt, and clay in the soil was 74.72, 10.67 and 14.61%, respectively, therefore, the soil texture was adjudged as sandy loam following the USDA soil taxonomy. The soil was weakly acidic with a pH of 6.16, the EC was 0.18 (dS m⁻¹) while the available P₂O₅ was 498.81 (mg kg⁻¹). C content was 0.07% but N was not detected. Exchangeable cations amounted to 0.23, 9.64 and 6.06 (cmol_c kg⁻¹) of K⁺, Ca²⁺ and Mg²⁺, respectively.

As indicated in Table 2, biochar yield decreased with the increasing pyrolysis temperatures with the yield amounting to 52.40, 27.30 and 24.19% of the BSG biomass when pyrolyzed at 300, 500 and 700 °C, respectively. On the other hand, pH of the biochar BB₃₀₀, BB₅₀₀ and BB₇₀₀ increased to 6.02, 7.10 and 7.82, respectively from 5.20 in BSG. EC of the biochar in the current study decreased from 2.69 in BSG to 0.73, 0.41 and 0.21 (dS m⁻¹) at 300, 500 and 700 °C, respectively. The T-P contents were in the order of BB₅₀₀ (3.75%) > BB₇₀₀ (3.39%) > BB₃₀₀ (1.57%) > BSG (1.01%). C and C/N increased with the increasing pyrolysis temperatures while the N content was highest in the BB₅₀₀ (4.87%) followed by BB₃₀₀ (4.47%), BSG (3.96%) and BB₇₀₀ (3.79%). Inorganic contents of K₂O, CaO and MgO were highest in the BB₅₀₀ with concentrations standing at 0.24, 1.26 and 1.35%, respectively.

Chemical properties of the soil after leaf lettuce growth

The results of the chemical properties of the soil after the growth of the leaf lettuce on BSG and BB_{XXX} amended soils are shown in Table 3. While the pH of the control experiment declined to 6.06, those of BSG and BB_{XXX} amended soils increased although the increments were higher in the 5% than in the 2% amended soils except for BSG treatment. The application of BSG to the soil augmented its EC while BB_{XXX} amendments produced opposite effects on the soil reflecting their corresponding low EC values. In addition, the EC values were higher in soils amended with BB_{XXX} 5% than those with 2% for all the treatments.

The available P₂O₅ increased in all the BB_{XXX} amended soils compared with the control but BB₃₀₀ 5% registered a value less than that of the control. Soil C content

increased in all the BB_{XXX} amended soils and the increments were magnified with the application of biochar pyrolyzed at higher temperatures and at a higher application rate of 5%. Consequently, the amount of organic matter in the soil also increased in the same fashion with a maximum value of 6.35% obtained with the application of BB₇₀₀ at 5%. The soil N content in the control was not detected as was the case at the beginning of the experiment while its concentration was higher in the 2% than in the 5% BSG and BB_{XXX} treatments, with a maximum value of 0.20% recorded in BB₅₀₀ 5% and BB₇₀₀ 5% amendments. The highest values for the exchangeable cations were 0.24, 9.44 and 6.21 (cmol_c kg⁻¹) for K⁺ in BSG 5%, Ca²⁺ in BB₅₀₀ 2% and Mg²⁺ in BB₃₀₀ 2% amendments, respectively.

Growth characteristics of leaf lettuce

Photographs of leaf lettuce grown on each BSG and BB_{XXX} amended soils are shown in Fig. 2 while their growth characteristics are indicated in Table 4. The BB₅₀₀ 5% treatment produced the most stunning results across all the growth characteristics measured except for the number of leaves. In that regard, BB₅₀₀ 5% treatment outperformed the rest of the amendments and was closely followed by BB₅₀₀ 2% treatment while BSG 5% treatment elicited adverse effects on leaf lettuce growth. Typically, the fresh shoot weight of the lettuce (107.24 g plant⁻¹) produced from a BB₅₀₀ 5% amended soil was approximately 1.44 times higher than that of the control (74.29 g plant⁻¹), whereas the fresh shoot weight of the BSG 5% treatment (32.78 g plant⁻¹) was approximately 0.44 times lower than the control. It is thus evident that BB_{XXX} amendments were largely beneficial while the pristine BSG amendment was mainly detrimental. Also, higher application rates of BB_{XXX} (5%) led to lower lettuce yields than the 2% amendment except for BB₅₀₀ amendment as shown in Table 3.

The fresh roots of BB₅₀₀ and BB₇₀₀ treatments were heavier than the control. The heaviest fresh roots of 76.04 g were obtained from BB₅₀₀ 5% amendment, a value approximately 1.67 times higher than the fresh root weight of the control (45.60 g) while the fresh root weight of the BSG 5% treatment (15.56 g) was about 2.9 times lower than that of the control. The average water content of the plant shoots of all the treatments was recorded at about 92% with no significant differences amongst the treatments while the average value of water content in the plant roots stood at approximately 86% with no significant differences among the treatments.

Table 2 Physicochemical properties of dried BSG, and BBxxx produced at the different pyrolysis temperatures

Sample	Yield (%)	pH (1:10 H ₂ O)	EC (dS m ⁻¹)	T-P (%)	Elemental content (%)		C/N ratio	Inorganic contents (%)		
					C	N		K ₂ O	CaO	MgO
BSG	–	5.20 ± 0.01	2.69 ± 0.01	1.01 ± 0.01	51.04 ± 1.37	3.96 ± 0.20	12.91 ± 0.33	0.08 ± 0.01	0.40 ± 0.01	0.41 ± 0.01
BB ₃₀₀	52.40 ± 1.42	6.02 ± 0.03	0.73 ± 0.00	1.57 ± 0.10	66.43 ± 1.88	4.47 ± 0.25	14.89 ± 0.83	0.15 ± 0.04	0.73 ± 0.02	0.71 ± 0.03
BB ₅₀₀	27.30 ± 0.73	7.10 ± 0.00	0.41 ± 0.00	3.75 ± 0.14	73.40 ± 1.64	4.87 ± 0.14	15.06 ± 0.46	0.24 ± 0.01	1.26 ± 0.02	1.35 ± 0.02
BB ₇₀₀	24.19 ± 1.41	7.82 ± 0.03	0.21 ± 0.00	3.39 ± 0.08	76.07 ± 1.74	3.79 ± 0.05	20.09 ± 0.40	0.22 ± 0.00	1.18 ± 0.04	1.35 ± 0.02

BSG brewer's spent grain, BBxxx brewer's spent grain biochar (pyrolyzed at xxx°C), EC electrical conductivity, T-P total phosphorus, C carbon, N nitrogen, OM organic matter

Table 3 Chemical properties of the soils under the different amendments at the end of the leaf lettuce growing season

Treatment	pH (1:5 H ₂ O)	EC (dS m ⁻¹)	Av. P ₂ O ₅ (mg kg ⁻¹)	Element content (%)		OM (%)	Exchangeable cations (cmol _c kg ⁻¹)		
				C	N		K ⁺	Ca ²⁺	Mg ²⁺
Control	6.06 ± 0.02 ^c	0.26 ± 0.00 ^{bc}	532.2 ± 19.4 ^f	0.06 ± 0.01 ^c	0.00 ± 0.00 ^c	0.10 ± 0.01 ^d	0.15 ± 0.01 ^b	8.40 ± 0.20 ^c	5.21 ± 0.13 ^c
BSG 2%	6.53 ± 0.16 ^a	0.37 ± 0.07 ^b	876.2 ± 24.1 ^c	0.34 ± 0.01 ^c	0.04 ± 0.01 ^c	0.59 ± 0.02 ^d	0.18 ± 0.04 ^{ab}	8.94 ± 0.63 ^{abc}	5.79 ± 0.41 ^b
BSG 5%	6.35 ± 0.08 ^b	1.12 ± 0.21 ^a	603.6 ± 14.2 ^d	0.66 ± 0.38 ^c	0.09 ± 0.06 ^b	1.13 ± 0.66 ^d	0.24 ± 0.02 ^a	8.68 ± 0.35 ^{bc}	5.77 ± 0.21 ^b
BB ₃₀₀ 2%	6.29 ± 0.06 ^b	0.22 ± 0.02 ^{bc}	580.3 ± 13.3 ^e	0.61 ± 0.10 ^c	0.04 ± 0.01 ^c	1.06 ± 0.17 ^d	0.14 ± 0.01 ^b	9.42 ± 0.38 ^a	6.21 ± 0.27 ^a
BB ₃₀₀ 5%	6.38 ± 0.11 ^{ab}	0.26 ± 0.03 ^{bc}	452.0 ± 11.4 ^g	1.40 ± 0.07 ^b	0.10 ± 0.01 ^b	2.41 ± 0.12 ^c	0.18 ± 0.04 ^{ab}	9.01 ± 0.07 ^{ab}	6.14 ± 0.09 ^{ab}
BB ₅₀₀ 2%	6.30 ± 0.07 ^b	0.21 ± 0.00 ^c	890.5 ± 15.2 ^b	1.67 ± 0.23 ^b	0.12 ± 0.02 ^b	2.88 ± 0.40 ^c	0.20 ± 0.09 ^{ab}	9.44 ± 0.07 ^a	6.13 ± 0.11 ^{ab}
BB ₅₀₀ 5%	6.41 ± 0.07 ^{ab}	0.24 ± 0.01 ^{bc}	907.3 ± 17.4 ^a	2.96 ± 0.77 ^b	0.20 ± 0.06 ^a	5.10 ± 1.33 ^b	0.18 ± 0.03 ^{ab}	8.94 ± 0.19 ^{abc}	6.14 ± 0.12 ^{ab}
BB ₇₀₀ 2%	6.32 ± 0.09 ^b	0.19 ± 0.01 ^c	573.4 ± 18.4 ^e	1.82 ± 0.32 ^b	0.10 ± 0.03 ^b	3.13 ± 0.56 ^c	0.17 ± 0.02 ^{ab}	9.15 ± 0.21 ^{ab}	6.11 ± 0.09 ^{ab}
BB ₇₀₀ 5%	6.42 ± 0.02 ^{ab}	0.25 ± 0.01 ^{bc}	566.1 ± 9.8 ^e	3.68 ± 0.36 ^a	0.20 ± 0.03 ^a	6.35 ± 0.63 ^a	0.18 ± 0.01 ^{ab}	8.96 ± 0.25 ^{abc}	5.74 ± 0.23 ^b

The letters denote significant differences amongst the different treatments based on the results of the Tukey posthoc tests

BSG brewer's spent grain, BBxxx brewer's spent grain biochar (pyrolyzed at xxx°C), EC electrical conductivity, Av. P₂O₅ available P₂O₅, C carbon, N nitrogen, OM organic matter

Quality characteristics of leaf lettuce

The quality characteristics of leaf lettuce grown on BSG and BBxxx amended soils are shown in Table 5. Sweetness degree of the leaf lettuce was highest in the crops grown on the BB₇₀₀ 5% amendment (3.83 Brix) whereas the lowest value of 1.67 (Brix) came from the BB₃₀₀ 5% amendment. The nitrate-nitrogen content was generally high in lettuce grown on BB₅₀₀ and BB₇₀₀ amended soils, with the highest value of 2200 (mg kg⁻¹) obtained from BB₅₀₀ 2% treatment while the lowest nitrate-nitrogen content was recorded in the control (773.33 mg kg⁻¹). Chlorophyll content was highest in the lettuce grown on BB₅₀₀ 2% treatment (33.73 SPAD) and lowest in the crops grown on the BB₇₀₀ 5% treatment (25.47 SPAD). The anthocyanin content was highest with the BB₇₀₀ 2% treatment (0.28 mg g⁻¹) and lowest in the BSG 5% (0.15 mg g⁻¹).

Discussion

As indicated above, the yield of biochar decreased with increasing pyrolysis temperatures which accorded with the observations made by Woo [4] and Lim et al. [25] that the higher the pyrolysis temperature of biomass,

the lower the biochar yield. The pH of the biochar BB₃₀₀, BB₅₀₀ and BB₇₀₀ rose with increasing pyrolysis temperature. The concentration of alkaline salts increased along pyrolysis temperatures, an observation consistent with the previous studies by Ahmed et al. [26], Shinogi and Kanri [27] and Oh et al. [28]. EC of BB_{xxx} decreased with the increasing pyrolysis temperatures contrary to most previously conducted studies by Cantrell et al. [29], Lim et al. [25, 30] who reported increased EC upon increasing the pyrolysis temperatures. Mussatto et al. [15] reported that the hot water extraction drains a lot of components out of the malt while Lee et al. [16] indicated that due to the extraction of beer components, brewer's spent grain contains lower ash content than other biomass which may explain the decreasing trend of EC values observed. Additionally, Woo [4] revealed that the ratio of stable aromatic hydrocarbons increases as the pyrolysis temperature of the biochar increases. Hence, since the hot water extraction of malt during the beer production process had already extracted a lot of components, producing lignin-rich brewer's spent grain, pyrolysis of BSG increases the aromatic hydrocarbons, stabilizing the biochar and lowering the EC of the resultant BB_{xxx}. The

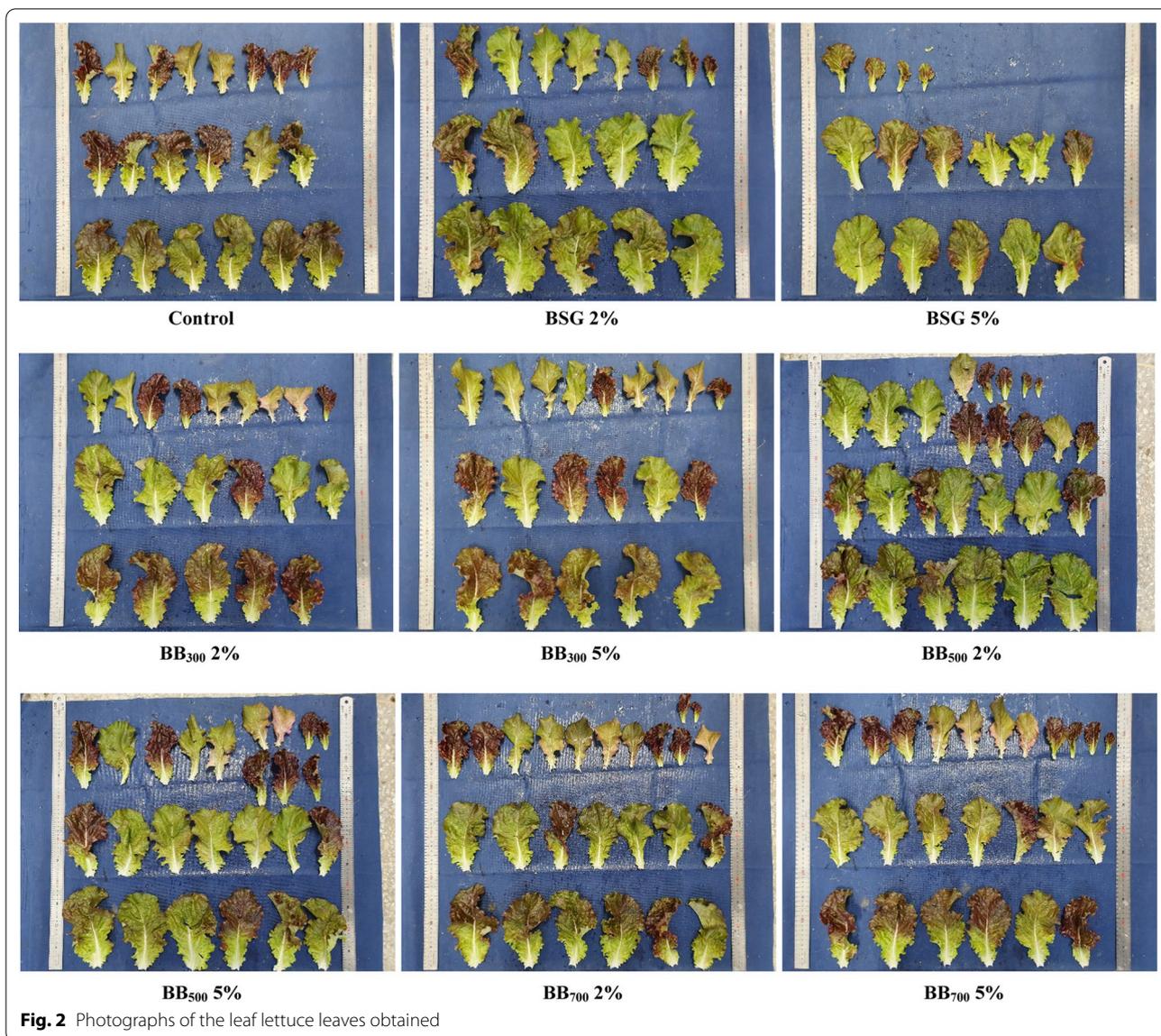


Fig. 2 Photographs of the leaf lettuce leaves obtained

Table 4 Yield components of the leaf lettuce

Treatment	Fresh shoot (g plant ⁻¹)	Fresh root (g plant ⁻¹)	Dry shoot (g plant ⁻¹)	Dry root (g plant ⁻¹)	Number of leaves (ea plant ⁻¹)	Leaf length (cm)	Leaf width (cm)	Weight per leaf (g plant ⁻¹)
Control	74.29 ± 2.99 ^b	45.60 ± 3.50 ^{bc}	6.79 ± 1.12 ^{bc}	8.07 ± 0.23 ^{bcd}	19.00 ± 1.00 ^{bcd}	16.01 ± 0.19 ^{bc}	10.05 ± 0.48 ^{abc}	24.61 ± 2.50 ^{ab}
BSG 2%	67.05 ± 19.25 ^{bc}	26.19 ± 2.66 ^{de}	6.18 ± 2.21 ^c	4.36 ± 0.49 ^{ef}	15.67 ± 2.08 ^{de}	14.88 ± 2.27 ^{cd}	12.41 ± 2.86 ^a	28.58 ± 10.68 ^a
BSG 5%	32.78 ± 7.74 ^d	15.56 ± 4.48 ^e	2.92 ± 0.90 ^d	2.67 ± 0.53 ^f	12.33 ± 2.52 ^e	12.88 ± 2.54 ^d	8.37 ± 2.28 ^c	14.33 ± 2.81 ^c
BB ₃₀₀ 2%	79.82 ± 9.17 ^b	39.18 ± 9.20 ^{bc}	6.48 ± 0.89 ^c	5.93 ± 1.26 ^{de}	19.67 ± 0.58 ^{bcd}	16.65 ± 2.28 ^{bc}	11.79 ± 0.45 ^{ab}	30.46 ± 3.60 ^a
BB ₃₀₀ 5%	53.38 ± 12.65 ^c	37.44 ± 9.35 ^{cd}	5.06 ± 1.68 ^c	6.25 ± 1.56 ^{cde}	18.00 ± 2.65 ^{cd}	15.44 ± 2.17 ^{cd}	9.39 ± 1.61 ^{bc}	16.86 ± 4.29 ^{bc}
BB ₅₀₀ 2%	106.87 ± 4.73 ^a	51.41 ± 8.88 ^b	8.79 ± 0.99 ^{ab}	9.08 ± 1.89 ^b	24.00 ± 2.00 ^a	19.13 ± 1.31 ^{ab}	12.42 ± 1.12 ^a	34.34 ± 6.60 ^a
BB ₅₀₀ 5%	107.24 ± 3.05 ^a	76.04 ± 6.42 ^a	9.31 ± 0.69 ^a	11.44 ± 0.79 ^a	23.00 ± 2.00 ^{ab}	19.72 ± 0.72 ^a	12.67 ± 0.30 ^a	31.41 ± 3.71 ^a
BB ₇₀₀ 2%	77.07 ± 7.31 ^b	47.62 ± 6.88 ^{bc}	6.20 ± 0.78 ^c	7.85 ± 1.10 ^{bcd}	23.00 ± 3.46 ^{ab}	16.30 ± 0.96 ^{bc}	10.10 ± 0.69 ^{abc}	27.27 ± 3.23 ^a
BB ₇₀₀ 5%	72.97 ± 0.82 ^b	46.87 ± 9.06 ^{bc}	6.99 ± 0.50 ^{bc}	8.53 ± 2.46 ^{bc}	21.67 ± 2.08 ^{abc}	16.44 ± 0.37 ^{bc}	10.60 ± 0.43 ^{abc}	24.99 ± 0.29 ^{ab}

The letters denote significant differences amongst the different treatments based on the results of the Tukey posthoc tests

BSG brewer's spent grain, BBxxx brewer's spent grain biochar (pyrolyzed at xxx°C)

Table 5 Quality characteristics of leaf lettuce produced with the different amendments

Treatment	Sweetness degree (Brix)	Nitrate-nitrogen (mg kg ⁻¹)	Chlorophyll (SPAD)	Anthocyanin (mg g ⁻¹)
Control	3.30 ± 0.36 ^b	773.3 ± 198.6 ^d	31.03 ± 1.50 ^{abc}	0.25 ± 0.05 ^a
BSG 2%	2.47 ± 0.50 ^{de}	1236.6 ± 398.8 ^c	28.60 ± 2.23 ^{bcd}	0.25 ± 0.11 ^{ab}
BSG 5%	3.17 ± 0.25 ^{bc}	1466.6 ± 152.8 ^{bc}	26.33 ± 1.40 ^{cd}	0.15 ± 0.07 ^b
BB ₃₀₀ 2%	2.67 ± 0.06 ^{cd}	1333.3 ± 321.5 ^c	27.93 ± 2.36 ^{cd}	0.21 ± 0.07 ^{ab}
BB ₃₀₀ 5%	1.67 ± 0.35 ^f	1073.3 ± 241.1 ^{cd}	28.87 ± 3.95 ^{abcd}	0.26 ± 0.02 ^a
BB ₅₀₀ 2%	2.07 ± 0.25 ^{ef}	2200.0 ± 264.6 ^a	33.73 ± 4.16 ^a	0.26 ± 0.00 ^a
BB ₅₀₀ 5%	3.00 ± 0.20 ^{bcd}	1466.6 ± 251.7 ^{bc}	33.27 ± 2.94 ^{ab}	0.26 ± 0.03 ^a
BB ₇₀₀ 2%	3.07 ± 0.23 ^{bc}	1866.6 ± 152.8 ^{ab}	29.63 ± 2.46 ^{abcd}	0.28 ± 0.04 ^a
BB ₇₀₀ 5%	3.83 ± 0.31 ^a	1466.7 ± 152.8 ^{bc}	25.47 ± 1.36 ^d	0.26 ± 0.03 ^a

The letters denote significant differences amongst the different treatments based on the results of the Tukey posthoc tests

BSG brewer's spent grain, BB_{xxx} brewer's spent grain biochar (pyrolyzed at xxx°C)

observed increase in carbon content of the BB₃₀₀, BB₅₀₀ and BB₇₀₀ with increasing pyrolysis temperature corresponded well to a previous study by Lim et al. [25]. Woo [4] reported that depending on the temperature at which biochar is produced, the higher the pyrolysis temperature, the higher the carbon content and recalcitrance of the resultant biochar.

The changes in soil pH showed closeness to the intrinsic pH values of BB_{xxx} but BSG amended soil surprisingly registered a high increase in pH although BSG had an inherently low pH. The latter could have been due to evolution of ammonia from the decomposition of uncharred organic waste (BSG) as was elaborated by Yang et al. [31]. Available P₂O₅ of the amended soils increased with increasing pyrolysis temperatures. The increased available P₂O₅ may be due to the rise in pH of the soil elicited by BB_{xxx} amendments. Previous studies by Kim et al. [17] and Kang and Hong [32] reported that the available P₂O₅ concentration increased by up to 1.7 times when the soil pH increased from 4.0 to 8.0 and 3.5 times above pH 8.0. Additionally, due to the hydrolysis of phosphoric acid compounds and reduction of iron oxides [32], the available P₂O₅ is expected to increase. The observed increases in exchangeable cations in all the amended soils were in agreement with previous observations by Lee et al. [33, 34] who observed similar increments in soil exchangeable cation concentrations upon biochar addition to the soil. This result is judged to have been affected by inorganic contents contained in the biochar.

Biochar has recently drawn significant attention from many researchers for its potential as a soil amendment to improve crop growth [35, 36]. In our current study, the BB₃₀₀ 2%, BB₅₀₀ 2%, BB₅₀₀ 5% and BB₇₀₀ 2% showed higher growth results than the control, an observation that accorded with previously concluded studies by Lee et al. [34] and Luyima et al. [37] who reported improved

plant growth upon biochar addition to the soil. The fresh roots of BB₅₀₀ and BB₇₀₀ treatments were heavier than the control something that concurred with Chan et al. [38] who delineated that biochar increases the growth of plant roots. Therefore, biochar amendments had positive effects on the growth of crops with the most outstanding result coming from the BB₅₀₀ amendment. Lee et al. [33] reported that biochar amended soil affects nitrate nitrogen contents in crops. This study showed that biochar improved the absorption of nitrate-nitrogen possibly through aiding proper root growth in the BB₅₀₀ and BB₇₀₀ amended soils and was in agreement with a study by Oh et al. [5] who reported that biochar amendments support proper root growth which in turn improve the plants' ability to absorb nitrate nitrogen. Additionally, all biochar amendments boosted anthocyanin production in leaf lettuce save for BB₃₀₀ 2%. This result indicates that anthocyanin development is favored by biochar addition into the soil in accordance with several previous studies for example by Hilioti et al. [39].

The current study was conducted to elucidate the most suitable option of recycling brewer's spent grain through pyrolysis for optimized agricultural productivity. The experimental results clearly show that the biochar pyrolyzed at 500 °C is the most suited for the soil amendment role. Except the BB₅₀₀ treatment which performed relatively well at both application rates even though the BB₅₀₀ 5% produced the most outstanding results of the two, all the remaining amendments produced better growth results when applied at a rate of 2%. Additionally, application of pristine BSG to the soil should be avoided if maximum agronomic benefits are to be realized. In summary, therefore, biochar production from brewer's spent grain looks a sound way of recycling the waste but since this study only looked at the effects on soil chemical properties and agronomic performance of the leaf lettuce

grown, more studies are required to unlock the unknown merits of biochar derived from brewer's spent grain.

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Authors' contributions

J-HY conceived and designed the experiment, collected samples, performed part of the laboratory analysis and drafted the manuscript. DL edited the manuscript to improve the English language quality. J-HL and S-YP performed the laboratory analyses of plant samples while J-WY and J-YA carried out the soil laboratory analyses. Y-UY and T-KO supervised the study and provided technical guidance. All authors read and approved the final manuscript.

Data availability

The data to support the conclusions made in the study are included in the manuscript while small amounts of the produced biochars can be provided to anyone upon request.

Competing interests

The authors declare that they have no conflict of interest.

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