

Spectrometric evaluation of biotransformed agro-industrial residues and their humic substances by UV–visible and infrared spectroscopy and their effect on winter wheat productivity

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Abstract

Purpose Agro-industrial residues could be bio-transformed to improve crop production and reduce environmental risk. Application of humic substances (HS) extracted from residues could be an alternative fertilization strategy. The objectives were: to characterize different bio-transformed agro-industrial residues (BR) and their HS by combining UV-vis and FT-IR techniques, and to assess the effects of their application on wheat crop development and production.

Method Two experiments were performed with winter wheat. The residues used were sunflower shells biotransformed by *Pleurotus ostreatus* fungus, alperujo naturally biotransformed by microorganisms in the environment and solid municipal waste biotransformed by Californian worms and composting. The HS were extracted from these residues. A pot experiment was performed with solid residue incorporated into the soil (Petrocalcic Haplustoll) using four nitrogen (N) levels calculated as the equivalent amount of N considering the amount of organic N (0, 50, 100 and 150 kg N ha⁻¹). A Petri dish experiment was performed to evaluate the effect of an application of HS extracted from each BR on early wheat development.

Results In general, application of BR produced a negative crop response regardless of the residue and rate. The IR spectra of three BR showed similar absorption zones but with different intensities.

Conclusion The application of BR in terms of the type of residue and rate had variable, mostly negative, effects on the wheat crop. However, HS extracted from BR and the subsequent application favored crop development. Spectrometric techniques are useful to verify the potential effect of HS as biostimulants of plant growth.

Keywords Residue valorization, Biotransformation, Biostimulant substances, Fourier Transform Infrared Spectroscopy, Crop production

Introduction

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The Argentine Pampas region is known as one of the most important grain-producing areas in the world, with wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and soybean (*Glycine max* L. Merr.) as its main crops (Martínez et al. 2017a). The wheat crop is the basis of the production system in a large region in

the southwest of the Argentine Pampas (Martínez et al. 2015). This region is characterized by a semi-arid climate, *i.e.*, rainfall is scarce and the climate is seasonally variable. Enhancing nitrogen (N)-use efficiency is also essential, particularly in semi-arid areas where fertilizers are quite expensive and their efficiency is limited by low water availability (Martínez et al. 2018).

Over recent decades, production in agrosystems has been simplified by converting mixed production systems (cattle-grain) into purely crop systems (Kramer et al. 2017), which has led to soil degradation (Duval et al. 2016). In addition, it is well known that agricultural crop systems cause soil degradation in semi-arid environments (Duval et al. 2020). The environmental and economic consequences of soil degradation force the adoption of technologies and management practices to reduce or reverse these deleterious effects. One alternative could be the use of amendments and/or residues in the agricultural agrosystems, either alone or in combination with mineral fertilizers (Mandal et al. 2007).

It is important to note that this region generates a large amount of residues coming from different agro-industrial activities. Many of these residues are difficult to break down, causing contamination and health problems. Among them, sunflower shells (SS) from the oil industry are difficult to break down due to their high lignin content and quality (high C:N ratio= 72:1) (Curvetto et al. 2005). Moreover, residues from olive oil production, called alperujo (AL), have organic compounds (mainly phenolic compounds) that cause phytotoxicity (Morillo et al. 2009). An alternative that takes advantage of these resources is bio-transformation (Rajkhowa et al. 2019). The bio-process of growing fungi on conditioned substrates is technically known as a solid-state fermentation

(SEF). This process is efficient for the growth of microorganisms when it is necessary to use large amounts of biomass, as it has the particularity that the substrate is also the matrix where the microorganisms grow (Woiciechowski et al. 2014). SEF and basidiome production together generate important changes in the substrate, such as a reduction in the density and interstitial spaces, a reduction in lignin content and carbohydrates, whereas other biological processes such as mineralization increase. The quality of the initial substrate before fungi inoculation differs completely from the substrate after degradation (Postemsky and Lopez Castro 2016).

Currently, foliar application of humic substances (HS) is being performed as an alternative among the different fertilization strategies (Balmori et al. 2019; Bettoni et al. 2016; Rose et al. 2014). Humic substances increase water retention in the leaves, photosynthetic metabolism, increases the root length and leaf area index of several crops (Qin and Leskovar 2020) and also don't have any adverse impact on environmental quality (Senn 1991). Moreover, the application of HS has been shown to enhance plant growth and improve plant stress tolerance (Tan 2003). In semi-arid conditions, the foliar application of HS can represent an alternative to conventional fertilization, playing a major role in the improvement of crop growth by stimulating elongation of root systems and enhancing resistance to stress conditions. In addition, several studies (Espinosa-Loréns et al. 2012) showed that HS can be obtained from solid residues. However, since HS depend largely on the composition of the original material and the extractant (Timofeevna Shirshova et al. 2005), it is necessary to know the composition of the HS obtained from the residues to verify their potential for improving wheat crop development.

At present, there are rapid and low-cost analytical techniques that infer the structural information of different materials, facilitating interpretation of their effect on plants and the soil (Iocoli et al. 2017). The development of different spectral techniques - such as ultraviolet-visible (UV-vis) spectroscopy and Fourier Transform Infrared Spectroscopy (FT-IR), among others, has made it possible to assess and understand the chemical structure of HS (Mosquera et al. 2007). Although UV-vis spectroscopy cannot be used to identify functional groups in the samples, the spectrum obtained can be used to estimate the degree of aromaticity of the organic matter (Wang et al. 2018). On the other hand, infrared spectroscopy can identify functional groups and has become a very useful tool to study the nature, reactivity, and structural arrangement of components (Hou et al. 2018). This is a highly effective technique, although its information is basically qualitative, and it has been successful in studying the degradation processes of organic matter and mineralization (Chefetz et al. 1996). The objectives of this study were i) to characterize different biotransformed agro-industrial residues and their HS by combining UV-vis and FT-IR techniques, and ii) to assess the effects of their application on wheat crop development and production.

Material and methods

Study site

In 2017, a pot trial was carried out under controlled conditions in greenhouse at the Departamento de Agronomía- Universidad Nacional del Sur, Buenos Aires, Argentina (38° 41' 48.2" S; 62° 15' 0.17" W). The arable soil used in this experiment was collected from the campus at a depth of 0-20 cm. The

soil is classified as Petrocalcic Haplustoll (Soil Survey Staff 2010) with a sandy loam texture. Each square pot, 12 cm high and 9 cm wide by 9 cm long, was filled with 300 g of soil and it was then sown by hand in July with the wheat cultivar *ACA 303*. Eight seeds were placed in each pot for harvesting once germination had occurred, leaving an average of four plants per pot. A completely randomized experimental design with three replicates was used. When the wheat was sown, each residue milled with a mesh of 1 mm in diameter was incorporated into the soil, using four N levels as dosage criteria: control without application of residue (T) and the equivalent amounts of 50 (1), 100 (2) and 150 (3) kg N ha⁻¹. BR rates were calculated as the equivalent amount of N considering its organic N composition.

The residues used were sunflower shells biotransformed by *Pleurotus ostreatus* fungus (SS_{ple}), residue from the production of olive oil, called *alperujo*, naturally biotransformed by microorganisms from the environment (AL) and solid municipal waste biotransformed by the action of Californian worms and composting (SW_{wp}). At 55 days of the crop cycle, the total aerial biomass of wheat was harvested. The samples were placed in an oven at 60°C until a constant mass was achieved, and the aerial dry matter (DM) was determined.

At the same time, another trial was performed in Petri dishes to evaluate the application of HS extracted from each BR on early wheat development. The wheat seeds, previously disinfected with bleach (1:3) were washed in sterile distilled water for two minutes and then soaked in HS solutions for 12 hours at two concentrations, 1:10 and 1:20. Ten seeds were placed in each Petri dish and incubated under conditions of darkness, controlled moisture, and constant temperature at 25°C for 7 days.

After this time, the total wheat biomass was collected. The samples were placed in an oven at 60°C until a constant weight was achieved, and the aerial (ADM) and root dry matter (RDM) were determined.

Soil chemical and physical analyses

The soil was air-dried, sieved, and homogenized to 2 mm and the retained plant residues were discarded. The soil sample was chemically analyzed to determine: soil organic matter (SOM) by the loss-on-ignition method, and then, organic carbon (SOC) was estimated using a conversion factor of 2.2 (Martínez et

al. 2017b), soil organic N (SON) by the micro-Kjeldahl method (Bremner 1996) and inorganic N as nitrates (NO₃) by steam distillation (Mulvaney 1996); total phosphorus (Pt) was determined by the procedure of Sommers and Nelson (1972), extractable P (Pe) with the Olsen method (Olsen et al. 1954); assimilable potassium (K) by extraction with ammonium acetate; pH in soil suspension: water (1:2.5); and EC in saturated paste. Also, soil texture was estimated by touch. The soil analytical data are detailed in Table 1.

Table 1 Soil chemical and physical properties

Depth (cm)	pH	EC dS m ⁻¹	SOM	SOC g kg ⁻¹	Nt	N-NO ₃	Pt	Pe mg kg ⁻¹	K _{assim}	Texture
0-20	8.2	0.54	41	19	2.3	32	250	21	773	Sandy loam

Electrical conductivity (EC), Soil organic matter (SOM), Soil organic carbon (SOC), Total nitrogen (Nt), Inorganic nitrogen (N-NO₃), Total phosphorus (Pt), Extractable phosphorus (Pe), Assimilable potassium (assim K).

Chemical analysis of BR

The following chemical determinations were made on the residues: total organic matter (OM) by the loss-on-ignition method at 550°C for 4 h (Martínez et al. 2017b); total organic carbon (OC) with a Leco Truspec automatic C analyzer (Leco Corporation, St Joseph, MI); total nitrogen (Nt) by the micro-Kjeldahl method (Bremner 1996). In addition, other nutrients (phosphorus, sulfur, magnesium, potassium, manganese, and iron) were determined by wet digestion (nitric and perchloric acid 2:1) with subsequent determination by plasma emission spectrometry (ICP-OES) and also pH and electrical conductivity (EC) by a potentiometric method with a soil-water ratio of 1:5 volume/ volume (v/v) (Bárbaro et al. 2011).

Humic substances

Humic substances (HS) were extracted from each BR using the International Society for Humic Substances (IHSS) method (Senesi et al. 1989), with 0.1M KOH as an extractant. The HS treatments were defined as HS_{SS}, HS_{AL} HS_{SW} depending on the BR considered. With the HS extracted from the residues, an incubation chamber test was carried out.

Fourier transform infrared spectroscopy (FT-IR)

To determine the structural/functional differences responsible for the contrasting responses observed for each BR and its HS, spectra within the mid-infrared range (4000 - 400 cm⁻¹) were performed with 64 scanning scans and 8 cm⁻¹ with a Thermo Scientific Nicolet iS50 FT-IR spectrometer. The solid samples

(SS_{ple}, AL, SW_{wp}) were prepared as Merck Uvasol 1% potassium bromide tablets (1.8 mg dry sample in 180 mg KBr). The liquid samples (HS) were also recorded as tablets, which were obtained by incorporating 0.30 mL HS in 180 mg KBr, to achieve a dry-base concentration of 0.5-1.0%.

UV-vis spectroscopy

The HS was subjected to a UV-Visible spectroscopic scan at 20 wavelengths between 250 and 665 nm with a T60, PG instruments, UV-Visible spectrophotometer. The samples were diluted in distilled water from 1:25 to 1:100 depending on the HS concentration, to obtain a complete spectroscopic scan, without signal saturation. Each recording was made in triplicate. In addition to the analysis of the obtained spectra, the absorption ratios 280 to 465 nm, 280 to 665nm, and 465 to 665 nm (coefficients E2/E4, E2/E6 and E4/E6 respectively) were calculated from the HS absorbances.

Statistical analysis

An analysis of variance (ANOVA) was performed to compare the effect of different treatments (BR and rates) on the wheat DM production, and to evaluate the effect of the HS on aerial and root dry matter. The means of the effects were compared using the least significant difference test when the differences between treatment means were significant ($P < 0.05$). A Pearson's correlation analysis was performed to assess the relationship between wheat DM and BR chemical properties. One way to see the relationships of all variables in different dimensions is by using the principal component analysis (PCA). The objective of the multivariate analysis was to analyze all the variables correlated together and the relationships between them, and how they affected the wheat produc-

tion. Also, this analysis was employed as a data-reduction tool to select the most appropriate factors, through which the number of independent variables could be reduced (Li et al. 2013). Within each principal component (PC) variables receiving weighted loading values within 10% of the highest weighted loading were selected for each PC (Martinez et al. 2018). The variables incorporated in the PCA were the chemical properties of residues using wheat DM as classification criteria: pH, electrical conductivity (mS cm^{-1}), organic matter (g kg^{-1}), organic C (g kg^{-1}), organic N (g kg^{-1}), C:N ratio, phosphorus (g kg^{-1}), sulfur (g kg^{-1}) and potassium (g kg^{-1}). The statistical analysis was carried out with Infostat software (Di Rienzo et al. 2018).

Results and discussion

Bio transformed residue characterization

Important differences were observed in the characterization of BR (Table 2). The pH values were in the range of 5.3 to 7.9, which do not affect plant growth (Hogg et al. 2002). EC presented values greater than 2.5 dS m^{-1} , which was defined as the tolerable level for plants of medium sensitivity (Lasaridi et al. 2006). On other hand, OC was high in SS_{ple} and AL, according to the criteria of the European Union (Commission decision 2001/688/EC), which establishes the requirements for using residues without a negative impact on human and environmental health. The Nt values of BR were in the reference range ($10\text{--}20 \text{ g kg}^{-1}$) suggested by Alexander (1994). Regarding the C:N ratio, SW_{wp} showed the lowest C:N ratio, whereas SS_{ple} and AL residues showed a higher C:N ratio ($\text{C:N} > 60$). Sunflower shells have a high proportion of lignin and more than 40% of C as part of the structural compounds (Curvetto et al. 2005). The C:N ratio can be used as a residue quality index and it has an important role in N dynamics in the soil-

plant system. The C:N ratio regulates the occurrence of mineralization or immobilization processes of available N during its decomposition. According to Allison (1966) when the C:N ratio is between 25 and 30, there is a balance between mineralization and immobilization. However, the higher the C:N ratio, the greater the biological blocking effect of assimilable N in the soil by microorganisms, generating N deficiencies for the plant to the detriment of its performance. In the case of AL, the high K content is noteworthy, coinciding with the results of Albuquerque et al. (2004) which indicated that AL is especially rich in K, a common characteristic of residues from

oil mills, and low in Mg content, compared to other residues.

Relationship between BR application and wheat crop productivity

The application of SS_{ple} did not generate any significant differences in biomass production at the lowest rates (N1 and N2) ($P>0.05$). This is in contrast to N3 that produced a decrease in the wheat biomass ($P<0.05$) (Fig. 1). In the case of AL, wheat DM decreased significantly at all three N levels ($P<0.05$). In the case of SW_{wp}, no significant differences were found for the different N levels ($P>0.05$).

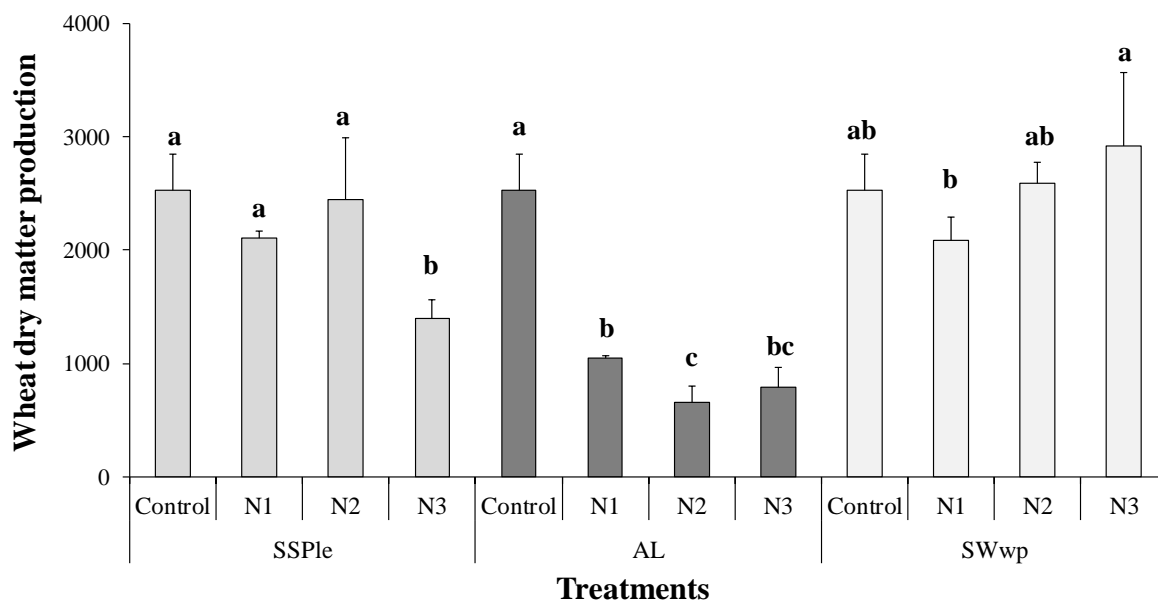


Fig. 1 Dry matter production of wheat according to treatments: control, sunflower shells with *Pleurotus ostreatus* (SSple), alperujo (AL) and worm compost (SWwp)

Different letters indicate significant differences between N rates for each residue ($P<0.05$). Vertical bars indicate the standard deviation.

Based on Pearson's correlations (Table 3), scarce, significant relationships ($P<0.05$) were found between wheat DM and individual chemical properties of residues.

A significant positive relationship was detected with pH, whereas significant negative correlations were

detected with EC, OM, OC, and the C:N ratio. The lack of high correlations between wheat DM and chemical BR properties suggests that productivity may be controlled by different sets of factors (Turner et al. 1997).

Table 2 Chemical characterization of biotransformed residues

Biotransformed Residue	pH	EC	OM	OC	Nt	C:N	P	S	Mg	K
		[mS cm ⁻¹]	[g kg ⁻¹]				[g kg ⁻¹]			
SS _{ple}	5.3	4.47	878.6	569.2	9.05	62.9	0.44	15.0	3.4	12.9
AL	5.4	6.95	932.9	659.8	10.10	65.3	1.28	0.7	0.9	24.7
SW _{wp}	7.9	3.36	268.5	185.8	18.14	10.2	1.95	1.3	6.6	6.7

Sunflower shells with *Pleurotus* (SS_{ple}), Alperujo (AL), Worm compost (SW_{wp}). Electrical conductivity (EC), Organic matter (OM), Organic carbon (OC), Total nitrogen (Nt), C:N ratio (C:N), Phosphorus (P), Sulphur (S), Magnesium (Mg), Potassium (K).

Table 3 Pearson's correlations (r) between crop variables and BR chemical properties

r	pH	EC	OM	OC	Nt	C:N ratio	P	S	K
wheat DM	0.65	-0.74	-0.71	-0.73	0.24	-0.69	0.27	0.13	0.25

In bold letter correlations significant at $P < 0.01$. r, Coefficient of correlation See abbreviations in Table 2.

Understanding the function of each chemical property and their interrelationships with other properties may improve our ability to predict wheat crop production. Therefore, the results obtained were evaluated using a PCA. The PCA showed eigenvalues >1 for the first two PCs, which accounted for 89% of the variance. PC1 and PC2 contribute 62% and 27% of the variance, respectively (Table 4). The first PC had high positive loadings for OM, OC, and the C:N ratio, and a negative loading with pH. The second PC had a positive loading for Nt. These results may indicate that there are several factors, - i.e., OM, OC, C:N ratio, pH and Nt- explaining the variance of the wheat crop, all of them mainly related to the organic quality of the residue.

Effect of Humic substances on early wheat development

The application of HS from AL modified the shoot:root ratio during early wheat development (AL= 1.32**) with respect to the other treatments (SS_{ple}=1.09; SW_{wp}= 1.19) and the control (T= 1.15) ($P > 0.001$) (Fig. 2).

Table 4 Results of principal component analysis for wheat DM production

	Wheat DM	
	PC1	PC2
Eigenvalue	5.60	2.44
Proportion of variance	0.62	0.27
Total variance	0.62	0.89
Variable	Eigenvectors	
OM	0.40	-0.19
OC	0.40	-0.06
Nt	-0.05	0.63
C:N ratio	0.40	-0.18
pH	-0.40	0.17
EC	0.35	0.15
P	-0.28	-0.46
K	-0.27	-0.48
S	0.20	-0.19

Electrical conductivity (EC), Organic matter (OM), Organic carbon (OC), Total nitrogen (Nt), C:N ratio (C:N), Phosphorus (P), Sulphur (S), Potassium (K).

When analyzing the HS results (Fig. 2) no differences were observed between the N application rates ($P > 0.05$), so the treatments were analyzed together.

In RDM, no significant differences were found between the BR ($P>0.05$). On the contrary, ADM showed significant differences in SW_{wp} , and in particular, the highest values were with HS extracted from AL.

This result coincides with those found by Ayuso et al. (1996), who observed similar results when evaluating the effect of HS from unprocessed and composted urban waste on barley crop growth, where HS only stimulated the development of the aerial part.

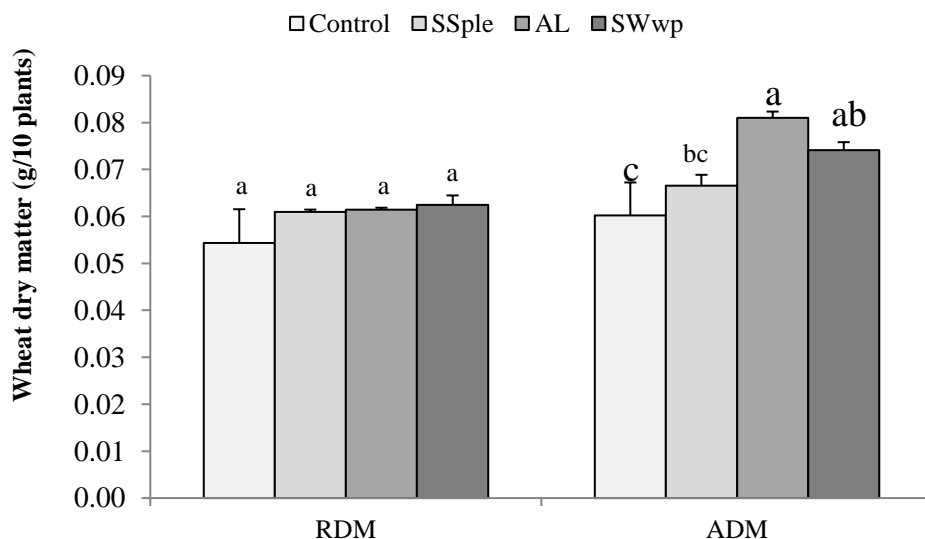


Fig. 2 Root dry matter (RDM) and aerial dry matter (ADM) of wheat according to treatments: control (T), sunflower shells with *Pleurotus ostreatus* (SSple), alperujo (AL) and worm compost (SWwp). Different letters indicate significant differences ($P<0.05$). Vertical bars indicate the standard deviation.

Fourier transform infrared spectroscopy (FT-IR)

The IR spectra of three BR show similar absorption zones (Table 5) but with different intensities (Fig. 3) (Iocoli et al. 2019). The bands observed at about $2920\text{--}2850\text{ cm}^{-1}$ could be assigned to the aliphatic C–H group (Kapoor and Viraraghavan 1997; Uddin et al. 2009). At wave number 1733 cm^{-1} a shoulder is observed which may be due to the carbonyl stretch of carboxyl. The intensity of the band at 1648 cm^{-1} (C=C; C=O) together with the small band at 1515 cm^{-1} (lignin) allows us to infer a high proportion of aromatic compounds and also, that part of the lignin was fragmented into aromatic compounds of lower molecular weight by the action of fungal enzymes. A peak associated with carbonates was observed close

to 1315 cm^{-1} , possibly because important quantities of calcium sulfate and/or calcium carbonate (CaSO_4 , CaCO_3) are incorporated for nutritional purposes, pH buffering, colloid flocculation and fruit induction during the preparation of the substrate for the cultivation of any species of fungus (Postemsky and Lopez Castro 2016). The great intensity of the bands between 1250 and 1000 cm^{-1} (C–O–C; C–O), together with the band between 3600 and 3100 cm^{-1} (O–H), suggests the presence of polysaccharides, probably associated with the content of cellulose and hemicellulose and aliphatic chains, derived from the partial degradation of lignin. In the AL spectrum, the presence and intensity of the 3006 cm^{-1} band, corresponding to the stretching of the C–H bond in unsaturated compounds (=CH–); the 2930 and 2857 cm^{-1} bands,

corresponding to the symmetric and asymmetric vibrations of the C-H bond of methyl and methylene; the 1745 cm^{-1} band, corresponding to the asymmetric vibration of the C=O double bond of the carbonyl group of esters; the 1465 cm^{-1} band, due to bending of the C-H bond of methylene groups; the small band at 1412 cm^{-1} , associated with C-H stretching in unsaturated fatty acids; and the 1238 cm^{-1} band associated with the elongation of the C-O bond, infer that a high content of fatty acids remain from the olive oil extraction process. The overlapping bands between 1650 and 1600 cm^{-1} (C=C), together with the 1515 cm^{-1} band, associated with the presence of ortho and para substituted aromatic rings, confirm the presence of lignin (Niemeyer et al. 1992; Zambrano et al. 2011). The band at 1745 cm^{-1} , together with the bands between 1650 and 1600 (C=O), the bands at 1238 and 1165 cm^{-1} (C-OH), and the low intensity of the band between 3100 -3600 cm^{-1} (OH), infer the presence of condensed, high molecular weight phenolic compounds (polyphenols and tannins). This is probably due to the fact that most of the low molecular weight phenolic compounds are extracted with oil (Servili and Montedoro 2002). Additionally, polyphenols can be associated with the lignin structure (Panettieri et al. 2022).

In the SW_{wp} spectrum, the highest relative intensity of the band centered at 1642 cm^{-1} , and the lower intensity bands at 1550 and 1515 cm^{-1} , lead us to assume a high proportion of condensed aromatic compounds. The bands at 870, 800 and 782 cm^{-1} may indicate substitutions in the aromatic compounds. The band between 1250 and 900 cm^{-1} , with peaks at 1088 and 1041, may correspond to the C-O-C and C-O bonds of polysaccharides, esters, and hydroxylated compounds, which may constitute aliphatic chains of aromatic compounds. In this same region, the stretching of the C-H bond in the aromatics is observed and bands of the aromatic substituents overlap, which, although they are usually of low intensity, they are increased by the presence of polar groups. Additionally, the bands between 3600 and 3100 cm^{-1} , the zone of 1600 cm^{-1} overlapped by the band at 1642 cm^{-1} , the band at 1550 cm^{-1} corresponding to the stretching of the N-H bond, the band at 1384 cm^{-1} (N-O), and the band at 800 cm^{-1} (N-O and NH_2) indicate a higher proportion of nitrogenous compounds than in AL and SS_{ple} . These observations could be evidence of the transformation and humification process of the SW_{wp} .

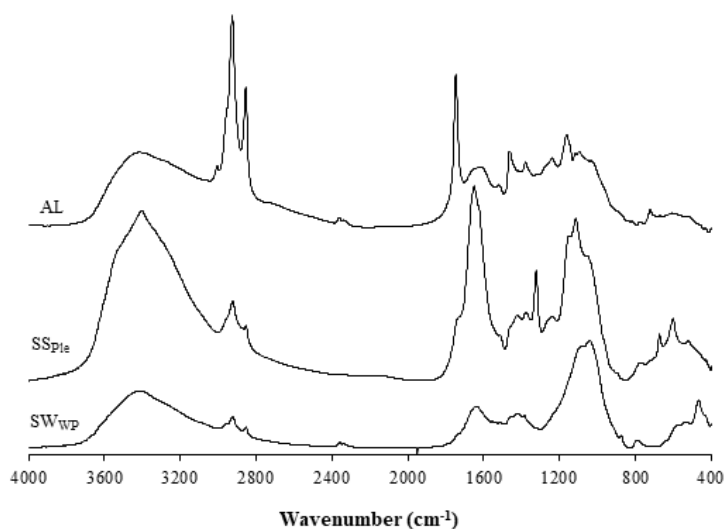


Fig. 3 Fourier transform infrared spectra (FT-IR) of BR: SS_{ple} (sunflower shells bio-transformed by *P. ostreatus* fungus), AL (alperujo with natural biotransformation) and SW_{wp} (urban solid waste worms)

Table 5 Location of the principal indicator bands and assignment to functional groups

Location wave number (cm ⁻¹)	Vibration	Functional group
3100-3600	O-H stretch	<i>Bonded and non-bonded hydroxyl groups. Phenols, alcohols and carboxylic acids.</i>
	N-H stretch	<i>Primary and secondary amides and amines.</i>
2920, 2850	C-H stretch	<i>Aliphatic hydrocarbon structures and alkyl substitute groups</i>
1740-1720	C=O	<i>Aldehyde, ketone, carboxylic acids and esters</i>
1640	C=O	<i>Amide I, carboxylates</i>
	C=C	<i>Aromatic skeleton</i>
1600	N-H in plane	<i>Amines</i>
1600-1590	C=C	<i>Aromatic skeleton</i>
1570-1540	N-H in plane	<i>Amides II</i>
1515-1505		<i>Higher weight and hydrophobic aromatic and olefinic compounds.</i>
		<i>Lignin.</i>
1460	C-H in plane	<i>Aliphatic groups</i>
1425	COO ⁻ stretch	<i>Carboxylic acids</i>
	N-O stretch	<i>Nitrates and nitrites</i>
1384-1400	NH ₄	<i>Free ammonium</i>
	C-H in plane	<i>Methyls and methylenes</i>
1270-1220	C-O stretch	<i>Carboxylic acids, esters, phenols and alcohols.</i>
	C-N stretch	<i>Amide III</i>
1250-900	C-O-C, C-O	<i>Polysaccharides, phenols, esters and hydroxy compounds</i>
	C-O-P	<i>Phosphodiesterases</i>
875	C-H in plane	<i>Aromatic and alkenes</i>
	C-O out of plane	<i>Carbonate</i>
850-750	NH ₂ out of plane	<i>Primary amine group</i>
	N-O stretch	<i>Nitrates and nitrites</i>
750-700	C-H out of plane	<i>Substituted and condensed aromatic systems</i>
	N-H wag	<i>Secondary amine group</i>

Due to the contrasting results obtained by the application of AL and its HS on the wheat crop (Figs. 1 and 2), both were analyzed and compared with the FT-IR technique (Fig. 4). In the AL spectrum, there

was a peak in the band close to 1270 cm⁻¹, corresponding to the C- OH vibration of phenolic OHs (Niemeyer et al. 1992), although it was absent in HS. In HS, an increase in the band between 3600 and

3100 cm^{-1} (O-H and N-H) was observed, with lower intensity in the 2920 and 2850 cm^{-1} bands (C-H in CH₂ and CH₃) and 1745 cm^{-1} (C=O). The bands between 1650 and 1600 cm^{-1} were shifted and defined in a much more intense peak at 1595 cm^{-1} (C=C). Moreover, a higher intensity was also observed for HS in the band between 1380 and 1400 cm^{-1} , a region related to a deformation of the O-H, C=O stretch of phenols, COO⁻ anti-symmetric stretch and also to a C-H deformation in aliphatic (Suñer et al. 2018). The peaks at 1238 and 1162 cm^{-1} were reduced. The latter was reduced to a shoulder from the peak at 1079 cm^{-1}

¹. It is important to note that the band at 1079 cm^{-1} may correspond to the C-N bending vibration in linear and cyclic (C-N) amines. These changes indicate a higher proportion of hydroxyl groups and nitrogenous functional groups with a reduction in the level of fatty acids. Also, there was a relative increase in aromatic compounds, but with a lower proportion of phenolic compounds, and an increase in carboxylic groups, probably as substituents in aromatic compounds. Additionally, the presence of cyclic amines, aromatic compounds and carboxylic groups suggests the presence of auxins.

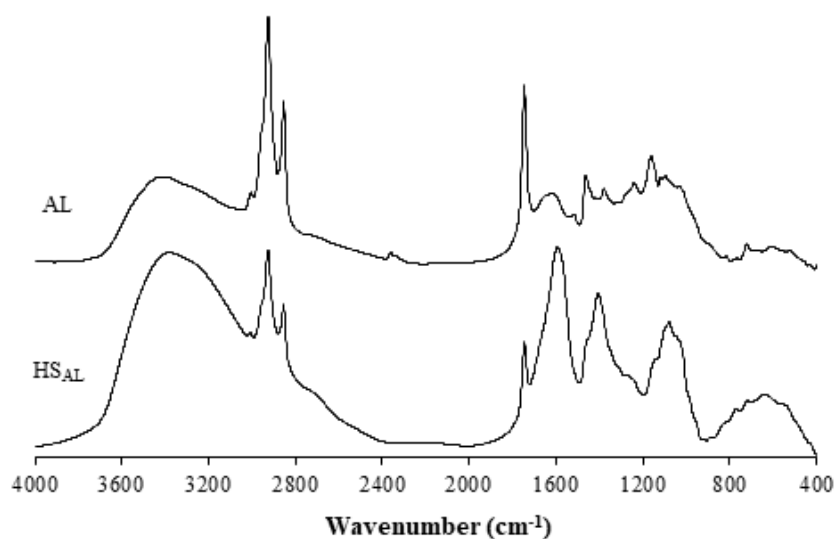


Fig. 4 Fourier transform infrared spectra (FT-IR) of alperujo (AL) and its HS (HS_{AL})

The differences observed in the effect of AL and its HS, together with the analysis of the IR spectra, may infer that phenolic compounds are not taken up from the residue during the HS extraction process or that there is a modification in the molecular structure by the extractant, which possibly diminishes the negative effect of AL on plant growth. Moreover, the greater molecular weight observed in the HS with respect to AL suggests the presence of a greater proportion of indole acetic acid (IAA), biostimulant

structures (Nardi et al. 2021) and auxin (Ferro et al. 2006). In this regard, Mora et al. (2010) showed that HS extracted from leonardite increased the development of the aerial part as a result of increased activity of the enzyme H⁺-ATPase in the root, which modified the N distribution, being higher in the aerial part and lower in the roots. According to Albuquerque et al. (2004) 13% of the organic matter in AL is composed of fatty acid content. Piccolo et al. (2002) indicated that hydrophobic humic components derived

from plant degradation and microbial activity are capable of incorporating more random polar molecules, thus increasing protection against degradation. Spaccini et al. (2002) showed that labile organic compounds are incorporated in the hydrophobic domains of organic matter and that this hydrophobic protection mechanism prevents rapid microbial degradation, thus improving the persistence of organic matter in the soil. The process of molecular entrapment in the hydrophobic domain of humic compounds suggests that the greater the hydrophobicity of HS, the greater the potential for incorporating hydrophilic molecules (Canellas et al. 2012). However, the mechanisms by which the HS act are not clearly understood. The difference in the effect observed in the application of HS obtained from different residues could then be due to a larger molecular size or a higher content of hydrophobic molecules. Moreover, the application of AL to the soil could generate soil N immobilization, because of the main fatty acids in its composition (Meneses and Cerda 2016).

Spectrometric evaluation of HS by UV- visible

The UV-vis spectra of HS -regardless of their origin (SS_{ple} , AL and SW_{wp})- were characterized by the absence of defined maxima, thereby absorption decreases continuously as the wavelength increases (Fig. 5), which coincides with that reported by several authors (Chen et al. 2002; Domeizel et al. 2004; Albrecht et al. 2011; Iocoli et al. 2017). In the region between 240 and 280 nm electronic transition occurs for phenolic compounds, benzoic acids, aniline derivative, polyenes and polycyclic aromatic hydrocarbons with two or more rings (Hansima et al. 2022). Specifically, in the residues and terrestrial HS the absorbance in this region could be due to lignin and quinone moieties (Zbytniewski and Buszewski 2005).

The higher value for SS_{ple} in comparison with SW_{wp} could indicate the beginning of organic matter transformation (Ukalska-Jaruga et al. 2021). In AL the absorbance in this region could be associated with phenolic compounds. The absorbance at 465 nm indicates the proportion of low molecular aliphatic HS with a low degree of transformation, whereas the absorbance at 665 nm shows the proportion of macromolecular or condensed HS, *i.e.*, strongly humidified material (Ganguly and Chakraborty 2009).

The E2/E4 ratio indicates the hydrocarbon structure of conjugated double bonds and the auxochromic groups. This ratio in SS_{ple} presented the highest value (36.9) in comparison with AL and SW_{wp} , (6.3 and 9.4, respectively) and could be related to a high degree of aromaticity of soluble organic matter and to the intensity of UV absorbing and colored functional groups in this organic fraction (Table 6). The E4/E6 ratio of AL (1.8) was lower than SS_{ple} (8.6) and SW_{wp} (7.4). This ratio indicates the presence of highly conjugated structures, capable of absorption even in the visible region of the electromagnetic spectrum (He et al. 2009). Higher values of this relationship could be attributed to a decrease in absorption within the frequency range between 500 and 800 nm wavelength. This decrease is produced by the presence of molecules in which there is no extensive conjugation (Iocoli et al. 2017). The reduction in the E4/E6 ratio means an increase in molecular size and a lower proportion of carboxylic acids (Heymann et al. 2005). Finally, the E4/E6 parameter indicates the grade of humification (decomposition of organic matter) for the humic substances in the soil, whereas progressive humification is indicated by decreasing E4/E6 ratios. However, these HS extracted from SS_{ple} and AL are not comparable to the soil HS, because they are different substances. In the case of SW_{wp} this coefficient indicates a low degree of humification in relation to

the soil HS with a high proportion of fulvic acids (Ukalska-Jaruga et al. 2021). The high coefficient E2/E6 (317.6) of SS_{ple} could indicate the presence a high proportion of lignin structures without transformation and probably aromatic compounds from fungi mycelium, *e.g.* ergosterols. The low coefficient for AL could probably be a consequence of the high content of phenolic compounds of high molecular weight capable of being absorbed in the visible zone (665 nm). In the case of SW_{wp} the coefficient E2/E6

(69.8) could indicate a high proportion of fulvic acids (Ukalska-Jaruga et al. 2021).

Table 6 Absorbance ratios for HS extracted from BR by UV-visible

Absorbance ratio	E2:E4	E4:E6	E2:E6
$HS_{SS_{ple}}$	36.9	8.6	317.6
HS_{AL}	6.3	1.8	11.5
$HS_{SW_{wp}}$	9.4	7.4	69.8

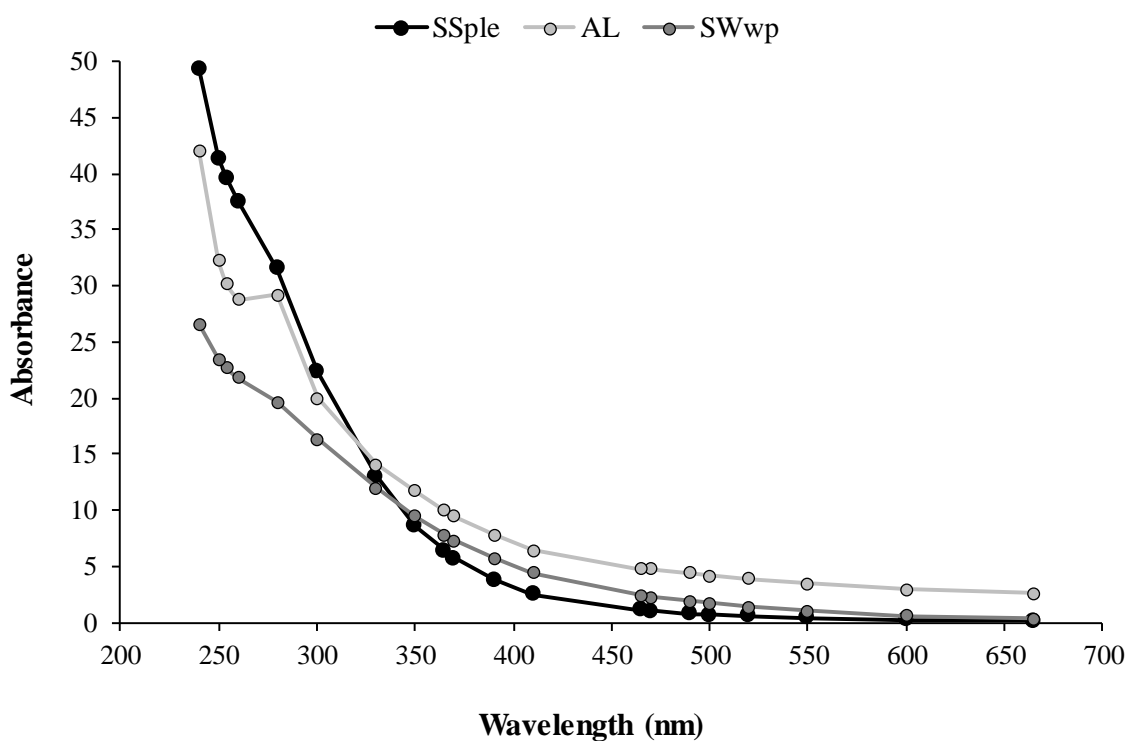


Fig. 5 Variation of absorbance by HS extracted from biotransformed residues: sunflower shells with *Pleurotus ostreatus* (SS_{ple}), alperujo (AL) and worm compost (SW_{wp})

Conclusion

The application of BR presents variable, mostly negative, effects on the wheat crop regardless of the residue and application rate. Wheat production seems to be related mainly with the organic component of residues, *i.e.* OM, OC, C:N ratio, pH and Nt. In spite of

this result, the extraction of HS from BR and its subsequent application favored crop development, especially with HS from AL. The extraction of HS could represent a residue management alternative for enhancing wheat crop production with less environmental risk. Spectrometric techniques, such as UV-Visible and FT-IR, are useful for a better understanding of the characterization of organic materials, and

to verify the potential effect of their application -as agro-industrial residues and HS- as biostimulants of plant growth.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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