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### **ORIGINAL ARTICLE**

# Wood distillate (pyroligneous acid) boosts nutritional traits of potato tubers

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### Abstract

Potato is the fourth most widely consumed staple food in the world. This study investigated the effectiveness of 0.2% wood distillate (WD), a biostimulant derived from the pyrolysis of waste plant biomass, in boosting the nutritional quality of potato tubers. The results showed that application of WD significantly increased the content of soluble sugars (sucrose +56.3%; glucose +44.9%; fructose +62.2%). starch (+35.1%) and total carbohydrates (+16.8%). Antioxidants (total antioxidant power, polyphenols, flavonoids) and most mineral elements (K, Mg, Ca, Na, Fe, Zn) were not affected. A lower content of Cu (-17.8%) and P (-24.5%) was found in WD-treated potato.

### KEYWORDS

potato, pyroligneous acid, soluble sugars, starch, wood vinegar

#### 1 INTRODUCTION

Potato (Solanum tuberosum L.) is the fourth most widely consumed staple food in the world after maize, wheat and rice (Chandrasekara & Kumar, 2016) with a production of about 360 million tons per year (FAOSTAT, 2022). Potato is easy to grow and produces more food, faster and with less land use than any other crop species (FAO, 2008). Currently, the production and consumption of this food take place mainly in Africa and Asia (+50% since the 1970s), given the growth in population and the low cost of purchase (Wijesinha-Bettoni & Mouille, 2019), while in Europe and the USA there is a decrease due to the mainstream of low-carb diets (Beals, 2019). Besides high starch and carbohydrate content, potato is a rich source of sugars, fibre, vitamins, phenols and minerals (Burlingame et al., 2009) and plays a fundamental role in human nutrition (Deußer et al., 2012). Potato, besides being recognized as a functional food because of its high content of nutrient-rich carbohydrates, is also used in the food industry. Indeed, the starch in potato can be used to modify the rate of digestion, creating healthier foods such as fat-free meats and gluten-free products (Dupuis & Liu, 2019).

Several studies have shown that the use of organic fertilizers in place of chemical ones in potato cultivation produces healthier food, which in some cases has however a lower sugar, vitamin, protein, and mineral content (Bártová et al., 2013; Hamouz et al., 2005; Warman & Havard, 1998). Following the guidelines of the 2030 Agenda for Sustainable Development endorsed by the member states of the United Nations in 2015 (United Nations, 2015), it is mandatory to find valid alternatives to the use of chemical fertilizer in agriculture, improving crop yield and nutritional quality without harming the surrounding environment.

One of the most recent bio-based products available on the market is wood distillate (WD), also known as wood vinegar or pyroligneous acid. WD is a by-product of the pyrolysis of waste plant biomass used for bioenergy production (Grewal et al., 2018). Recent literature has given evidence of the biostimulant effects of WD on crop plants (Fedeli, Vannini, Celletti, et al., 2022; Mungkunkamchao et al., 2013; Ofoe et al., 2022; Yuan et al., 2022), as a result of its >200 biologically active compounds (Wei et al., 2010). Furthermore, the use of WD has been shown to be safe for humans (Filippelli et al., 2021) and sensitive non-target organisms such as lichen, moss and fern, as well as for arable plants growing with crops (Fačkovcová et al., 2020a, 2020b; Fanfarillo

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et al., 2022). Starting from 2018, in Italy, WD has been identified as a corroborant and is presently listed as products that can be used in organic farming (Italian Ministerial Decree, 2018). It can be provided by foliar application or directly into the soil by fertigation.

Given the lack of data on the effects of WD on potato, the purpose of this study was to investigate whether WD may boost nutritional parameters of potato tuber.

#### 2 MATERIALS AND METHODS

#### 2.1 Experimental

The planting material, that is, potato tubers of similar size with sprouts, was buried in March 2020 following a randomized complete block design with 10 blocks (replicates) and two treatments. Within each block, two parcels of  $1 \text{ m} \times 1 \text{ m}$ , 3 m away each other, were set. One treatment consisted of water only (control) and the other of 0.2% sweet chestnut (Castanea sativa) WD (BioDea®). Analysis of WD provided by the producer indicates pH in the range 3.5-4.5, density = 1.05 kg/L, acetic acid in the range 2%-2.3%, polyphenols in the range 22-25 g/L. At 'sowing', the soil was fertigated either with water (control) or with 0.2% WD. Fertigation was repeated two more times when potato plants reached a height of 15-20 cm and had 5-6 leaves. Plants were sprayed every 10 days either with water (control) or 0.2% WD until tuber harvest. In August tubers were randomly harvested in the middle of the experimental plots, washed, peeled and prepared for the analysis.

#### 2.2 Starch

The technique outlined by Loppi et al. (2021) was used to determine the starch content. Fifty milligrams of the samples were emulsified in 2 mL of dimethyl sulfoxide. As a result, 0.5 mL of 8 M HCl was added, and the samples were then heated to 60°C in an oven for 30 min. Seven millilitres of deionized water and 0.5 mL of 8 M NaOH were added after cooling. The samples were then centrifuged at 4000 rpm for 5 min, after which 0.5 mL of the supernatant was added to 2.5 mL of Lugol's solution (0.05 M HCl, 0.03% I<sub>2</sub>, and 0.06% Kl). Using a UV-VIS spectrophotometer (8453, Agilent, Santa Clara, CA, USA), the samples were read at 605 nm after 15 min. Utilizing a calibration curve (10-400 g mL<sup>-1</sup>) produced with pure starch, the quantification was performed.

#### 2.3 Soluble sugars and total sweetness index

The method outlined by Fedeli, Vannini, Guarnieri, et al. (2022) was used to determine the amount of soluble sugars (sucrose, glucose and fructose). About 1 g of samples were homogenized in 2 mL of deionized water and centrifuged for 5 min at 15,000 rpm. Syringe filters were used to filter the supernatant down to 0.45  $\mu$ m, and an HPLC (Waters 600 system, MA, USA) fitted with a Waters 2410 refractive index detector was used to examine the results. Deionized water was

used as the mobile phase, eluted at 0.5 mL min  $^{-1}\!,$  and a Waters Sugar-Pak I ion-exchange column (6.5  $\times$  300 mm) kept at 90°C using an external temperature controller (Waters Column Heater Module, MA, USA) to allow sugars to separate. Using calibration curves made by combining analytical sugars with deionized water at concentrations ranging from 0.1 to 20 mg mL $^{-1}$ , sugars were quantified.

The formula proposed by Clarke (1995) was used to calculate the total sweetness index (TSI):

 $\mathsf{TSI} = (1.00 \times [\mathsf{sucrose}]) + (0.76 \times [\mathsf{glucose}]) + (1.50 \times [\mathsf{fructose}]).$ 

#### 2.4 Total carbohydrates

By adding the entire amount of soluble sugar and starch, the total amount of carbohydrates was determined (Vemmos, 2005).

#### 2.5 Antioxidants

#### 2.5.1 Flavonoids

The technique suggested by Heimler et al. (2005) was used to measure flavonoids. After homogenizing samples (about 1 g) in 2 mL of 80% ethanol/water, they were centrifuged at 15,000 rpm for 5 min. A total of 300 µL of deionized water and 500 µL of the supernatant were combined with 45  $\mu$ L of a 5% NaNO<sub>2</sub> solution. A total of 300  $\mu$ L of a 1 M NaOH solution, 300 µL of deionized water, and 45 µL of a 10% AICl<sub>3</sub> solution were then added. After that, a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA) was used to read the samples at 510 nm. Quercetin  $(5-200 \text{ g mL}^{-1})$  calibration curve was used for quantification.

#### 2.5.2 Polyphenols

With a few minor adjustments, the technique suggested by Henriquez et al. (2010) was used to quantify polyphenols. One gram of the samples were homogenized in 4 mL of a 70% acetone/water solution. After that, 0.950 mL of deionized water, 0.750 mL of saturated NaCO<sub>3</sub> solution, and 0.125 mL of Folin-Denis reagent (Sigma-Aldrich, USA) were added to the extract (0.5 mL). After being centrifuged once again, the resultant solution was kept at 36°C for 30 min. The samples were then read using a UV spectrophotometer (8453, Agilent, Santa Clara, CA, USA) at 750 nm. Gallic acid was used as a calibration curve ( $30-300 \text{ g mL}^{-1}$ ).

#### 2.5.3 Total antioxidant power

The technique suggested by Vannini et al. (2022) was used to measure the total antioxidant power. After homogenizing the samples (about 1 g) in 2 mL of 80% ethanol/water, they were centrifuged at 15,000 rpm for 5 min. A DPPH solution was made by dissolving

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3.9 mg of 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, USA) in 100 mL of an 80% methanol/water solution. The supernatant 2.7 3

(100 µL) was then added to this solution. The samples were exposed to darkness for 1 h, and a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA) was used to measure their absorbance at 517 nm. One hundred microlitres of an 80% ethanol/water solution were dissolved in 1 mL of an 80% methanol/water solution to create a blank. The outcomes were presented with the formula as follows:

 $ARA\% = 100 \times [1 - (control absorbance/sample absorbance)],$ 

where control indicates the absorbance of reagents only.

#### 2.6 Mineral elements

Samples were lyophilized using a freeze-dryer Lio SP (5Pascal) fitted with an Edwards RV3 oil vacuum pump under the following working conditions:  $T = -50^{\circ}C$ , P 0.2 mbar, before being frozen at  $-80^{\circ}$ C for 24 h prior to analysis. With a few minor adjustments, the technique suggested by Lamaro et al. (2023) was used to measure the mineral elements. Samples (about 250 mg) were solubilized using a microwave digestion device (Milestone Ethos 900), 3 mL of 67% HNO<sub>3</sub>, and 1 mL of 30% H<sub>2</sub>O<sub>2</sub>. After being digested, the samples were filtered, and then 50 mL of ultrapure water was added. Inductively coupled plasma mass spectroscopy (ICP-MS, NexION 350 Perkin-Elmer) was used to determine the amounts of potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium

(Na), iron (Fe), zinc (Zn) and copper (Cu). The certified reference materials GBW 07604 (Poplar leaves) and GBW 07603 (Branches leaves) were used to validate the analytical quality. Recovery rates were between 95% and 107%. The coefficient of variation of five replicates was used to determine analytical precision, which was consistently more than 97%.

# Statistical analysis

The Shapiro-Wilk test was used to confirm the normality of the data. A Student t-test at p < .05 was used to assess the significance of differences between samples treated with WD and controls. The mean and standard error for each result are shown. R program, was used to conduct the statistical analysis (R Core Team, 2020).

# RESULTS

Potato tubers from plants treated with 0.2% WD showed a significant increase in soluble sugars, that is, sucrose (+56.3%), glucose (+44.9%), fructose (+62.7%), as well as in their TSI (+54.2%) (Figure 1). Additionally, increases in WD-treated plants were observed also for starch (+35.1%) and the total carbohydrate content (+16.8%).

Flavonoids, polyphenols and the overall antioxidant capacity were not altered by WD treatment (Figure 2).

The content of mineral elements did not differ statistically between controls and plants treated with 0.2% WD, with the

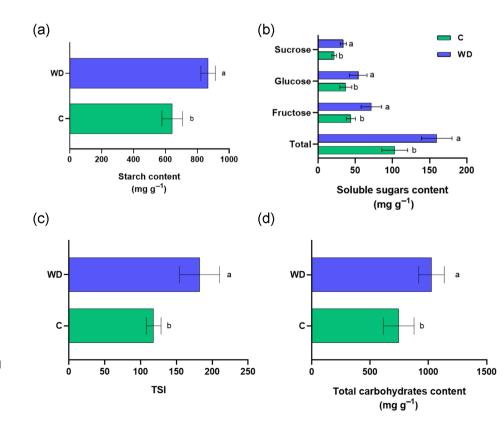
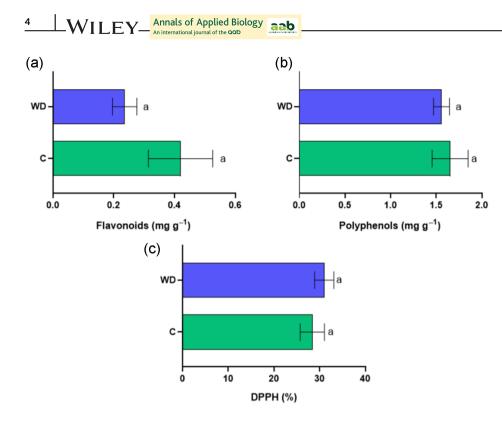


FIGURE 1 Content of starch (a) and soluble sugars (b). Total Sweetness Index (TSI) (c), total carbohydrates content (d) of potato tubers. C = control plants;WD = plants treated with 0.2% wood distillate. Different letters indicate statistically significant (p < .05) differences between treatments.



**FIGURE 2** Content of flavonoids (a), polyphenols (b) and total antioxidant power (DPPH) (c) in potato tubers. C = control plants; WD = plantstreated with 0.2% wood distillate. Different letters indicate statistically significant (p < .05) differences between treatments.

FEDELI ET AL.

exceptions of P and Cu, that were lower (-24.5% and -17.8%, respectively) in tubers of plants treated with WD (Table 1).

# 4 | DISCUSSION

The results of our study showed clear benefits of the use of WD in enhancing some nutritional parameters of potato tubers. A remarkable 35% increase in the content of starch was found upon treatment with WD. Starch, which is composed of amylose and amylopectins (Zeeman et al., 2010), is the main energy reserve form of plants that is stored mainly in tubers, such as potatoes and tapioca (Burlingame et al., 2009; Yuan et al., 2007). In potato tubers, starch is one of the main components (De Meulenaer et al., 2016) that is stored and used during plant growth when needed (Fincher, 1989). Studies on how WD affects starch content are very scanty, however, our results are in agreement with those reported by Jee and Cho (2005) and Fedeli, Vannini, Guarnieri, et al. (2022) which showed increases in the starch content of Neofinetia falcata roots and lettuce (Lactuca sativa L.) leaves, respectively. The increased starch content in potato is of remarkable importance, as it plays a fundamental role in the nutrition of people worldwide (Reyniers et al., 2020), especially in underdeveloped countries, where it is consumed as a basic food (Wijesinha-Bettoni & Mouille, 2019). Energetically, the increase found in this study corresponds to about 70 kcal (about 300 kj) every 100 g of consumed potato, which is a notable amount. Moreover, potato starch is widely used also in the food industry because of its exceptional capacity to thicken and gel, especially when used to make soups, sauces and dressings, and its capacity to bind a lot of water (Bortnowska et al., 2013, 2014).

**TABLE 1** Content (mg kg<sup>-1</sup> dw) of potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn) and copper (Cu) in potato tubers.

Element	с	WD
К	16,798 ± 1446 <sup>a</sup>	13,801 ± 265 <sup>a</sup>
Р	$3033 \pm 148^{a}$	$2289 \pm 182^{b}$
Mg	$1011 \pm 38^{a}$	$930 \pm 32^{a}$
Ca	$544 \pm 33^{a}$	$512 \pm 29^{a}$
Na	97 ± 16 <sup>a</sup>	$119 \pm 28^{a}$
Fe	$24.3 \pm 1.8^{a}$	$23.2 \pm 0.9^{a}$
Zn	$14.5 \pm 0.7^{a}$	$14.4 \pm 0.9^{a}$
Cu	$7.2 \pm 0.4^{a}$	$5.9 \pm 0.8^{b}$

*Note*: C = control plants; WD = plants treated with 0.2% wood distillate.Different letters indicate statistically significant (p < .05) differences between treatments.

The sugar content of potato tubers is an important component of their quality. Although a high content of sugars can lead to the formation of acrylamide due to the Maillard reaction during cooking (Shallenberger et al., 1959), there is however very limited evidence of negative health outcomes (Liska et al., 2015). Our results showed increased levels of glucose, fructose and sucrose in WD-treated plants, but with values still in the range commonly reported for potato tubers (Duarte-Delgado et al., 2016; Saar-Reismaa et al., 2020). To the best of our knowledge, this is the first report of the effects of WD on sugar content in potato tubers, but our results are consistent with those obtained for other crop species such as sweet potato (*Ipomoea batatas* (L.) Lam.) (Dou et al., 2012), eggplant (*Solanum melongena* L.) (Zhou et al., 2013), tomato (*Solanum lycopersicum* L.) (Zhou et al., 2011) and black pepper (Piper nigrum L.) (Jeong et al., 2006). This significant increase in sugars could reflect the general well-being of the plant, as it has been hypothesized that an increase in soluble sugar content is likely related to an increase in photosynthetic performance and subsequent plant yield (Fedeli, Vannini, Celletti, et al., 2022). These considerations are supported by scientific evidence showing that in plants WD stimulates chlorophyll production and photosynthetic activity, as reported for several crop species, with important positive consequences on the fruits (Berahim et al., 2014; Grewal et al., 2018; Theerakulpisut et al., 2016).

The rise in starch and soluble sugar content in tubers of WDtreated plants is reflected in an increase in the total carbohydrate content. From a nutritional point of view, this augmentation is of remarkable importance because potato is an affordable food option (Wijesinha-Bettoni & Mouille, 2019), and is essential for human health in underdeveloped countries (Furrer et al., 2018).

Treatment with WD did not affect the mineral content of potato tubers for most analysed elements, which were all, including' Cu and P that were slightly lower in WD treated plants, within the ranges commonly reported in the literature (Ekin, 2011; Lewu et al., 2010; Lombardo et al., 2014; Saar-Reismaa et al., 2020; Warman & Havard, 1998).

#### CONCLUSION 5

This study showed that treating potato plants with 0.2% WD may boost nutritional parameters of potato tuber. A remarkable increase in the content of soluble sugars, starch and total carbohydrates was found, which is notable from a nutritional point of view for the wide consumption that is made of this affordable food, but also for the industrial sector where higher starch and carbohydrate contents reflect lower material consumption for the processing of different types of products.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The raw data presented in this study are available on request from the corresponding author.

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