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Potential of Pre-Harvest Wastes of Tobacco (*Nicotiana tabacum* L.) Crops, Grown for Smoke Products, as Source of Bioactive Compounds (Phenols and Flavonoids)

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Abstract: Tobacco cultivation is characterized by high amounts of waste biomasses whose disposal frequently represents a complex and expensive problem. A study was conducted to evaluate the potential of pre-harvest light air-cured (Burley) and dark fire-cured (Kentucky) tobacco waste biomasses as a source of bioactive compounds (nutraceutical ingredients) such as polyphenols. Pre-harvest waste materials (topping fresh materials and residual stalks at final harvest) were collected to determine dry matter, total polyphenols content (TPC; Folin assay), and DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,20-azino-bis(3-ethylbenzothiazoline-60-sulfonic acid) diammonium salt) antioxidant capacity. Polyphenols qualitative profiles obtained by Orbitrap Q Exactive of both tobacco types were also determined. Total pre-harvest waste biomass amounted to 3956.9 and 1304.4 kg d.w. ha⁻¹ in light air-cured (Burley) and dark fire-cured (Kentucky) tobacco types, respectively. Polyphenols content, expressed as g kg⁻¹ dry weight (d.w.), ranged between 4.6 and 15.7 g kg⁻¹ d.w. and was generally greater in leaves than in stalks. Considering both leaves and stalks, the light air-cured (Burley) tobacco crop yielded 22.1 kg ha⁻¹ of polyphenols, while the dark fire-cured (Kentucky) tobacco yielded 12.0 kg ha⁻¹. DPPH and ABTS were significantly greater in leaves than in stalks waste biomass in both types of tobacco. The most abundant components were quinic and chlorogenic acids, rutin, and luteolin rutinoside.

Keywords: antioxidant capacity; chlorogenic acid; dark fire-cured tobacco; leaves; light air-cured tobacco; polyphenols; rutin; stalks

1. Introduction

Tobacco is a cash crop widely cultivated over the world (3.4 million ha) [1] with 2.0 million ha in Asia, 0.6 million ha in America, 0.6 million ha in Africa, and 0.1 million ha in Europe [1]. Despite of the contraction of the cultivated areas in last 30 years (there were 4.2 million ha just in 2001) [1], it has great economic and social importance in several countries [2], and it is yet considered the most important non-food crop in the world.

At present, 15–7 thousand ha of different types of tobacco are cultivated in Italy [1,3] with the following regional distribution: (i) flue-cured type in Veneto and Umbria (4105 and 5178 ha, respectively; [3,4]), (ii) flue-cured and dark fire-cured types in Tuscany and Lazio (1646 and 411 ha, respectively) [3], and (iii) light air-cured, dark air-cured, and dark fire-cured types in Campania (1911 ha at Caserta province and 1399 ha at Benevento

province) [3,4]. Currently, Italian tobacco represents 25% of total European production and 1% of world production [1,4].

In Campania region (Southern Italy), tobacco is a crop of the tradition (i.e., cultivation of light air-cured Burley type is reportedly started already in 1891), and it has contributed over time to the income, employment, and cultural heritage of entire generations. It has also deeply characterized agricultural landscapes. Despite the alternate but always unfavorable trends of the last two to three decades, tobacco growers continued to cultivate this crop thanks to general good prospects of specific smoke products (i.e., American cigarettes for light air-cured product and Toscano® cigar for dark fire-cured product). Nevertheless, they urgently need to find technical solutions to maintain high-quality standards while adequately covering the high production costs (it is a labor-intensive crop), and it should be possible through additional incomes.

Tobacco cultivation is characterized by high amounts of waste biomasses [5–8]. Zi et al. [9] reported that more than 68 million tons of tobacco stalks were disposed of worldwide in 2010. Tobacco waste biomasses are currently not fully exploited but, by contrast, their disposal frequently represents a complex problem for growers in a social/economic/environmental key [8].

Tobacco cultivation produces two kind of wastes depending on when they are generated (i.e., in pre- or post-harvest period), which specifically include (i) green materials like discarded leaves, suckers (leaves and stalks), and stalks at the end of the field growing period (pre-harvest waste) or (ii) cured materials like mid ribs, cured leaf, and dust (post-harvest waste).

Differently from energy production, several other environmentally sustainable and profitable plans for using agricultural residual biomasses should be considered as source of additional income [8,10]. Some of these plans recommend waste biomass be used as a source of bioactive and healthy compounds, so that a more efficient (sustainable) use of resource factors (fertilizers, water, etc.) could be also achieved. In the case of tobacco, e.g., with the same amount of resource factors (N fertilizers, irrigation water, etc.), plant extracts could be also produced together with the cured leaves for smoke products. As a result, both the efficiency of N and water use could be roughly doubled.

Tobacco biomass contains several useful chemical components [11–14], like alkaloids, proteins and amino acids, phenols, polyphenols, etc. Therefore, tobacco has always been considered a plant with extraordinary properties [12,15]. In particular, its alkaloids were studied for their well-known effect against insect [16,17] or in the control of neurological diseases like Alzheimer [18] and Parkinson [19]. Tobacco leaf extracts were also reported to have insecticides effects against mites [20] and larva of the dengue vector mosquitos [21] or antifungal activity against *Fusarium* spp. [22]. Tobacco stalk extracts showed antibacterial activity [23]. Tobacco biomass also contains high-quality soluble proteins [12,24]. Considering that it generates significant quantities of biomass in a short period of time, it is generally considered an interesting biological reactor [12,24].

Among several chemical components of plant biomass, polyphenols are receiving increasing interest in agriculture, since they protect plants against ultraviolet radiation and some pathogens [25–27] and in addition, they interact with nutritive plants cycling [28]. Polyphenols are also very interesting for food industries, since they show antioxidant capacity, which improve the quality of food products [29–32]. Several studies [33,34] reported that polyphenols have not only simple antioxidant action but could also exert positive modulatory effects in cells through selective activity on pathways involved in the pathogenesis of degenerative diseases. Overall, they improve the wellness state of body.

Polyphenols profile of tobacco is considered relatively simple and well defined [27,35,36]. In particular, tobacco extracts are rich in phenolic acids, mainly chlorogenic acid [5,27,37], and flavonoids represented by rutin [27,37]. Polyphenols profile appears strictly related to different types of tobacco, i.e., Virginia tobacco, Oriental tobacco [38], *Nicotiana glauca*, or *Nicotiana glauca* [39]. In addition, the polyphenols profile depends on altitude, latitude [40], intensity, quality of light [41,42], age of plant parts [43,44], and water stress [45].

No previous study has been conducted to assess polyphenols yield by pre-harvest waste materials (leaves and stalks) of light air-cured (Burley) and dark-fire-cured (Kentucky) tobaccos as well as little information is available on their polyphenol profiles. Considering that (i) there is a potential great amount of pre-harvest waste of both light air-cured (Burley) and dark-fire-cured (Kentucky) tobaccos, (ii) a more efficient and sustainable use of resources is currently highly desirable for these crops, as well as (iii) additional incomes should be needed to cover the high production costs. The basic aim of this research was to determine polyphenols yield and profile and antioxidant properties of both kind of tobacco crops, which are widely cultivated for smoke products in Mediterranean region (Southern Italy).

2. Materials and Methods

2.1. Plant Materials and Samplings

The experiment was conducted in Campania region (Southern Italy) during 2017 on light air-cured (Burley, cv. PMSP that is local ecotype) and dark fire-cured (Kentucky, cv. KTD8) tobacco crops. Plant materials were collected in a field at the Experimental Station Parco Gussone (Department of Agricultural Sciences, University of Napoli Federico II) located in Portici (40°48.870' N; 14°20.821' E; 70 m a.s.l.). Plants of both kind of tobaccos were grown between May and September. They were regularly fertilized, topped and fully irrigated up to final (commercial) harvest according to standard practices [46,47]. Topping fresh materials (leaves and stalks) of light air-cured (Burley) tobacco and leaves of dark fire-cured (Kentucky) tobacco, the latter of which emerged after topping despite of treatment for sprouts control and that are currently considered out of commercial product, were sampled and weighted after oven dried at 60 °C up to constant weight to determine dry matter content. Each topping dry material was then separately prepared to determine total polyphenols and flavonoids content and antioxidant capacity. At the end of the growing period, after the last harvest of commercial leaves, residual stalks were also collected in both tobacco crops, weighted after oven drying at 60 °C for dry matter content, and prepared for the already reported analytical determinations.

2.2. Ultrasound-Assisted Extraction of Polyphenolic Compounds

The extraction of polyphenols was carried out by ultrasound-assisted extraction on lyophilized samples following the procedure reported in Banožić et al. [14] with a few modifications. Before extraction, lyophilized samples were ground in a mill IKA A11 (IKA-Werke, Staufen, Germany). In particular, 3 g of dried sample were extracted with 30 mL of ethanol/water (50:50 *v/v*), the mixture was vortexed intensively for 1 min and sonicated in the dark, at room temperature, for 30 min. After centrifugation (4000 rpm/min) at 4 °C for 10 min, samples were filtered through 0.22 µm nylon filters (Phenomenex, Castel Maggiore, Italy) and then used for high-resolution mass spectrometry analysis, total polyphenolic content, and antioxidant activity assay. The extraction procedure was repeated three times.

2.3. Total Polyphenolic Content Assay (Folin)

Folin assay was used to estimate total polyphenolic content (TPC) in the tobacco leaves and stalks extracts. In this assay, the Folin reagent reacts with phenolic compounds forming a blue complex due to electron transfer [48]. In brief, 125 µL of extract properly diluted with extractive mixture [14] was mixed with the same volume of Folin–Ciocalteu reagent and 500 µL of deionized water; then, it was incubated at room temperature for 6 min. Further, 1250 µL of a 7.5% sodium carbonate solution were added to the mixture, and the volume was adjusted to 3 mL with water. The absorbance was measured at 760 nm after 90 min at room temperature. Results were expressed as gallic acid equivalent (g GAE kg^{−1} d.w.).

2.4. Determination of Antioxidant Activity (ABTS and DPPH Assay)

The 2,20-azino-bis(3-ethylbenzothiazoline-60-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used as standards.

The antioxidant potential of the extracts with DPPH was measured according to the procedure described by Brand-Williams et al. [49], with minor modifications. In brief, methanolic DPPH solution (4 mg/10 mL) was diluted with methanol to obtain an absorbance of 1.000 ± 0.020 at 517 nm (working solution). Afterward, 1000 μL of this solution was added to 200 μL of the studied extract, mixed, and incubated at room temperature for 10 min. The absorbance was monitored at 517 nm, and results were expressed as Trolox equivalents ($\text{mmol TE kg}^{-1} \text{ d.w.}$).

To evaluate the antioxidant activity of the tobacco extracts, the ABTS method was also applied according to the procedure described by Re et al. [50], with minor modifications. In particular, a 7 mM solution of ABTS in 2.45 mM aqueous potassium persulfate was prepared, and after 16 h of incubation in the dark at room temperature, the solution was diluted with ethanol to obtain an absorbance of 1.000 ± 0.020 at 734 nm. Then, 100 μL of the extract was added to 1000 μL of this solution. Measurements of absorbance were taken at 734 nm after 2.5 min of incubation. In addition, Trolox was used as the reference and activities were expressed as Trolox equivalents ($\text{mmol TE kg}^{-1} \text{ d.w.}$).

2.5. Orbitrap High-Resolution Mass Spectrometry Analysis

The Orbitrap analysis was used to determine polyphenols profile of both kind of tobaccos applying conditions as reported in Graziani et al. [51]. Chromatographic analysis was carried out through an UHPLC system (UHPLC, Thermo Fisher Scientific, Waltham, MA, USA), equipped with a Dionex Ultimate 3000 Quaternary pump and a thermostated (25 °C) Kinetex 1.7 μm biphenyl (10 mm $\times$ 2.1 mm) column (Phenomenex, Torrance, CA, USA), with the following analytical conditions: solvent A, water/formic acid (99.9:0.1); solvent B, methanol/formic acid (99.9:0.1); flow rate, 0.2 mL/min.; and injection volume, 2 μL . The auto-sampler and column temperatures were set at 10 and 25 °C, respectively. A gradient elution program was applied as follows: 0 min, 5% of phase B; 1.3 min, 30% of phase B; 9.3 min, 100% of phase B; 11.3 min, 100% of phase B; 13.3 min, 5% of phase B; 20 min, 5% of phase B. The mass spectrometry analysis was facilitated by a Q Exactive Orbitrap LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA) that was equipped with an electrospray (ESI) source operating in negative ion mode (Thermo Scientific, Bremen, Germany). The acquisitions were conducted by setting a Full MS/AIF mode that uses a full MS scan (without HCD fragmentation), followed by an all-ion fragmentation (AIF) scan (with a fragmentation energy applied). Full MS experiments were carried out with settings: microscans, 1; AGC target, 1×10^6 ; maximum injection time, 200 ms; mass resolution, 35,000 FWHM at m/z 200, whereas the AIF scan conditions were: microscans, 1; AGC target, 1×10^5 ; maximum injection time, 200 ms; mass resolution, 17,500 FWHM at m/z 200; HCD energy, at 10, 20, and 45. In both cases, the instrument was set to spray voltage, 3.5 kV; capillary temperature, 275 °C; sheath gas, 45 (arbitrary units); auxiliary gas, 10 (arbitrary units); m/z range, 80–1200; data acquisition, profile mode. The accuracy of MS analysis was ensured by calibrating the detector using the commercial calibration solutions that were provided by the manufacturer. Mass tolerance was kept at 5 ppm in both full-scan MS and AIF modes. Xcalibur software v. 3.1.66.10 (Xcalibur, Thermo Fisher Scientific, Waltham, MA, USA; v. 3.0.63) was used to perform data analysis and processing. Polyphenols content, as g kg^{-1} , was obtained by summing contents of different components within each profile.

2.6. Statistical Analyses

All results, separately for each tobacco type, were subjected to ANOVA [52] with a one-factor complete randomized block design, and means were separated by least significant differences (LSD) test.

3. Results and Discussion

3.1. Yield in Waste Biomass and Polyphenols

Polyphenols have several health or nutraceutical properties that are well accepted by the scientific community. In particular, with regard to food application, a diet rich in polyphenols is recommended to improve the quality of life and reduce the risk of chronic diseases [29,33]. Considering the increasing interest for such substances, more and more attention is being given to new natural sources of such compounds.

As previously reported, in this study, we used the ultrasound-assisted extraction, which allows to use less organic solvent but gives greater extraction yields as compared to conventional techniques [14]. In addition, it also permits to shorten the extraction times [14].

Total pre-harvest waste biomass of the present experiment (sprouts materials and residual stalks) amounted to 3956.9 and 1304.4 kg d.w. ha⁻¹ in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos, respectively (Table 1), in agreement with previously reported for both types of tobacco [46,47]. In particular, those previous field studies, conducted in the same cultivation areas, showed that it accounts for about 50% of the total biomass usually produced by both crops grown for smoke products [46,47]. Higher planting density usually applied in the field for light air-cured (Burley) than dark fire-cured (Kentucky) tobacco (30,000 vs. 10,000 plants ha⁻¹, respectively), due to different plant structure and shape (slim and cylindrical, respectively, in light air-cured tobacco; robust and conic, respectively, in dark fire-cured tobacco), was the main reason of greater pre-harvest biomass, and consequently, greater wastes, produced per land area by Burley than Kentucky crops.

Table 1. Waste biomass, polyphenols content, and yield in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos.

		Waste Biomass (kg d.w. ha ⁻¹)		Polyphenols Content ¹ (g kg ⁻¹ d.w.)		Polyphenols Yield (kg ha ⁻¹)	
		Burley	Kentucky	Burley	Kentucky	Burley	Kentucky
Topping materials	Leaf	482.7 B	191.6	12.6 a	15.7	6.1 B	3.0
	Stalk	254.5 B	–	5.2 b	–	1.3 B	–
Residual biomass ²	Stalk	3219.7 A	1112.8	4.6 b	8.1	14.7 A	9.0
Significance		**	NS	*	NS	**	NS

¹ Orbitrap; ² at final harvest. Different letters within columns indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters) between different plant materials. NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; d.w., dry weight.

Different plant parts were compared and most of the pre-harvest waste biomass in both tobacco types came, as expected, from residual stalks (Table 1).

Zi et al. [9] reported a pre-harvest stalks waste of about 25% of commercial leaves output. Zhang et al. [53] measured, during cigarette processing, a residual leaves waste of more than 20%. Selvamuthukumaran and Shi [54] also measured similar percentage amounts of waste materials for other species, which, nevertheless, were by-products of industrial processing (i.e., post-harvest waste biomasses). In particular, in their review, they cited experiments that reported percentages of by-products both equal or slightly lower (40% in agave or 15–40% in potato) and slightly greater (60% in artichoke or about 65% in orange) than those we found in tobacco.

Regardless of tobacco types, the polyphenols content, expressed as g kg⁻¹ d.w., ranged between 4.6 and 15.7 g kg⁻¹ and was generally greater in leaves than in stalks but significantly only in light air-cured (Burley) tobacco (Table 1). Regardless of different organs, polyphenols content of the present experiment for both types of tobacco was lower than that found by Leffingwell [11], which ranged between 1.78% and 2.05% or between 2.78% and 3.64%, in Burley or dark fire-cured tobaccos, respectively. In the present experiment,

light air-cured (Burley) tobacco did not show significant difference in polyphenols content between stalks sampled at topping or those collected at final harvest (Table 1).

Comparing with other species, tobacco polyphenols content, averaged on leaves and stalks, was close to the values reported for grapes (5–25 mg g⁻¹ d.w.) [55] and pepper (1.7 g 100 g⁻¹ d.w.) [56], greater than that of apple fruit (3467.47 mg kg⁻¹ d.w.) [57] but lower than that found in artichoke (7564.7–9861.6 mg 100 g⁻¹ d.w. in primary heads and 3174.8–5309.8 mg 100 g⁻¹ d.w. in secondary heads) [32] or seeds and flowers of safflower (40–126 mg g⁻¹ d.w.) [57].

Interestingly, when we consider the yield of polyphenols as kilogram per hectare, we found greater values in stalks than in leaves (16 vs. 6.1 kg ha⁻¹ and 9 vs. 3 kg ha⁻¹ in light air-cured and dark fire-cured tobaccos, respectively; Table 1). This result was due to the previously reported greater amount of stalk than leaf waste biomass produced by crops. Therefore, it should be convenient to collect stalks after the final harvest to have extracts rather than to bury them, as usually occurs [8], even because their burial could increase the risk of epidemics for many diseases due to survival of some infectious microorganisms on those biomasses [58].

On the whole, by summing yield of polyphenols from leaves and stalks, the light air-cured (Burley) tobacco crop yielded 22.1 kg ha⁻¹, while the dark fire-cured (Kentucky) tobacco yielded 12.0 kg ha⁻¹ (Table 1). Market prices of commercial plant extract polyphenols powders ranged, on average, between 10 and 100 USD per kg (rarely more) [59] depending on their purity or percentage concentration of polyphenols. Then, the potential extra gross income could range between 221 and 2210 USD (about 184–1840 €) for light air-cured (Burley) type and 120 and 1200 USD (about 101–1010 €) for dark fire-cured (Kentucky) type. Therefore, these extras should be relevant for tobacco growers even though potential costs to prepare waste biomasses for further industrial processing and other potential additional costs have to be also considered.

TPC, expressed as g GAE kg⁻¹ d.w., is reported in Table 2. The leaf content of both tobacco types was close to that reported by Ru et al. [60] (23.2 mg GAE g⁻¹).

Table 2. Total polyphenols content (TPC, g GAE kg⁻¹ d.w.) in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos.

		TPC (g GAE kg ⁻¹ d.w.)	
		Burley	Kentucky
Topping materials	Leaf	18.1 a	22.4 A
	Stalk	5.6 b	—
Residual biomass ¹	Stalk	3.8 b	8.1 B
Significance		*	**

¹ At final harvest. Different letters within columns indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters) between different plant materials. *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; d.w., dry weight; GAE, gallic acid equivalent.

Regardless of tobacco types, TPC was higher in leaves than in stalks (Table 2) according to that reported by Sheen [61] and Ben Nasr et al. [43]. In particular, Ben Nasr et al. [43] measured amounts of leaf and stalk TPC similar to those of the present experiment (14.46 and 23.05 mg GAE g⁻¹ in leaves of adult and young plants, respectively; 5.70 and 5.33 mg GAE g⁻¹ in stalks of adult and young plants, respectively). In addition, in our experiment, light air-cured (Burley) tobacco did not show significant difference in TPC between stalks harvested at topping and those collected at final harvest (Table 2). Finally, when we compared different tobacco types, we found that both plant parts (leaves and stalks) of dark fire-cured (Kentucky) tobacco contained more TPC than light air-cured (Burley) one (Table 2).

3.2. Radical Scavenging Activity (DPPH, ABTS, and % Inhibition)

As already reported, phenolic compounds are known to possess biological properties including antioxidant activity, which aims to scavenge free radicals or preventing their formation. In the present experiment, the radical scavenging of tobacco extract (leaf and stalk, separately) was determined using two well-known spectrophotometric assays by determining DPPH and ABTS free radical scavenging activity. The results are reported in Table 3.

Table 3. DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,20-azino-bis(3-ethylbenzothiazoline-60-sulfonic acid) diammonium salt) radical-scavenging activity in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos.

		DPPH (mmol TE kg ⁻¹ d.w.)		ABTS (mmol TE kg ⁻¹ d.w.)	
		Burley	Kentucky	Burley	Kentucky
Topping materials	Leaf	46.3 a	56.4 a	61.0	70.7 A
	Stalk	7.7 b	—	10.0	—
Residual biomass ¹	Stalk	11.2 b	13.1 b	7.8	7.5 B
Significance		*	*	NS	**

¹ At final harvest. Different letters within columns indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters) between different plant materials. d.w., dry weight. *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$. TE, Trolox equivalent.

Overall, the antioxidant capacity was higher in the leaves than in the stalks in both types of tobacco (Table 3) and the dark fire-cured (Kentucky) extracts showed a higher antioxidant activity compared to light air-cured (Burley) ones (Table 3). Ru et al. [60] reported that flavonoids of tobacco leaves were good antioxidants with strong DPPH radical scavenging activity.

Rodu and Ou [62] showed that the antioxidant activity of commercially tobacco products, measured as ORAC, varied from 66 to 230 $\mu\text{mol TE g}^{-1}$ on a dry weight basis. Our results are in line with these results regarding the leaves while lower antioxidant activity values were obtained for stalks by both DPPH and ABTS methods. Interestingly, the range of activity in the tobacco leaves was similar to that reported for many fruits and vegetables [63].

Results of antioxidant activity were consistent with those of Folin assay, since there was a good linear and highly significant correlation between the TPC and the antioxidant capacity (Figure 1; $r = 0.901$ and $r = 0.913$, for DPPH and ABTS respectively; $n = 28$) without appreciable differences between DPPH and ABTS. This result showed that polyphenols greatly contributed to the antioxidant activity of tobacco extracts. Strong correlations between both DPPH and ABTS and TPC were also found in artichoke [32], Thymus species [64], and mango fruits [65].

The percentage of inhibition of free radicals of leaves and stalks was similar in both kind of tobaccos, with average values of 45.1 (± 3.4 standard error) and 19.7 (± 2.9 standard error) in leaf and stalk, respectively, for DPPH, and of 32.9 (± 2.1 standard error) and 17.3 (± 0.5 standard error) in leaf and stalk, respectively, for ABTS. These values were lower than those reported by Sharma et al. [7] who found in tobacco a percentage of inhibition of free radicals ranging between 43.8 and 70.9.

Figure 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,20-azino-bis(3-ethylbenzothiazoline-60-sulfonic acid) diammonium salt) antioxidant activity versus TPC. TPC, total polyphenols content; TE, Trolox equivalent; GAE, gallic acid equivalent; d.w., dry weight. Equations: $Y = -4.03 + 2.64 X$, $r = 0.901$ ** (DPPH); $Y = -14.01 + 3.87 X$, $r = 0.913$ ** (ABTS). **, significant at $p \leq 0.01$. o, stalks; ●, leaves.

3.3. Phenolic Profile: Phenolic Acids and Flavonoids

Tobacco phenolic profiles are reported in Tables 4 and 5. In both tobacco types, the most abundant components were quinic and chlorogenic acids (Table 4) and rutin and luteolin rutinoside (Table 5). Leaves were richer in phenolic acids and flavonoids compounds than in stalks and differences were usually significant in both tobacco types (Tables 4 and 5).

Table 4. Phenolic acids (mg kg^{-1} d.w.) in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos.

Burley		Quinic Acid	Chlorogenic Acid	Caffeic Acid	5-O-p-Coumaroyl Quinic Acid	Feruloyl Quinic Acid Isomer 1	3-O-p-coumaroyl Quinic Acid	Feruloyl Quinic Acid Isomer 2
Topping materials	Leaf	3926.2	2339.9	6.87	53.5 A	45.3 a	64.0 a	20.8 a
	Stalk	4398.0	491.8	1.31	5.8 B	2.2 b	4.6 b	1.9 b
Residual biomass ¹	Stalk	3153.0	1198.0	3.58	5.5 B	2.1 b	5.0 b	2.0 b
Significance		NS	NS	NS	**	*	*	*
Kentucky		Quinic Acid	Chlorogenic Acid	Caffeic Acid	5-O-p-Coumaroyl Quinic Acid	Feruloyl Quinic Acid Isomer 1	3-O-p-Coumaroyl Quinic Acid	Feruloyl Quinic Acid Isomer 2
Topping materials	Leaf	1980.0	4777.5 a	14.2 a	88.7 a	69.8	63.3 a	23.7 a
	Stalk	6925.5	1998.6 b	1.7 b	9.5 b	4.7	6.5 b	3.9 b
Residual biomass ¹								
Significance		NS	*	*	*	NS	*	*

¹ At final harvest. Different letters within columns indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters) between different plant materials. NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; d.w., dry weight.

Table 5. Flavonoids (mg kg^{−1} d.w.) in light air-cured (Burley) and dark fire-cured (Kentucky) tobaccos.

Burley		Scopoletin	Isoscapoletin	Rutin	Isoquercetin	Luteolin Rutinoside
Topping materials	Leaf	15.2	10.7	4830.3	44.6	1250.0 a
	Stalk	108.2	48.3	150.5	1.4	14.3 b
Residual biomass ¹	Stalk	45.1	15.9	117.1	0.8	9.2 b
Significance		NS	NS	NS	NS	*
Kentucky		Scopoletin	Isoscapoletin	Rutin	Isoquercetin	Luteolin Rutinoside
Topping materials	Leaf	48.0	30.0	7401.1 A	75.3 A	1152.2 A
Residual biomass ¹	Stalk	177.7	108.3	127.8 B	1.2 B	16.8 B
Significance		NS	NS	**	**	**

¹ At final harvest. Different letters within columns indicate least significant differences at $p \leq 0.05$ and $p \leq 0.01$ (capital letters) between different plant materials. NS, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; d.w., dry weight.

In dark fire-cured (Kentucky) tobacco, the chlorogenic, caffeic acids, rutin, isoquercetin, and luteolin rutinoside contents were significantly higher in leaves than in stalks (Tables 4 and 5) as well as luteolin rutinoside content in light air-cured (Burley) tobacco (Tables 4 and 5). Leaves of both tobacco types were also significantly richer than stalks in 5-O-p-coumaroyl quinic acid and 3-O-p-coumaroyl quinic acid contents (Table 4).

Regardless of tobacco type, Wang et al. [27] also reported that the dominant polyphenols in tobacco leaves were chlorogenic acid and rutin. Similar profile was also found in Virginia and Oriental tobaccos [66] and in light air-cured (Burley) tobacco [67]. Chlorogenic acid and rutin contents in both light air-cured (Burley) and dark fire-cured (Kentucky) tobacco of our experiment were comparable to that reported for other kind of tobacco (Oriental) [68] or for some medicinal species [69]. Both compounds are of relevant interest for industry.

Rutin is frequently used as cosmetic ingredients [70–72]. Chlorogenic acid, which is a major component of coffee [73] but also present in several fruits and vegetables (apple, tomato, potato, eggplant, etc.), is reported to reduce blood pressure and weight [74].

4. Conclusions

The quality of tobacco waste extracts appeared very interesting because their contents of functional ingredients such as phenolic acids or flavonoids as rutin were sufficiently high to consider the wastes of tobacco as economic and convenient natural sources of these salutistic compounds.

It should be considered that the trend of the market of food integrator, nutraceuticals, or functional ingredients is continuously raising up despite the economic crisis and the COVID-19 pandemic. Food transformer industries use different kinds of extracts to fortify the conventional foods by phenolic compounds and often they use synthetic phenolic antioxidants permitted in foods (i.e., butylated hydroxyanisole, BHA, butylated hydroxytoluene, BHT, propyl gallate, PG, tertiary-butylhydroquinone, TBHQ, etc.). Nevertheless, due to safety concerns and consumer request of natural products, natural antioxidants obtained not only from edible materials and edible by-products but also from residual sources are receiving increasing interest, and thus, the market is going toward natural polyphenols.

Food industries are always in search of new natural ingredients or, alternatively, the same molecules but much more economic ones; in this view, considering results of the present study, new good opportunities should be opened for tobacco crops.

We consider of great importance the high percentage of pre-harvest wastes produced by both light air-cured and dark fire-cured tobacco crops and the good quality of their extracts. Such wastes should be conveniently, and also preferably, used as source of polyphenols.

nols, and sustainable goals of improving the efficiency of use of crop resources factors (water, N, etc.) would be also achieved. Moreover, after the extraction, the residual nicotine-free organic matter could be recovered for the production of bio-amendments (i.e., from microorganisms and/or pyrolysis), allowing additional favorable environmental effects.

Further investigations will be necessary to correctly quantify the economic advantage of farmers due to the new products, taking into account the costs of pre-treatments (drying, cutting, etc.) and of delivery of waste biomasses for subsequent industrial extraction.

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