High Voltage Electric Discharge for Recovery of Chlorogenic Acid from Tobacco Waste

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Abstract: Three fractions of tobacco waste (scrap, dust and midrib) were subjected to a high voltage electric discharge (HVED) assisted extraction procedure under different experimental conditions: solvent:solid ratio (300, 500, 700 mL/g), frequency (40, 70, 100 Hz) and treatment time (15, 30, 45 min), in order to study the influence of these conditions on the content of chlorogenic acid. The content of chlorogenic acid ranged from 1.54 to 3.66 mg/100 g for scrap, from 1.90 to 2.97 mg/100 g for dust, and from 2.30 to 3.38 mg/100 g for midrib extract, showing a strong dependence on the applied process parameters. The temperature change and the change in pH and electrical conductivity of the extracts after high voltage discharge treatment were also observed. The studied process parameters showed a statistically significant effect on the chemical and physical properties of the extracts from tobacco waste as well as on the content of chlorogenic acid, indicating the potential of HVED assisted processes in the separation of chlorogenic acid from tobacco industry waste. Multiple regression analysis was used to fit the results for the chlorogenic acid to a second order polynomial equation and the optimum conditions were determined.

Keywords: tobacco waste; high voltage electric discharge; extraction; chlorogenic acid

1. Introduction

The narrative of the 21st century production is related to the resource sustainability where development of new products delivered from the biomass resources came under the spotlight. The climate changes, industrialization and intensive agriculture production cause an enormous amount of the biomass waste materials [1]. Harvesting of tobacco, as the important economic and agricultural crop, and production and consummation of tobacco products across the world led to the massive generation of agricultural, industrial and consumer waste. This problem has forced the industry and the science community to seek solutions to decrease pollution rates and improve cost-effectiveness of tobacco production processes [2]. There are two main directions in tobacco waste management: the energy production and the extraction of bioactive compounds [3]. Utilization of tobacco waste for energy production causes loss of bioactive compounds which have higher economical value than energy produced [2,4]. On the other hand, the tobacco waste is an abundant industrial waste material, rich in nicotine and phenolic compounds, mainly the chlorogenic acids (chlorogenic acid, neochlorogenic acid and cyriptochlorogenic acid) and rutin as the dominant flavonoid. The tobacco waste is typically subjected to the production of reconstituted tobacco or landfilling as industrial waste material, which is unfortunately not appropriate due to its high nicotine content [4–6]. Recently, the reuse of tobacco waste for the recovery of bioactive compounds has been extensively researched, which offers some new environmentally friendly methods for the reuse of tobacco waste. Some of the proposed extraction methods for the separation of active compounds from tobacco waste are ultrasound-assisted extraction [7,8], microwave-assisted extraction [6], subcritical water

extraction [9], supercritical fluid extraction [10,11], and eutectic solvent extraction [12]. Tobacco industrial wastes can be divided into three groups, namely scrap, dust and midrib, depending on the separation method during tobacco processing and particle size. Scrap consists of small leaf lamina parts, that are crushed and separated during processing, dust is considered as fine powder particles, while midrib is a part that extends from the plant stem and is separated from the leaf lamina [4]. Nowadays, there is a global trend in exploring the nonconventional green extraction techniques for separation of bioactive compounds from the various plant materials. The pharmaceutical and food industries are also challenged to make technological improvements and develop the new nonthermal extraction techniques, such as the pulsed electric field, high hydrostatic pressure, high-intensity ultrasound, and nonthermal plasma. These techniques are effective at room temperature or slightly elevated temperatures, which allows lower energy input while avoiding the thermal degradation of thermolabile compounds [13,14]. High voltage electrical electric discharge (HVED) assisted extraction represents the green extraction technique with many advantages, including the low energy input, short treatment time, low applied temperatures and higher recovery of bioactive compounds. Extraction assisted with electrical discharge has been studied by many authors [15–18] reporting the mentioned advantages. The principle of HVED assisted extraction is based on the electrohydraulic discharge (electric discharges generated directly in water), which induces the chemical and physical reactions in water. The energy is injected directly into an aqueous solution through a plasma channel formed by the high voltage electrical discharge between two submersed electrodes. There are several theories regarding the reactions. Some of the proposed reactions include the generation of localized high-temperature plasmas, the emission of high-intensity UV light, the generation of shock waves, and the generation of free radicals produced by the photodissociation of water [18,19]. Increased shock waves and explosions of cavitation bubbles can decrease particle size and influence fragmentation of cell membranes. Additionally, generation of very reactive species such as molecular oxygen and hydrogen, hydrogen peroxide, hydroxyl radicals, and oxygen radical ions were reported [20]. All of these processes lead to cell disruption, intensified mass transfer and increased release of intracellular compounds into the solvent [21]. However, some problems and questions regarding the role of electrical discharge in the extraction of bioactive compounds remain unanswered. Moreover, after the extraction processes, the residual bioactive compound-free materials could be recovered for the aerobic and anaerobic treatment where additional favorable environmental effects could be achieved [3]. Treatment of tobacco waste such as recycling and reusing are an imperative today due to rigorous environmental protection legislation and novel extraction techniques could contribute to effective tobacco waste utilization.

The aim of this study was to evaluate the potential of HVED in the extraction of chlorogenic acid (CA), as the main phenolic compound from tobacco waste. This is the first time that HVED has been applied for the separation of any bioactive compounds from tobacco industry waste. Three extraction parameters (solvent:solid ratio, frequency and extraction time) were studied and optimized using response surface methodology (RSM). The results were compared for all three types of tobacco industry wastes. Additionally, results were compared with conventional extraction process (stirring) with the same solvent (water) at the same process condition (solvent:solid ratio and time).

2. Materials and Methods

2.1. Material

Three types of dried tobacco industry waste (scrap, midrib and dust) were provided by tobacco factory ("Fabrika duhana Sarajevo", Bosnia and Herzegovina). The tobacco waste came from the processing of an original Virginia tobacco blend, which consisted of 26.7% Oriental tobacco and 73.3% Virginia tobacco. The dried material was ground to a plant particle size of less than 2 mm using a laboratory mill (MRC Sample mill C-SM/450-C, Holon, Israel).

2.2. Chemicals

DPPH and Folin-Ciocalteu's phenol reagent chemicals were purchased from the Sigma-Aldrich Chemie (Steiheim, Germany). The standards used with indicated purity were chlorogenic acid (98%), purchased from Sigma-Aldrich (Steiheim, Germany) and gallic acid (97%), purchased from Acros Organics (Geel, Belgium). All other chemicals were of the analytical grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA).

2.3. High Voltage Electric Discharge-Assisted Extraction

Extraction process was performed using water as solvent. HVED assisted extraction was performed with frequency (40, 70, 100 Hz), solvent:solid ratio (300, 500, 700 mL/g) and treatment time (15, 30, 45 min) using custom-built equipment constructed by Ingeniare CPTS1 at the Faculty of Food Technology Osijek, Croatia, and described in detail elsewhere [20]. The device is equipped with a high-voltage pulse generator of 30 kV, a magnetic stirrer and a treatment chamber in which two electrodes in the form of a cylindrical stainless steel needle (diameter 2.5 mm) and the ground electrode in the form of a plate (diameter 45 mm) were placed (Figure 1).
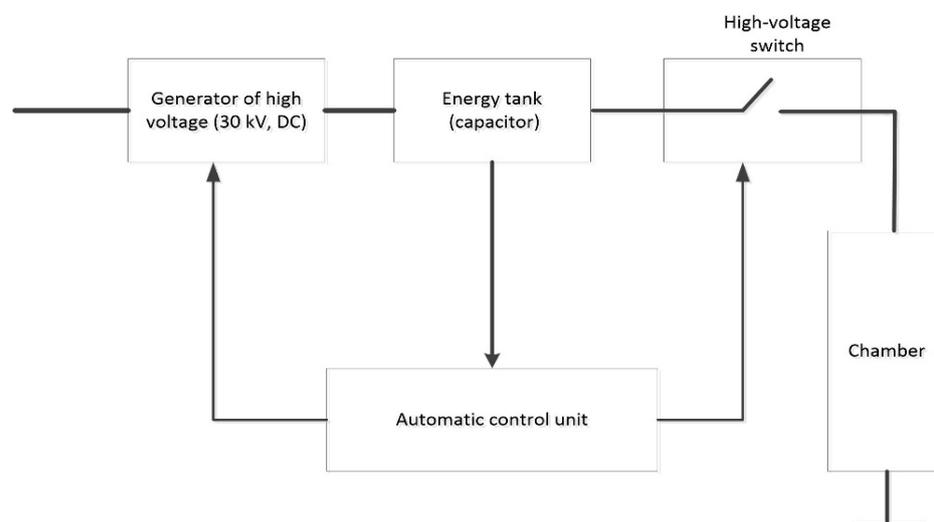

Figure 1. Scheme of HVED generator [20].

The distance between the electrodes was 5 mm in all treatments. Dispersions consisting of 1 g of tobacco waste material and a volume (according to the experimental design) of water were prepared and treated with HVED with different frequency and during the time as indicated in the experimental design (Table 1).

2.4. Reference Extraction Procedure

A reference extraction of untreated samples (tobacco waste/water dispersions) was performed at different solvent:solid ratios (1/300, 1/500, 1/700) at 20 °C for 15, 30 and 45 min with mechanical stirring (same as for HVED assisted extractions) for further comparison of obtained results and for estimating extraction efficiency. All experiments were performed in triplicate.

2.5. Determination of the Physical Properties of Extracts (pH, Conductivity and Temperature)

The physical properties were measured before and after extraction process using a multiparameter (EC/pH/°C) device HI2030 (Hanna Instruments, Woonsocket, RI, USA). Before each measurement, the HVED treatment was stopped and measurements were recorded after 3 min to avoid interference with other phenomena. All measurements were performed in duplicate.

Table 1. Box-Behnken experimental design for HVED assisted extraction of tobacco waste.

Run	Solvent:Solid Ratio (mL/g)	Frequency (Hz)	Time (min)
1	700	40	30
2	300	40	30
3	300	100	30
4	500	40	45
5	700	70	45
6	500	100	45
7	500	70	30
8	500	100	15
9	500	70	30
10	500	70	30
11	500	40	15
12	500	70	30
13	700	100	30
14	300	70	45
15	500	70	30
16	700	70	15
17	300	70	15

According to the previous studies [22–24] the cell designation index Z was calculated by measuring the electrical conductivity before and after HVED treatment and using Equation (1):

$$Z = (\sigma - \sigma_i)/(\sigma f - \sigma_i), \tag{1}$$

where σ_i electrical conductivity before HVED treatment, σ after HVED treatment. The value of σ_i was estimated by measuring final electrical conductivity of each tobacco waste type. The above equation gives $Z = 0$ for the untreated samples and $Z = 1$ for the maximally disintegrated tobacco waste sample. All measurements were performed in triplicate.

2.6. Spectrophotometric Analysis

The radical scavenging activity and total phenolic content (TPC) of HVED extracts were determined by photometric assays as explained in detail by Molnar et al. [25]. Radical scavenging activity results were expressed as percentage (%) and TPC results as mg of gallic acid per 100 g of dry plant material (mg GAE/100 g). All measurements were performed in triplicate.

2.7. High Performance Liquid Chromatography (HPLC)

Separation, identification and quantification of chlorogenic acid (CA) in extracts from tobacco waste were performed using an HPLC instrument, Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany). This instrument is equipped with a binary pump, an auto-sampler, a column heater and a variable wavelength detector. The used HPLC method is explained in our previously published work [7]. All measurements were performed in triplicate.

2.8. Experimental Design and Statistical Analysis

Statistical analysis and experimental design were performed using Design Expert® commercial software (Ver. 9, Stat-Ease Inc., Minneapolis, MN, USA). Box-Behnken design (BBD) was used to determine the optimal HVED assisted extraction parameters according to work published by Bas and Boyaci [26]. The HVED assisted extraction parameters, solvent:solid ratio (X_1), frequency (X_2), and treatment time (X_3) were independent variables tested to optimize the extraction process with the aim to obtain the higher amount of CA. A total of 17 experiments were proposed by BBD, which include five center points at the following conditions: 500 mL/g, 70 Hz and 30 min, which was used to check the reproducibility and stability of the results. Additionally, all measurements were performed

in triplicate. The experiments proposed by BBD are shown in Table 1. Experimental data were fitted with second-order response surface models with interactions and quadratic terms included. The analysis of variance (ANOVA) was used to evaluate the quality of the proposed models. Testing for a statistically significant difference was based on total error criteria with a confidence level of 95.0%. The least square method was used to estimate regression coefficients, which are used in model fitting. The adequacy of the fitted models was tested and evaluated through Lack of Fit, F-value and p-value of the ANOVA.

3. Results and Discussion

HVED was applied at different solvent:solid ratios (300–700 mL/g), frequencies (40–70 Hz), and times (15–45 min), resulting in 17 combinations of these variables for each tobacco waste type (scrap, dust, and midrib). The objective of the present study was to determine the potential of HVED assisted extraction of CA from tobacco waste compared to conventional extraction, and also to determine the influence of process parameters, and optimize the extraction process.

3.1. Influence of HVED Treatment on Physical Properties of Tobacco Waste Extracts

Locke [27] recommended a procedure for HVED treatments in which pH, temperature, and conductivity data should be measured and reported to make an appropriate comparison and analyze the role of HVED chemistry. These changes, in turn, affect radical reactivity or post-plasma reactions, where degradation continues for long periods of time, more than several hours, after the plasma is turned off. Table 2 shows the results of pH, conductivity and temperatures before and after HVED treatment for our study. The maximum temperature reached during the extractions was 45 °C for scrap extracts, 43 °C for tobacco dust, and 42.4 °C for midrib extracts, and the maximum difference between final and initial temperatures during HVED treatment was 22.0, 20.1, and 21 °C for scrap, dust, and midrib, respectively. Poojitha et al. [28] reported that the increase in temperature during HVED treatment is caused by an increase in ion mobility due to structural changes in biological tissues such as degradation of cell wall protopectin, decrease in viscosity of aqueous phase and nonconductive gas bubbles expulsion.

All maximum temperature values were reached after 45 min of treatment, indicating that treatment duration correlates positively with temperature elevation. At shorter treatment duration of 15 min, the differences between the final and initial temperature were less than 10.6 °C for all the extracts, while for the control samples (stirring without heating), the temperature increased up to 3 °C (Table S1). Poojitha et al. [28] reported that an increase in temperature was related to a change in conductivity. However, we found no such correlations. ANOVA of the experimental results obtained for the physical properties of tobacco waste samples treated with HVED are given in the Supplementary Materials Table S2).

All three process parameters studied, solvent:solid ratio ($p < 0.05$), frequency ($p < 0.0001$) and time ($p < 0.0001$), significantly affected the temperature changes of the scrap samples. The similar results were found for the tobacco dust where solvent:solid ratio ($p < 0.05$), frequency ($p < 0.05$) and time ($p < 0.0001$) influenced the temperature changes. Only for the midrib samples, the solvent:solid ratio showed no significant influence, while frequency ($p < 0.05$) and time ($p < 0.0001$) influenced the temperature changes. In addition to the influence of the linear terms, the RSM also shows the influence of the interactions of the process parameters. Most of the interactions between the HVED process parameters showed no significant influence on the temperature changes, except for the scrap, where the interaction between the solvent:solid ratio and time, and the interaction between frequency and time showed a significant influence ($p < 0.05$) on the temperature changes. Moreover, all quadratic terms of the studied parameters had a significant effect on the temperature changes for the scrap samples, while for the midrib, quadratic changes of frequency and time had a significant effect ($p < 0.05$) on the temperature changes. However, nonsignificant heating during the 15 min long HVED treatment (up to 10.5 °C) confirms

HVED as a nonthermal technology. Similarly, Nutrizio et al. [29] found that the maximum temperatures reached during HVED treatment of rosemary were below 30 °C and the separation process was classified as "nonthermal".

Table 2. Values of temperature (°C), pH and conductivity (μS/cm) before and after HVED treatment of tobacco waste *.

Tobacco Waste	Run	Starting Temperature (°C)	Final Temperature (°C)	pH-Value before Treatment	pH-Value after Treatment	Conductivity (μS/cm) before Treatment	Conductivity (μS/cm) after Treatment	Z Value
Scrap								
	1	22.5	32.1	5.96	6.46	298	310	0.14
	2	22.8	33.0	6.17	6.15	307	329	0.28
	3	22.8	39.5	6.02	6.08	336	355	0.38
	4	23.1	38.2	6.46	6.60	216	236	0.12
	5	22.5	40.7	6.52	6.75	181	201	0.10
	6	23.0	45.0	6.61	6.55	220	243	0.14
	7	22.4	36.8	6.60	6.80	231	251	0.17
	8	23.3	32.3	6.66	6.62	220	237	0.10
	9	22.4	37.4	6.72	6.76	220	230	0.12
	10	22.3	37.7	6.61	6.55	219	234	0.09
	11	22.8	28.8	6.5	6.59	242	258	0.11
	12	22.7	38.7	6.51	6.58	216	234	0.11
	13	22.7	37.0	6.89	6.00	159	187	0.12
	14	22.6	43.4	6.43	6.49	339	361	0.47
	15	22.5	38.0	6.60	6.55	216	235	0.11
	16	23.4	31.0	6.00	6.22	172	188	0.07
	17	22.8	31.0	6.61	6.45	371	386	1.00
Dust								
	1	22.9	34.4	6.27	6.72	187	207	0.05
	2	22.9	34.4	6.39	6.80	292	316	0.09
	3	21.1	38.9	6.47	6.77	305	325	0.08
	4	22.4	38.6	6.21	6.91	186	209	0.06
	5	22.8	41.5	6.31	6.81	146	170	0.06
	6	22.4	41.7	6.23	6.88	332	370	0.17
	7	22.4	38.3	6.38	6.89	347	382	0.16
	8	22.7	33.2	6.34	6.55	188	213	0.07
	9	22.5	37.4	6.46	6.87	335	395	0.27
	10	22.8	38.4	6.57	6.79	389	421	0.19
	11	22.8	29.8	6.31	6.52	189	206	0.05
	12	22.8	37.3	6.31	6.75	190	215	0.17
	13	23.1	37.0	6.34	6.64	256	301	0.05
	14	22.9	43.0	6.29	6.76	507	560	1.00
	15	22.9	40.1	6.27	6.83	162	171	0.21
	16	22.5	22.7	6.19	6.31	150	184	0.08
	17	22.4	30.7	6.59	6.56	312	343	0.13
Midrib								
	1	22.3	36.8	5.84	5.85	458	607	0.34
	2	22.6	36.5	5.71	5.71	748	845	0.67
	3	22.6	38.1	5.55	5.79	755	819	0.47
	4	22.7	38.3	5.48	5.71	851	892	1.00
	5	23.1	42.4	5.63	5.87	396	451	0.11
	6	22.3	41.6	5.71	5.93	368	441	0.14
	7	23.7	39.2	5.44	5.70	564	651	0.27
	8	22.4	30.5	5.71	5.70	397	490	0.19
	9	22.6	38.5	5.65	5.63	476	515	0.09
	10	22	37.7	5.66	5.70	435	490	0.12
	11	22.4	28.1	5.35	5.68	460	514	0.13
	12	20.9	36.2	5.84	5.75	403	479	0.16
	13	21.1	36.1	5.72	5.7	348	410	0.11
	14	21.4	42.4	5.42	5.71	760	842	0.62
	15	21.7	38.2	5.47	5.75	490	532	0.10
	16	21.5	28.6	5.64	5.75	346	371	0.05
	17	21.9	29.5	5.4	5.54	675	761	0.40

* all data in table represent average value of three replicates.

Initial pH values of tobacco waste were between 5.96 and 6.89 for the scrap solutions, 6.19–6.59 for the dust solutions and 5.35 and 5.93 for the midrib, which is consistent with data reported in other studies [30,31]. The changes in pH values were very small, up to ± 0.89, ± 0.7, ± 0.29 for the scrap, dust and midrib extract, respectively, and the tobacco waste samples were slightly acidic before and after HVED treatment. The results obtained were similar to results for control samples. Poojitha et al. [28] reported that changes in pH are caused by interactions (electrochemical reactions) between the electrode and the plant material. However, the changes in pH values were too small to affect the extraction process.

The increase in electrical conductivity was in the range 12–28 µS/cm for the scrap extract, 9–60 µS/cm for the dust extract and 25–97 µS/cm for the midrib extract. The highest changes in electrical conductivity had the scrap extract obtained under solvent:solid ratio of 700 mL/g, frequency of 100 Hz and time of 30 min, while for the dust extract the highest changes in electrical conductivity were observed for the extract obtained under solvent:solid ratio of 500 mL/g, frequency of 70 Hz and the time of 30 min. In the case of midrib extract, the highest changes in electrical conductivity were observed for run 2 (300 mL/min, 40 Hz, 30 min). The electrical conductivity data were used for establishing Z values, which represents the cell disintegration index (Table 2). The highest disintegration index ($Z = 1$) had scrap extract obtained under the following condition solvent:solid ratio 300 mL/g, frequency 70 Hz and time 15 min. For the tobacco dust samples, the highest Z value ($Z = 1$) was obtained at solvent:solid ratio 300 mL/g, frequency 70 Hz and at time 45 min, while for midrib, the highest Z value ($Z = 1$) was obtained for sample obtained under following condition solvent:solid ratio 500 mL/g, frequency 40 Hz and time 45 min. For the scrap and dust samples, the linear and quadratic term of solvent:solid ratio significantly ($p < 0.05$) influenced Z value, while for midrib samples all linear terms, quadratic terms of frequency and interaction between frequency and time significantly ($p < 0.05$) influenced Z value.

3.2. Influence of HVED Treatment on Total Phenol Content and Radical Scavenging Activity of HVED Extracts

The previous studies had found that tobacco polyphenols possess the various biological activity, including radical scavenging activity [32]. In the present study, the radical scavenging activity expressed as DPPH (%) ranged from 49.28 to 63.59% for scrap extracts, from 41.05 to 56.19% for tobacco dust extract and from 45.29 to 65.24% for midrib extract (Table 3). The highest values for scrap and dust extract were obtained under the same condition (500 mL/g, 70 Hz, 30 min), while for midrib the highest radical scavenging activity had extract obtained under following condition: 500 mL/g, 100 Hz, 15 min. The obtained values were significantly lower than in our other studies, where the other extraction techniques were applied on tobacco waste [7,9,12]. Since the radical scavenging activity of tobacco waste is attributed to the polyphenol content, the degradation of polyphenols could lead to lower radical scavenging activity.

Bogomaz et al. [33] found that although electrical discharges lead to enhanced extraction of polyphenols, sometimes the electrical discharge also leads to the formation of hydroxyl radicals during photodissociation of water, atomic hydrogen, and ozone. These oxidative chemical reactions may damage the extracted polyphenols. Similar findings were made by Chen et al. [34], where the degradation of polyphenols was caused by the OH radical reaction with phenols, resulting in the formation of phenoxyl radical. Moreover, the phenoxyl radical can also be formed directly under the influence of ultraviolet light generated by the electrical discharge in liquid. TPC expressed as equivalents of gallic acid per 100 g of dry material were in the range 17.56–49.41 mg GAE/100 g for scrap extracts, 21.02–78.95 mg GAE/100 g for dust extracts and 7.25–28.86 mg GAE/100 g for midrib extracts. The highest TPC values for all three types of waste were obtained for solvent:solid ratio of 700 mL/g, while the lowest values were obtained for 300 mL/g, implying that solvent:solid ratio has the greatest influence on TPC. This was confirmed with statistical analysis presented in the Supplementary Materials Table S3). The linear term of solvent:solid ratio had significant ($p < 0.05$) influence on TPC of scrap, while other

parameters did not exhibit a significant influence. The linear term of solvent:solid ratio and frequency, interaction between solvent:solid ratio and frequency and between solvent:solid ratio and time had significant ($p < 0.05$) influence on the TPC of tobacco dust. In the case of midrib extract, the linear term of solvent:solid ratio exhibited highly significant influence ($p < 0.0001$) on TPC while the linear term of frequency and time, interaction between solvent:solid ratio and frequency, and the interaction between frequency and time exhibited significant influence ($p < 0.05$) on TPC. Compared to other published studies [9], the results for TPC were significantly lower than those obtained with subcritical water extraction and slightly lower compared to tobacco waste extract obtained with deep eutectic solvents [12]. The amounts of phenolic compounds in midrib are similar with the amounts of TPC obtained from tobacco root in the study by Nasr et al. [35].

Table 3. Total phenol content and radical scavenging activity of HVED tobacco waste extracts *.

| | Tobacco Waste | | | | | |
| | Scrap | | Dust | | Midrib | |
RUN	TPC (mg GAE/100 g)	DPPH (%)	TPC (mg GAE/100 g)	DPPH (%)	TPC (mg GAE/100 g)	DPPH (%)
1	36.49	53.54	48.16	48.83	28.86	49.39
2	17.56	49.28	28.95	44.75	8.64	45.29
3	84.64	59.41	21.02	46.19	12.64	45.67
4	43.63	58.62	42.99	53.00	27.09	48.71
5	40.98	50.68	50.49	48.32	26.62	47.19
6	38.24	53.68	52.09	39.40	12.99	47.58
7	34.14	50.27	32.09	49.38	19.40	49.20
8	33.50	52.49	65.04	49.90	15.68	65.24
9	27.73	49.90	43.76	50.38	16.58	49.77
10	25.17	50.82	46.19	56.10	18.24	47.15
11	29.40	54.84	41.32	50.02	14.53	47.72
12	30.04	52.15	45.55	51.71	17.22	50.00
13	49.41	52.83	75.62	48.35	20.34	47.38
14	22.25	52.59	28.33	46.43	10.18	51.40
15	23.37	49.86	52.47	41.05	16.45	50.27
16	41.87	50.58	78.85	47.91	19.44	49.89
17	23.87	51.53	31.10	47.29	7.25	48.25

* data in table represent average value of three replicates.

3.3. Influence of HVED Treatment on Extraction of CA from Tobacco Waste

Previous studies showed that the major tobacco phenols are CA, and its isomers—neochlorogenic and cyrptochlorogenic acids. The CA is a member of the group caffeoylquinic acids, commercially delivered from *Lonicera japonica Thunb* and *Eucommia* ulmoides Oliver plants [6]. Several authors confirmed tobacco leaves and related materials as a rich source of CA [6,9,36,37]. In order to evaluate HVED as green technique for separation of CA from tobacco waste, CA was determined and quantified (Table 4). In the scrap extract content of CA ranked from 1.54 to 3.66 mg/100 g, in the dust extract from 1.90 to 2.97 mg/100 g and in the midrib extract from 2.30 to 3.38 mg/100 g. Obtained results are in good agreement with those obtained for tobacco waste extract with microwave assisted extraction from Chen et al. [6] and ultrasound-assisted extraction by Banožić et al. [7]. Interestingly, different from other studies [7,9,12] content of CA in midrib was a little bit higher than in dust. That is probably due differences in the waste material. Dust is primary delivered from leaf lamina, while midrib is directly extended from the tobacco stalk [4].

Table 4. Content of CA in tobacco waste extracts obtained with HVED assisted extraction *.

RUN	Tobacco Waste		
	Scrap	Dust	Midrib
	CA (mg/100 g)	CA (mg/100 g)	CA (mg/100 g)
1	3.66	2.36	3.14
2	2.69	2.38	2.48
3	1.54	2.43	2.51
4	3.33	2.14	3.38
5	2.93	2.45	2.65
6	2.23	1.97	2.30
7	2.81	2.10	2.77
8	2.28	2.31	2.48
9	2.63	2.06	2.53
10	2.32	2.08	2.37
11	3.38	2.38	2.44
12	2.54	2.11	2.39
13	3.28	2.43	2.55
14	2.88	2.41	2.53
15	2.45	1.90	2.60
16	3.41	2.96	2.53
17	2.84	2.90	2.35

* data in table represent average value of three replicates.

The effects of HVED on the content of CA are summarized in Supplementary Materials Table S4). The linear term of solvent:solid ratio and frequency had significant influence ($p < 0.05$) on CA content in scrap extracts, while the other factor did not exhibit any significant influence. The linear term of time, and quadratic terms of time showed significant influence ($p < 0.05$) on dust extract while quadratic terms of solvent:solid ratio exhibited highly significant influence ($p < 0.0001$) on CA. In midrib extracts, linear term of frequency and interaction between frequency and time showed to have significant influence on CA content.

Concentrations of CA in scrap, dust and midrib, respectively, are presented by quadratic models without transformation in Equations (2)–(4). Obtained models are significant ($p < 0.05$), with the nonsignificant lack of fit relative to the pure error (Table S4). The determination coefficients (R^2) were 0.8369, 0.9485 and 0.8546 for scrap, dust and midrib, respectively, showing the adequacy of the model and good fit of the model to the experimental data (Table S4).

$$Y_{SCRAP} = 2.55 + 0.416 \times 1 - 0.466X_2 - 0.067X_3 + 0.23X_1^2 + 0.016X_2^2 + 0.24X_3^2 + 0.19X_1X_2 - 0.13X_1X_3 - 5.61 \times 10^{-17}X_2X_3 \quad (2)$$

$$Y_{DUST} = 2.05 + 0.01X_1 - 0.015X_2 - 0.20X_3 + 0.41X_1^2 - 0.06\ X_2^2 + 0.22X_3^2 + 5 \times 10^{-3}X_1X_2 - 5 \times 10^{-3}X_1X_3 - 0.025X_2X_3 \quad (3)$$

$$Y_{MIDRIB} = 2.53 + 0.13X_1 - 0.20X_2 + 0.13X_3 + 1.5 \times 10^{-3}X_1^2 + 0.014X_2^2 + 0.018X_3^2 - 0.16X_1X_2 - 0.15X_1X_3 - 0.28X_2X_3 \quad (4)$$

To visualize the influence of process parameters on CA content, 3D plots were constructed (Figures 2–4). For scrap samples, CA increased with increasing of solvent:solid ratio and decreased with increasing of frequency. Additionally, with increasing of treatment time, CA decreased, probably due to the degradation of CA. Increasing of solvent:solid ratio showed dual effect on CA of tobacco dust. Content of CA in dust extract decreased with increasing solvent:solid ratio from 300 to 500 mL/g and increased from 500 to 700 mL/g. For dust extract treatment time showed negative influence from 15 to 33 min, and after 33 min CA, negative influence was not significant and CA content stayed almost constant. For the midrib extracts, increase of frequency showed negative influence on CA within investigated experimental range, while with an increase of time and solvent:solid ratio, CA content slightly increased.
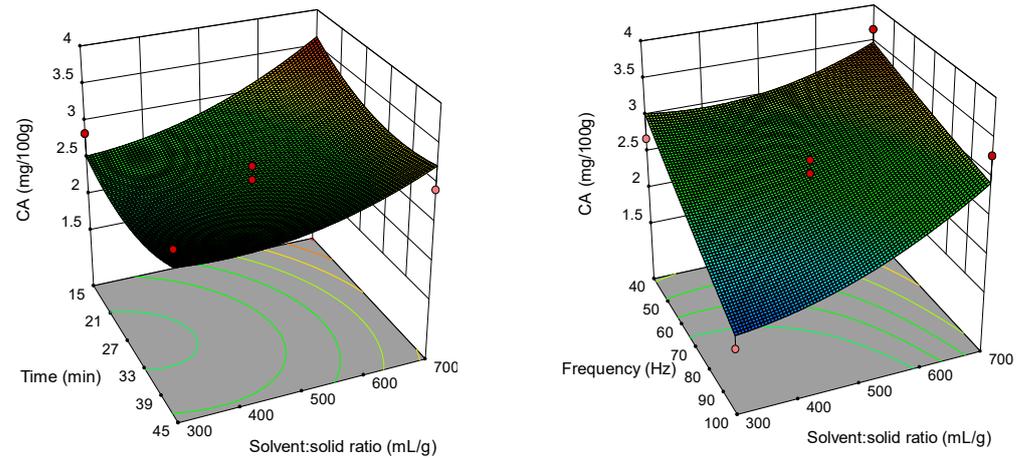
Figure 2. 3D plot showing the combined effects of HVED assisted extraction process on chlorogenic acid content of scrap.
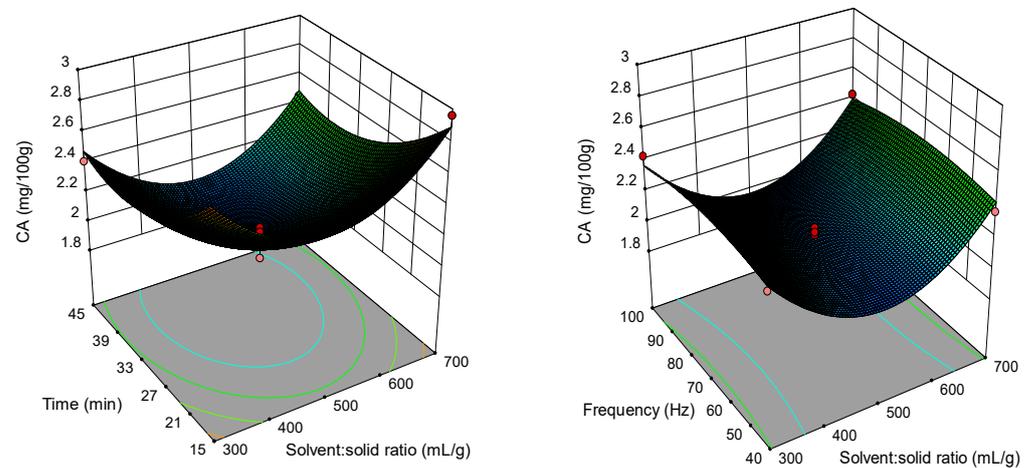

Figure 3. 3D plot showing the combined effects of HVED assisted extraction process on chlorogenic acid content of dust.
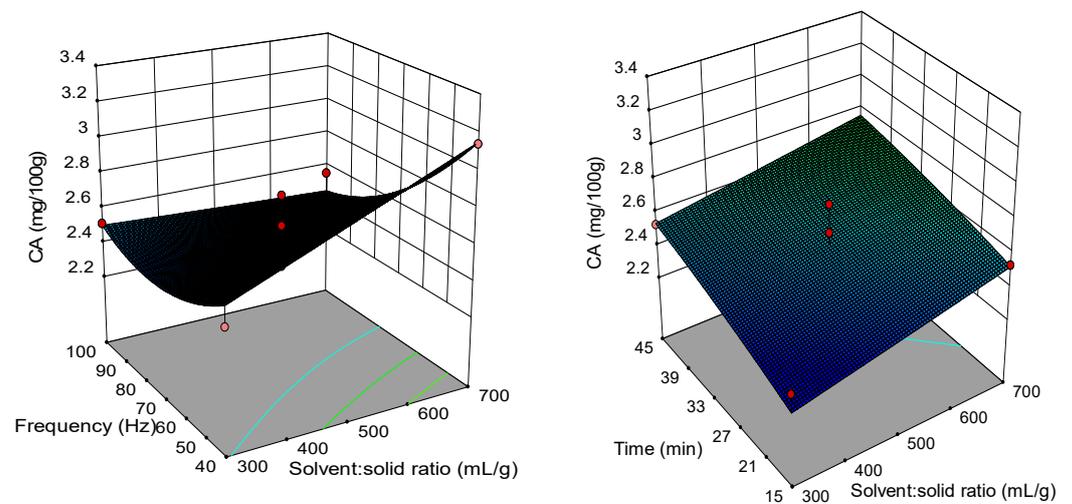

Figure 4. 3D plot showing the combined effects of HVED assisted extraction process on chlorogenic acid content of midrib.

3.4. Comparison of HVED Assisted Extraction Efficiency with Conventional Extraction Technique

In order to compare extraction efficiency, a reference extraction technique (stirring without heating) was performed at the same solvent:solid ratios and extraction times and results are presented in Table 5.

Table 5. Results for TPC, DPPH and CA obtained with conventional extraction of tobacco waste *.

Tobacco Waste		Scrap			Dust			Midrib		
Extraction Parameters		TPC (mg-GAE/100 g)	DPPH (%)	CA (mg/100 g)	TPC (mg-GAE/100 g)	DPPH (%)	CA (mg/100 g)	TPC (mg-GAE/100 g)	DPPH (%)	CA (mg/100 g)
C1	1/300 mL/g, 15 min	17.21	41.01	2.09	15.85	39.54	2.20	5.01	31.05	1.71
C2	1/300 mL/g, 30 min	18.13	42.05	2.11	16.01	40.01	1.89	5.66	37.15	1.79
C3	1/300 mL/g, 45 min	19.21	49.97	2.48	16.55	42.55	2.12	5.91	44.07	2.02
C4	1/500 mL/g, 15 min	19.38	33.80	2.18	11.13	21.22	1.81	4.15	17.71	1.78
C5	1/500 mL/g, 30 min	22.58	42.21	2.31	14.72	29.87	1.98	8.01	25.96	1.81
C6	1/500 mL/g, 45 min	32.22	48.78	2.98	29.87	37.11	2.02	9.58	34.21	1.85
C7	1/700 mL/g, 15 min	27.15	31.78	1.81	25.87	26.87	1.74	10.87	29.52	1.51
C8	1/700 mL/g, 30 min	37.42	39.56	2.01	31.47	21.78	1.80	11.01	31.45	1.89
C9	1/700 mL/g, 45 min	38.47	41.74	2.62	37.95	39.99	2.22	25.65	38.03	2.13

* data in table represent average value of three replicates.

When compared to HVED assisted extraction, values obtained after 15 and 30 min of conventional extraction process were significantly different. At these treatment times, obtained values for TPC, DPPH and CA were significantly lower. However, on treatment time of 45 min, differences are significantly lower, probably due to the negative HVED effects explained previously. It can be concluded that prolonged HVED assisted extraction process (above 30 min) does not influence positively on CA and that HVED treatment time should be properly optimized.

3.5. Optimization of HVED Parameters and Validation of Proposed Models

Using desirability function the optimal HVED assisted extraction conditions to maximize CA were established and experimentally verified. The optimal extraction conditions for the separation of CA from different types of tobacco waste are given in Table 6.

Table 6. Optimal HVED assisted parameters for waste obtained by RSM.

Optimal Parameters and Results	Tobacco Waste		
	Scrap	Dust	Midrib
Solvent:solid ratio (mL/g)	695.24	700.00	700.00
Frequency (Hz)	47.16	58.5	40.01
Time (min)	15.06	15.0	15.00
Predicted CA content (mg/mL)	3.82	2.88	2.73
Obtained CA content (mg/mL)	3.91	2.79	2.68
Predicted temperature changes (°C)	5.97	1.99	6.21
Obtained temperature changes (°C)	6.02	2.01	2.05

3.6. Role of Novel Extraction Techniques in Tobacco Sustainability Production

In the last decade, the tobacco industry had gone through different cycles of changes and it is clearer that the tobacco industry is not just "cigarette production". Despite dramatic declines in smoking, the tobacco industry continues to rebrand already existing

and develop new products, like flavor capsules and heated tobacco products. Using a combination of strategies, including environmental sustainability and promotion of reduced-risk tobacco products this industry continues growing among new markets and new consumers. Despite the economic importance, tobacco production causes some great environmental and social concerns, associated with deforestation, accumulation of agricultural, industry and consumer waste [38–41]. All problems addressed above clearly present that global sustainability politics do not favor global tobacco companies and that tobacco companies will have to make more effort to reach greener production and less harmful products. One of the possible paths is the implementation of new processes or valorization and reutilization of already generated waste. Chemat et al. [42] stated that in the global industry extraction techniques are "mature but underused" implying that more effective research in this area could lead to a more secure market and high added-value products. Extraction techniques are already implemented in tobacco production, mostly due to nicotine extraction for further incorporation into other non-tobacco products. On the other hand, after removing nicotine, there is a significant content of other bioactive compounds remaining (such as CA) in the material which can also be extracted and reused. Furthermore, novel extraction techniques such as HVED assisted extraction are considered as low-energy input and nonthermal technologies which are in accordance with the principles of green chemistry. This approach could be characterized as both, economically viable for the tobacco industry, and safe and harmless for the environment. Additionally, this process could be easily applicable for other bioactive compounds in tobacco-related materials as well as other industry-less-utilized wastes and by-products.

4. Conclusions

This is the first reported study on HVED assisted extraction of CA from tobacco industry waste material. The obtained results showed the potential of HVED assisted extraction recovery improvement of CA from tobacco waste (scrap, dust and midrib). At the same process conditions (treatment time and solvent:solid ratio), HVED assisted extraction was demonstrated to be more effective than the conventional extraction in terms of TPC, radical scavenging activity and CA content, respectively. Based on these observations, a predictive model that correlates solvent:solid ratio, frequency and time of HVED assisted extraction was proposed. Based on findings from present study, it can be concluded that HVED is reliable nonthermal technology for recovery CA from tobacco waste. In summary, the high demand for CA, the limited natural resources for its production, and the generation of tobacco waste justify the reasons for seeking new techniques for the recovery of bioactive compounds from tobacco waste. The proposed method (HVED-assisted extraction) provides useful insights to the separation of bioactive compounds from tobacco waste and could contribute in development of new added value products and lower environmental impact. In addition, the use of harmful organic solvents was avoided throughout the process, which makes this whole process completely harmless and environmentally friendly. The present findings could be transferred into other plant materials, especially to waste materials which contain chlorogenic acid or other phenolic compounds. Additionally, presented results could have implications for solving increased amount of tobacco waste with the contribution in sustainable waste managing. However, the feasibility of the application of HVED assisted extraction of CA from tobacco waste at pilot or industrial scales is still unknown. Nevertheless, tobacco wastes represent a great source of CA that could be recovered and used for many purposes as nutraceutical or food additive.

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