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Extraction of bioactive compounds from tobacco waste using deep eutectic solvents

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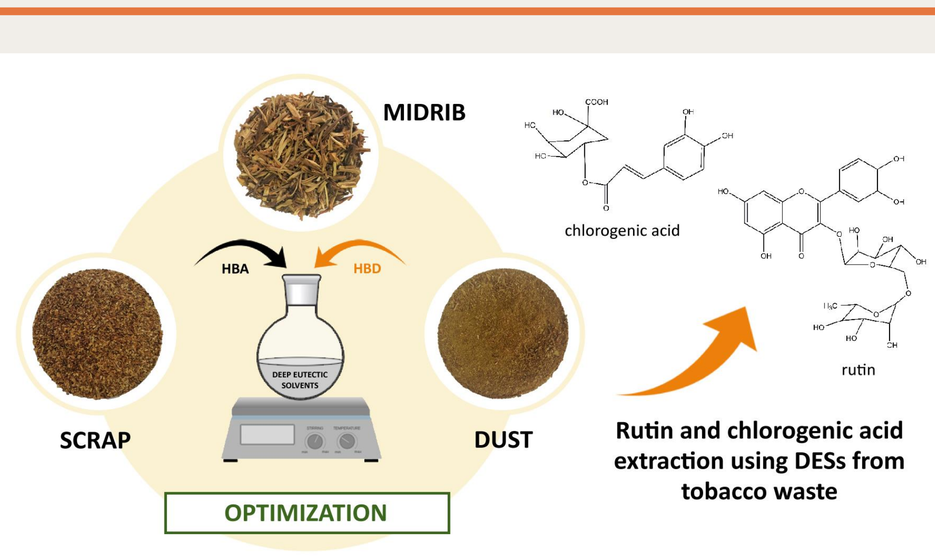
INTRODUCTION

Tobacco waste is a solid waste generated during leaf processing. Tobacco leaves processing generates three fraction of tobacco waste, namely scrap, midrib and dust, with differences in granulation, generation place during processing, and moisture content. The objective of these study was to investigate the efficacy of eutectic solvents in the extraction of bioactive compounds from tobacco waste. Influence of extraction parameters on the properties of the obtained extracts (total phenol, antioxidant activity, chlorogenic acid and rutin) has been determined. Using response surface methodology optimal extraction parameters were defined. Extraction with deep eutectic solvents on tobacco waste has not yet been sufficiently investigated and proposed research represents an innovative approach in extraction of bioactive compounds from mentioned material.



MATERIALS

TOBACCO WASTE

Tobacco industrial waste (dust, midrib and scrap) were obtained in 2018 from tobacco processing factory "Fabrika duhana Sarajevo" (Bosnia and Herzegovina).
Tobacco waste was obtained in dry condition, after industry processing. All samples were kept at ambient temperature at dark and dry place before the extraction. Tobacco leaves and waste were pulverized before the extraction (MRC Sample mill C-SM/450-C. Holon, Israel).

Figure 1. Tobacco waste (scrap, dust and midrib)

DEEP EUTECTIC SOLVENTS

DES PREPARATION EXTRACTION

DESs were prepared by mixing choline chloride (ChCl) as HBA with 15 different HBDs in certain molar ratio as specified in Table 1. The each mixture was heated to 80°C under constant stirring until a stable homogeneous liquid was formed. Dried and milled tobacco waste of each type (scrap, dust and midrib) were weighted (50 mg) and mixed with 1 mL of the mixture of DES with specific amount of distilled water. The mixture of DES and 20 % of added water (v/v) was then stirred at 50°C for 30 min. After the extraction, the mixture was centrifuged at 6000 rpm for 5 min and then decanted. The supernatant of 200 µL was then diluted with 800 µL methanol and filtered through a PTFE 0.45 µm filter before HPLC analysis. The extraction was performed in duplicate.

SCREENING

Table 1. List of DESs prepared and tested for the extraction of phenolic compound from tobacco waste

Hydrogen Bond Acceptors (HBAs)	Hydrogen Bond Donors (HBDs)	Mole Ratio (HBA: HBD)
Choline chloride	Urea	1:2
Choline chloride	N-methylurea	1:3
Choline chloride	Glucose	1:1
Choline chloride	Xylitol	1:1
Choline chloride	Sorbitol	1:1
Choline chloride	Butan-1,4-diol	1:2
Choline chloride	Etan-1,2-diol	1:2
Choline chloride	Glycerol	1:2
Choline chloride	Acetamide	1:2
Choline chloride	Malic acid	1:1
Choline chloride	Citric acid	1:2
Choline chloride	Malonic acid	1:1
Choline chloride	Oxalic acid	1:1
Choline chloride	Lactic acid	1:1
Choline chloride	Levulinic acid	1:1

Best DES → Choline chloride: Etan-1,2-diol

Most suitable material → Scrap

ANALYSIS

Antioxidant activity (DPPH) and total phenol content was determined using UV-visible spectrophotometer. Identification and quantification of bioactive compounds was performed by high performance liquid chromatography with diode array detection (HPLC/DAD)

Table 2. Experimental design used in RSM with experimental values for total phenol content, DPPH and Chlorogenic acid and rutin content

RUN	Time (min)	Temperature (°C)	Water content (%)	Total phenol content (mg GAE/L)	% DPPH	Klorogenska kiselina (µg/mL)	Rutin (µg/mL)
1.	60	30	30	90.21	36.97	6.56	17.85
2.	60	50	20	67.52	29.45	5.27	14.85
3.	30	70	20	86.10	30.03	8.07	14.04
4.	90	50	30	113.41	44.68	-	17.85
5.	30	50	10	63.54	29.32	6.86	9.27
6.	60	30	10	52.89	31.42	8.78	10.42
7.	90	50	10	80.08	22.56	7.84	13.67
8.	90	30	20	69.95	31.83	6.92	14.16
9.	90	70	20	125.08	44.34	-	15.82
10.	60	70	30	147.26	41.60	-	17.67
11.	60	70	10	105.97	44.24	4.66	12.06
12.	30	30	20	67.51	30.18	6.66	12.24
13.	60	50	20	64.41	26.98	4.29	14.81
14.	60	50	20	68.52	26.73	6.70	14.79
15.	60	50	20	67.55	25.44	5.69	14.92
16.	60	50	20	66.51	24.05	5.71	14.86
17.	30	50	30	102.77	39.40	8.26	14.31

OPTIMIZATION

Table 3. Analysis of variance (ANOVA) of second-order polynomial models for tobacco waste (scrap)

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	*p*-value	Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	*p*-value
Total phenol content						**Chlorogenic acid**					
Model	11404.09	9	1267.12	52.73	< 0.0001	Model	124.76	9	13.86	15.09	0.0008
X_1 – Time	754.66	1	754.66	31.40	0.0008	X_1 - Time	28.46	1	28.46	30.97	0.0008
X_2 - Temperature	4663.37	1	4663.37	194.06	< 0.0001	X_2 - Temperature	32.76	1	32.76	35.66	0.0006
X_3 – Water content	2848.24	1	2848.24	118.53	< 0.0001	X_3 - Water content	22.18	1	22.18	24.13	0.0017
X_1X_2	520.75	1	520.75	21.67	0.0023	X_1X_2	17.35	1	17.35	18.88	0.0034
X_1X_3	8.70	1	8.70	0.3621	0.5663	X_1X_3	21.34	1	21.34	23.23	0.0019
X_2X_3	4.39	1	4.39	0.1826	0.6819	X_2X_3	1.49	1	1.49	1.62	0.2438
X_1^2	190.83	1	190.83	7.94	0.0258	X_1^2	0.4053	1	0.4053	0.4410	0.5279
X_2^2	1053.08	1	1053.08	43.82	0.0003	X_2^2	0.7776	1	0.7776	0.8462	0.3882
X_3^2	1122.78	1	1122.78	46.72	0.0002	X_3^2	0.0440	1	0.0440	0.0479	0.8330
RESIDUAL	168.21	7	24.03			RESIDUAL	6.43	7	0.9189		
LACK OF FIT	166.94	3	55.65	175.17	0.0001	LACK OF FIT	3.40	3	1.13	1.50	0.3439
PURE ERROR	1.27	4	0.3177			PURE ERROR	3.03	4	0.7580		
TOTAL	11572.31	16				TOTAL	131.20	16			
R^2	0.9855					R^2	0.9510				
DPPH						**Rutin**					
Model	779.01	9	86.56	3.93	0.0423	Model	85.88	9	9.54	14.58	0.0009
X_1 - Time	26.21	1	26.21	1.19	0.3113	X_1 - Time	16.94	1	16.94	25.88	0.0014
X_2 - Temperature	111.08	1	111.08	5.05	0.0595	X_2 - Temperature	3.03	1	3.03	4.62	0.0686
X_3 - Water content	154.09	1	154.09	7.00	0.0331	X_3 - Water content	61.94	1	61.94	94.64	< 0.0001
X_1X_2	40.07	1	40.07	1.82	0.2192	X_1X_2	0.0049	1	0.0049	0.0075	0.9335
X_1X_3	36.24	1	36.24	1.65	0.2402	X_1X_3	0.1849	1	0.1849	0.2825	0.6115
X_2X_3	16.77	1	16.77	0.7620	0.4116	X_2X_3	0.8281	1	0.8281	1.27	0.2977
X_1^2	15.18	1	15.18	0.6898	0.4336	X_1^2	2.39	1	2.39	3.65	0.0978
X_2^2	176.05	1	176.05	8.00	0.0255	X_2^2	0.0033	1	0.0033	0.0050	0.9454
X_3^2	170.38	1	170.38	7.74	0.0272	X_3^2	0.4258	1	0.4258	0.6506	0.4464
RESIDUAL	154.04	7	22.01			RESIDUAL	4.58	7	0.6545		
LACK OF FIT	148.50	3	49.50	35.69	0.0024	LACK OF FIT	4.57	3	1.52	602.27	< 0.0001
PURE ERROR	5.55	4	1.39			PURE ERROR	0.0101	4	0.0025		
TOTAL	933.06	16				TOTAL	90.46	16			
R^2	0.8349					R^2	0.9494				

The best-fitted models for obtained values were quadratic models without response transformation:

Total phenol content= $66.89 + 9.71X_1 + 24.14X_2 + 18.87X_3 + 6.73X_1^2 + 15.81X_2^2 + 16.33X_3^2 + 11.41X_1X_2 - 1.48X_1X_3 + 1.05X_2X_3$

DPPH = $25.73 + 1.81X_1 + 3.73X_2 + 4.39X_3 + 1.9X_1^2 + 6.47X_2^2 + 6.36X_3^2 + 3.16X_1X_2 + 3.01X_1X_3 - 2.05X_2X_3$

Chlorogenic acid = $5.53 - 1.89X_1 - 2.02X_2 - 1.67X_3 + 0.31X_1^2 - 0.43X_2^2 - 0.1X_3^2 - 2.08X_1X_2 - 2.31X_1X_3 - 0.61X_2X_3$

Rutin = $14.85 + 1.46X_1 + 0.62X_2 + 2.78X_3 - 0.75X_1^2 - 0.028X_2^2 - 0.32X_3^2 - 0.0035X_1X_2 - 0.21X_1X_3 - 0.46X_2X_3$

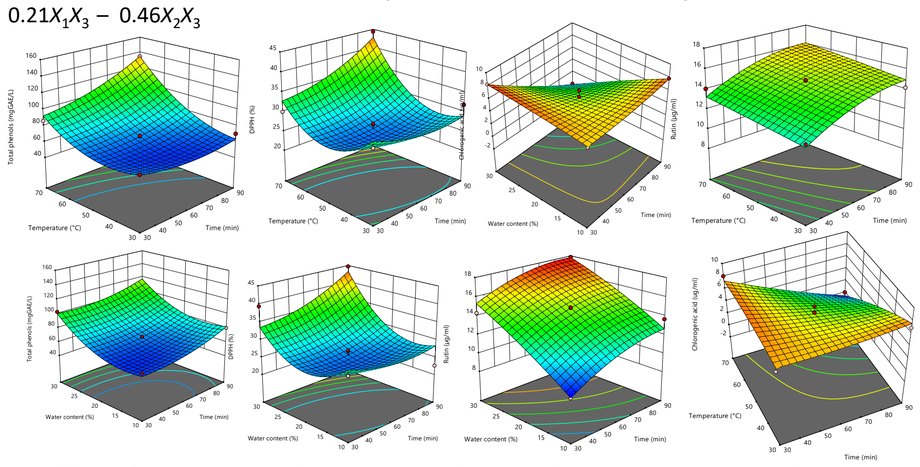


Figure 2. Response surface plots showing the effects of temperature, time and water content on DES extraction of tobacco waste: Scrap

Table 4. Optimal DES extraction parameters and predicted response data for tobacco waste obtained by RSM

Time (min)	30
Temperature (°C)	70
Water content (%)	29.99
Predicted total phenol content (mg GAE/L)	130.177
Predicted DPPH value (%)	38.13
Predicted chlorogenic acid content (µg/ml)	7.29
Predicted rutin content (µg/ml)	15.48
Desirability	0.767

Predicted data were experimentally verified with good agreement between predicted and experimental values with a deviation of ± 5 %, which confirms significance of proposed model.

CONCLUSIONS

The use of DES extraction for the purpose of isolation of bioactive compounds from tobacco waste proved to be highly efficient. The screening showed that DES mixture choline chloride: Etan-1,2-diol and tobacco waste: scrap was the most suitable for the extraction.
Using response surface methodology optimal parameters were defined (30 min, 70°C, 29.99 % water). The meaning of the proposed research is reflected in the maximum utilization of tobacco by-product in order to obtain extracts that can be implemented in other products and processes.

ACKNOWLEDGEMENT

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