Green extraction techniques of nicotine from tobacco waste: possibilities, limitations and potential application

Zelene tehnike ekstrakcije nikotina iz duhanskog otpada: mogućnosti, ograničenja i potencijalna primjena

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ByProExtract

Uspostavni istraživački projekt:

„Primjena inovativnih tehnika ekstrakcije bioaktivnih komponenti iz nusproizvoda biljnoga podrijetla"

(2018-2023)

Voditeljica projekta: prof. dr. sc. Stela Jokić

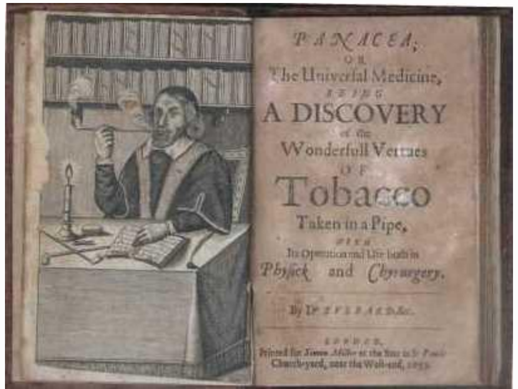
*(Iznos financiranja: **1.607,708.72 HRK**)*






TOBACCO WASTE AND NICOTINE

- Tobacco waste rich source of nicotine
- Nicotine is a plant alkaloid, found in the tobacco plant, and addictive central nervous system (CNS) stimulant
- Act as an agonist at the nicotinic cholinergic receptors in the autonomic ganglia, at neuromuscular junctions, and in the adrenal medulla and the brain.



Tobacco Leaf, Smoke and Smoking, MAO Inhibitors, Parkinson's Disease and Neuroprotection; Are There Links?

Kay Castagnoli, Thangaraju Murugesan

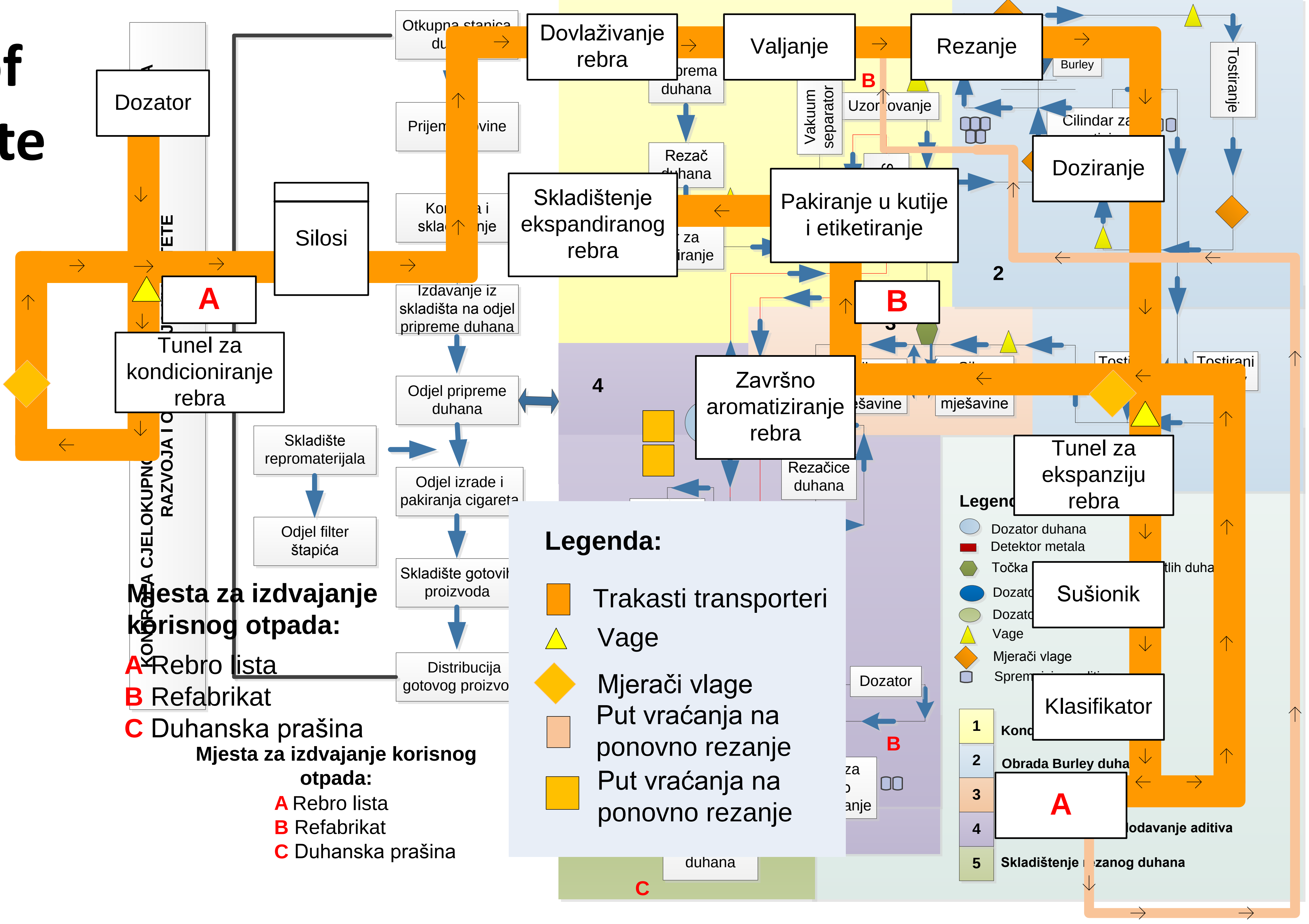
Show more

https://doi.org/10.1016/S0161-813X(03)00107-4

Material

Tobacco processing factory
„Fabrika duhana Sarajevo” (BiH), British American Tobacco

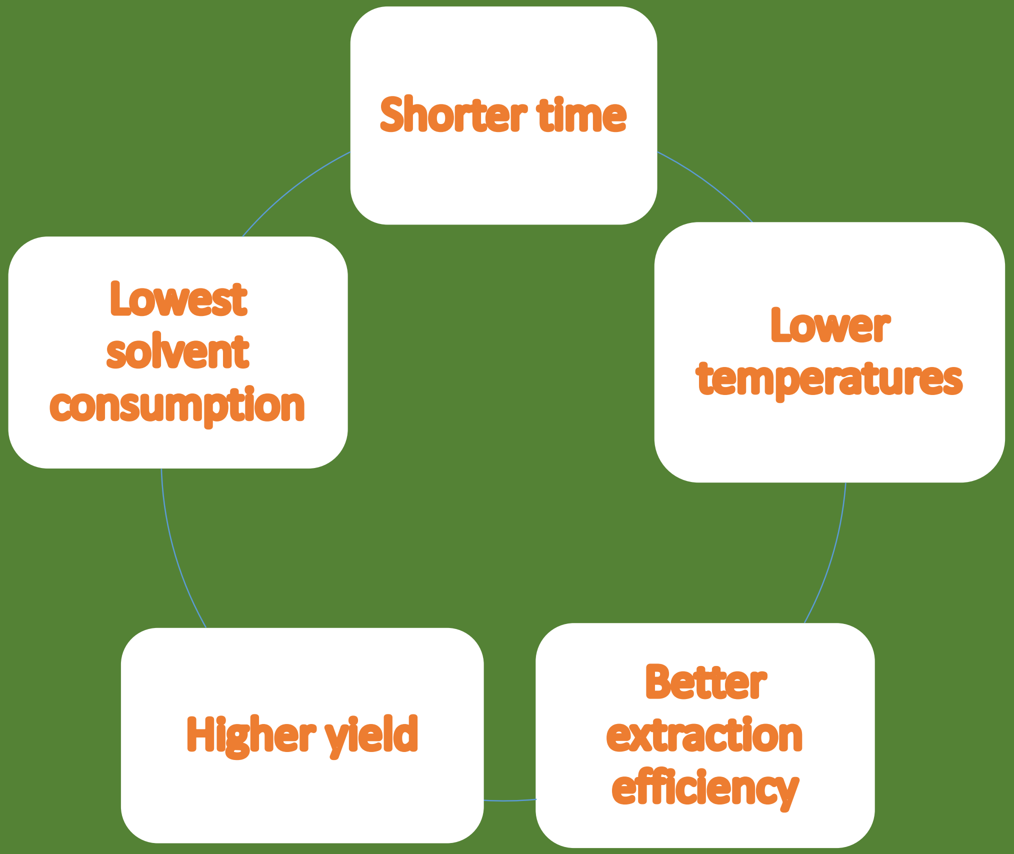
Separation of tobacco waste during leaf processing



Mjesta za izdvajanje korisnog otpada:

A Rebro lista
B Refabrikat
C Duhanska prašina

GREEN EXTRACTION TECHNIQUES

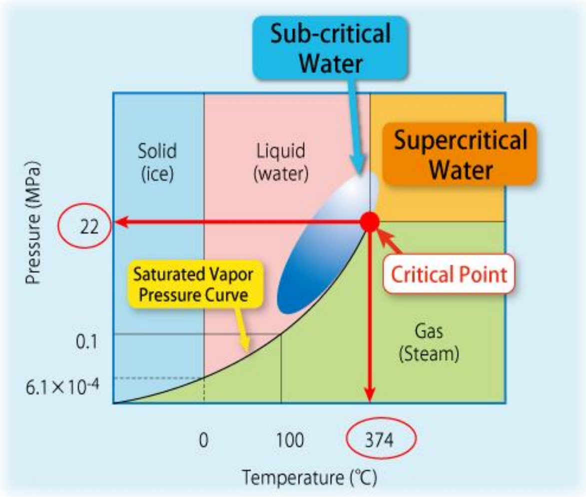


- Cavitation phenomena,
- Implosion of cavitation (surface peeling, erosion and particle breakdown)
- Macro-turbulences
- Micro mixing

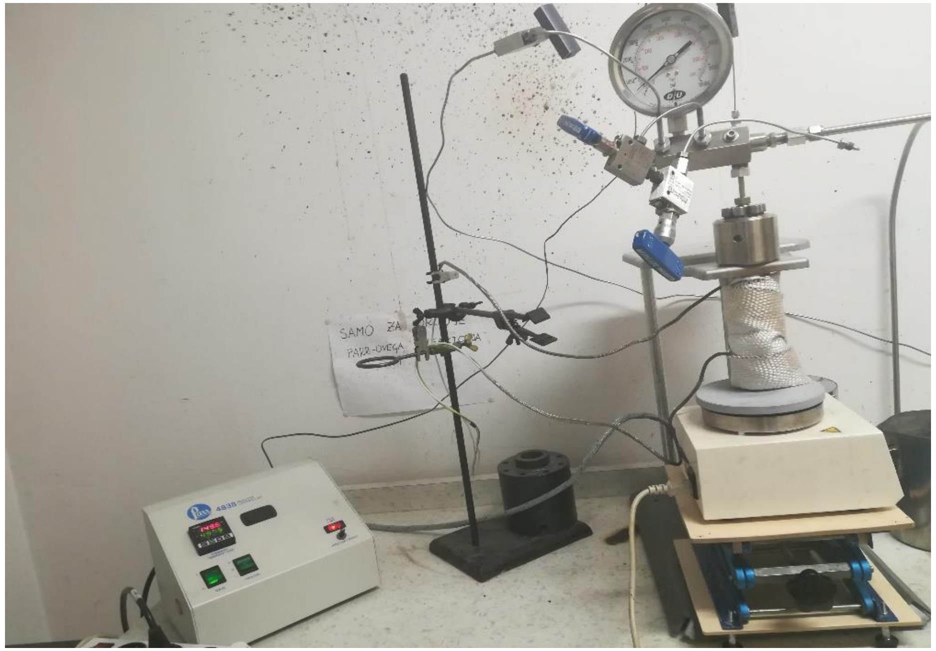
ULTRASOUND-ASSISTED EXTRACTION

- Solvent is heated far above its boiling point
- Intensive disruption of tissue structure under microwave irradiation
- Ionic conductance and dipole rotation

MICROWAVE-ASSISTED EXTRACTION

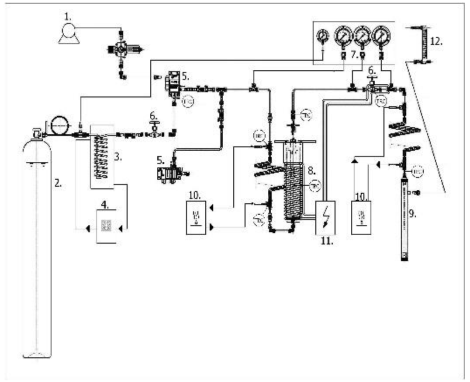


- Safe, non-toxic, non-flammable and environmentally friendly solvent
- Available and cheap solvent
- Obtained extracts are safe
- high diffusion into the plant matrix
- increased mass-transfer properties
- Can be applied for extraction of low-polar and non-polar compounds



SUBCRITICAL WATER EXTRACTION

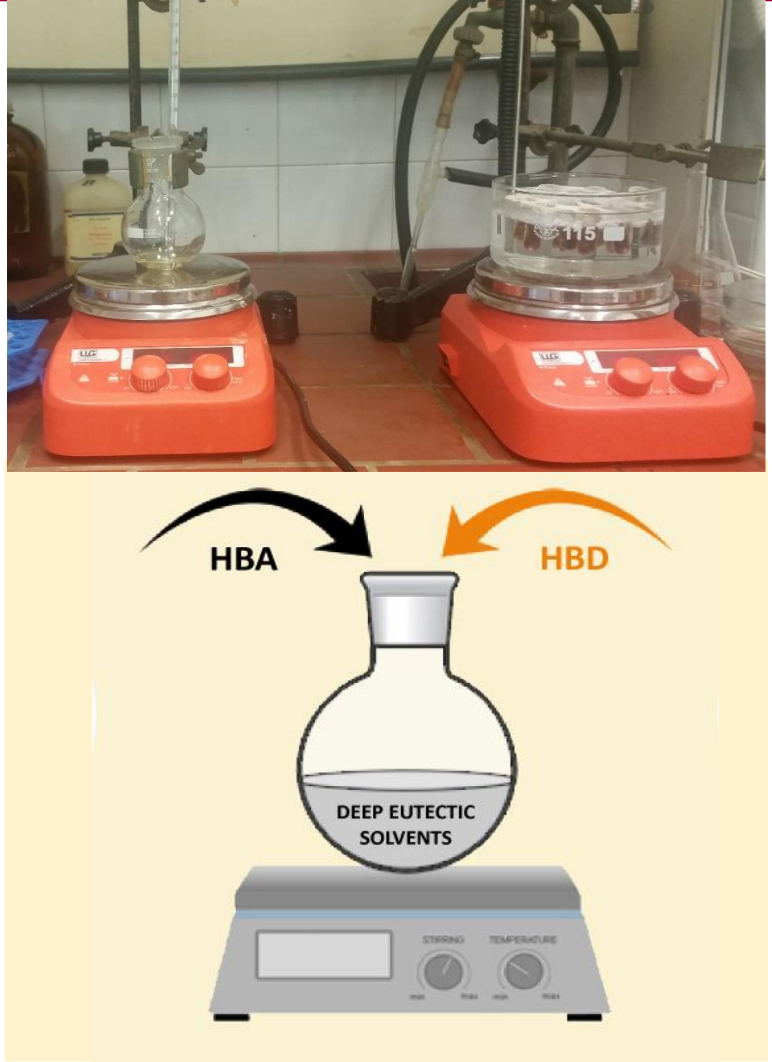
- Elimination of organic solvents
- Shorter time
- Lower temperatures
- Complete separation of solvent from extracts
- Easy solvent recovery



- High pressures
- High investment cost
- High polar compounds are insoluble

SUPERCRITICAL CO_2 EXTRACTION

HIGH VOLTAGE ELECTRIC DISCHARGE ASSISTED EXTRACTION



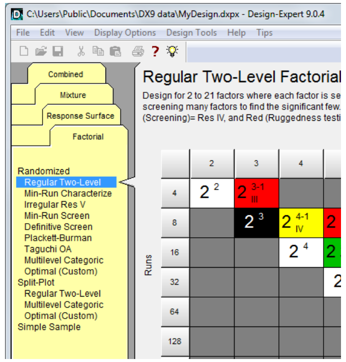
EXTRACTION WITH DEEP EUTECTIC SOLVENTS

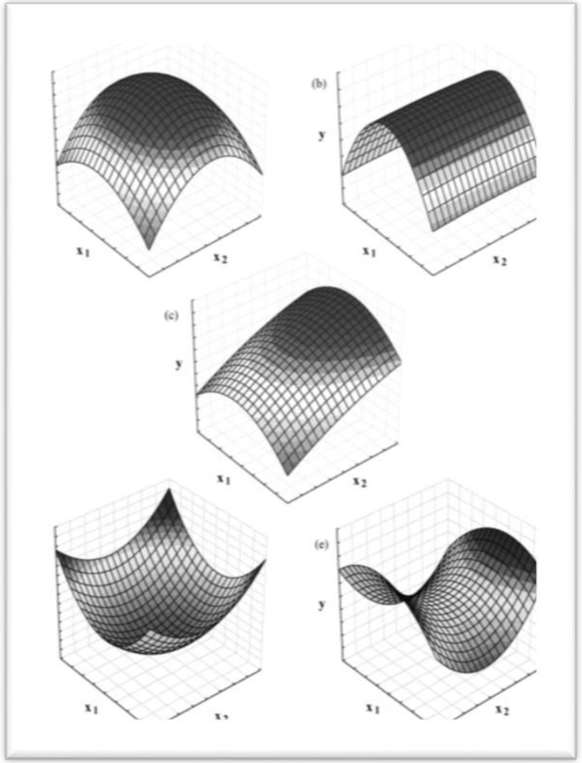
PULSED ELECTRIC FIELD-ASSISTED EXTRACTION

Analysis

Gas Chromatography coupled to Mass Spectrometry (GC-MS)
Gas Chromatography with Flame-Ionization Detection (GC-FID)
High Performance Liquid Chromatography (HPLC)
Spectrophotometric analysis

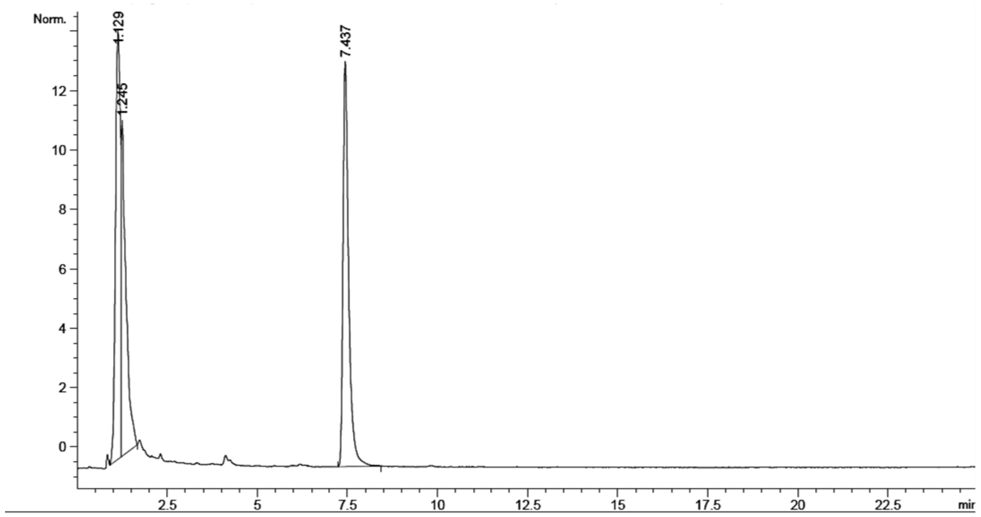
Optimization (response surface methodology) Design *Expert*®9, (Stat-Ease .Inc., USA)





NEW HPLC METHOD

• Suitable for adoption by laboratories to determine the actual content and
stability of nicotine-containing products
• Simple high-performance liquid chromatography (HPLC) method to determine
nicotine content in nicotine-containing products
• HPLC method performed strongly and was validated according to
international guidelines



METHOD:

- Mobile phase: Carbonate-bicarbonate buffer (30 mM, pH 10): Acetonitrile (80:20)
- Used colon: Proshell Infinity lab 120 C-C18 (4.6x150 mm, 4um)
- Flow rate of 1 mL/min
- 10 µL injection volume
- Wavelenght 259 nm

RESULTS

Publications

Open Access | Article

Optimization of Ultrasound-Assisted Extraction of Some Bioactive Compounds from Tobacco Waste

by Marija Banožić, Ines Banjari, Martina Jakovljević, Drago Šubarić, Srećko Tomas, Jurislav Babić and Stela Jokić *

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Separation of active compounds from tobacco waste using subcritical water extraction

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Journal of the Science of Food and Agriculture SCI

Research Article | Full Access

Volatile organic compounds of tobacco leaves *versus* waste (scrap, dust, and midrib): extraction and optimization

Marija Banožić, Krunoslav Aladić, Igor Jerković, Stela Jokić

First published: 07 September 2020 | https://doi.org/10.1002/jsfa.10796

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Prihvaćeno 19. svibnja 2020.

Ekstrakcija bioaktivnih spojeva iz duhanskog otpada primjenom eutektičkih otapala

M. Banožić,[a] M. Matić,[b] S. Šafranko,[a] A.-M. Cikoš,[a] M. Jakovljević,[a] M. Molnar[a] i S. Jokić[a]*

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Open Access | Review

Carbohydrates—Key Players in Tobacco Aroma Formation and Quality Determination

by Marija Banožić [1], Stela Jokić [1,*], Đurđica Ačkar [1], Marijana Blažić [2] and Drago Šubarić [1]

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Recent advances in extraction of bioactive compounds from tobacco industrial waste-a review

Marija Banožić, Jurislav Babić, Stela Jokić

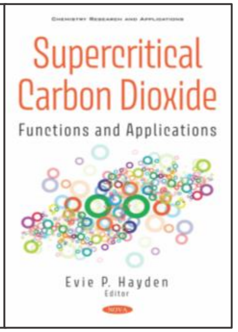
Overview on the Application of Supercritical CO2 Extraction of Active Compounds from Tobacco and Tobacco Waste

March 2020

In book: Supercritical Carbon Dioxide: Functions and Applications · Publisher: Nova Science Publishers, Inc.

Project: Application of innovative techniques of the extraction of bioactive components from by-products of plant origin

Marija Banožić · Senka Vidovic · Jelena Vladic · Stela Jokic



U: Neke mogućnosti iskorištenja nusproizvoda prehrambene industrije – Knjiga 2.

ISBN:

Urednici: Drago Šubarić
Jurislav Babić

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Poglavlje 13

NEKE MOGUĆNOSTI ISKORIŠTENJA NUSPROIZVODA DUHANSKE INDUSTRIJE

Marija Banožić[1], Elma Nukić-Hrastovina[2], Stela Jokić[1*]

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ULTRASOUND-ASSISTED EXTRACTION

Tested variables	Time (min)	Temperature (°C)	Solvent/solid ratio (mL/g)
Experimental range	5-25	30-70	10-30
Detected compounds	**Nicotine**, volatile organic compounds, neophytadiene, 4,8.,3-duvatriene-1,3-diol		
Optimal conditions for non-polar compounds	Scrap: 70 °C. 50 min. 12.74 mL/g	Dust: 70 °C. 45 min. 10 mL/g	Midrib: 70 °C. 20.19 min. 10 mL/g

Run	NICOTINE(mg/g_{dw})		
	SCRAP	DUST	MIDRIB
1	0.6347	0.4689	0.2165
2	0.4482	0.3040	0.1488
3	0.6465	0.4248	0.1838
4	0.1029	0.1809	0.1197
5	0.4631	0.4132	0.2480
6	0.5238	0.3882	0.2634
7	0.5841	0.4797	0.3314
8	0.5802	0.5274	0.4701
9	0.3127	0.0603	0.1204
10	0.3292	0.3372	0.1260
11	0.1935	0.1060	0.1010
12	0.5139	0.4883	0.1628
13	0.4812	0.2956	0.1955
14	0.3208	0.0238	0.1509
15	0.4611	0.3002	0.1716
16	0.3976	0.3380	0.1547
17	0.1523	0.1172	0.0719

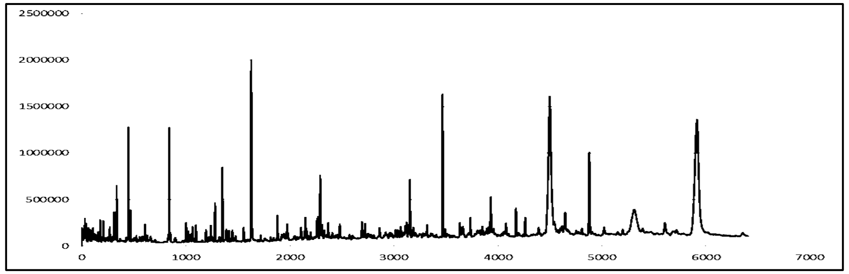


Figure 1: GC-MS midrib chromatogram obtained under UAE condition of 50 °C, 10 min, 10 mL/g

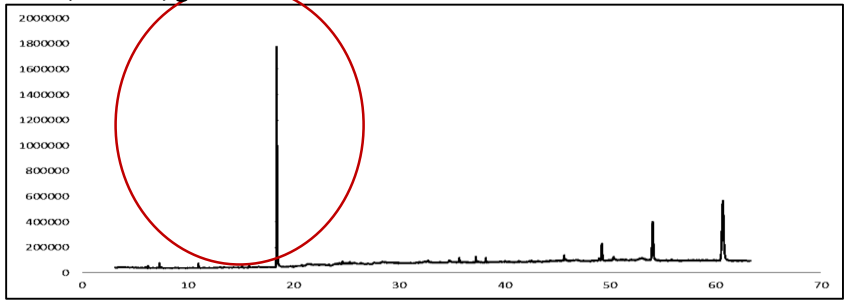


Figure 2: GC-MS midrib chromatogram obtained under UAE condition of 70 °C, 30 min, 30 mL/g

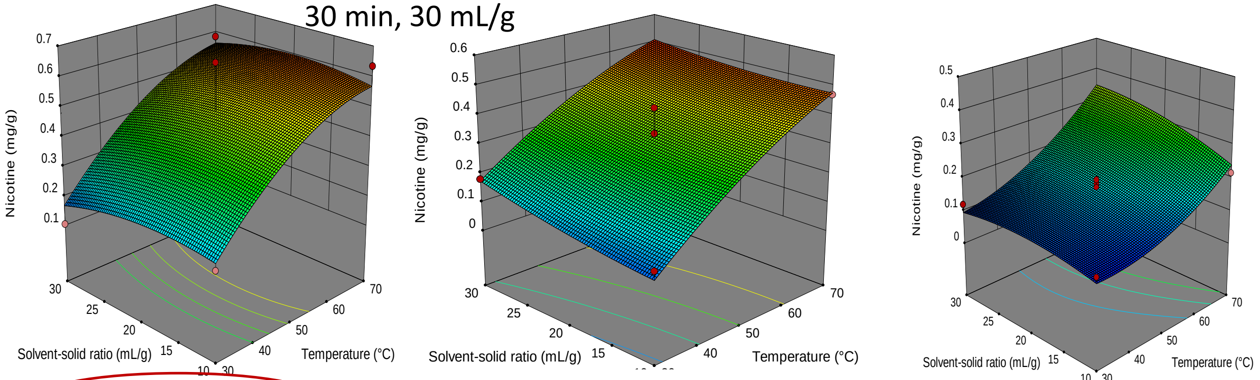


Figure 3: 3D plots showing the influence of the independent variables on the response values

Proposed models:

Scrap $_{\text{Nicotine content}} = 0.4869 + 0.1866X_1 + 0.0852X_2 - 0.0143X_3 - 0.0745X_1^2 - 0.0333X_2^2 - 0.0337X_3^2 - 0.0129X_1X_2 + 0.01X_1X_3 - 0.0016X_2X_3$

Dust $_{\text{Nicotine content}} = 0.3325 + 0.1827X_1 + 0.0625X_2 + 0.0328X_3 - 0.0359X_1^2 - 0.0262X_2^2 + 0.0122X_3^2 + 0.0519X_1X_2 - 0.0016X_1X_3 - 0.0943X_2X_3$

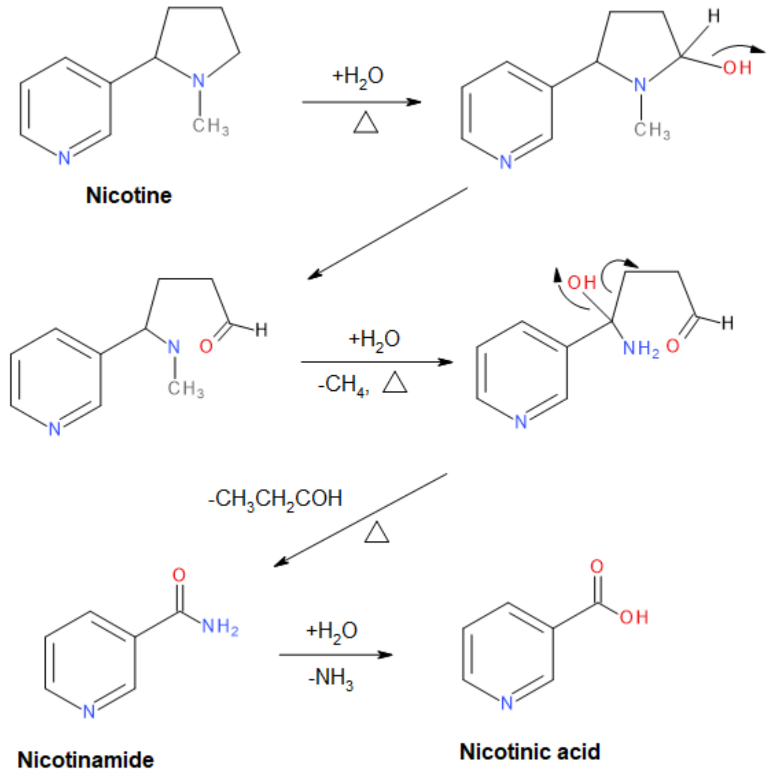
Midrib $_{\text{Nicotine content}} = 0.1709 + 0.1028X_1 + 0.0601X_2 + 0.03X_3 + 0.0442X_1^2 + 0.0202X_2^2 - 0.0229X_3^2 + 0.0358X_1X_2 - 0.0240X_1X_3 - 0.0237X_2X_3$

SUBCRITICAL-WATER EXTRACTION

Tested variables	Time (min)	Temperature (°C)	Solvent/solid ratio (mL/g)	
Experimental range	5-25	150-250	10-30	
Detected compounds	Phenolic compounds. carbohydrates. chlorogenic acid. rutin. nicotine. 3.4 DHBA. nicotinic acid. nicotinamide. 5-HMF. furfural and 5-MF			
Optimal conditions	Scrap: 150 °C. 23 min. 28 mL/g	Dust: 160 °C. 20 min. 10 mL/g		Midrib: 150 °C. 25 min. 30 mL/g

Nicotine mg/g			
	SCRAP	DUST	MIDRIB
1	26.6	15.0	9.07
2	44.3	22.8	19.7
3	46.1	23.1	12.3
4	29.9	18.0	10.2
5	33.5	27.2	13.0
6	51.4	27.5	17.7
7	32.5	21.5	11.0
8	42.4	32.2	17.1
9	40.5	24.3	14.8
10	42.5	29.5	15.7
11	30.6	21.7	12.5
12	39.4	23.6	14.7
13	32.01	26.9	13.0
14	37.1	28.0	15.2
15	32.4	26.4	13.3
16	32.4	24.5	14.1

Proposed degradation mechanism:



Proposed models:

$$(Y_N)_L^{-3} = 0.049 - 0.055X_1 - 0.013X_2 + 9.606\cdot10^{-3}X_1X_2 + 0.016X_1^2 + 0.018X_3^2$$

$$(Y_N)_S^{-3} = 0.028 - 6.209\cdot10^{-3}X_1 - 4.699\cdot10^{-3}X_2 + 3.932\cdot10^{-3}X_3 + 0.017X_1X_2$$
$$-4.131\cdot10^{-3}X_2X_3 - 8.489\cdot10^{-3}X_3^2$$

$$(Y_N)_D^{-3} = 0.053 - 0.024X_1 - 0.023X_2 + 0.012X_3 + 0.076X_1X_2 - 0.017X_1X_3$$
$$+0.046X_1^2 + 0.059X_2^2 - 0.042X_3^2$$

$$(Y_N)_M^{-3} = 0.38 - 0.20X_1 + 0.072X_3 + 0.40X_1X_2 - 0.069X_1X_3 + 0.20X_1^2$$
$$+0.16X_2^2 - 0.18X_3^2$$

SUPERCRITICAL CO_2 EXTRACTION			
Tested variables	Pressure (bar)	Temperature (°C)	Time (min)
Experimental range	100-300	40-80	5-120
Detected compounds	Fatty acids, nicotine, volatile organic compounds		
Optimal conditions	Type: scrap, 120 min, 300 bar and 61.22 °C		

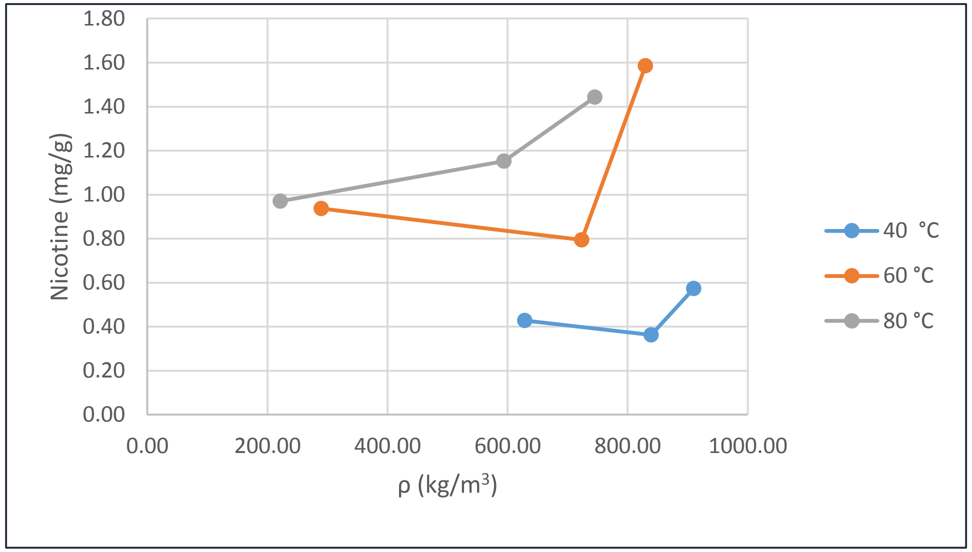


Figure 4: Nicotine content of tobacco waste *vs*. solvent density (ρ) at different temperatures during SC-CO_2 extraction

RUN	Pressure (bar)	Temperature (°C)	Nicotine (mg/g)	Other detected compounds
E1	100	40	4.28	*Duva-4.8.13-triene-1.3-diol*
E2	300	40	5.74	*2.3'-Dipyridyl*
E3	100	80	9.71	*3-Oxo-α-ionol*
E4	300	80	14.43	*Cotinine*
E5	100	60	9.37	*Solavetivone*
E6	300	60	15.85	*Neophytadiene*
E7	200	40	3.62	*Hexahydrofarnesyl acetone*
E8	200	80	11.52	*(E.E)-Farnesyl acetone*
E9	200	60	9.03	*Hexadecanoic acid*
E10	200	60	7.96	*Thunbergol*
E11	200	60	7.62	*Phytol*
E12	200	60	8.31	Fatty acids (palmitic acid. stearic acid. linoleic acid. linolenic acid. oleic acid
E13	200	60	6.76	
Soxhlet			8.34	

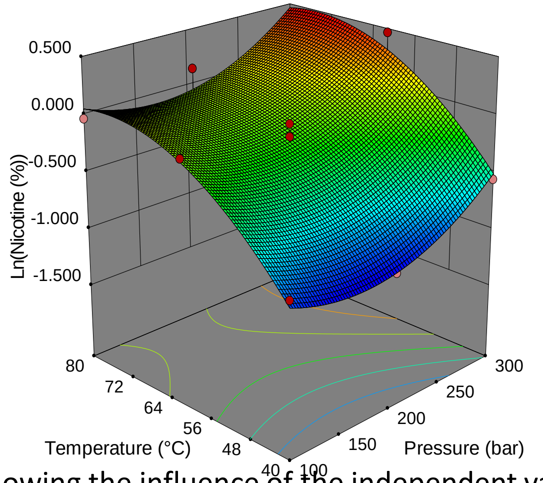


Figure 5: 3D plots showing the influence of the independent variables on the response values

Proposed model:

$$\ln(Y_2) = -0.19 + 0.20X_1 + 0.48X_2 + 0.30{X_1}^2 - 0.33{X_2}^2$$

Nicotine forms: why and how do they matter

El-Hellani i sur..2015

Nicotine has two basic nitrogen groups in its chemical structure ($pka_1 = 3.12$, $pka_2 = 8.02$), and thus it can exist in three forms (namely free-base (Nic), monoprotonated ($NicH^+$) or diprotonated salt ($NiCH_2^+$) depending on the pH of the matrix [7-9]. This intrinsic characteristic is quite important since it controls the "bioavailability" of nicotine [10]. In particular, Nic is more volatile than its protonated forms and it is thought to be the only one that diffuses through epithelial tissues in human body [11, 12]. Thus, all else being equal, an ECIG with a higher proportion of Nic will deliver a larger effective nicotine dose to the user

Gholap i sur..2020

Expert opinion

The protonated form of nicotine is being correlated with the smooth sensory effects and high nicotine absorption as compared to free base nicotine. With the introduction of nicotine salts, which yield mostly the protonated form, the youth popularity of e-cigarettes has spiked exponentially. While it is important to control nicotine levels in e-cigarette products, attention should also be given to the nicotine forms present in these products in order to address nicotine addiction in the population.

John i sur.. 2018

Foremost in importance among the organic compounds in MS tobacco smoke is nicotine, which is predominantly in the particle phase and amounts to ~5–10% by weight of the smoke particles. Nicotine is recognised as a major factor in the sensory effects and addictive properties of tobacco smoking. It can exist in unprotonated ("free-base"), monoprotonated and diprotonated forms, although the fraction in the diprotonated form is practically negligible in MS cigarette smoke. The non-volatile, protonated forms of nicotine reside only in the particle phase, whereas the volatile unprotonated form is present in both the particle (liquid) and gas phase, and essentially this latter form of nicotine determines the rate and extent of uptake of nicotine during smoking (Ingebrethsen, 2006).

Xiao i sur.. 2015

There is a vast literature on nicotine, the main alkaloid in tobacco. Nicotine from smoking is associated with development of some diseases such as lung cancer, and recent studies have also found that it may have a certain effect on treatment of Alzheimer's disease.[1] Nicotine is a weak diacidic base, with two protonation sites at the pyrrolidine nitrogen and pyridine nitrogen ($pK_{a1} = 8.02$, $pK_{a2} = 3.12$). There are three different forms of nicotine (Scheme 1), and their proportions vary with the pH conditions. At pH 2.0–2.7 and 4.5–7.0, the main forms are diprotonation and monoprotonation, respectively.[2] At pH 7.0–8.0 (close to pK_{a1}), there are changes in protonation of the acid–base functions in the pyrrolidine moiety. At pH > 9, free base is the main form.[3] Nicotine can be oxidized by oxygen in air. Suffredini *et al.* found that the oxidation mechanism of nicotine at a boron-doped diamond electrode in alkaline solutions involves formation of methanol and substitution of CH_3 to OH in the tertiary nitrogen of pyrrolidine ring with two-electron transition.[4]

DeCarlo i sur.. 2015

Reactive uptake of free-base nicotine into the particle phase will be dominant until pH 7 when the ratio of protonated to nonprotonated nicotine will be 10:1. The acidic nature of ambient outdoor-originated aerosols therefore provides a sink for any gas-phase nicotine, nicotine by-products, and other alkaloid species that participate in acid-base chemistry. The mass spectral signature of the THS factor, shown in fig. S2, is consistent with RdNS commonly found in cigarette smoke (*8*, *9*). However, mass spectral similarity to pure nicotine is poor, indicating chemical modification of the deposited ETS. The chemical signature of THS therefore changes with time.

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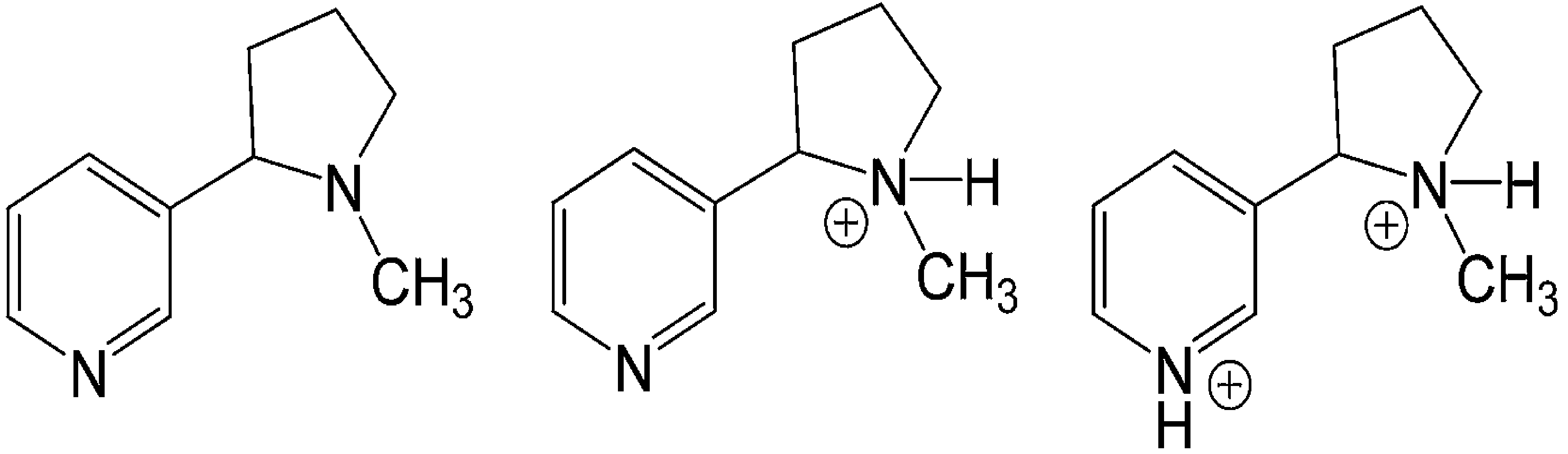
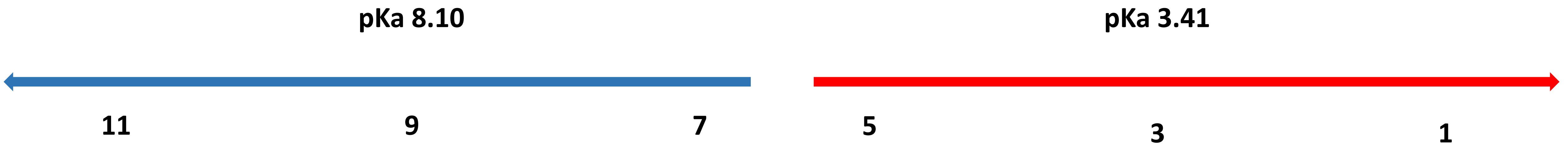


(a) *Free base* (b) *Monoprotonated form* (c) *Diprotonated form*



SEQUENCE OF SUPERCRITICAL CO_2 EXTRACTION AND SUBCRITICAL WATER EXTRACTION

Tested SFE variables	Pressure (bar)	Temperature (°C)	Time (min)
Experimental range	100-300	40-80	5-120
Tested SWE variables	Temperature (°C)	Time (min)	Solvent: solid ratio (mL/g)
Experimental conditions	150	23	28
Detected compounds	Nicotine, Phenolic compounds, nicotinic acid, nicotinamide,5-HMF. furfural and 5-MF		
Optimal conditions	Type: scrap, SFE 120 min. 300 bar and 61.22 °C		

RUN	Extraction yield (%)	Nicotine (mg/g)	Nicotinamide (mg/g)	Nicotine acid (mg/g)
R1	54.08	46.70	4.02	2.39
R2	59.15	42.10	3.71	1.87
R3	58.34	29.30	3.04	1.43
R4	74.05	16.10	1.98	1.18
R5	52.47	42.90	4.28	2.22
R6	65.83	21.10	2.58	1.33
R7	51.39	45.60	4.25	2.19
R8	54.50	19.50	3.03	1.47
R9	53.62	31.40	3.46	2.05
R10	56.50	36.40	3.65	1.72
R11	60.42	30.00	3.07	1.62
R12	52.68	30.20	3.29	1.59
R13	54.77	28.70	3.03	1.54
Raw material	54.57	38.10	3.15	22.58

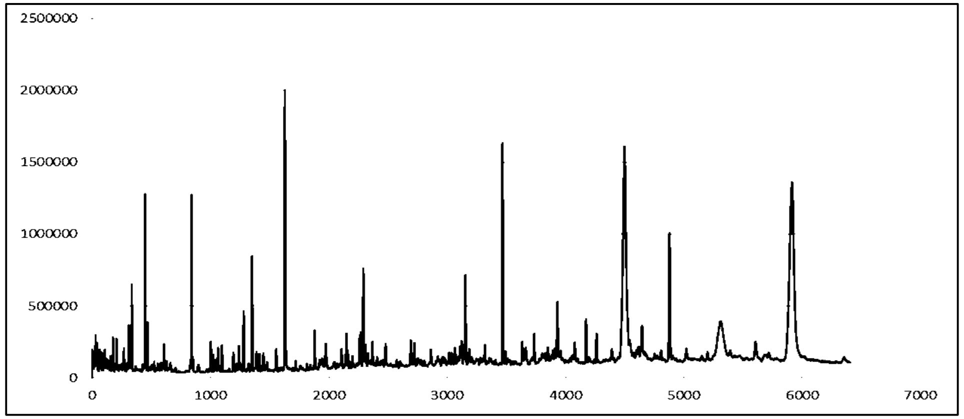


Figure 6: GC-MS midrib chromatogram obtained after SFE

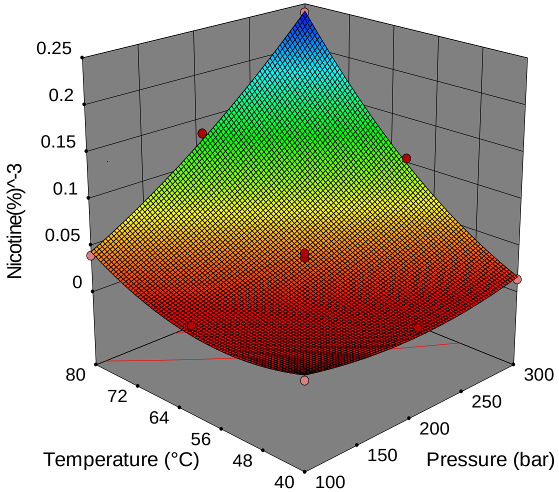


Figure 7: 3D plots showing the influence of the independent variables on the response values

Proposed model:

$$Y_1^{-3} = 0.0369 + 0.0495X_1 + 0.0634X_2 + 0.0491X_1X_2 + 0.0148X_1^2 + 0.0279X_2^2$$

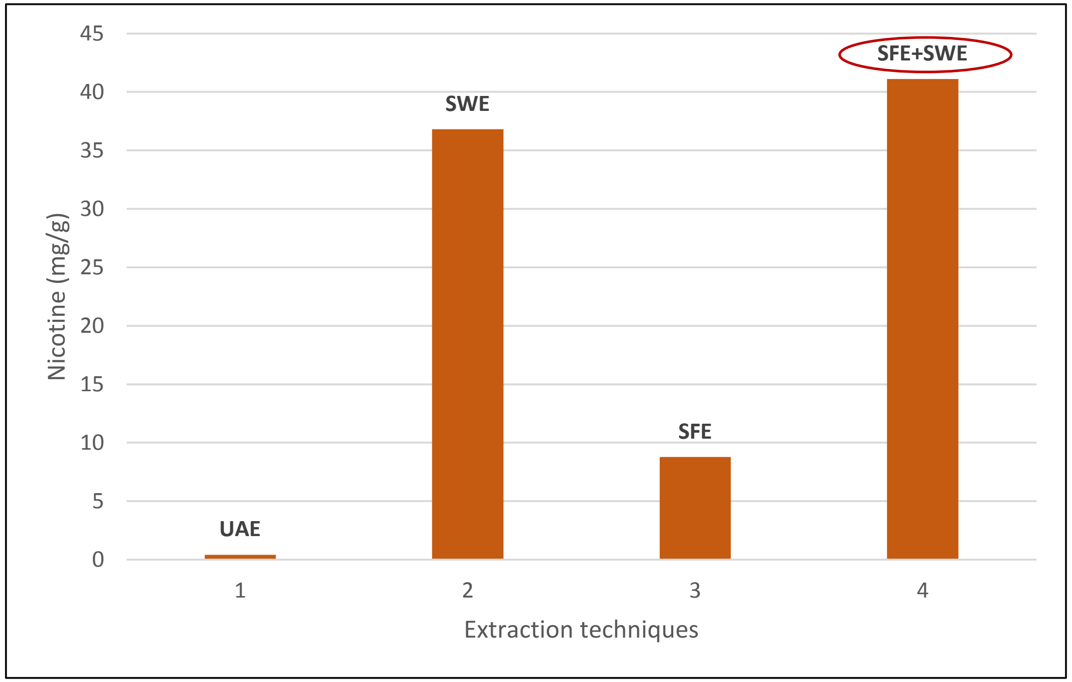


Figure 8: Comparison of different methods in extraction of nicotine form tobacco waste

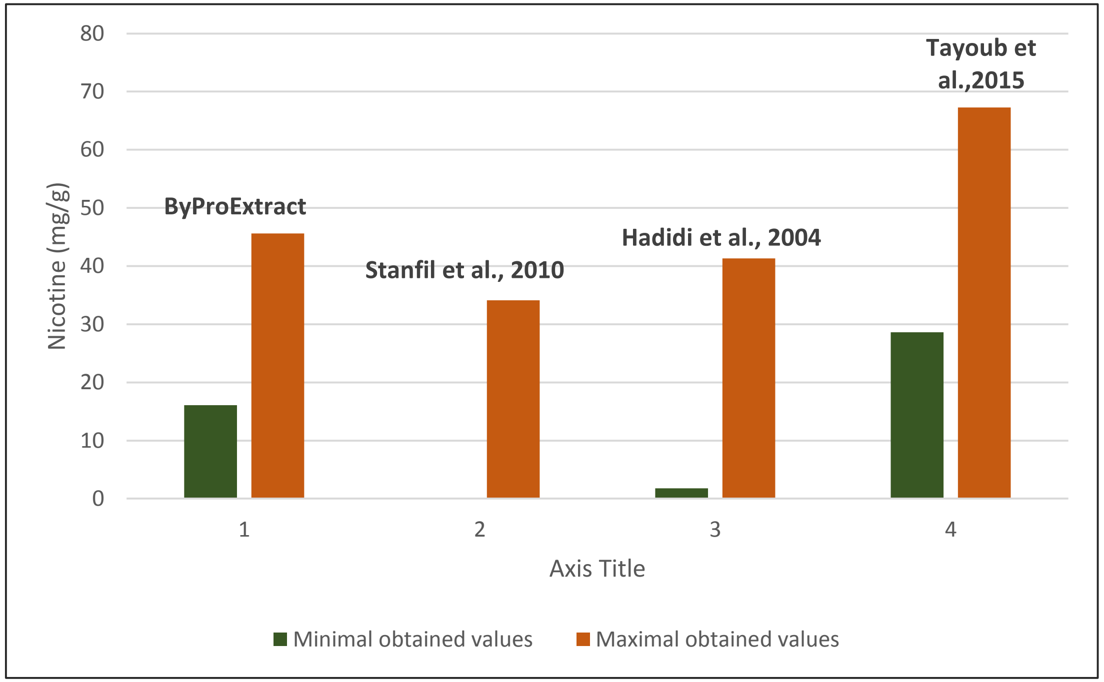


Figure 9: Comparison of results of nicotine extraction with literature reported results

- 11.6% higher nicotine yield
- Very high pH coresponded high with deprotonated nicotine

Stanfill, S. B., Connolly, G. N., Zhang, L., Jia, L. T., Henningfield, J. E., Richter, P., … Watson, C. H. (2010). *Global surveillance of oral tobacco products: total nicotine, unionised nicotine and tobacco-specific N-nitrosamines. Tobacco Control, 20(3), e2–e2.* doi:10.1136/tc.2010.037465 Malson JL, Sims K, Murty R*, et al*
Comparison of the nicotine content of tobacco used in bidis and conventional cigarettes
Tobacco Control 2001;**10:**181-183.
K.A Hadidi, F. Mohammed, Nicotine content in tobacco used in hubble-bubble smoking 2004, Saudi Medical Journal 25(7):912-7
Tayoub, G., Sulaiman, H., & Alorfi, M. (2015). *Determination of nicotine levels in the leaves of some Nicotiana tabacum varieties cultivated in Syria. Herba Polonica, 61(4), 23–30.* doi:10.1515/hepo-2015-0028

POTENTIONAL APLLICATION OF NICOTINE EXTRACTED FROM TOBACCO WASTE

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Review

Application of nicotine enantiomers, derivatives and analogues in therapy of neurodegenerative disorders

Dariusz Pogocki [a, b], Tomasz Ruman [a], Magdalena Danilczuk [c], Marek Danilczuk [b, d], Monika Celuch [b], Elżbieta Wałajtys-Rode [a]

Show more

- Nicotine pouches
- Nicotine snus pouches
- The e-liquid and e-cigarette market

Final remarks

- Two-stage extraction process (SC-CO_2 extraction followed by SWE) can enhance the extraction efficiency due to the elimination of fats during SC-CO_2 extraction which enables better dissolution of the other compounds in subcritical H_2O.
- Tobacco nicotine is found in three forms in leaves, as a free base, monoprotonated (+1) and diprotonated (+2) respectively. Free base has non polar character and can be easily volatilized.
- Protonated forms of nicotine have polar character and are nonvolatile . So, free base form of nicotine volatilizes at lower temperatures than that required to volatilize the charged form (monoprotonated (+1) and diprotonated (+2) of nicotine.
- Low applied temperatures and non-polar features of SC-CO_2 influenced the extraction of only free form of nicotine, while at higher temperatures of SWE, charged forms (which are also present in higher amounts than free base) were extracted.

Thank you for your attention!

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