

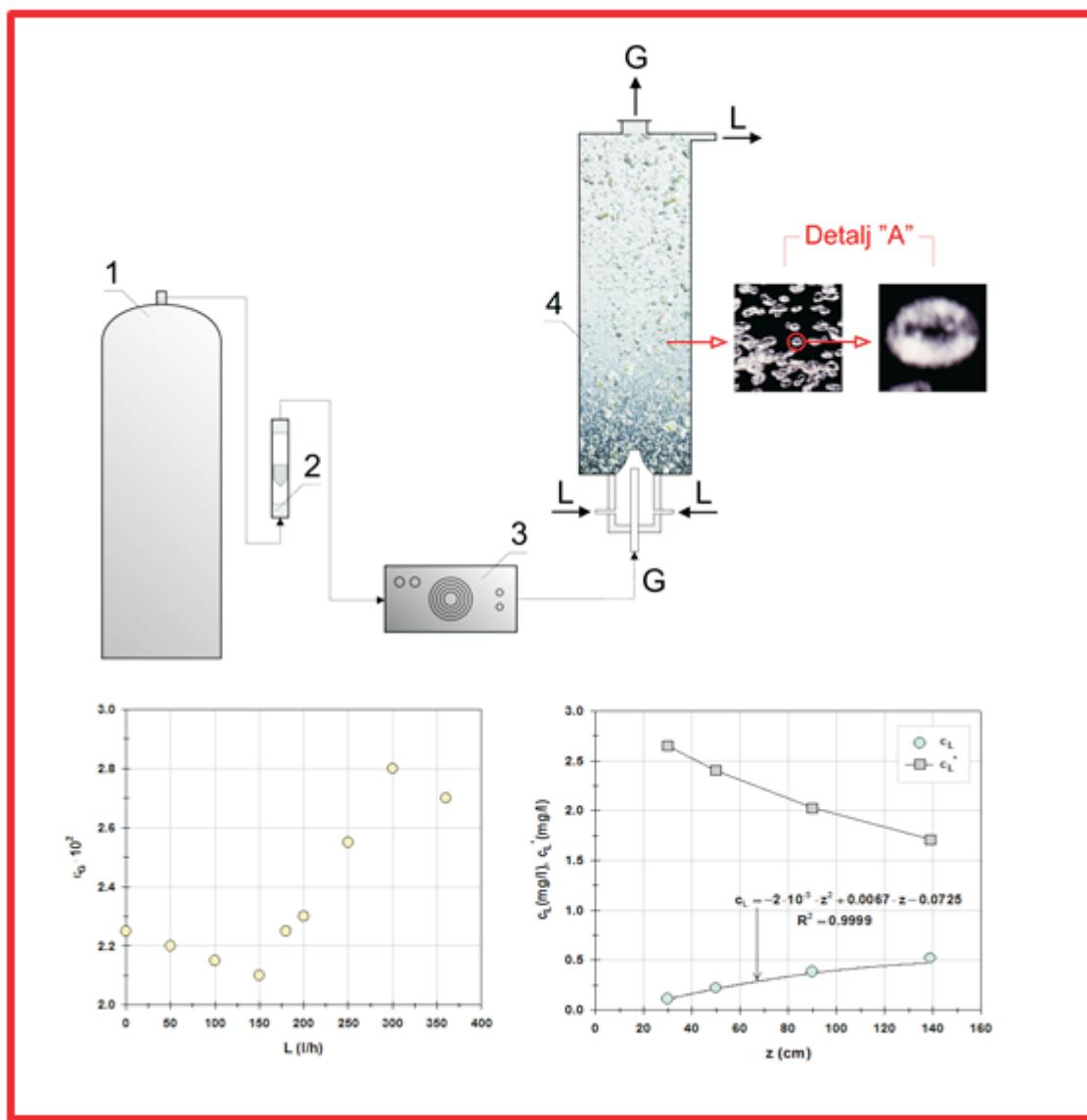
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# Hemijiska industrija

Vol. 69

asopis Saveza hemijskih inženjera

Chemical Industry



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# Višekriterijumska analiza kod izbora optimalne varijante snabdevanja apsorbentom termoelektrane Ugljevik

Cvjetko P. Stojanović

Mješoviti Holding „Elektroprivreda Republike Srpske“ Matično preduzeće a.d. Trebinje i Zavisno preduzeće Rudnik i Termoelektrana Ugljevik a.d., Ugljevik, Republika Srpska, Bosna i Hercegovina

## Izvod

U ovom radu dat je istorijski prikaz razvoja projekta odsumporavanja dimnih gasova (ODG) termoelektrane Ugljevik, sa specifičnostima izabrane tehnologije, te prikaz metodologije kod izbora najpovoljnije opcije za snabdevanje apsorbentom kao najznačajnijim faktorom u projekciji operativnih troškova rada ODG postrojenja. Primenjeni višekriterijumski postupak kod donošenja odluke razvijen je pomoću analitičkog hijerarhijskog postupka ocene (AHP). Proces donošenja odluke kao i korišćeni kriterijumi i podkriterijumi predstavljeni su kroz primere rađene u okviru različitih studija za potrebe definisanja, kako same tehnologije, tako i pitanja obezbeđenja apsorbenta za postupak odsumporavanja dimnih gasova. Takođe, dat je prikaz ekonomskih efekta odabranog rešenja.

**Ključne reči:** višekriterijumska analiza, analitički hijerarhijski postupak, desumporizacija, apsorbent.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Danas je zaštita okoline međunarodni standard i obaveza. Usvajanjem Direktive o integrisanoj kontroli i prevenciji zagađenja (IPPC direktiva – IPPC, *Integrated Prevention Pollution Control* 96/61/EC) [1], Evropska Unija je postavila ambiciozni cilj visokog nivoa zaštite okoline u celini i uvela koncept održivog razvoja za široki spektar zagađujućih industrijskih aktivnosti uključujući rudarstvo i metalurgiju, energetiku, i niz drugih oblasti.

Fundamentalni i esencijalni element IPPC direktive predstavlja upotreba najboljih raspoloživih tehnika (*Best Available Techniques* – BAT) koje čine najefikasnije i najnaprednije faze u razvoju aktivnosti i njihovih operativnih metoda.

Termoelektrana Ugljevik (TE Ugljevik), instalisane snage 300 MW, kao gorivo koristi rovni mrki ugalj, koji se eksploatiše iz površinskog kopa Bogutovo Selo. Ugalj sadrži visok nivo sumpora (približno 5–6%) i ima kaloričnu vrednost od 10.000–13.000 kJ/kg.

Prolazeći kroz jedan veoma dug period, od skromno prezentovanih prvih razmišljanja o potrebi zaštite vazduha i životne sredine, pa sve do aktivnosti koje sasvim realno vode ka ostvarenju ove ideje, konstantno je prisutan dualizam neophodnosti preduzimanja ovih mera i činjenice da je odsumporavanje dimnih gasova jedan skup proces, koji trajno opterećuje proizvodnu cenu električne energije. Poslednji period, obeležen trendom globalizacije, kao i jasno opredjeljenje za evropskom budućnošću zemalja regiona, donose i obaveze koje

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više nije moguće izbeći ni u jednoj sferi života, a pogotovo ne u zaštiti životne sredine, i koje se nalaze visoko na lestvici prioriteta EU.

Sve navedeno, bilo je zamajac za konačno finaliziranje ideje, o izgradnji postrojenja za odsumporavanje dimnih gasova, pogotovo što je postojala, i postoji, opasnost zaustavljanja rada i pre isteka životnog veka Termoelektrane.

U cilju rešavanja ovog pitanja, obezbeđen je kredit japanske vlade, odnosno od *Japan International Cooperation Agency* (JICA), urađena je konceptualna studija, Studija izvodljivosti o izgradnji sistema za odsumporavanje dimnog gasa, kao i sproveden postupak pretkvalifikacije potencijalnih izvođača za izgradnju postrojenja za odsumporavanje dimnih gasova (ODG), a u završnoj fazi je izrada tenderskog dokumenta za prikupljanje ponuda iz reda kvalifikovanih ponuđača.

Konceptualnom studijom, Odsumporavanje dimnih gasova Termoelektrane Ugljevik, *Japan International Cooperation Agency* (JICA) iz 2006. godine, odabran je mokri postupak, a kao mogući apsorbent krečnjak ili kreč, sa gipsom kao finalnim proizvodom. Zbog izuzetno visokog sadržaja sumpora u uglju, zaključeno je da ostali procesi nisu izvodljivi te su navedenom studijom razmatrani samo mokri procesi.

Vlažni postupak ODG trenutno se smatra najefikasnijim i u svetu se najviše koristi u termoenergetskim objektima. Ovaj postupak zasniva se na raspršivanju suspenzije krečnjaka u dimni gas, s ciljem konverzije SO<sub>2</sub> u potencijalno komercijalan krajnji proizvod. Princip rada je jednostavan: nakon što je leteći pepeo u najvećoj meri odstranjen iz dimnog gasa u elektrofiltrerskom postrojenju, sorbent, koji uglavnom pred-

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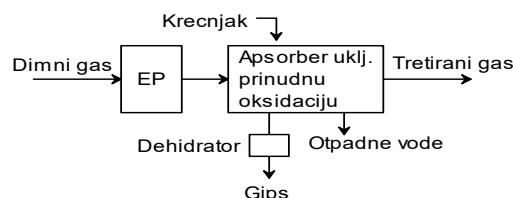
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stavlja suspenziju krečnjaka, se unutar apsorbera raspršuje u dimni gas. Sorbent reaguje sa  $\text{SO}_2$  u struji gasa formirajući nusproizvod koji je sulfatnog karaktera, kalcijum-sulfat dihidrat ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) tj. gips. Kalcijum-sulfat se iz pomenutog produkta taloži, dok se većina vode reciklira.

Na slici 1 data je pojednostavljena tehnološka šema postupka odsumporavanja dimnih gasova mokrim postupkom, a na slici 2 tehnološka šema sa glavnom opremonom.



Slika 1. Tehnološka šema postupka odsumporavanja dimnih gasova mokrim postupkom.

Figure 1. Process flow diagram of wet Flue Gas Desulphurisation process.

Prema navedenoj studiji, glavna oprema sistema za odsumporavanje dimnih gasova mokrim postupkom sadrži:

- sistem dimnog gasa,
- sistem za dopremanje krečnjaka,
- sistem za apsorpciju i oksidaciju – apsorber,
- sistem za suspenziju gipsa,
- pomoći sistemi,
- sistem za pražnjenje,
- sistem za ispuštanje dimnog gasa – dimnjak,
- sistem deponovanja gipsa i tretiranje otpadnih voda i
- sistem kontrole i upravljanja.

U Tabeli 1 dat je prikaz osnovnih karakteristika procesa odsumporavanja dimnih gasova mokrim krečnjackim postupkom.

U cilju dizajniranja sistema za dugoročno snabdevanje apsorbentom detaljno su analizirani brojni ulazni podaci, a nakon razmatranja niza uticajnih faktora izvršen je konačan izbor apsorbenta – drobljeni karbonat, sa veličinom frakcije, maksimalne krupnoće 20–30 mm, koja bi se dopremala u krug termoelektrane, dok bi se finalna mikronizacija obavljala u mlinskom postrojenju kao integralnom delu ODG postrojenja.

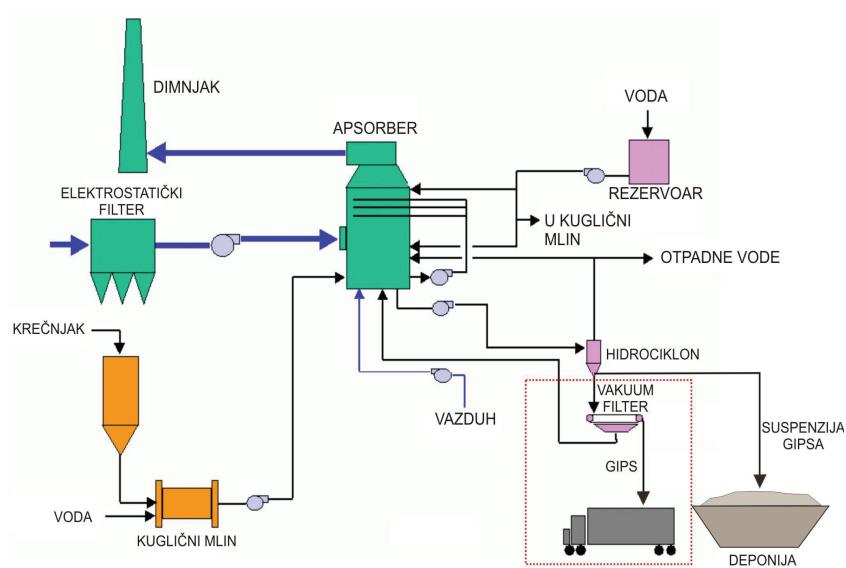
Ovakvo rešenje je nastalo kao plod sagledavanja niza faktora od kojih su, kao najuticajni, izdvojeni sledeći:

– Primarna prerada van termoelektrane bi u znatnoj meri smanjila uticaj pripreme apsorbenta na životnu sredinu, odnosno olakšala aktivnosti na ispunjavanju ekoloških normi.

– Mlinsko postrojenje, za finalnu mikronizaciju karbonata, već je definisano kao integralni deo ukupnog ODG postrojenja unutar Termoelektrane, kako u pogledu prostornog razmeštaja, tako i u smislu dostupnosti potrebne infrastrukture.

Sam izbor postupka proistekao je iz razrade operativnih troškova uz zadovoljenje osnovnog cilja, postizanja traženih izlaznih vrednosti dimnih gasova, odnosno koncentracije  $\text{SO}_2$  u istim. Pri tome, investicioni troškovi nisu procenjeni, jer ponude potencijalnih dobavljača postrojenja za ODG nisu dostupne u ovoj fazi projekta. Procenjeni su preliminarni troškovi rada kako bi se pronašlo optimalno rešenje između više mogućih varijanti.

Poseban akcenat stavljen je na dostupnost karbonata kao apsorbenta, uz činjenicu da kada se uzmu u obzir različite varijante razmeštaja i različita rešenja



Slika 2. Tehnološka šema postupka odsumporavanja dimnih gasova mokrim postupkom sa glavnom opremom [2].

Figure 2. Process flow diagram of wet Flue Gas Desulphurisation process with main equipment [2].

*Tabela 1. Osnovne karakteristike procesa odsumoravanja dimnih gasova mokrim krečnjačkim postupkom*  
*Table 1. Basic features of wet limestone Flue Gas Desulphurisation process*

Proces	Sistem krečnjak–gips
Prikaz	Sumpor-dioksid u dimnom gasu se apsorbuje u mulj koji se sadrži u krečnjaku kroz povezivanje sa dimnim gasom u upijaču/apsorberu. Gips se javlja kao nusprodukt u dehidratoru. Hemijska reakcija: $\text{SO}_2 + \text{CaCO}_3 + 2\text{H}_2\text{O} + 1/2\text{O}_2 \rightarrow \text{CaSO}_4 \cdot 2\text{H}_2\text{O} + \text{CO}_2$
Efikasnost	90–98%
Apsorbent	Krečnjak; lako se obezbeđuje i nabavlja
Nusproizvod	Gips; koristi se kao dodatak cementu, gipsu/gipsanim pločama, poboljšanju kvaliteta zemljišta, itd.
Lokacija postrojenja	Krug termoelektrane
Reprezentativna iskustva	Mnoga iskustva zbog raširenosti ODG tehnologije širom sveta
Specijalne osobine	– Jeftin apsorbent – Nusprodukt koji se može prodati
Prednosti	– Zahteva se tretiranje otpadnih voda – Zahteva se dogревač dimnog gasa
Mane	– Zahteva se tretiranje otpadnih voda – Zahteva se dogревač dimnog gasa
Primena	Široka primjena za postrojenja ODG gde se zahteva visoka efikasnost uklanjanja $\text{SO}_2$ i/ili relativno veći kapacitet, zbog lakog nabavljanja krečnjaka

ispuštanja dimnih gasova, kao najekonomičnije rešenje odabran je vlažni postupak sa krečnjakom kao apsorbentom.

Uzimajući u obzir minimalno potrebnu čistoću krečnjaka od 95,0%  $\text{CaCO}_3$ , procenjeno je da prosečna godišnja potrošnja ove sirovine može da dostigne i do 183.000 t/godišnje, saglasno ulaznim parametrima dimnih gasova u ODG postrojenje prema Tabeli 2.

*Tabela 2. Potrebne količine apsorbenta na osnovu količine polutanata [2]*

*Table 2. Required quantities of absorbent based on pollutant amount [2]*

Parametar	Polutant	Apsorbent
	$\text{SO}_2$	
Protok mase	16.600 kg/h	–
Efikasnost uklanjanja	98,5%	25.548 kg/h
	$\text{SO}_3$	
Protok mase	420 kg/h	–
Efikasnost uklanjanja	50%	263 kg/h
	HCl	
Protok mase	60 kg/h	–
Efikasnost uklanjanja	95%	78 kg/h
	HF	
Protok mase	10 kg/h	–
Efikasnost uklanjanja	95%	24 kg/h
Ukupnokarbonata( $100\%\text{CaCO}_3$ )	–	25.913 kg/h

Uvažavajući činjenicu da je udeo apsorbenta u ukupnim operativnim troškovima budućeg ODG postrojenja najdominantniji (50% i više), bilo je neophodno uraditi opsežna istraživanja u smislu pribavljanja relevantnih informacija kao što su: potencijalni dobavljači,

rezerve i kvalitet mineralne sirovine, cena, pouzdanost snabdevanja, logistika, itd.

U okviru razmatranja idejnih rešenja ukazala se potreba za identifikacijom ležišta krečnjaka, odnosno potencijalnih dobavljača koji mogu zadovoljiti specifične kriterijume za odabranu tehnologiju odsumopravanja.

#### Opis primenjene metodologije

Savremeni pristup procesu izbora optimalne varijante podrazumeva postupak višekriterijumske odlučivanja sa konačnim brojem alternativa, koje moraju biti kategorisane s obzirom na mnogo različitih kriterijuma.

Prednost ovih metoda je u tome što one mogu istovremeno da razmatraju i finansijske i nefinansijske faktore pri postupku izbora. Najpoznatije od ovih metoda su modeli ocenjivanja, analitički hijerarhijski proces – AHP, analitičke mreže procesa – ANP, TOPSIS, ELECTRE i PROMETHEE.

Pre nego što se izabere i primeni odgovarajuća metoda višekriterijumske odlučivanja za izbor optimalne varijante, neophodno je detaljno razmotriti sve elemente i faktore vezane za konkretnu situaciju [3, 4].

U procesu višekriterijumske odlučivanja adekvatno određivanje značaja kriterijuma je veoma bitno. Pretходno navedene metode, osim AHP metode, ne uključuju postupak za određivanje značaja kriterijuma. Bitarafan i Ataei [5] koristili su različite fazi metode u cilju grupisanja kriterijuma pri procesu donošenja odluka.

U cilju rešavanja postavljenog zadatka bilo je neophodno kreirati odgovarajući hijerarhijski postupak ocene na osnovu koga bi se izvršilo rangiranje odnosno izvršio izbor najpovoljnije varijante. U nastavku rada dat je prikaz višekriterijumskega postupka kod donošenja odluke pri izboru optimalne varijante snabdevanja

apsorbentom za potrebe odsumporavanja dimnih gasova TE Ugljevik korišćenjem analitičkog hijerarhijskog postupka (AHP).

#### Kratak opis AHP metode

U stručnoj terminologiji, veoma poznat izraz, multi kriterijumski način donošenja odluke (*Multi Criteria Decision Making – MCDM*) u osnovi počiva na korišćenju AHP metode.

Može se slobodno reći da je jedan od najčešće korišćenih metoda koji se koriste prilikom donošenja odluka o različitim pogodnostima je „*Analytic Hierarchy Process*“ (AHP), odnosno Analitički hijerarhijski postupak ocene. Ovaj metod razvijen od strane Satija [6] kao moćan alat za višekriterijumsko odlučivanje.

Lee i sar. definišu AHP kao kvantitativnu tehniku koja omogućava strukturisanje složenog višekriterijumskog problema i kao metodologiju koja ima široku primenu pri donošenju odluka [7].

AHP uključuje dekompoziciju složenog problema višekriterijumskog odlučivanja u višedimenzionalnu hijerarhijsku strukturu ciljeva, kriterijuma i alternativa. Dekompozicija se vrši na bazi prethodnih studija, istraživanja i empirijskog iskustva. Nakon što je hijerarhija razvijena, vrši se procena uticaja kriterijuma, onda se upoređuju alternative u odnosu na svaki kriterijum i utvrđuje ukupni prioritet za svaku alternativu i konačno rangiranje alternativa [8].

S obzirom na to da su principi AHP metode poznati stručnoj javnosti i dostupni na brojnim internet stranicama u tekstu koji sledi dat je kraći prikaz primene AHP metode kod izbora optimalne varijante snabdevanja krečnjakom za potrebe odsumporavanja dimnih gasova TE Ugljevik. Zbog obimnosti materijala prikazan je samo deo kalkulacija sa konačnim rezultatima izbora optimalne varijante.

#### Kratak prikaz AHP kalkulacija

Prilikom postupka ocene pogodnosti ležišta krečnjaka, odnosno potencijalnog snabdevača apsorbentom, osnovni kriterijumi podeljeni su na tehničke, ekonomski i kriterijume pouzdanosti, koji su dalje podeđeni na više podkriterijuma kako je to prikazano na slici 3.

Tehnički podkriterijumi su uglavnom diktirani ograničenjima koja su postavljena usvojenom tehnologijom odsumporavanja. U tom smislu bilo je neophodno detaljno poznavanje problema tehnološkog postupka kako bi se identifikovali kriterijumi i podkriterijumi, za svako od ležišta krečnjaka, odnosno potencijalnog dobavljača.

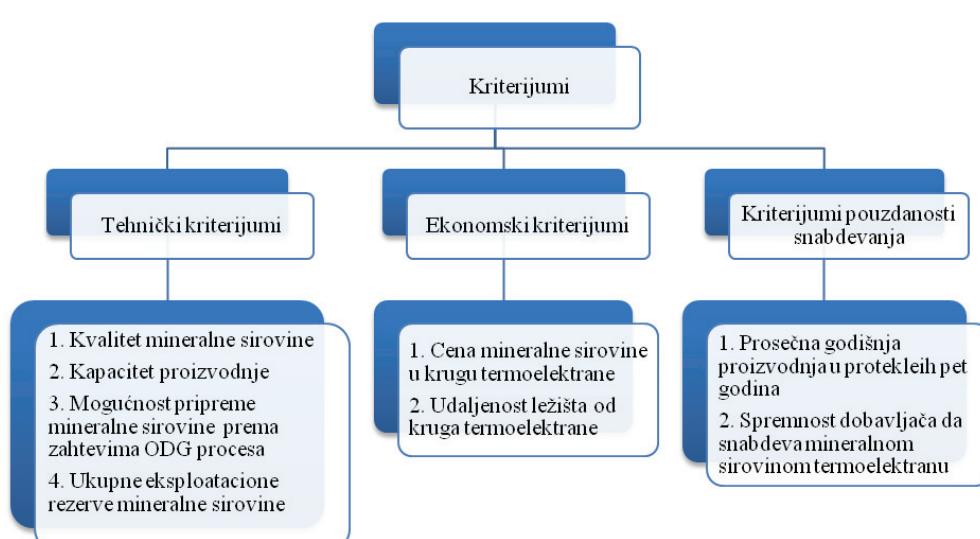
Imajući u vidu da različite tehnologije odsumporavanja postavljaju različite zahteve u pogledu ispunjenja tehničkih kriterijuma granični uslovi vezani za kvalitet krečnjaka za odabranu tehnologiju odsumporavanja, dati su u tabeli 3.

*Tabela 3. Zahtevani kvalitet krečnjaka za odabranu tehnologiju [2]*

*Table 3. Required limestone quality for technology selected [2]*

Komponenta	Granične vrednosti, %
CaCO <sub>3</sub>	Min. 96,0
MgO	Maks. 3,0
SiO <sub>2</sub>	Maks. 2,0
Al <sub>2</sub> O <sub>3</sub>	Maks. 0,5
HCl	Maks. 3,0
Fe <sub>2</sub> O <sub>3</sub>	Maks. 0,1
Vлага	Maks. 0,5

Vrednost svakog od podkriterijuma za pojedinačno ležište dobijena je na osnovu ocene ispunjenosti postavljenih graničnih uslova kako je to dato u Tabeli 4.



*Slika 3. Osnovni kriterijumi sa podkriterijumima.  
Figure 3. Basic criteria with subcriteria.*

*Tabela 4. Ocena kriterijuma  
Table 4. Criteria rating*

Ispunjenošć uslova	Definicija
0	Eliminatori kriterijum nije ispunjen
1	Kriterijum je ispunjen
2	Kriterijum je ispunjen sa značajnom sigurnošću
3	Kriterijum je ispunjen višestruko

Iz Tabele 3 vidljivo je da je kvalitet krečnjaka, neophodan za odabranu tehnologiju, definisan graničnim vrednostima (minimumima i maksimumima) koje je vrlo lako u okviru ocene definisati kao eliminatore u pogledu ispunjenja zahtevanog kvaliteta.

Prilikom razmatranja potrebnih količina mineralne sirovine neophodno je bilo razmatrati rezerve ležišta sa stanovišta ukupnih potreba za period od 25 godina (planirani životni vek Termoelektrane do 2039. godine) kao i proizvodnih mogućnosti potencijalnih dobavljača.

Svakom od navedenih podkriterijuma dodat je i težinski faktor te je na taj način dobijena konačna ocena ležišta sa aspekta potencijalnog snabdevača, tabela 5.

*Tabela 5. Težinski faktor podkriterijuma eliminatori kriterijumi  
Table 5. Weight factor of subcriteria, elimination criteria*

Podkriterijum	Težinski faktor	Eliminatori kriterijum
T1	0,25	Da
T2	0,10	Ne
T3	0,05	Da
T4	0,25	Ne
E1	0,10	Ne
E2	0,05	Ne
P1	0,15	Ne
P2	0,05	Da

Podkriterijum i pripadajući težinski faktor razložen je na sve elemente koji grade razmatrani podkriterijum. Npr. tehnički podkriterijum T1, čiji težinski faktor iznosi 0,25, razložen je na kriterijume zahtevanog kvaliteta datih u tabeli 3.

Ukoliko je neki od eliminatornih podkriterijuma ocenjen sa nula (0) krajnji rezultat je eliminacija potencijalnog snabdevača.

Konačna ocena potencijalnog snabdevača dobijena je primenom formule:

$$P_{uk} = \sum P_k \times T_f \quad (1)$$

gde su:  $P_{uk}$  – ukupna pogodnost snabdevača,  $P_k$  – ocena snabdevača po određenom kriterijumu i  $T_f$  – težinski faktor po određenom kriterijumu.

Nakon okončanog postupka evaluacije prema opisanoj metodologiji izvršeno je rangiranje potencijalnih snabdevača prema broju bodova.

#### **Opis analitičkog postupka ocene za definisane kriterijume**

##### *Tehnički kriterijumi (T)*

*T1. Kvalitet krečnjaka.* U okviru podkriterijuma T1 obrađeni su svi elementi iz tabele 5, a težinski faktor (0,25) je raspoređen između njih. Ukoliko bilo koji od kriterijuma kvaliteta nije ispunjen potencijalni snabdevač je eliminisan iz dalje analize.

*T2. Kapacitet proizvodnje.* U zavisnosti od potreba termoelektrane izvršena je minimalna procena potencijalne proizvodnje na osnovu instalisanih kapaciteta za svako od analiziranih ležišta.

*T3. Mogućnost pripreme mineralne sirovine na potrebnu granulaciju.* Imajući u vidu da je u okviru idejnih rešenja predviđena instalacija postrojenja za pripremu apsorbenta u krugu termoelektrane (fina mikronizacija) svi potencijalni snabdevači imali su isti status kod ocene, a zahtevana granulacija apsorbenta je u granicama od 20–30 mm.

*T4. Ukupne rezerve ležišta.* Ukupne rezerve su razmatrane za period planiranog radnog veka termoelektrane od minimalno 25 godina i prosečnim radnim satima od 7.000 h godišnje. Za ocenu ovog kriterija, u obzir su uzete eksploataционе rezerve mineralne sirovine.

##### *Ekonomski kriterijum (E)*

*E1. Cena.* Jedan od najvažnijih kriterijuma, pored kvaliteta i pouzdanosti u snabdevanju, je svakako cena krečnjaka sa isporukom u krug Termoelektrane. Što se transporta mineralne sirovine tiče, od mesta proizvodnje do termoelektrane, isti se može obavljati isključivo drumskim saobraćajem, jer ne postoji drugi oblik (železnički kolosek niti plovni put).

*E2. Udaljenost ležišta od termoelektrane.* Ocena je izvršena na osnovu udaljenosti ležišta od termolektrane i to na način da je sa maksimalnom vrednošću ocenjeno ležište koje je najbliže, dok se ocena ostalih ležišta zasnivala na odnosu najbližeg ležišta i razmatranog ležišta.

##### *Kriterijum pouzdanosti (P)*

*P1. Prosečna godišnja proizvodnja.* Kriterijum pouzdanosti je razmatran sa aspekta mogućnosti ostvarenja kontinuiteta isporuke u skladu sa potrebama termoelektrane za mineralnom sirovinom (krečnjakom).

*P2. Spremnost potencijalnih dobavljača da snabdeva mineralnom sirovinom termoelektranu.* S obzirom da pojedini potencijalni dobavljači imaju već obezbeđeno tržište isti su tretirani za nijansu niže od onih koji imaju potencijal, ali ne i razvijeno tržište, tj. imaju primaran interes da snabdevaju Termoelektranu sa sirovinom.

## REZULTATI OCENE

Na osnovu sprovedene detaljne analize izvršeno je rangiranje potencijalnih dobavljača, kako je dano u tabeli 6. Kao što se može videti kao najpovoljnija opcija pokazala se varijanta novog površinskog kopa krečnjaka u okviru poslovnog sistema Rudnik i termoelektrana Ugljevik (RiTE Ugljevik).

*Tabela 6. Konačna ocena potencijalnih snabdevača krečnjakom*  
*Table 6. Final evaluation of prospective limestone suppliers*

Snabdevač	Konačno rangiranje
Novi površinski kop ZP RiTE Ugljevik	1
RUDING AD Ugljevik	2
RAVNAJA Mali Zvornik	3
CARMEUSE Doboј	4
NEXE JELEN DO AD Jelen Do	5

Ovako značajna količina (26 t/h, odnosno blizu 5.000 tona nedeljno potrebnog apsorbenta u krugu termoelektrane), kao i veoma uticajan faktor transporta, sa aspekta ukupnih troškova, nametnule su potrebu da se rešenje pronađe, na užem prostoru Ugljevika. Ovo pogotovo zbog činjenice proistekle iz zaključka idejne studije o podobnosti karbonata sa širem prostora eksplotacionog polja Rudnika za potrebe odsumporavanja dimnih gasova TE Ugljevik.

U zaključku Elaborata o kvalitetu i rezervama karbonata Ugljevika kao sirovinske baze za proces odsumporavanja dimnih gasova iz TE Ugljevik naglašeno je da na prostoru Ugljevika postoji više karbonatnih ležišta koja kvalitativnim i kvantitativnim karakteristikama predstavljaju realnu osnovu za eksplotaciju u svrhu snabdevanja termoelektrane apsorbentom.

Za svako potencijalno odabrano ležište postoje osnovne pozitivne postavke, koje bi se trebale detaljno razraditi i na kraju dokazati istražnim geološkim radovima.

Uvažavajući niz bitnih faktora (udaljenost, naseljenost, povezanost sa već otvorenim površinskim kopom i odlagalištima, transport, itd.) izvršen je izbor lokacije ležišta karbonata, za koje se smatra da može zadovoljiti zahteve za potrebnim apsorbentom budućeg postrojenja za odsumporavanje dimnih gasova TE Ugljevik.

Na osnovu raspoloživih podataka, posebno obrađenih u Elaboratu o kvalitetu i rezervama karbonata Ugljevika kao sirovinske baze za proces odsumporavanja dimnih gasova iz TE Ugljevik, sa sigurnošću se može reći da istraživani deo terena, odnosno rudno telo sadrži oko  $20 \times 10^6 \text{ m}^3$  karbonata.

Prema preliminarnim ispitivanjima izabrano ležište sadrži visok procenat korisne mineralne komponente ( $\text{CaCO}_3$ ), visoku reaktivnost i male vrednosti štetnih primesa, kao i relativno nizak indeks meljivosti (Bondov

Index) koji je ispod 10 KWh/t, što je sa ekonomskog aspekta od izuzetnog značaja.

Usled specifičnosti projekta dugoročnog snabdevanja apsorbentom za potrebe TE Ugljevik, isključiva uloga novog površinskog kopa karbonata je obezbeđivanje potrebnih količina za nesmetano funkcionisanje ovog procesa.

Iz navedenih razloga projektovana dobit na godišnjem nivou iskazana je na ostvarenoj uštedi, koja provodi iz razlike između procenjene proizvodne cene karbonata na novom površinskom kopu, i najpovoljnije trenutne cene u okruženju i šire.

Na osnovu prikupljenih ponuda potencijalnih dobavljača utvrđeno je da je najniža cena za isporuku u krugu TE Ugljevik iznosi 11 konvertibilnih maraka po toni. Tehnoekonomskom analizom ukupnih troškova [8] novog površinskog kopa karbonata došlo se do jedinične cene od 5,5 KM/t ili 2,8 evra po toni (odnos 1 evro = 1,955 KM).

Kao što se može videti, razlika između najpovoljnije cene potencijalnih snabdevača i cene karbonata iz „vlastitih izvora“, iznosi 5,5 KM. Na osnovu potrebne količine karbonata za ODG sistem, od oko 200.000 t godišnje, došlo se do godišnje dobiti u iznosu od 1.100.000 KM godišnje ili oko 560.000 evra.

## Ocena efikasnosti projekta

Opšte je poznato, da se investicioni projekti ocenjuju na osnovu dva tipa ocene: prema statičkoj i dinamičkoj oceni efikasnosti projekta.

### Statička ocena efikasnosti

Statička ocena bazira se na pokazateljima koji se izvode iz podataka iz novčanih tokova (bilansa uspeha i finansijskog toka) u reprezentativnoj godini životnog veka projekta, a obično se uzima godina kada je postignut projektovani kapacitet.

U praksi postoji veliki broj pokazatelja, a za ovaj projekt dati su sledeći statički pokazatelji [8]:

- Rentabilnost = neto dobit/ukupne investicije = 12,3%
- Ekonomičnost = neto dobit/ukupan prihod = 40,9%
- Reproduktivnost = (neto dobit+amortizacija)/ukupne investicije = 12,3%
- Prosečna profitna stopa investicionog projekta: 14,4%

### Generalna ocena bilansa uspeha i finansijskog toka

Kako se radi o investicionom projektu koji je praktično vezan za snabdevanje poznatog potrošača (ODG postrojenje TE Ugljevik), a finansiraće se vlastitim sredstvima, nema negativnog finansijskog rezultata, u godinama intenzivnog ulaganja. Umesto toga postoje određena smanjenja neto dobiti zbog povećanih troškova.

### *Prag rentabilnosti*

Utvrdjeni prag rentabilnosti, uz proizvodne troškove od 5,5 KM/t, je godišnja proizvodnja od oko 60.000 tona karbonata.

Proizvodni troškovi mogu da porastu od 5,5 do 11 KM/t, za puni kapacitet, od 200.000 tona godišnje, a da projekat bude rentabilan.

Projekat je rentabilan i u slučaju da nabavna cena karbonata se smanji na nivo do 5,5 KM/t za puni kapacitet.

Investiciona ulaganja mogu da porastu i do 35%, a da projekat bude rentabilan.

Troškovi koncesione naknade za pravo korišćenja (jednokratna naknada) projektovani su u iznosu od 16.500 KM, a obračunati su na osnovu Pravilnika o načinu utvrđivanja visine koncesione naknade za korišćenje mineralne sirovine (Službeni glasnik RS, br. 5/03), dok su troškovi koncesione naknade za proizvedenu mineralnu sirovину (godišnji) obračunati u iznosu 4% od projektovanog prihoda (110.000 KM/god) i iznose 44.000 KM, za projektovani kapacitet.

### *Dinamička ocena efikasnosti investicije*

Kod dinamičke ocene efikasnosti investicije generalno postoje tri osnovne metode: Povraćaj (PB – Pay Back); Neto sadašnja vrednost (NPV – Net Present Value) i Interna stopa povraćaja (IRR – Internal Rate of Return). Sve analize zavise od predviđanja troškova i dobiti i dinamičkog plana trošenja i priliva novca, tako da se isti princip koristi bilo da se odluka odnosi na opravku ili zamenu opreme, povećanje proizvodnje ili bilo koje druge investicione aktivnosti.

#### *Metoda roka otplate – povratka ulaganja (Pay Back period – PB)*

Po ovoj metodi određuje se vreme za koje projekat vraća uložena sredstva, odnosno to je godina veka projekta u kojoj kumulativ neto priliva postaje pozitivan. Poželjno je da ovaj period bude što kraći, a ne sme biti duži od ekonomskog veka projekta. Obračun ovog pokazatelja je jednostavan: iznos ukupnih ulaganja se umanjuje za godišnje iznose neto-priliva iz ekonomskog toka.

Kao prvi korak potrebno je da se postavi plan sa jednakim vremenskim periodima, a ukupan broj vremenskih perioda treba da bude jednak veku projekta.

Kako je za ovu investiciju potrebno 7.300.000 KM, povraćaj se računa tako što se iznos investicije podeli sa godišnjim prosekom realizacije, tj. ostvarenom dobiti koja iznosi 1.100.000 KM, tj.  $7.300.000 \text{ (KM)} / 1.100.000 \text{ (KM/god.)} = 6,6 \text{ godina}$

U slučaju da postoji više alternativnih projekata kao najbolji uzima se onaj koji je po broju godina povraćaja najkraći.

Kao što se iz prikazanog primera može videti, ovo je relativno jednostavan i lako razumljiv kriterijum, pa se

vrlo često koristi u praksi, naročito kod manjih projekata, a isto tako može se koristiti i kod većih, gde predstavlja prvi kriterijum na koji se nadograđuju složeniji pokazateli. Analiza povraćaja odražava kratak rok i to joj je osnovna slabost. Sa povećanjem ulaganja i dužim rokom potrebno je razmatranje obima ulaganja i vremenske vrednosti novca. U tom cilju koristi se analiza Neto sadašnja vrednost, ili skraćeno NPV analiza.

#### *Neto sadašnja vrednost projekta (Net Present Value – NPV)*

Neto sadašnja vrednost predstavlja sposobnost projekta da vrati uložena sredstva. Finansijsko tumačenje je sledeće: kada investitor odlučuje o tome da li će da investira ili ne, on treba da odluči da li mu se više isplati da sredstva deponuje u banku i obezbedi određen siguran prinos ili da ih uloži u planirani projekat. Odluka će biti u prilog ulaganju u projekat samo ako on obezbedi prinos veći od kamate na koju može sa sigurnošću računati ukoliko su sredstva u banci. Po ovome diskontna stopa bi trebalo da bude najmanje jednakam kamatnoj stopi koja se sa velikom sigurnošću može obezrediti u pouzdanim bankarskim institucijama, uvećana za faktore rizika od ulaganja.

U tabeli 7 dat je primer samo sa dva slučaja, sa diskontnim stopama od 8 i 15%.

Navedeni broj godina predstavlja period od početka rada ODG sistema (2016. godina) do kraja životnog veka Termoelektrane (2039. godina).

Analizom tabele 7 može se zaključiti sledeće:

a) Kada se zahteva stopa povratka od 8%, investicija je opravdana, jer je sadašnja vrednost celog projekta veća od vrednosti početnog ulaganja za 4.108.165 KM.

b) Kada se zahteva stopa povratka od 15% investicija nije opravdana, jer je sadašnja vrednost celog projekta manja od vrednosti početnog ulaganja za 261.279 KM.

U suštini koncept sadašnje vrednosti znači da se za neki projekat svaki priliv po godinama, odnosno odliv, diskonтуje faktorima unapred zadate stope povratka i svodi na sadašnju vrednost. Ta stopa treba da bude tako određena da bi mogla da reprezentuje obim investicija, vrednost novca u funkciji vremena, kao i stepen poslovnog rizika projekta koji se razmatra.

Projekat se usvaja ako je sadašnja vrednost veća od nule, a odbija se ako je jednak nuli ili je manja od nule. Dakle, kod ovakvog pristupa vrlo je važno odabrati stopu povrata.

#### *Interna stopa povrata (IRR)*

Interna stopa povrata (Internal Rate of Return – IRR) ili interna stopa prinosa po definiciji je stopa koja sadašnju vrednost projekta svodi na nulu. To praktično znači da bi se izračunala IRR neophodno je naći diskontnu stopu koja će izjednačiti sadašnju vrednost

Tabela 7. NPV Analiza  
Table7. NPV analysis

Godina	Finansijski rezultat (Dobit) $\times 10^{-3}$	Diskontna stopa 8%	Sadašnja vrednost budućih dobitaka (KM $\times 10^{-3}$ )	Diskontrastopa 15%	Sadašnja vrednost budućih dubitaka (KM $\times 10^{-3}$ )
1.	1100	0,925925926	1018,519	0,86956522	956,522
2.	1100	0,85733882	943,073	0,75614367	831,758
3.	1100	0,793832241	873,215	0,65751623	723,268
4.	1100	0,735029853	808,533	0,57175325	628,929
5.	1100	0,680583197	748,642	0,49717674	546,894
6.	1100	0,630169627	693,187	0,4323276	475,560
7.	1100	0,583490395	641,839	0,37593704	413,531
8.	1100	0,540268885	594,296	0,32690177	359,592
9.	1100	0,500248967	550,274	0,28426241	312,689
10.	1100	0,463193488	509,513	0,24718471	271,903
11.	1100	0,428882859	471,771	0,21494322	236,438
12.	1100	0,397113759	436,825	0,18690715	205,598
13.	1100	0,367697925	404,468	0,16252796	178,781
14.	1100	0,340461041	374,507	0,14132866	155,462
15.	1100	0,315241705	346,766	0,12289449	135,184
16.	1100	0,291890468	321,080	0,10686477	117,551
17.	1100	0,270268951	297,296	0,09292589	102,218
18.	1100	0,250249029	275,274	0,08080512	88,886
19.	1100	0,231712064	254,883	0,07026532	772,92
20.	1100	0,214548207	236,003	0,06110028	67,210
21.	1100	0,198655748	218,521	0,05313068	58,444
22.	1100	0,183940507	202,335	0,04620059	50,821
23.	1100	0,170315284	187,347	0,04017443	44,192
Ukupno			11.408,165		7.038,721
I Varijanta			11.408,165 – 7.300 = 4.108,165 $\times 10^3$ KM, prihvatljiva varijanta		
II Varijanta			7.038,721 – 7.300 = -261,279 $\times 10^3$ KM, neprihvatljiva varijanta		

očekivanih troškova sa sadašnjom vrednošću očekivanih koristi. Ukoliko je neto sadašnja vrednost projekta pozitivna, jasno je da će ova stopa biti veća od diskontne stope.

Interna stopa povrata projekta izračunava se metodom pokušaja i greške. Pretpostavi se jedna diskontna stopa i na osnovu nje se izračuna neto sadašnja vrednost projekta. Ako se dobije pozitivna vrednost, postupak se nastavlja tako što se diskontna stopa povećava sve do momenta dok se ne dobija negativna neto sadašnja vrednost projekta, zatim se interpolacijom u okviru ove dve diskontne stope traži interna stopa povrata koja neto sadašnju vrednost projekta svodi na nulu.

Koristi se poznata formula za interpolaciju:

$$IRR = d_p + (d_n - d_p)(NPV_p/(NPV_p - NPV_n)) \quad (2)$$

gde su:  $d_p$  = diskontna stopa koja daje pozitivnu neto sadašnju vrednost projekta,  $d_n$  = diskontna stopa koja daje negativnu neto sadašnju vrednost projekta,  $NPV_p$  = pozitivna neto sadašnja vrednost projekta i  $NPV_n$  = negativna neto sadašnja vrednost projekta.

Za investicioni projekat proizvodnje karbonata potrebnog za dugoročno snabdevanje ODG postrojenja na slici 4 izračunata je interna stopa povrata –  $IRR = 14,38\%$ .

## ZAKLJUČAK

Uspeh svakog investicionog projekta procenjuje se njegovom efikasnošću u određenom vremenskom periodu koja proizilazi iz odnosa koristi i troškova projekta.

Da bi se izrazila i komparirala efikasnost pojedinih projekata, koriste se različiti investicioni kriterijumi. Pored kriterijuma, neophodno je definisati i adekvatne metode za utvrđivanje rentabilnosti investicionih projekata, te utvrditi način na koji će se izvršiti izbor između više mogućih varijanti pri datom kriterijumu.

U ovom radu, primenjena je višekriterijumska metoda donošenja odluke u cilju izbora optimalne varijante snabdevanja apsorbentom (krečnjakom) za potrebe osumporavanja dimnih gasova TE Ugljevik. Izbor optimalne varijante snabdevanja apsorbentom je jedna od najznačajnijih odluka u toku implementacije ovog

Godina	Očekivana dobit	Diskontni faktor za diskontnu stopu 14%		Diskontni faktor za diskontnu stopu 14,5%		Diskontni faktor za diskontnu stopu 14,383453%	
		Sadašnja vrednost	Diskontni faktor za diskontnu stopu 14%	Sadašnja vrednost	Diskontni faktor za diskontnu stopu 14,5%	Sadašnja vrednost	Diskontni faktor za diskontnu stopu 14,383453%
1	1.100.000	0,877193	964.912	0,873302	960.699	0,874252	961.678
2	1.100.000	0,789468	846.414	0,782762	839.038	0,784317	840.749
3	1.100.000	0,674972	742.469	0,666168	732.784	0,668206	735.027
4	1.100.000	0,592080	651.288	0,581806	639.986	0,584181	642.599
5	1.100.000	0,519369	571.308	0,508127	558.940	0,510721	561.793
6	1.100.000	0,455587	501.145	0,443779	488.157	0,446499	491.149
7	1.100.000	0,399637	439.601	0,387580	426.338	0,390363	429.388
8	1.100.000	0,350559	385.615	0,338498	372.348	0,341267	375.394
9	1.100.000	0,307508	338.259	0,295631	325.195	0,298354	328.189
10	1.100.000	0,289744	296.718	0,285193	284.013	0,280836	288.920
11	1.100.000	0,238617	280.279	0,225498	248.048	0,228037	250.840
12	1.100.000	0,207559	228.315	0,196940	216.634	0,199362	219.298
13	1.100.000	0,182069	200.276	0,172000	189.200	0,174292	191.722
14	1.100.000	0,159710	175.681	0,150218	165.240	0,152376	167.613
15	1.100.000	0,140096	154.108	0,131195	144.315	0,133215	146.536
16	1.100.000	0,122892	135.181	0,114581	126.039	0,116463	128.110
17	1.100.000	0,107800	118.580	0,100071	110.078	0,101818	112.000
18	1.100.000	0,094561	104.017	0,087398	96.138	0,089015	97.918
19	1.100.000	0,082948	91.243	0,076330	83.963	0,077821	85.604
20	1.100.000	0,072762	80.038	0,066664	73.330	0,068036	74.839
21	1.100.000	0,063826	70.209	0,058222	64.044	0,059480	65.428
22	1.100.000	0,055988	61.587	0,050849	55.933	0,052001	57.201
23	1.100.000	0,049112	54.023	0,044409	48.850	0,045462	50.008
Ukupno:	25.300.000		7.471.262		7.249.309		7.300.000
Sadašnja vrijednost ulaganja			7.300.000		7.300.000		7.300.000
Neto sadašnja vrijednost projekta			171.262		-50.691		0
Određivanje IRR interpolacijom			=14+(14,5-14)*(171.262/(171.262-50.691))= 14,3858067%				
Diskontna stopa određena uz pomoć excel formule			= 14,383453%				

Slika 4. Kalkulacije za određivanje Interne stope povrta – IRR.

Figur 4. IRR calculations.

projekta koji za preduzeće ima strateški karakter. Sam proces izbora zahteva, pre svega, definisanje mogućih varijanti sa poređenjem primenljivih opcija u skladu sa odgovarajućim kriterijumima kako bi se došlo do optimalnog rešenja.

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## SUMMARY

### MULTI CRITERIA ANALYSIS WITH REGARDS TO SELECTING OPTIMAL ABSORBENT SUPPLY OPTION TO UGLJEVIK THERMAL POWER PLANT

Cvjetko P. Stojanović

*Mixed Holding „Power Utility of the Republic of Srpska“ joint stock company Trebinje and Subsidiary Company „Mine and Thermal Power Plant“ Ugljevik*

(Professional paper)

Concept of sustainable development implies an effective long-term development, both in developed and developing countries, and must be based on three pillars: environmental protection, economic development and social cohesion, both on the state and on a global level. This clear definition of sustainable development suggests a necessity of a combined action on environmental protection, economic development and the achievement of social cohesion. As a rule, by investing in environmental protection projects, power generation companies implement previously defined growth targets, development policies and business strategies. Investment projects for the given coal type are considered as the capital ones, for they use great financial resources, significant resources and are long-lasting. The companies undertake investing in such projects to be affirmed in the new business environment, adapt to anticipated changes or to overcome the handicaps of their current position and thus adjust their production capabilities with the opportunities offered by the market. All the above implies that environmentally based capital project investments are the prerequisite of existence, sustainable growth and the acquisition of competitive advantages. However, investing in such projects is a very complex process comprised of multidimensional testing activities of all relevant determinants of future conditions and changes that the project entails. Bearing in mind that such projects are associated with the company's strategic development objectives, in the realization of such projects all the internal and external factors of development shall be taken into account, as well as a number of constraints arising from the socio-economic environment.

**Keywords:** Multi criteria analysis • Analytical Hierarchy Process • Desulphurization • Absorbent

# Acid leaching of oxide–sulfide copper ore prior the flotation – A way for an increased metal recovery

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## Abstract

The copper mine Cerovo – East Serbia, as well as the other ore bodies in its vicinity, contains a significant amount of oxide copper minerals in their upper layers (>40%). Processing of such mixed ores by the existing concentration technologies leads to a substantial copper losses (<60%). Reduction of “oxide copper”, by acid leaching prior the flotation concentration, can increase the overall copper efficiency up to more than 70% in the single-stage leaching, achieving an efficiency in the flotation concentration stage higher than 75%. Based on the performed experimental results, the flow sheet for processing of the mixed oxide-sulfide copper ore is proposed.

**Keywords:** oxide–sulfide copper ore, leaching, flotation, increase of copper recovery.

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Many copper mines contain, beside sulfide ore as a main quantity in deeper layers, a certain amount of oxide deposit in upper layers, as well as oxide–sulfide blend in the middle of an ore body. In order to recover the copper metal oxide ore parties are usually processed by leaching, followed by solvent extraction, and electrowinning, while the rest of ore deposit is processed by flotation, having considerable technological problems, and usually a low percent of copper recovery may be achieved. Hence, the major fraction of oxide and a part of sulfide minerals may be lost and thus making the process economy less advantageous. Such case is found in the copper ore deposit “Kraku Bugaresku”, an ore field in Eastern Serbia, a property of Mining and Smelting Copper Co., Bor, Serbia. This ore field contains several smaller ore bodies, named “Cerovo – C<sub>1–4</sub>”, having oxide cap on the top, followed by a layer of oxide-sulfide mixture, before coming deeper into the main sulfide ore deposit. So far, only the ore body C<sub>1</sub>-Copper Mine Cerovo is opened, this body is under exploitation. The majority of oxide–sulfide batches from “Cerovo – C<sub>1–4</sub>” ore bodies contain carbonate minerals, with some cuprite and chrysocolla which may easily leach. From all ferrous minerals the hematite and limonite are predominant, including some parts of lodestone. The oxide batches are placed on the surface, and upon the body opening they are the first to be excavated. Potential content of copper oxide batches in the C<sub>1</sub> to C<sub>4</sub> ore bodies is estimated to

be 18000 t of copper, including a part that is already extracted during the ten years of C<sub>1</sub> ore body (Open pit Cerovo) exploitation [1].

Three sources of copper losses are noticed during the exploitation of C<sub>1</sub> ore body, which also generates technical and environmental problems that will appear in each of the other ore bodies:

- Barren surface layer and associated gangue material, with copper content below 0.2%;
- Oxide–sulfide ore from the current ore excavation containing various percentages of oxide and sulfide minerals, in a range from few percents up to 60% of oxide;
- Mine water collected at the open pit bottom, as well as the one drained as an acid mine drainage (AMD), due to a spontaneous self-leaching of the out of balance ore stored as a dump at the open pit edge, as is illustrated in Figure 1.



Figure 1. A photograph of the dump, formed of out of balance ore (top part), and the pond (lower part of the photo), formed by collecting the AMD.

Each of these copper sources needs a different approach related to their technological treatment. Flo-

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tation of sulfide minerals from copper ore, containing a high percent of oxide copper, generates technical problems in the concentration process, decreasing the level of copper content, which is already low. In order to increase the overall efficiency of copper concentration process from such ore blend it is necessarily to treat the part of copper oxide either by leaching of grinded ore prior the flotation or by leaching the flotation tailing coming out from the concentrator.

Direct leaching process of copper oxides or complex ores attracts considerable scientific and engineering attention, which plays an important role in the sustainable development of copper industry. Sulphuric, hydrochloric and nitric acid, as well as the ammonia are usually used as the leaching agents in a various copper ore leaching processes [2–12]. Copper oxides easily react with sulphuric acid solutions at the room temperature producing  $\text{Cu}^{2+}$  in the aqueous phase. Energy consumption can also become an important factor of economy when the leaching process occurs, considering the heating and agitation of the input suspension. The kinetics of copper minerals leaching was recently published by several authors, for example Sokić *et al.* kinetics of copper leaching from chalcopyrite [7], Bingol and Canbazoglu kinetics of copper leaching from malachite [10,11], while Quast has published the kinetics of copper leaching from atacamite [12]. Also some researchers studied the processing of copper oxides wrapped by silicate and limonite [13,14]. This kind of ore is usually processed by mill running and then smelting, and the copper recovery is about 35% during the mill running process. Sun *et al.* [14] performed the copper leaching from low grade copper oxide ore by sulphuric acid, and they have obtained leaching percent of copper over 90%. Compared with the copper leaching efficiency of about 35% in the mill running technology the leaching process has achieved a better economic result.

In the present study the leaching of oxide-sulfide copper ore was considered, and a modification of the existing process was performed by inserting the leaching process between the ore milling and the flotation sections. Therefore, a higher overall efficiency of the copper removal, from such minerals blend, was obtained. Through this study, the influence of some parameters, affecting the leaching process, and the flotation process of the leach residue were defined. Finally, the oxide-sulfide copper ore, used in the experimental part, was taken from the current mine works in the Copper Mine Cerovo.

## EXPERIMENTAL

### Equipment and chemicals

The leaching experiments were carried out in a glass reactor, presented in Figure 2, equipped with the

teflon mechanical stirrer, the condenser, the thermometer, the glass funnel for feeding a solid sample and the sampling device. Such set-up provides stable hermetic conditions, and allows leaching at the chosen constant temperature as well as at the constant stirring rate.

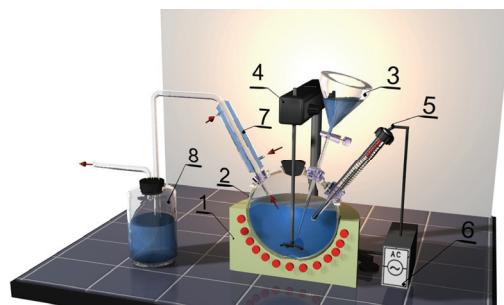


Figure 2. Experimental set-up for the leaching process at atmospheric pressure: 1 – electrical heater, 2 – glass reactor, 3 – glass funnel, 4 – mechanical stirrer, 5 – contact thermometer, 6 – cut off relay, 7 – condenser, 8 – absorber.

Sulphuric acid, analytical grade was used to prepare the leach solution by dissolving it in distilled water. Since the initial  $\text{H}_2\text{SO}_4$  concentration was the key parameter of leaching, the stock solution with the concentration of 0.3 M was prepared and further diluted to get the desired initial concentration of  $\text{H}_2\text{SO}_4$ .

In flotation concentration of the leaching residue standard chemicals were used, save as in the concentration plant Veliki Krivelj. These are the potassium-ethyl-xanthate (PEX) as the collector, and the DOW 250 as the frothing agent. These chemicals were of a technical grade. Lime milk (technical grade) was used for adjusting the pH value of suspension before the flotation.

Time was measured by a chronometer. Atomic absorption spectrophotometer (AAS – Perkin Elmer, model Analyst 300) was used for chemical analysis of the leaching samples. Chemical analysis of the ore samples, as well as of the leaching residue, the concentrate, and the flotation tailing were all analyzed in laboratories of the Institute of Mining and Metallurgy Bor.

### Leaching procedure

Ore samples, at first were disintegrated and then sieved through a set of standard laboratory sieves. The fraction  $<74 \mu\text{m}$  was used in the leaching experiments. Prior the leaching the chemical analysis was done in order to determine the initial concentrations of oxide, sulfide and total copper. Also, the percent of moisture, the contents of Fe, S, and  $\text{SiO}_2$  were determined.

The measured volume of the leaching solution with a chosen initial  $\text{H}_2\text{SO}_4$  concentration was poured into the reactor. The weighted mass (1372 g) of disintegrated and sieved ore was joined to the leaching solu-

tion ( $2.5 \text{ dm}^3$ ), and the time of leaching was measured. The solution samples were taken in a predetermined time intervals for chemical analysis on  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$ . After completing the leaching process, solid residue was filtered, than washed and further processed by flotation in order to separate and concentrates copper sulfide minerals from gangue minerals.

Leaching time was in a range of 20 to 240 min, while the initial  $\text{H}_2\text{SO}_4$  concentration in the leaching solution varied from 0.05 M to 0.3 M. The particle size was 90% –74  $\mu\text{m}$ ; stirring velocity 300 rpm, while the phase ratio was S:L = 1:2. These three process parameters were kept constant in all experiments. All experiments were performed at the room temperature.

The leach residue, that followed each of leach experiments, was conducted in the laboratory flotation cell of the following characteristics: Denver DR12, 1350 rpm, a small impeller and average air consumption. All experimental parameters were scaled down, adding the same flotation agents to the conditioned suspension, and the proportional quantities as they are used at the industrial scale. Also, the process time of each flotation stage was the same as it is on the industrial scale in the concentrator "Veliki Krivelj". The aim was to get an insight how high is the copper recovery from the ore, liberated from oxide minerals, in the leaching stage. Potassium ethylxanthate (PEX) was used as a flotation reagent, while as an aero promoter AP 3418 was used, both as collectors, while as a frothing agent DOW 250 was used. After completing both leaching and flotation processes, chemical analysis of the obtained products – leach solution, concentrate and flotation tailing, was carried out and the mass balance was evaluated. Finally, the copper distribution along the both stages was determined.

## RESULTS AND DISCUSSION

Oxide-sulfide copper ore was sampled from the ore body Cerovo C<sub>1</sub> (south deposit) and used in this study. The chemical composition of the ore sample is presented in Table 1. From Table 1, it can be seen that the high copper concentration is in the sample, as well as the high copper oxide content, while the sulfide copper concentration is lower. The iron concentration is also high, what may be related to the presence of hematite and other iron oxides. Low sulphur content indicates a low content of pyrite. Also, the content of impurities in the ore, such as zinc, lead, arsenic and cadmium is low, which is very convenient for its processing.

Table 1. Chemical composition of the ore sample (%)

Cu	$\text{Cu}_{\text{ox}}$	$\text{Cu}_{\text{sulf}}$	Fe	Zn	Pb	As	Cd	S	$\text{SiO}_2$
0.68	0.45	0.23	4.37	0.016	0.023	0.004	<0.0025	1.33	60.36

## Leaching of oxide-sulfide copper ore

The influence of time and  $\text{H}_2\text{SO}_4$  concentration on the leaching degree of oxide copper, leached out from the ore sample, was determined and presented in Figure 3.

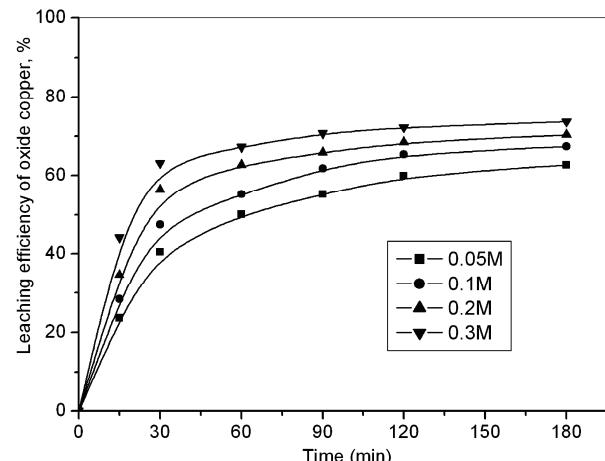


Figure 3. Leaching efficiency vs. process time for different  $\text{H}_2\text{SO}_4$  concentrations in the leaching solution.

As expected, a copper extraction increases with the process time, tending to achieve a plateau value after approximately two hours for all here used initial concentrations of  $\text{H}_2\text{SO}_4$  in the leaching solution. The higher concentrations the higher copper leaching percentages were obtained. The highest extraction percentage of 73.8% was achieved at the  $\text{H}_2\text{SO}_4$  concentration of 0.3 M. One can see that after 180 min increment of leaching degree is almost negligible and the process occurs very slowly. From an engineering point of view, the optimal process time could be chosen between 1.5 and 3 h, depending on the used initial concentration of  $\text{H}_2\text{SO}_4$ . However, increasing the concentration of  $\text{H}_2\text{SO}_4$  over 0.2M is not justified due to its higher consumption in undesired reactions with earth-alkali carbonates present in the ore, as well as with higher acidity of the leach solution. The leach solution has to be adjusted to a desired value (pH 2), to be prepared for further processing by ion exchange and/or solvent extraction [15].

The chemical composition of the leach liquor and leach residue, obtained after leaching of the ore sample at 120 min and 0.2 M of  $\text{H}_2\text{SO}_4$ , is presented in Table 2.

Obtained copper concentration in the leach solutions was usually in the range 1.2–1.7 g/dm<sup>3</sup>, for other experimental conditions. Concentration of leached fer-

Table 2. Chemical composition of the leach liquor and leach residue

Leach part	Cu	Cu <sub>ox</sub>	Cu <sub>sulf</sub>	Fe	Zn	Moisture
Liquor	1.62 g/l	—	—	1.39 g/l	7.75 mg/l	—
Residue	0.41%	0.176%	0.23%	4.06%	0.013%	22.24%

rous/ferric ions is approximately equal to the copper ions concentration, while the concentration of zinc ions was for more than two orders of magnitude less.

#### Flotation of the leach residue

In the residual solid part, after completing the leaching process and phase separation by filtration, lime milk was added in order to adjust the pH value up to 9.5. Then after, the residue was processed by flotation in order to separate the copper sulfide minerals from the gangue and produce the concentrate. Frothing agent D 250 was used as needed while the consumption of PEX collector was 40 g/t. Figure 4 presents the influence of flotation time on the flotation efficiency of sulfide copper, for five experiments with the different amount of the PEX collector of 15, 20, 25, 30 and 35 g/t. The maximum sulfide copper removal in the flotation process was 78%, for 20 min of treating, and amount of the PEX collector of 30 g/t. Similar results were obtained by Chen *et al.* [16]. They have studied chalcopyrite recovery from cleaner flotation as a function of collector dosage, and their result showed that with the increase of collector, chalcopyrite recovery slightly increase and then decrease. Besides the valorization of oxide copper, the utilization of sulfide copper in flotation process increases 2–4% by introducing the leaching process, because it has a positive effect on the surface of the sulfide mineral grains.

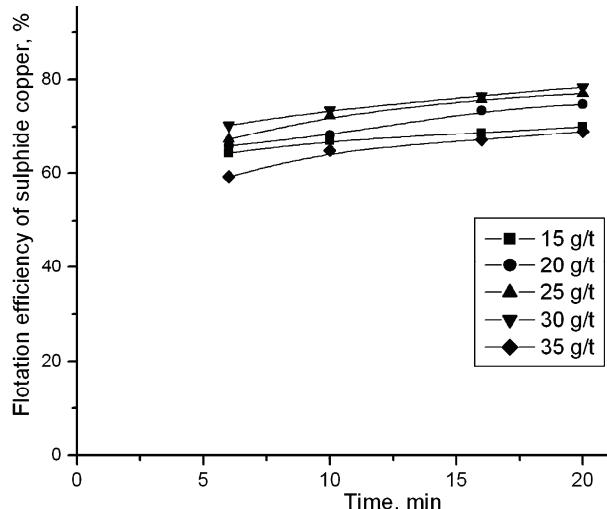


Figure 4. Influence of the time of treatment on the sulfide copper flotation efficiency, from leach residue, for five experiments with the different amount of the PEX collector.

The chemical composition of the sulfide copper concentrate and the flotation outflow are presented in Table 3. Base concentrate with a 5% of Cu was obtained, and after a three-stage rinsing the final concentrate with over 20% of Cu, for smelting process, was obtained. In the base concentrate total copper recovery, during the ore processing (leaching and flotation), is approximately 75%.

Table 3. Chemical composition (%) of the flotation products obtained at the content of 30 g/t PEX

Flotation product	Cu <sub>total</sub>	Cu <sub>ox</sub>	Cu <sub>sulf</sub>
Base concentrate	5.06	0.6	4.46
Outflow	0.23	0.16	0.07

#### Process proposal

Based on these, preliminary, experimental results, on an enlarged laboratory scale the schematic flow sheet of mixed oxide-sulfide copper ore processing was proposed and presented in Figure 5. Also, the copper distribution and its recovery, along some particular stages, are shown.

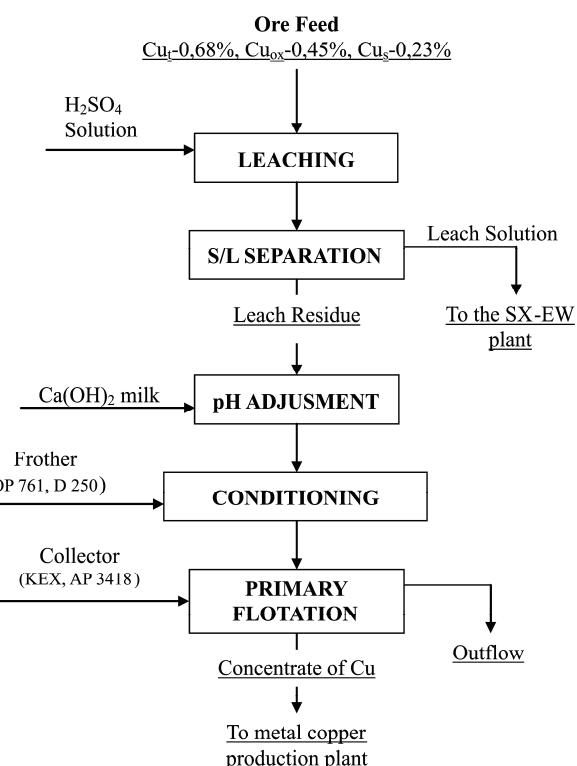


Figure 5. Schematic flow sheet of the oxide-sulfide copper ore processing.

## CONCLUSION

Copper Mine Cerovo, as well as the other ore bodies in its vicinity, contains significant amount of oxide copper minerals (>40%) in the upper layers. Processing of such mixed ores by the existing concentration technologies leads to the oxide copper losses. In order to increase an overall degree of copper utilization, a study of the copper oxides leaching from oxide-sulfide ore followed by flotation of copper sulphide minerals from the leach residue was presented. Maximum copper oxide extraction during the single leaching stage was 73% with 0.3 M H<sub>2</sub>SO<sub>4</sub> leaching solution and after 180 min. Maximum sulfide copper extraction in the flotation process was 78% for 20 min and amount of the PEX collector of 30 g/t. Achieved total copper recovery in the ore leaching and flotation process is about 75%. Introducing the leaching process prior to the flotation process is the main reason for increases in the total copper recovery. This is due to the valorization of most of the oxide copper, and improved efficiency of the sulfide copper flotation. Copper concentration in the leach solutions was in the range 1.2–1.7 g /dm<sup>3</sup>, and content of copper in the concentrate after flotation was about 5%.

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## IZVOD

### KISELO LUŽENJE OKSIDNO–SULFIDNE RUDE BAKRA PRE FLOTACIJE – NAČIN ZA POVEĆANJE ISKORIŠĆENJA METALA

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(Stručni rad)

Rudnik bakra Bor, lokalitet Cerovo, sadrži više rudnih tela koja sadrže značajnu količinu oksidnih minerala bakra u svojim površinskim slojevima (> 40%). To su najvećim delom karbonatni minerali, uz nešto kuprita i hrizokole, a od minerala gvožđa preovlađuju hematit, limonit i nešto magnetita. Prerada mešanih sulfidno-oksidnih ruda stvara tehnološke probleme pri flotiranju, smanjujući ionako nizak stepen iskorišćenja, pa ukupni gubici na bakru dostižu i do 60%. U cilju povećanja ukupnog stepena iskorišćenja bakra, prilazani su rezultati luženja oksidnog bakra iz oksidno-sulfidne rude i flotacije sulfidnog bakra iz čvrstog ostatka luženja. Maksimalno iskorišćenje oksidnog bakra tokom jednostepenog luženja sa 0,3 M rastvorom H<sub>2</sub>SO<sub>4</sub> i vremenu od 180 min je 73%, a sulfidnog bakra u procesu flotacije je 78%, za 20 min i dodatak PEX kolektora od 30 g/t koncentrata. Ukupno iskorišćenje bakra u procesima luženja i flotacije rude iznosi oko 75%. Koncentracija bakra u lužnom rastvoru iznosi 1,2–1,7 g / dm<sup>3</sup>, a sadržaj bakra u koncentratu nakon flotacije je oko 5%. Na osnovu izvršenih eksperimentalnih rezultata predložena je tehnološka šema za preradu mešovite oksidno-sulfidne rude bakra.

*Ključne reči:* Oksidno–sulfidna ruda bakra • Luženje • Flotacija • Povećanje iskorišćenja bakra

# Upotrebna vrednost i akumulacija teških metala u krmnim travama odgajenim na pepelištu termoelektrane

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## Izvod

Pet krmnih trava, ježevica (*Dactylis glomerata* L.), francuski ljlj (*Arrhenatherum elatius* Presl.), italijanski ljlj (*Lolium multiflorum* Lam.), crveni vijuk (*Festuca rubra* L.) i visoki vijuk (*Festuca arundinacea* Schreb.) su ispitivane na nezagađenom njivskom zemljištu na oglednom dobru „Radmilovac“ u Beogradu, i na odlagalištu pepela termoelektrane “Nikola Tesla A” (TENT A) u Obrenovcu. Merene su koncentracije As, Pb, Cd, Zn, Ni, Fe i Cu u zemljištu i pepelu, kao i u tkivima ispitivanih vrsta trava u cilju utvrđivanja zdravstvene bezbednosti u ishrani domaćih životinja. Uzorci trava su prikupljani u fazi klasanja, u punom vegetativnom razvoju. Skidana je nadzemna biomasa u 3 ponavljanja na visini od 3–5 cm, oponašajući ispašu ili kosidbu. Analiza je pokazala visok sadržaj As i Ni u pepelu dok je sadržaj svih elemenata u zemljištu bio u dozvoljenim koncentracijama. Koncentracije As, Cd, Fe i Ni su bile više u travama sa pepelišta, koncentracije Pb i Cu u travama sa njivskog zemljišta, dok su koncentracije Zn bile slične u uzorcima sa oba lokaliteta. Rezultati ispitivanja ukazuju da krma trava sa obe lokacije ne akumulira metale iznad tolerantnog nivoa za ishranu goveda, ali da se mora oprezno pristupiti mogućnosti korišćenja trava sa pepelišta za krmu.

**Ključne reči:** odlagalište pepela, krmna biomasa, teški metali, trave.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Rezultat sagorevanja uglja u termoelektranama su velike količine pepela i šljake koje je potrebno bezbedno odložiti. Trajno odlaganje treba da spreči štetan uticaj deponovanog pepela i šljake na životnu sredinu. Termoelektrana “Nikola Tesla A” u Obrenovcu sagoreva lignit i nastali pepeo i šljaka se transportuju do odlagališta u neposrednoj blizini termoelektrane koju okružuju površine obradivog zemljišta. Najopasnije dejstvo odlagališta pepela na prirodno okruženje nastaje usled eolske erozije pepela, koja se odvija i pri vrlo slabim vazdušnim strujanjima. Radi sprečavanja raznošenja pepela vrši se biološka rekultivacija površina odlagališta setvom trava na nasipima aktivnih i pasivnih kaseta, kao i setvom na ravnim delovima pasivnih kaseta, uz sadnju različitih vrsta drveća i žbunova. Iako je reč o hroničnom i synergističkom delovanju stresnih faktora (vodni deficit, nepovoljne fizičke i hemijske karakteristike pepela, teški metali u višku) na pepelištu, veliki broj različitih vrsta biljaka toleriše oštре ekološke uslove, i sa manje ili više teškoća opstaje i širi se na pepelu [1,2]. Pepeo ima veliku sposobnost infiltracije

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vode i u letnjim mesecima ne zadržava vlagu, tako da se biljke moraju prilagoditi sušnom okruženju i generalno slabije napreduju nego u prirodnom okruženju, pa se vrši plansko navodnjavanje. Sam proces setve zbog toga zahteva unošenje velikih količina semena trava i pojačano đubrenje sa većim normama azota u odnosu na klasičan pristup u poljoprivredi [1]. Rekultivacija je često diktirana i dostupnošću semenskog materijala specifičnih višegodišnjih travnih vrsta, otpornih na ekstremne agroekološke prilike odlagališta pepela.

Akumulacija teških metala u biljkama ne zavisi samo od ukupnog sadržaja u zemljištu, nego i afiniteta vrste, kao i individualnog ili interaktivnog dejstva raznih zemljišnih svojstava [3]. Neki teški metali (Cu, Zn, Mn, Fe i Mo) u nižim koncentracijama su neophodni za rastenje i razviće biljaka i smatraju se mikroelementima, dok su u visokim koncentracijama toksični za biljke [4]. U njihovom nedostatku biljke ne mogu da završe svoj životni ciklus, a toksični efekti su mnogostruki jer deluju na biljke na različitim organizacionim i funkcionalnim nivoima u isto vreme i reflektuju se vidljivim strukturalnim oštećenjima (hloroza). Njihov uticaj se reflektuje i na poremećaje u mineralnom režimu biljaka kompeticijom sa ostalim elementima, čineći ih nedostupnim biljakama [5–7], što predstavlja dodatni ograničavajući faktor za vegetaciju na pepelu. Visoki vijuk ima veći

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potencijal za usvajanje olova u odnosu na zubaču (*Cynodon dactylon*), ali se akumulacija obavlja na nivou korena [8]. Na drugu stranu, zubača akumulira više nikla i to u nadzemnom delu. Međutim, obe vrste ne pokazuju sposobnost hiperakumulacije metala na zemljišta sa osrednjom pristupačnošću metala. Visoki vijuk može biti efikasan u akumuliranju Pb, uz primenu fitoekstracionih strategija, kao što je niža pH ili primena helata [9]. Engleski ljlj i crveni vijuk se smatraju najtolerantnijim travnim vrstama za gajenje na pepelištima [10].

Kako su odlagališta pepela ogromne površine, često nedovoljno obezbeđene, postoje rizici da različita divljač ili nekontrolisane domaće životinje konzumiraju travnu biomasu sa rekultivisanih površina. Radi utvrđivanja upotrebljene vrednosti i akumulacije teških metala u pojedinačnim krmnim travama sa pepelišta, potrebno je poređenje sa istim vrstama dobijenim na prirodnom nezagadenom zemljištu. Najčešće korišćena vrsta trave sa odlagališta pepela u Srbiji, kao i u rekultivaciji je crveni vijuk (*Festuca rubra* L.) [11–14], ekonomski značajna zbog pogodnosti za ishranu domaćih životinja. Crveni vijuk je tolerantan na visoke koncentracije metala, i može biti pionirska vrsta za revitalizaciju–remedijaciju-bioško obnavljanje industrijskih površina ili odlagališta pepela zagađenih teškim metalima [13,15,16]. U flori Srbije je sa 86 lokacija prikupljeno i determinisano 200 autohtonih populacija crvenog vijuka različite ploidnosti [17]. Značaj vijuka ogleda se u meliorisanju prirodnih travnjaka, u zasnivanju sejanih livada i pašnjaka i proizvodnji semena [18], a kao dugotrajna vrsta pogodan je za javno zelenilo i hortikulturu [19]. Druga značajna trava za rekultivaciju pepelišta je italijanski ljlj (*Lolium multiflorum* Lam.); kratkotrajna trava poželjnih agronomskih kvaliteta koja se brzo zasniva, ima dugu vegetacionu sezonu, daje visok priнос pod povoljnim ekološkim uslovima i može se koristiti ispašom ili za seno [19]. U svetu se koristi u uslovima kada je potrebno brzo snabdevanje stočnom hranom ili sigurno pokrivanje terena u borbi protiv erozije. Visoki vijuk (*Festuca arundinacea* Schreb.) je travna vrsta sa širokom ekološkom amplitudom, te je pogodan za gajenje u toplim i sušnim regionima. Osim krmne proizvodnje, pogodan je za zaštitu od erozije na nasipima i obodima kanala. Francuski ljlj (*Arrhenatherum elatius* Presl.) i ježevica (*Dactylis glomerata* L.) su takođe višegodišnje vrste pogodne za krmu, za zatravljivanje nasipa, ekstenzivne travnjake pored puteva i na kosinama. Setva trave na odlagalištu pepela TENT A u Obrenovcu se izvodi najčešće smešom višegodišnjih vrsta ili pojedinačnih vrsta otpornih na nepovoljne uslove uspevanja na pepelištu. Upotreba krme sa takvog staništa u kome se mogu nalaziti toksični teški metali predstavlja kompleksno pitanje, kako zbog potencijalne mogućnosti njihovog uključivanja u lanac

ishrane, tako i zbog interakcija između pojedinih teških metala i minerala, što može povećati ili sniziti granice toksičnosti. Na primer, kada se u travi nalazi količina bakra oko  $10 \text{ mg kg}^{-1}$ , koncentracija Mo se mora povećati na oko  $20 \text{ mg kg}^{-1}$  da bi došlo do ispoljavanja toksičnosti, ali kada u travi ima  $3\text{--}5 \text{ mg kg}^{-1}$  Cu, štetan uticaj ispoljiće se već pri  $3\text{--}7 \text{ mg kg}^{-1}$  Mo [20]. Koncentracija Cd se smanjuje u krvi, jetri, bubrežima i mišićnom tkivu goveda uz prisutan Zn u ishrani u odnosu na ishranu gde je samo prisutan Cd [21].

Razlike u ispoljavanju simptoma toksičnosti se mogu javiti kod različitih vrsta domaćih životinja, različitih kategorija i faza proizvodnog ciklusa grla. Najdrastičnija razlika u toleranciji između vrsta se može primetiti u odnosu na bakar, tako kod goveda štetne doze iznose oko  $100 \text{ mg kg}^{-1}$ , a kod ovaca  $20 \text{ mg kg}^{-1}$ . Takođe, dugotrajna ishrana hranivima sa akumuliranim teškim metalima, čak i ispod dozvoljene koncentracije, može izazvati nakupljanje ovih elemenata u jetri i kasnije dovesti do trovanja. Telad su najosetljivije domaće životinje na trovanje olovom [22]. Oovo se akumulira u bubrežima, jetri i kostima, i izaziva smanjeni rast, dijareju, slabost, drhtanje mišića, anemiju, slepilo, dovodeći i do smrti. I pored visokih koncentracija olova u pojedinim organima životinja, meka tkiva i mleko su bez povećanog sadržaja Pb. Zato se u literaturi sreću kritične koncentracije za pojedine elemente prvenstveno u jetri. Na nivo toksičnosti teških metala utiče i forma (organski ili neorganski oblik) i jedinjenje u kom se nalaze. Tako arsen u neorganskoj formi je toksičniji od organski vezanog arsena [23]. Vrlo često povećanje koncentracije teških metala u hrani ne mora dovesti do trovanja već se ispoljava u padu proizvodnih i reproduktivnih sposobnosti, što onemogućava postavljanje dijagnoze.

Ispitivanja su obavljena sa ciljem da se utvrdi nivo akumulacije teških metala u krmnim vrstama trave koje rastu na nezagadenom zemljištu i na odlagalištu pepela termoelektrane sa visokim sadržajem metala. Ispitivanjem individualnih razlika u akumulaciji teških metala svake ispitivane vrste analiziran je njihov potencijalni rizik za ishranu preživara.

## EKSPERIMENTALNI DEO

Prema FAO (Food and Agriculture Organization) sistemu klasifikacije zemljišta [24], zemljište na oglednom dobru „Radmilovac“ je tipa izluženi černozem, obrazovan na lesu. Vrednosti pH u zemljištu i u pepelu su određene kod odnosa 1:2,5 u 1 M KCl rastvoru. Merenje koncentracija makroelemenata (N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O i Ca) u zemljištu i u pepelu izvedeno je nakon alkalnog topljenja sa litijum-boratom, litijum-tetraboratom i litijum-jodidom, na temperaturi od 1000 °C, a merenje koncentracija mikroelemenata i teških metala (As, Cd, Cu, Fe, Ni, Pb i Zn) nakon digestije sa HNO<sub>3</sub> i H<sub>2</sub>O<sub>2</sub>. Koncen-

tracije navedenih makro, mikroelemenata i teških metala u zemljištu i u pepelu su određene metodom ASTM D6349-01 [25] tehnikom atomske emisione spektrometrije, sa induktivno spregnutom plazmom (ICP-AES) na uređaju Perkin–Elmer, model ICP/6500.

Za ispitivanje koncentracija pojedinih metala i mikroelemenata u biljnog materijalu prikupljeni su uzorci 5 krmnih trava (ježevica, francuski i italijanski ljlj, crveni i visoki vijuk) sa odlagališta pepela „TENT A“ u Obrenovcu i sa oglednog dobra Poljoprivrednog fakulteta „Radmilovac“. Uzorci ispitivanih trava su prikupljeni u maju 2005. godine, u punom vegetativnom razvoju. U tri ponavljanja je skidana nadzemna biomasa na visini 3–5 cm. Biljni materijal je osušen na temperaturi od 25 °C.

Merenje koncentracija mikroelemenata i teških metala (Cd, Cu, Fe, Ni, Pb i Zn) u prikupljenom biljnog materijalu izvedeno je nakon digestije sa  $\text{HNO}_3$  i  $\text{H}_2\text{O}_2$ . Koncentracije su određene metodom AOAC 986.15 [26], tehnikom atomske emisione spektrometrije, sa ICP-AES. Koncentracije arsena (As) u biljnog materijalu metodom AOAC 985.01 [26], hidridnom tehnikom atomske emisione spektrometrije (HG-AAS) na uređaju Perkin–Elmer, model MHS-10/5000. Prag detekcije za merenja koncentracija u zemljištu, pepelu i biljnog materijalu bio je za azot,  $\text{K}_2\text{O}$  i Ca  $\leq 0,03\%$ ; za Fe  $< 0,5 \text{ mg kg}^{-1}$ ; za koncentracije  $\text{P}_2\text{O}_5 < 1,0 \text{ mg kg}^{-1}$ ; Cu, Ni, Pb i Zn  $\leq 0,5 \text{ mg kg}^{-1}$ ; Cd  $\leq 0,02 \text{ mg kg}^{-1}$ ; As  $\leq 0,01 \text{ mg kg}^{-1}$ .

Sedam eksternih krivih je konstruisano korišćenjem referentnih standarda za koncentracije As, Cd, Cu, Fe, Ni, Pb i Zn u analiziranim uzorcima zemljišta, pepela i biljnog materijala. Kalibracione krive su izvedene sa pet različitih koncentracija. Izračunat je prosek koncentracija iz tri ponavljanja za svaku analiziranu biljnu vrstu, kao i standardna devijacija ( $SD$ ).

## REZULTATI I DISKUSIJA

Oba supstrata, pepeo sa odlagališta pepela i zemljište sa oglednog dobra su neutralne reakcije, s tim što je na pepelištu nizak sadržaj ukupnog fosfora ( $\text{P} < 1 \text{ mg kg}^{-1}$ ) i azota, a visok sadržaj kalijuma (Tabela 1). Koncentracije As i Ni na pepelištu su nadmašili maksimalno dozvoljene količine [27], potvrdivši da su među najčešćim polutantima u Srbiji [28].

Koncentracije metala i mikroelemenata u biljnog materijalu pet travnih vrsta su upoređene sa referentnim vrednostima za listove većine zeljastih biljaka [29], sa kritičnom koncentracijom u biljnog tkivu koja može uticati na produkciju biomase ili izazvati njen smanjenje do 10% [30], i maksimalnom koncentracijom pojedinih elemenata u krmi koja ne deluje negativno na ishranu domaćih životinja (Tabela 2).

Tabela 2. Granične koncentracije teških metala i mikroelemenata u travama ( $\text{mg kg}^{-1}$ )

Table 2. Limit values of trace element concentrations in grasses ( $\text{mg kg}^{-1}$ )

Parametar	As	Pb	Cd	Zn	Ni	Fe	Cu
NN <sup>a</sup>	0,01–1	2–5	0,1–1	15–150	0,1–5	–	3–20
KK <sup>b</sup>	1–20	–	10–20	100–500	10–30	–	10–30
TN <sup>c</sup>	4	40	1	2000	50	1250	12–50

<sup>a</sup>Normalni nivo u lišću biljaka; Normal level in plant leaves [29]; <sup>b</sup>kritične koncentracije u biljnog tkivu pri kojima može doći do 10% gubitaka biomase; critical level in plant tissues for 10% yield loss [30];

<sup>c</sup>maksimalni tolerantni nivo za ishranu životinja; maximum tolerant level for fodder [23,32]

Francuski ljlj je akumulirao višestruko više As u odnosu na konvencionalno gajenje, dok je četiri puta manje akumulirao Pb na pepelištu. Koncentracija Fe je bila šest puta veća na pepelištu, dok su ostali elementi imali sličnu koncentraciju na obe lokacije. Slični odnosi u akumuliraju As, Pb i Fe su uočeni i kod ježevice, s tim

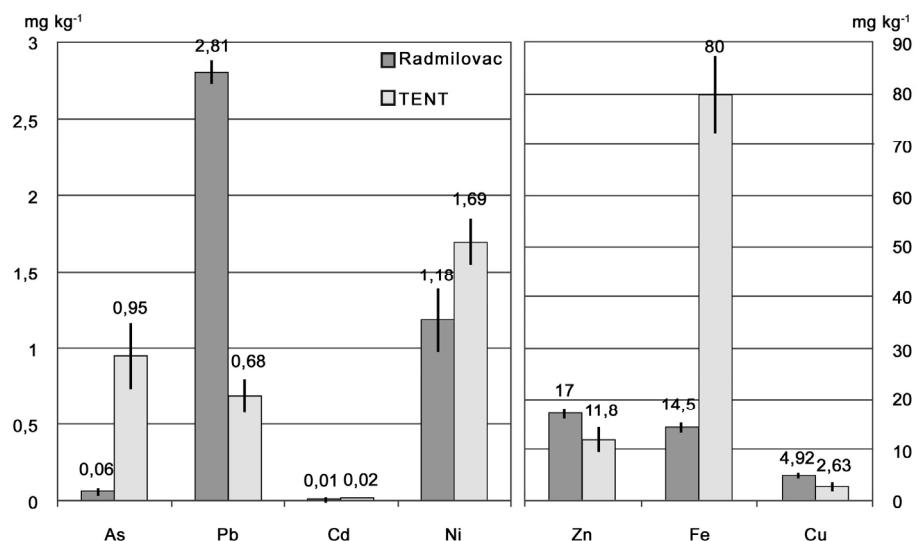
Tabela 1. pH vrednosti i hemijski sastav pepela i obradivog zemljišta u sloju 0–20 cm; MDK [25] – maksimalno dozvoljena količina  
Table 1. pH value and chemical composition of disposed ash and cultivated soil in layer 0–20 cm; MDK [25] – maximum tolerant amount

Parametar	TENT	Radmilovac	MDK
pH u KCl	7,03	6,8	–
N, %	0,04	0,11	–
$\text{P}_2\text{O}_5$ , $\text{mg kg}^{-1}$	<1,0	14,2	–
$\text{K}_2\text{O}$ , %	3,02	17,3	–
Ca, %	3,71	0,61	–
As, $\text{mg kg}^{-1}$	34,7	0,01	25
Pb, $\text{mg kg}^{-1}$	56,7	3,79	100
Cd, $\text{mg kg}^{-1}$	0,8	0,36	3
Zn, $\text{mg kg}^{-1}$	75,9	75,4	300
Ni, $\text{mg kg}^{-1}$	123	2,17	50
Fe, $\text{mg kg}^{-1}$	21,7	2,71	–
Cu, $\text{mg kg}^{-1}$	62,7	26,7	100

što je disproporcija manja u odnosu na francuski ljlj za As i Pb na različitim supstratima, ali je veća razlika u usvojenom gvožđu (Slika 1).

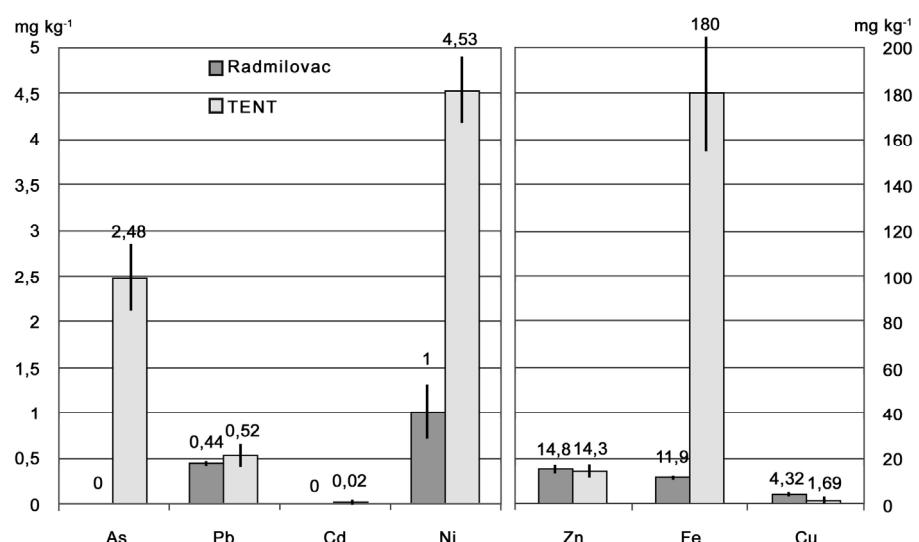
Italijanski ljlj je akumulirao As i Ni sa pepelišta dok u konvencionalnom gajenju nije uočeno usvajanje As i zabeležena je 4,5 puta manja koncentracija nego na pepelištu (Slika 2). U odnosu na ježevicu, francuski i italijanski ljlj i crveni vijuk, visoki vijuk je imao najveću akumulaciju As na pepelištu, neznatnu na imanju Radmilovac, višestruko veću količinu Fe u uzorcima sa pepelišta i značajno veću koncentraciju Cu u biljkama odgajenim na zemljištu (Slika 3). Crveni vijuk je imao sličnu disproporciju u akumulaciji As i Pb na različitim supstratima kao i ostale vrste (Slika 4).

Koncentracija Zn u pepelištu „TENT A” i obradivom zemljištu (75,9 i 75,4 mg kg<sup>-1</sup>, redom) je u granicama zemljišta u svetu (60–89 mg kg<sup>-1</sup>), a zbog neutralne reakcije pepelišta je prilično mala mobilnost cinka [31]. To se potvrđuje poređenjem koncentracija Zn u travama na dva supstrata koji se kreće u rasponu od 11,8 do 24,5 mg kg<sup>-1</sup>. Ovim ispitivanjima nisu potvrđena velika variranja Zn u krmi [32] i dobijeni rezultati ukazuju na ujednačenost koncentracija po vrstama i lokacijama. Sve ispitivane trave su imale koncentraciju Zn ispod maksimalnih za korišćenje kao krme, a takođe su i ispod kritične koncentracije koja bi mogla da utiče na zaustavljanje rasta i smanjenje biomase trave (Tabela 2).



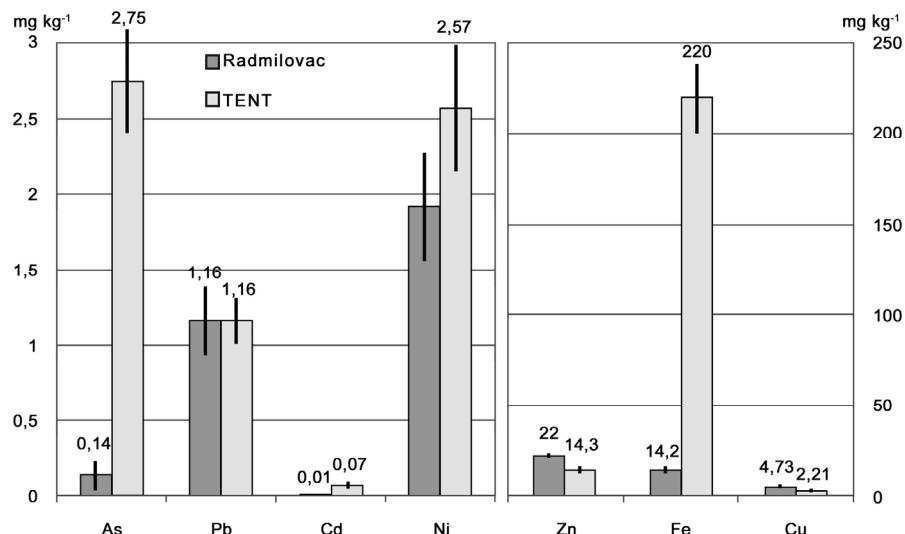
Slika 1. Koncentracije teških metala i mikroelemenata (srednje vrednosti i standardna devijacija) u francuskom ljlju (*Arrhenatherum elatius*).

Figure 1. Trace elements concentrations (mean values and standard deviation) in tall oatgrass (*Arrhenatherum elatius*).



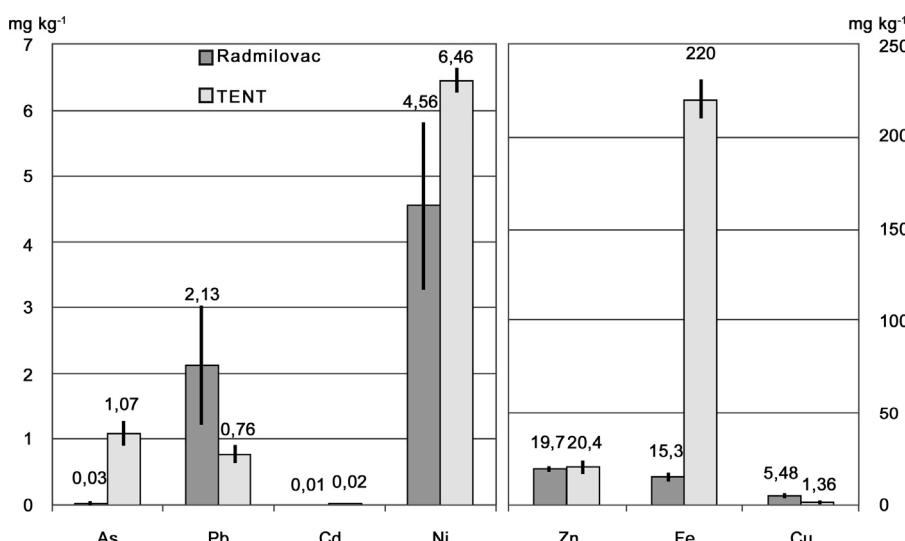
Slika 2. Koncentracije teških metala i mikroelemenata (srednje vrednosti i standardna devijacija) u italijanskom ljlju (*Lolium multiflorum*).

Figure 2. Trace elements concentrations (mean values and standard deviation) in Italian ryegrass (*Lolium multiflorum*).



Slika 3. Koncentracije teških metala i mikroelemenata (srednje vrednosti i standardna devijacija) u visokom vijuku (*Festuca arundinacea*)

Figure 3. Trace elements concentrations (mean values and standard deviation) in tall fescue (*Festuca arundinacea*).



Slika 4. Koncentracije teških metala i mikroelemenata (srednje vrednosti i standardna devijacija) u crvenom vijuku (*Festuca rubra*)

Figure 4. Trace elements concentrations (mean values and standard deviation) in red fescue (*Festuca rubra*).

Koncentracije Cu u kabastoj krmi mogu da variraju od 2 do 69 mg kg⁻¹ [32]. Ukoliko ga nema dovoljno u supstratu, neznatno je premeštanje iz korena u nadzemne organe, kao i iz starijih listova u mlađe. Ako je njegov udio u suvoj materiji lista manji od 4 mg kg⁻¹, smatra se da biljke nisu u dovoljnoj meri obezbeđene, dok sadržaj od 20 do 100 mg kg⁻¹ ukazuje na njegovu visoku koncentraciju [30]. Pri ispaši nekim travama životinje mogu imati nedostatak Cu, ali se simptomi nedostatka ne javljaju pri ishrani senom od istog travnog pokrivača [33]. Koncentracija bakra u ispitivanim travama je bila u normalnim granicama na Radmilovcu, dok je trava na pepelištu bila nedovoljno obezbeđena bakrom za normalni rast i razviće.

U prirodi se veoma retko može uočiti fitotoksično dejstvo visokih koncentracija arsena ili njegovo nepovoljno dejstvo na prinos biljaka. Količina arsena je različita u ispitivanim supstratima, pošto je u pepelištu iznad maksimalno dozvoljene i uslovjava koncentraciju u biljnog tkivu trave od 0,90 do 2,75 mg kg⁻¹ biljne mase. Ovo nisu kritične koncentracije za korišćenje kao krme, ali nepovoljno utiču na rast i razviće biljaka.

Olovo je najmanje pokretan element među mikroelementima zemljišta [30], slabo se usvaja i premešta u nadzemne organe biljaka, izuzev na kiselim zemljištima. Nivo olova je prosečno 2,1 mg kg⁻¹ za trave [30], a ispitivanjem na TENT A i Radmilovcu je izmerena koncentracija olova u travama od 0,44 do 2,81 mg kg⁻¹. Uprerdivši koncentracije olova po lokacijama, uočljive su

nešto veće koncentracije na obradivom zemljištu, što je verovatno posledica blizine prometne saobraćajnice.

Slično olovu, količina kadmijuma je mala u supstratu i niska u tkivu trava. Izmerene koncentracije kadmijuma su oko deset puta manje od maksimalno dozvoljenih u hrani za preživare. Pošto količina Cd u hrani nije kritična, ni eventualna akumulacija u jetri i bubrežima [20] ne bi ugrozila zdravlje domaćih životinja.

Nikl se uglavnom akumulira u korenju biljaka a pH vrednost zemljišta je glavni faktor koji određuje njegovo ponašanje i pristupačnost [35]. Odnos Ni:Fe je važniji od koncentracija ova dva elementa u biljkama radi objašnjenja toksičnosti Ni [36]. Količina nikla u pepelištu je bila 2,5 puta veća od dozvoljene, ali je zato u ispitivanom zemljištu skoro 50 puta manja u odnosu na pepelište. Smanjena pristupačnost nikla usled visoke pH pepelišta je uticala da sve koncentracije u bilnjom tkivu budu ispod kritičnih za normalan razvoj biljaka. Najmanje nikla na obe lokacije je akumulirao francuski Ijulj, dok je najvišu koncentraciju imao crveni vijuk, ali u prihvatljim granicama za ishranu životinja (Slike 1 i 4).

Gvožđe može da se akumulira u biljkama bez bilo kakvog štetnog uticaja [37] i zato nije neobično naći koncentraciju Fe koja premašuje granice maksimalno dozvoljenih količina. Na odlagalištu pepela vrednosti za gvožđe variraju u zavisnosti od vrste i kreću se od 80 do 280 mg kg<sup>-1</sup>, što je u skladu sa drugim istraživačima koji navode veliko variranje koncentracije gvožđa u krmi, od 32 do 1200 mg kg<sup>-1</sup>, sa prosekom 184 mg kg<sup>-1</sup> i velikom standardnom devijacijom [32]. Iako su količine Fe na pepelištu veoma velike, on je nepristupačan biljkama i koncentracije u bilnjom tkivu su veoma male. Sve vrednosti Fe u travama su u normalnim granicama [20].

Posmatrano po vrstama, italijanski Ijulj i visoki vijuk su akumulirali više arsena na pepelištu od drugih trava,

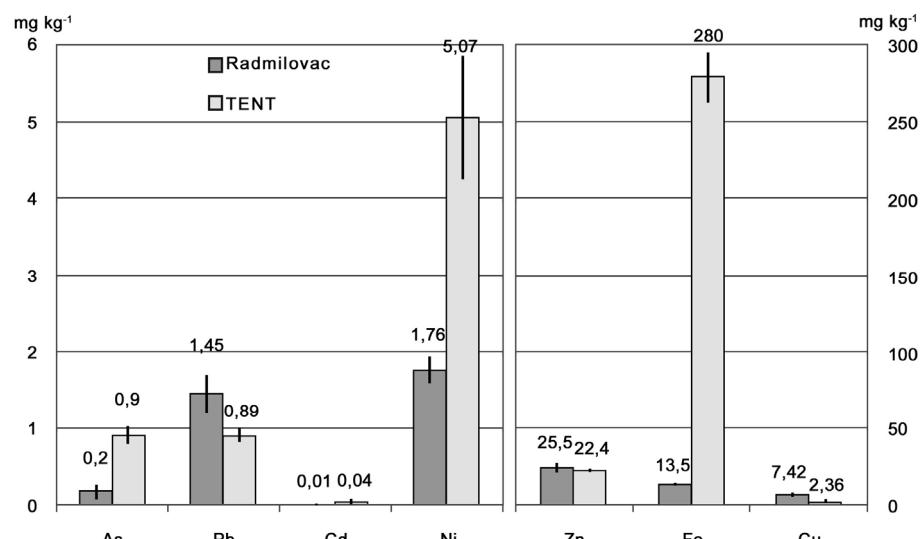
potvrdivši rezultate [38] na Ijulju sa pepelišta. Na drugu stranu, italijanski Ijulj nije nadprosečno akumulirao ostale teške metale, što je u saglasnosti sa prethodnim istraživanjima [38], da Ijulj ne akumulira Ni, Cu, Zn, Pb i Cd. Italijanski Ijulj je podložan sezonskim variranjima usvajanja Zn u stablu i listu [39]. Prinos italijanskog Ijulja gajenog na supstratu od pepela mnogo više zavisi od đubrenja i kalcizacije nego od tipa pepela ili smeše zemljišta i pepela [40], jer se đubrenjem sa N, P i K i unošenjem Ca smanjuje koncentracija teških metala ispod fitotoksičnog nivoa u lancu ishrane do čoveka.

Gajenjem na pepelištu visoki vijuk pokazuje bolje rezultate od drvenastih vrsta [41]. Sa rastućim koncentracijama nikla i olova u pepelu (0, 50 i 100 mg kg<sup>-1</sup>, redom), visoki vijuk povećava koncentraciju u bilnjom tkivu na 5, 16 i 18 mg kg<sup>-1</sup> Ni i 1, 8 i 12 mg kg<sup>-1</sup> Pb [8]. Ujednačene količine Pb i Ni u našim ispitivanjima ukazuju da visoki vijuk ne akumulira značajnije količine olova u nadzemnom delu, iako se značajne količine Pb skladište u korenju [8].

U našim istraživanjima, crveni vijuk je na odlagalištu pepela TENT imao sličnu koncentraciju As kao i u nekim prethodnim istraživanjima [12,14], ili veću [13]. Koncentracije Pb, Cd, Zn, Fe i Ni u crvenom vijuku su slične prethodnim ispitivanjima [12,14]. Crveni vijuk je na obe supstrata na kojima je gajen akumulirao najveću količinu Ni, ali daleko ispod maksimalno dozvoljenih količina u krmi.

Koncentracije svih ispitivanih elemenata u ježevici je u tolerantnim granicama za ishranu preživara, na obe ispitivane lokacije (slika 5).

Akumulacija Pb, Cu i Fe u bilnjom tkivu francuskog Ijulja, ježevice, visokog i crvenog vijuka može biti povećana usled niskog pH supstrata površinskog rudokopa [37]. Vrste gajene na odlagalištu pepela TENT imale su



Slika 5. Koncentracije teških metala i mikroelemenata (srednje vrednosti i standardna devijacija) u ježevici (*Dactylis glomerata*).  
Figure 5. Trace elements concentrations (mean values and standard deviation) in cocksfoot (*Dactylis glomerata*).

desetostruko veću količinu Pb i Cu nego vrste gajene na Radmilovcu. Razlog tome je veća koncentracija Cu u supstratu i veća pristupačnosti usled niskog pH zemljišta.

Standardna devijacija prosečnih koncentracija metala je bila veća u travama sa odlagališta pepela u odnosu na trave sa Radmilovca, izuzev crvenog vijuka. Niske koncentracije teških metala uz malu standardnu devijaciju kod crvenog vijuka potvrđuju njegovu pogodnost za rekultivaciju pepelišta.

Količine ispitivanih teških metala su ispod kritičnih toksičnih koncentracija za preživare prema stranim normama [23] i prema domaćem Pravilniku o kvalitetu hrane za životinje [34]. Iako se u depositu neki elementi nalaze iznad maksimalno dozvoljene koncentracije za zemljište i vode (As, Ni), nijedna od ispitivanih travnih vrsta, pa ni crveni vijuk kao najtolerantnija na prisustvo teških metala, nije bila značajan bioakumulator. Objasnjenje se nalazi i u pH vrednosti supstrata, koja je u sveže deponovanom pepelu vrlo visoka (pH~12), ali se vremenom smanjuje zbog ispiranja. Visoka pH vrednost smanjuje pokretljivost toksičnih elemenata u vodenom rastvoru i usvajanje od strane biljaka, što je potvrđeno i kod krmnih leguminoza [3]. Ovo je takođe u saglasnosti sa rezultatima [42] da se teški metali na pepelištu slabo transportuju u biljna tkiva. Teški metali se čvrsto vezuju za minerale gline i period njihove akumulacije na teškim, glinovitim zemljištima je dugotrajniji, dok je pepelište potpuno drugačije strukture.

## ZAKLJUČAK

Koncentracija pojedinih mikroelemenata i teških metala izmerena u travama sa pepelišta je u okvirima uobičajenih koncentracija za konvencionalnu proizvodnju na zemljišnom supstratu. Poređenje istih vrsta na različitim lokalitetima gajenja ukazuje da trave nisu značajni akumulatori (As, Pb, Cd, Zn, Ni, Fe i Cu) i da je krma proizvedena na obe lokacije sa dozvoljenim koncentracijama ovih elemenata, te da je zdravstveno ispravna za ishranu domaćih životinja.

Na osnovu dobijenih rezultata može se zaključiti da se na pepelištu termoelektrane „Nikola Tesla” u Obrenovcu može proizvesti trava bez toksičnog sadržaja pojedinih teških metala, slično travi proizvedenoj na obradivom zemljištu. Međutim, visoke koncentracije nekih elemenata u pojedinim uzorcima upućuju na oprez pri korišćenju ovako dobijene krme. Ježevica, francuski i italijanski ljlj, crveni i visoki vijuk nisu bili akumulatori većih količina istraživanih teških metala. Iako je koncentracija As i Ni u pepelištu bila iznad maksimalno dozvoljene koncentracije, sadržaj teških metala u krmi je bio ispod maksimalno dozvoljene koncentracije prema normativima za ishranu životinja. Eks-tremni uslovi uspevanja (nedostatak pojedinih makro-

elemenata, relativno visoka pH vrednost i loš fizički sastav supstrata) ometaju pravilan rast i razvoj trave. Analizirane krmne trave pokazuju zadovoljavajući kvalitet biomase sa stanovišta upotrebe vrednosti i akumulacije pojedinih teških metala, s obzirom na veoma stresne uslove gajenja na pepelištu termoelektrane.

## Zahvalnica

Rad je rezultat projekta TR 31016 „Unapređenje tehnologije gajenja krmnih biljaka na oranicama i travnjacima“ Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije.

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**SUMMARY****USABILITY VALUE AND HEAVY METALS ACCUMULATION IN FORAGE GRASSES GROWN ON POWER STATION ASH DEPOSIT**

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(Scientific paper)

The study of five forage grasses (*Lolium multiflorum*, *Festuca rubra*, *Festuca arundinacea*, *Arrhenatherum elatius* and *Dactylis glomerata*) was conducted on an uncontaminated cultivated land, of leached chernozem type, and on "Nikola Tesla A" (TENT A) thermal power station ash deposit. The concentrations of: As, Pb, Cd, Zn, Ni, Fe and Cu in grasses grown on two media were compared. Grass samples have been collected in tillering stage, when they were in full development. The aboveground biomass was cut in three replications during the vegetative period at about 3–5 cm height, imitating mowing and grazing. The concentrations of As and Ni were elevated in media samples collected from TENT A ash deposit, while the level of all studied elements in soil samples collected from cultivated land were within allowed limits. The variance of certain elements amounts in plant material collected from TENT A ash deposit was less homogeneous; the concentrations of As, Fe and Ni were higher in grasses collected from ash deposit, but Pb and Cu concentrations were higher in grasses grown on cultivated land. The concentrations of Zn were approximately the same in plants collected from the sites, whereas Cd concentrations were slightly increased in grasses grown on the ash deposit. In general, it can be concluded from the results of this study that the concentrations of heavy metals in plants collected from both sites do not exceed maximal tolerant levels for fodder. The use of grasses grown on ash deposit for forage production should be taken with reserve.

**Keywords:** Ash deposit • Heavy metals • Forage • Grasses



# Antioksidativna svojstva brašna od heljde – Doprinos funkcionalnosti pekarskih, testeničarskih i brašneno-konditorskih proizvoda

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## Izvod

Heljda se primarno uzgaja zbog zrna, od koga se, nakon ljuštenja, mlevenja i prosejavanja, proizvodi brašno od heljde, koga odlikuje zavidan sadržaj antioksidanata, pre svega polifenola i tokoferola. Visok sadržaj antioksidanata u zrnu heljde, pored drugih funkcionalnih komponenti, predstavlja osnov za korišćenje belog i integralnog heljdinog brašna kao substituenata pšeničnog brašna ili nekog drugog brašna od cerealija u formulacijama pekarskih, testeničarskih i brašneno-konditorskih proizvoda sa ciljem da se dobiju funkcionalni proizvodi sa dodatom vrednošću ili bezglutenski funkcionalni proizvodi. Tehnološki postupci proizvodnje navedenih grupa proizvoda i pojedini načini pripreme hrane utiču na sastav i sadržaj polifenola, pa posledično i na funkcionalnost finalnih proizvoda.

**Ključne reči:** belo i integralno heljokino brašno, antioksidanti, polifenoli, tokoferoli, proizvodi na bazi cerealija.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Heljda je pseudocerealija iz porodice Polygonaceae, koja se često izučava i poredi sa cerealijama zbog sličnosti u uzgajanju i primeni. Njena adaptibilnost uslovila je postojanje mnogih vrsta ove biljke širom sveta, od kojih je obična heljda (*Fagopyrum esculentum* Möench) najzastupljenija, sa 90% u ukupnoj svetskoj proizvodnji heljde, dok je tatarska ili gorka heljda (*Fagopyrum tartaricum*) prisutna uglavnom u planinskim regijama, pretežno na severnoj hemisferi [1–3].

Heljda se primarno uzgaja zbog zrna, od koga se, nakon ljuštenja, mlevenja i prosejavanja, proizvodi brašno od heljde, koje se širom sveta koristi u spravljanju mnogih tradicionalnih jela, od kojih su neka heljdopita, soba rezanci, beskvazni hleb *chapattis*, *pakora* i drugi [4]. Pored mogućnosti korišćenja brašna od heljde u proizvodnji pekarskih, testeničarskih i brašneno-konditorskih proizvoda, heljda pruža mogućnost za dobijanje niza proizvoda poput čaja od lista i cveta heljde [5], meda od heljde [6], klijanaca ili proizvoda sa klijancima heljde [7,8], hleba sa dodatkom lista heljde [9] i drugih.

Nutritivni profil oljuštenog zrna heljde varira u zavisnosti od vrste heljde, klimatskih uslova i uslova gajenja, ali ga, u proseku, karakteriše 55% skroba, 12% proteina, 4% lipida, 2% rastvorljivih ugljenih hidrata, 7% ukupnih prehrambenih vlakana, 2% pepela i 18% ostalih komponenti u koje se ubrajaju organske kiseline, polifenolna jedinjenja, tanini, nukleotidi i nukleinske kiseline [3,10]. Heljda ne sadrži gluten, što je čini siro-

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vinom podesnom u kreiranju bezglutenskih proizvoda, namenjenih obolelima od celjakije.

Nutritivni profil proteina heljde u poređenju sa žitaricama ocenjuje se kao visoko kvalitetan, s obzirom da ga odlikuje relativno visok sadržaj lizina [11,12]. Heljda sadrži visok nivo esencijalnih polinezasićenih masnih kiselina, kao što je linolna kiselina (18:2) [13–15]. Skrob i prehrambena vlakna su prisutni u sličnim količinama kao u žitaricama [16,17], dok je nivo rezistentnog skroba, D-*ribo*-inozitola i fagopiritola visok u zrnu heljde [18]. Ovu biljku karakteriše prisustvo nekoliko vitamina (B, C i E) [19], dok su minerali zastupljeni u izobilju [20]. S druge strane, heljda sadrži tanine, fitinsku kiselinu i inhibitore proteaza, koji mogu da prouzrokuju alergijske reakcije [19,21,22].

Interesovanje za uzgajanje heljde narasta, s obzirom na širinu palete proizvoda dobijenih korišćenjem ove biljne kulture, kao i porast broja informacija fokusiranih na njene funkcionalne karakteristike, koje obezbeđuju mnoge zdravstvene dobrobiti pri konzumirajući hrane koja sadrži heljdu, pre svega pri prevenciji i lečenju hroničnih bolesti [21].

Funkcionalna svojstva heljde obezbeđuju proteini i druge komponente ove biljne vrste, od kojih su svakako najdelotvorniji polifenoli, odnosno flavoni i flavonoidi, kao i tokoferoli, fitosteroli, fagopirini, tiamin-vezujući proteini i dijetna vlakna, prisutni kako u semunu tako i u drugim delovima biljke.

Proteine heljde odlikuju jedinstveni aminokiselinski sastav koji uslovljava specijalne biološke efekte, pre svega snižavanje nivoa holesterola, antihipertenzivno dejstvo i poboljšanje pri stanjima konstipacije i gojaznosti [21,23].

Kayashita i sar. [24] navode da se poboljšanje zdravstvenog stanja, primarno vezano za snižavanje nivoa

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holesterola i poboljšanja u digestiji, može pripisati delovanju dijetnih vlakana proizvoda od heljde u slučaju pacijenata koji su dugoročno orijentisani na hranu na bazi heljde.

Biološka i farmakološka svojstva heljde uslovljena su delovanjem polifenola, pre svega flavonoida heljde, koji ispoljavaju izrazita antioksidativna svojstva kao osnovu za farmakološke efekte [25]. Rutin, dominantni flavonoid heljde, ispoljava niz pozitivnih efekata na zdravlje, poput normalizacije povećane vaskularne permeabilnosti i fragilnosti, preventivnog delovanja u hipertenziji i kardiovaskularnim oboljenjima [26], kao i pozitivnog učinka u lečenju edema [5], hipotenzije [27] i inflamatornih stanja [28].

## ANTIOKSIDANTI HELJDE

### Polifenolna jedinjenja heljde

Heljda se može smatrati funkcionalnom sirovinom, ne samo zbog toga što sadrži visokovredne proteine, vitamine i prehrambena vlakna i ne sadrži gluten, već i zbog činjenice da obiluje antioksidantima [22]. Antioksidanti prisutni u zrnu heljde su tokoferoli i polifenolna jedinjenja i to flavan-3-oli, rutin, fenolne kiseline i njihovi derivati [25,29].

Polifenolna jedinjenja su supstance koje u strukturi imaju jedan ili više aromatičnih prstenova sa jednom ili više hidroksilnih grupa i obično se dele na fenolne kiseline, flavonoide, stilbene, kumarine i tanine [30].

Sadržaj polifenolnih jedinjenja u celom zrnu žitarica zavisi od vrste i sorte žitarica i dela zrna [31–34]. Ove supstance se uglavnom nalaze u obliku glikozida sa različitim šećernim ostacima ili u obliku kompleksa sa organskim kiselinama, aminima, lipidima, ugljenim hidra-

tima i drugim polifenolnim jedinjenjima. Polifenole žitarica primarno karakterišu fenolne kiseline [35,36].

Polifenole heljde reprezentuju kako fenolne kiseline [37], tako i flavonoidi [25,29,38]. Sadržaj flavonoida u heljadi zavisi od vrste heljde, lokaliteta i klimatskih uslova [39], čak i visinskih razlika na istom lokalitetu [40]. Na sadržaj ukupnih fenola, ukupnih flavonoida, kao i pojedinačnih polifenolnih jedinjenja heljde dominantno utiču uslovi gajenja i lokalitet, a najmanje sorta [2]. Oomah i Mazza [25] su na bazi rezultata četvoro-godišnjeg ogleda u kome su ispitali uticaj četiri sorte heljde, tri lokaliteta i sezone gajenja na sadržaj ukupnih flavonoida i rutina u semenu i ljusci ustanovili da je najveća varijacija u semenu izazvana razlikom u lokalitetu gajenja, a da je na sadržaj rutina u ljusci najviše uticala sezona gajenja. Dokazano je, takođe, da je sadržaj flavonoida direktno zavisao od UV zračenja i koncentracije ugljendioksida [41,42].

Obična heljda (*Fagopyrum esculentum* Möench), koja se najčešće koristi za proizvodnju belog i integralnog heljdinga brašna, znatno je siromašnija flavonoidima od tatarske heljde. Sadržaj ukupnih flavonoida u tatarskoj heljadi može iznositi i do 7%, mada retko, dok je njihov sadržaj u običnoj heljadi daleko niži (< 0,5%) [43].

Ispitujući sadržaj ukupnih flavonoida i rutina u 4 sorte *Fagopyrum esculentum*, 3 sorte *Fagopyrum homotropicum* i 4 sorte *Fagopyrum tataricum*, Jiang i sar. [44] su i ustanovili da se ispitane vrste heljde značajno razlikuju u sadržaju ukupnih flavonoida i rutina, dok su razlike između sorti iste vrste bile značajno izražene (tabela 1).

Sadržaj flavonoida u zrnu obične heljde zavisi od dela zrna. Stoga njihov sadržaj u oljuštenom zrnu

Tabela 1. Sadržaj ukupnih flavonoida i rutina u različitim vrstama heljde (%) [44]; vrednosti obeležene različitim slovima u istoj koloni su signifikantno različite kada se koristi Duncanov test višestrukih intervala ( $P < 0,05$ )

Table 1. Total flavonoids and rutin in different buckwheat species (%) [44]; values with different letters in the same column indicate significant difference when Duncan's multiple range test is used ( $P < 0,05$ )

Sorta/linija	Ukupni flavonoidi	Prosečna vrednost $\pm SD$	Rutin	Prosečna vrednost $\pm SD$
<i>F. esculentum</i>	–	0,037 $\pm$ 0,008 <sup>c</sup>	–	0,020 $\pm$ 0,004 <sup>c</sup>
Koto	0,042		0,017	
Mancan	0,044		0,025	
Manizoba	0,028		0,018	
Koban	0,033		0,020	
<i>F. homotropicum</i>	–	0,351 $\pm$ 0,061 <sup>b</sup>	–	0,101 $\pm$ 0,036 <sup>b</sup>
K980856	0,410		0,143	
K980855	0,354		0,088	
K980854	0,288		0,074	
<i>F. tataricum</i>	–	2,038 $\pm$ 0,083 <sup>a</sup>	–	1,669 $\pm$ 0,093 <sup>a</sup>
B930550	2,017		1,649	
B930586	2,153		1,795	
B930554	1,954		1,570	
B880276	2,028		1,664	

obične heljde iznosi oko 400 mg/100 g, odnosno oko 1300 mg/100 g u lјusci [25] ili može biti još niži [29,39]. Gallardo i sar. [45] navode da prerada, odnosno frakcionisanje zrna, značajno utiče na sastav polifenolnih jedinjenja finalnog mlinskog proizvoda. Tako, na primer, ispitujući distribuciju flavonoida u 16 frakcija heljdinog brašna dobijenih tokom mlevenja zrna heljde, Hung i Morita [46] zaključuju da se sadržaj flavonoida značajno smanjuje sa smanjenjem udela lјuske i spoljašnjih omotača zrna u frakciji mlevenja. Rezultati Oomah i Mazza [25], odnosno sadržaji flavonoida i rutina u semenu i lјusci četiri sorte heljde svedoče, takođe, o ustanovljenoj distribuciji (tabela 2).

Potpuniji uvid u distribuciju polifenolnih jedinjenja u zrnu heljde može se sagledati iz sadržaja polifenolnih jedinjenja u frakcijama mlevenja heljde prikazanih u tabeli 3 [10,47–51].

Osim flavonoida karakterističnih za heljdu, rutina i kvercetina, u svim frakcijama mlevenja heljde zabeleženo je prisustvo protokatehinske, siringinske, ferulne i sinapinske kiseljine, dok je vanilinska kiselina kvantifikovana samo u celom zrnu i lјusci (tabela 3). Najveći sadržaj svih identifikovanih jedinjenja, izuzev siringinske kiseljine, utvrđen je u lјusci heljde.

Iako su u zrnu i frakcijama mlevenja heljde identifikovane različite fenolne kiseljine, navodi o njihovom sadržaju variraju. Tako su, na primer, Hung i Morita [46] ustanovili da u različitim frakcijama heljdinog

brašna dobijenim tokom mlevenja zrna heljde dominira ferulna kiselina, dok su Alvarez-Jubete i sar. [52] utvrđili prisustvo siringinske i kafene kiseljine u zrnu heljde. Vanilinska, siringinska, ferulna i kumarinska kiselina su identifikovane u heljdinom grizu [53], a u aleuronском sloju heljdinog zrna, pored rutina i kvercetina, utvrđeno je prisustvo osam fenolnih kiselina: kafene, *o*-kumarinske, *p*-kumarinske, ferulne, galne, *p*-hidroksibenzoeve, siringinske i vanilinske kiseljine [54].

U flavonoidima heljde dominira rutin (slika 1). Dietrych-Szostak i Oleszek [39] su u celom zrnu obične heljde identifikovali šest flavonoida – rutin, orientin, viteksin, kvercetin, izoviteksin i izoorientin, dok su u oljuštenom zrnu utvrđili prisustvo rutina i izoviteksina, a u lјusci svih šest izolovanih flavonoida. Iako je heljda primarno bogata rutinom, u skupini njenih flavonoida značajno mesto pripada i kvercetinu (slika 1), hiperozidu (kvercetin 3-O-D-galaktozid) i kvercitrinu (kvercetin 3-O- $\alpha$ -L-ramnozid), kao i epikatehinu i već pomenjanim orientinu, viteksinu, izoviteksinu i izoorientinu [55,56]. Kvercetin i izokvercitrin su prekursori u biosintezi rutina, koji, najverovatnije, nastaje glikolizacijom kvercetina u položaju 3, praćenom ramnozilacijom izokvercitrina [57].

Danila i sar. [58] su, ispitujući heljdina brašna, ustanovili prisustvo katehina, epikatehina i epikatehin galata, dok su Verardo i sar. [59] identifikovali čak 30 polifenolnih jedinjenja u heljdinom brašnu, uključujući

Tabela 2. Sadržaj ukupnih flavonoida i rutina (mg/100 g) u semenu i lјusci heljde [25]; vrednosti obeležene različitim slovima u istoj koloni su signifikantno različite kada se koristi Duncanov test višestrukih intervala ( $P < 0,05$ )

Table 2. Total flavonoid and rutin contents (mg/100 g) of buckwheat seed and hulls [25]; values with different letters in the same column indicate significant difference when Duncan's multiple range test is used ( $P < 0,05$ )

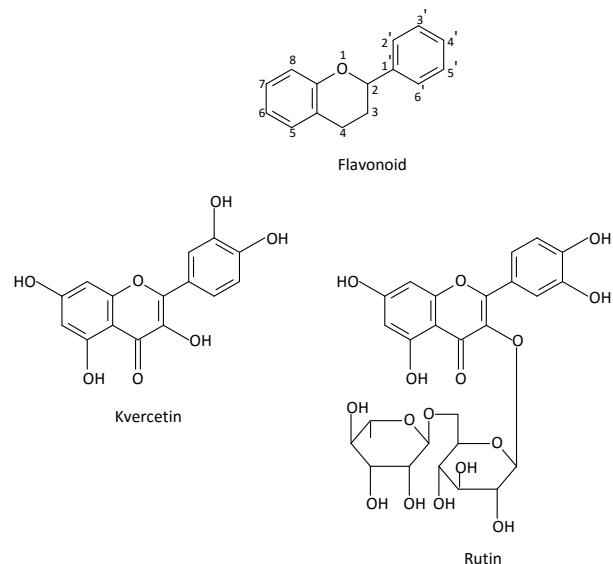
Sorta	Flavonoidi		Rutin		Udeo lјuske, %
	Seme	Lјuska	Seme	Lјuska	
AC Manisoba	372,8 <sup>b</sup>	1302,8 <sup>b</sup>	44,7 <sup>b</sup>	76,1 <sup>c</sup>	25,6 ± 2,7
BS85601	396,5 <sup>a</sup>	1277,7 <sup>b</sup>	46,4 <sup>b</sup>	85,3 <sup>b</sup>	25,7 ± 3,2
CM-15	371,5 <sup>b</sup>	1212,8 <sup>c</sup>	44,2 <sup>b</sup>	50,5 <sup>d</sup>	34,0 ± 2,1
Manor	407,5 <sup>a</sup>	1463,7 <sup>a</sup>	51,1 <sup>a</sup>	97,4 <sup>a</sup>	26,4 ± 3,0

Tabela 3. Polifenolna jedinjenja u frakcijama mlevenja heljde [10,48–51]; rezultati su prikazani kao srednja vrednost ± standardna devijacija ( $n = 3$ ); Vrednosti obeležene različitim slovima u istom redu su signifikantno različite ( $P < 0,05$ ); n.d. – nije detektovano

Table 3. Polyphenols in buckwheat milling fractions [10,48–51]; values are expressed as means ± standard deviation ( $n = 3$ ). Values with different letters in the same row are statistically different ( $P < 0,05$ ). n.d. – not detected

Polifenolna jedinjenja, µg/g	Celo zrno	Lјuska	Oljušteno zrno	Integralno heljdro brašno	Belo heljdro brašno
Protokatehinska kiselina	92,6 ± 0,94 <sup>c</sup>	168 ± 0,48 <sup>e</sup>	103 ± 1,21 <sup>d</sup>	78,0 ± 2,24 <sup>b</sup>	55,1 ± 0,56 <sup>a</sup>
Vanilinska kiselina	15,0 ± 0,46 <sup>a</sup>	37,1 ± 2,38 <sup>b</sup>	n.d.	n.d.	n.d.
Siringinska kiselina	52,3 ± 1,49 <sup>c</sup>	36,3 ± 1,6 <sup>a</sup>	63,5 ± 0,35 <sup>e</sup>	57,6 ± 2,03 <sup>d</sup>	42,4 ± 0,32 <sup>b</sup>
Ferulna kiselina	17,2 ± 0,13 <sup>a</sup>	17,5 ± 0,59 <sup>a</sup>	17,4 ± 0,21 <sup>a</sup>	14,4 ± 0,62 <sup>c</sup>	11,3 ± 0,13 <sup>b</sup>
Sinapinska kiselina	28,4 ± 1,68 <sup>a</sup>	29,8 ± 0,47 <sup>a</sup>	30,4 ± 3,44 <sup>a</sup>	18,8 ± 1,29 <sup>c</sup>	12,3 ± 0,26 <sup>b</sup>
Rutin	146 ± 4,56 <sup>c</sup>	225 ± 7,25 <sup>e</sup>	116 ± 0,26 <sup>b</sup>	179 ± 1,40 <sup>d</sup>	82,4 ± 0,76 <sup>a</sup>
Kvercetin	3,04 ± 0,14 <sup>ab</sup>	33,2 ± 1,74 <sup>d</sup>	2,48 ± 0,06 <sup>a</sup>	3,86 ± 0,40 <sup>b</sup>	1,35 ± 0,20 <sup>c</sup>
Ukupno	354,5	546,9	332,8	351,7	204,9
Ukupni flavonoidi, mg/100 g	0,07 ± 0,01 <sup>b</sup>	0,17 ± 0,02 <sup>c</sup>	0,06 ± 0,01 <sup>ab</sup>	0,06 ± 0,01 <sup>ab</sup>	0,04 ± 0,01 <sup>a</sup>

već navedena, ali i neka prvi put detektovana u ovoj vrsti brašna (2-hidroksi-3-O- $\beta$ -D-glukopiranozilbenzoeve kiseline, 1-O-kafeil-6-O- $\alpha$ -ramnopiranozil- $\beta$ -glukopiranozid i epikatehin-3-(3'-O-metil) galat). Sličan profil polifenolnih jedinjenja (28 identifikovanih) u heljdinim brašnima ustanovili su i Inglett i sar. [60], navodeći da neke flavonole i catechin glikozide nisu uspeli da identifikuju.



Slika 1. Strukture kvercetina i rutina.

Figure 1. Structures of quercetin and rutin.

Sadržaj rutina u zrnu heljde znatno varira u zavisnosti od vrste i sorte heljde, kao i od uslova okoline u kojima je biljka gajena [44]. Tatarska heljda može sadržati i do 100 puta veću količinu rutina od obične heljde [61]. Jiang i sar. [44] navode da je sadržaj rutina u tatarskoj heljadi 1,67%, dok je u običnoj heljadi niži – 0,02%, što je u saglasnosti sa sadržajem rutina u običnoj heljadi registrovanog od strane Dietrych-Szostak i Oleszek [39], koji iznosi 12,6–35,9 mg/100 g. Izuvez u heljadi, rutin nije detektovan u žitaricama i pseudocerealijama [62–64].

Ispitivanja zavisnosti hemijske strukture i antioksidativne aktivnosti pokazala su da su hidroksilna grupa u položaju 3 C prstena, 2,3 dvostruka veza u C prstenu i karbonilna grupa u položaju 4 C prstena odgovorni za antioksidativnu aktivnost flavonoida. Hidroksilne grupe u položajima 5 i 7 A prstena, kao i 3' i 4' B prstena doprinose, takođe, povećanju antioksidativne aktivnosti [44]. S obzirom na navedeno, rutin (3-O- $\beta$ -ramnoglukozidni oblik kvercetina) i kvercetin (3',4'-dihidroksiflavonol), polifenoli koji poseduju sve navedene strukturne karakteristike antioksidativno delotvornih flavonoida, spadaju u potentne antioksidante. Iako šećerni ostatak rutina smanjuje antioksidativnu aktivnost okolnih hidroksilnih grupa usled sternalih smetnji, on može biti hidrolizovan u svoj odgovarajući aglikon,

kvercetin, od strane mikroorganizama probavnog trakta, čime zadržava svoju *in vivo* aktivnost [65]. Potvrda razmatranja relacije hemijska struktura–antioksidativna aktivnost u slučaju antioksidativne aktivnosti rutina može se sresti u radovima Jiang i sar. [44], Yang i sar. [66], kao i Hsu i sar. [67], koji svedoče o izrazitoj anti-radikalскоj aktivnosti rutina na DPPH radikale (DPPH $^{\bullet}$ ). Kvercetin, jače nego rutin, deluje antioksidativno [68].

Rutin, osim antioksidativne aktivnosti, ispoljava i niz farmakoloških dejstava, kao što su antialergijsko [69], antiinflamatorno i antibakterijsko dejstvo [70]. Dokazano je, takođe, da rutin snižava krvni pritisak [71], smanjuje propustljivost krvnih sudova i rizik od ateroskleroze [72] i protektivno deluje u tretmanu gastričnih lezija indukovanih etanolom [73].

Kvercetin, jedan od najviše proučavanih flavonoida, pre svega zbog izražene antioksidativne aktivnosti, a potom i značajne apsorpcije u digestivnom traktu, drugi je značajan predstavnik antioksidativno delotvornih polifenola heljde. On, kao i ostali polifenoli, izuzetno moćno inhibira slobodnoradikalске procese u ćeliji, i to na tri različita nivoa: tokom faze inicijacije „hvata“ superoksid anjon radikale (O $_{2}^{\bullet}$ ) [74,75], tokom oksidacije lipida neutrališe dejstvo peroksil radikala [75,76] i inhibira stvaranje hidroksil radikala ( $^{\bullet}$ OH), verovatno heliranjem jona gvožđa [77]. Prepostavlja se da su biološki efekti kvercetina posledica njegovih antioksidativnih svojstava [78,79].

Iako se za većinu polifenola cerealija zna da egzistiraju u vezanom nerastvorljivom obliku (udeo u ukupnim fenolima > 97%) [31,32,80,81], relativno malo je poznato o oblicima polifenolnih jedinjenja u heljadi.

Guo i sar. [2] navode da najveći ideo ukupnih fenola (94–99%) i flavonoida (76–95%) tatarske heljde egzistira u slobodnom obliku, pri čemu su varijacije uslovljene sortom i lokalitetom (tabela 4). Ovakva distribucija značajno se razlikuje od one karakteristične za običnu heljadu, kod koje su, shodno rezultatima Hung i Morita [46], fenolna jedinjenja primarno zastupljena u slobodnoj formi, dok su flavonoidi u zrnu prisutni u nerastvorljivoj vezanoj formi (cca 50%), vezani za konstituente ćelijskog zida. Dominantnu zastupljenost slobodne forme ukupnih polifenola iz ljeske, makinja i brašna obične i tatarske heljde navode i Li i sar. [82], koja, bez obzira na vrstu heljde, iznosi 79,90, 92,89 i 94,07%, redom.

S obzirom na to da se obična heljda daleko najčešće koristi za proizvodnju heljdinog brašna, značajno je poznavati oblike u kojima egzistiraju polifenoli u heljdom brašnu. Stoga su Inglett i sar. [60] ispitali tri vrste brašna dostupna na američkom tržištu na sadržaje slobodnih i vezanih ukupnih fenola i flavonoida, poređivši njihovu distribuciju sa onom dobijenom za celo zrno heljde. Autori su ustanovili da ideo slobodnih polifenola varira u rasponu 48–64%, što je niže od već pri-

**Tabela 4.** Sadržaji ukupnih fenola i flavonoida tatarske heljde [2]; rezultati su prikazani kao srednja vrednost ± standardna devijacija ( $n = 3$ ); Vrednosti obeležene različitim slovima u istom redu su signifikantno različite ( $P < 0,05$ ); GAE – ekvivalenti galne kiseline; RE – ekvivalenti rutina; s.m. – suva materija

**Table 4.** Phenolic and flavonoid content of tartary buckwheat [2]; values are expressed as means ± standard deviation ( $n = 3$ ). Values with the different letters in the same row are statistically different ( $P < 0.05$ ). GAE – gallic acid equivalents; RE – rutin equivalents; s.m. – dry matter

Sorta	Lokalitet	Ukupni fenoli ( $\mu\text{mol GAE}/100 \text{ g s.m.}$ )			Ukupni flavonoidi ( $\mu\text{mol RE}/100 \text{ g s.m.}$ )		
		Slobodni	Vezani	Ukupni	Slobodni	Vezani	Ukupni
Xingku 2	Sichuan	9590 ± 428 <sup>a</sup>	71 ± 10 <sup>d</sup>	9660 ± 433 <sup>a</sup>	1980 ± 210 <sup>bc</sup>	97 ± 12 <sup>d</sup>	2077 ± 198 <sup>c</sup>
	Ningxia	8410 ± 621 <sup>b</sup>	353 ± 16 <sup>ab</sup>	8760 ± 614 <sup>abc</sup>	3014 ± 188 <sup>a</sup>	135 ± 23 <sup>d</sup>	3149 ± 187 <sup>a</sup>
	Gansu	4820 ± 260 <sup>d</sup>	333 ± 26 <sup>b</sup>	5150 ± 283 <sup>d</sup>	2161 ± 170 <sup>b</sup>	318 ± 14 <sup>c</sup>	2479 ± 157 <sup>bc</sup>
Diqing	Sichuan	7310 ± 412 <sup>c</sup>	394 ± 3 <sup>a</sup>	7700 ± 414 <sup>c</sup>	1719 ± 77 <sup>c</sup>	541 ± 5 <sup>a</sup>	2260 ± 81 <sup>bc</sup>
	Ningxia	8150 ± 337 <sup>bc</sup>	253 ± 13 <sup>c</sup>	8400 ± 342 <sup>bc</sup>	1871 ± 124 <sup>bc</sup>	593 ± 85 <sup>a</sup>	2464 ± 151 <sup>bc</sup>
	Gansu	8950 ± 138 <sup>ab</sup>	310 ± 20 <sup>b</sup>	9260 ± 118 <sup>ab</sup>	2109 ± 84 <sup>bc</sup>	425 ± 26 <sup>b</sup>	2534 ± 102 <sup>b</sup>

kazanih rezultata, pri čemu nisu ustanovili upečatljivu razliku između brašna i celog zrna heljde. Flavonoidi u brašnima egzistiraju u slobodnoj formi u rasponu 50–63%, dok su slabije zastupljeni u celom zrnu heljde (oko 40%).

#### Vitamin E

U antioksidante heljde, pored polifenola, spada i vitamin E, koji predstavlja opšti naziv koji se koristi za grupu od osam liposolubilnih antioksidanata koji se po strukturi dele na tokoferole ( $\alpha$ -tokoferol,  $\beta$ -tokoferol,  $\gamma$ -tokoferol i  $\delta$ -tokoferol) i tokotrienole ( $\alpha$ -tokotrienol,  $\beta$ -tokotrienol,  $\gamma$ -tokotrienol i  $\delta$ -tokotrienol).

Najvažnije uloge vitamina E su antoksidativna aktivnost, koja je posledica izrazite reaktivnosti tokoferola prema peroksil radikalima, perhidroksil radikalima i singletnom kiseoniku, kao i održavanje integriteta ćelijskih membrana.

Antioksidativna svojstva  $\alpha$ -tokoferola povezana su sa prevencijom kardiovaskularnih i neuroloških oboljenja, kancera i starenja [83]. Dokazano je, takođe, da vitamin E ima ulogu u funkcionisanju imunog sistema, u regeneraciji DNK i ostalim metaboličkim procesima [84].

Iz literature je poznato da su žitarice relativno dobar izvor vitamina E,  $\alpha$ -,  $\beta$ -,  $\gamma$ - i  $\delta$ -tokoferola i tokotrienola [32,85–88]. Tokoferoli su u zrnu primarno locirani u metabolički aktivnim delovima, u klici i aleuronskom sloju [89].

Prilikom obogaćivanja formulacija za pekarske, testeničarske i brašneno-konditorske proizvode, ukoliko je osnova formulacije pšenično brašno, dolazi do porasta sadržaja ukupnih tokoferola. Naime, prema navodima Mandić i sar. [10] i Sedej i sar. [48–50], sadržaj ukupnih tokoferola u frakcijama mlevenja pšenice kreće se u intervalu 1,13–19,1  $\mu\text{g/g}$ , dok se taj sadržaj u frakcijama mlevenja heljde kreće u intervalu 9,95–34,4  $\mu\text{g/g}$  [10,49,51] (tabela 5). Veći sadržaj ukupnih tokoferola ustanovljen je u frakcijama koje su bogatije spoljašnjim omotačima zrna [90]. Slične nivoje  $\alpha$ -tokoferola u celom zrnu pšenice (11,1  $\mu\text{g/g}$ ) i pšeničnom brašnu (7,8  $\mu\text{g/g}$ ) ustanovili su Engelsten i Hansen [89], dok su Moore i sar. [35] za sadržaj istog tokoferola u različitim sortama pšenice dobili vrednosti u opsegu 3,4–10,1  $\mu\text{g/g}$ . Više vrednosti sadržaja tokoferola u zrnu heljde u poređenju sa pšenicom ustanovili su i Alvarez-Jubete, Arendt i Gallagher [7] i Piironen, Syväoja, Varo, Salminen i Koivistoinen [90]. Razlike u navodima za sadržaj  $\alpha$ -,  $\gamma$ - i  $\delta$ -tokoferola u heljadi [14,86,91] posledica su različitog porekla i sorte heljde [92].

#### Antioksidativna aktivnost heljde

Prisustvo, odnosno delotvornost polifenola i tokoferola heljde obezbeđuje visok antioksidativni kapacitet proizvoda od heljde [2,10,39,48–51,93,94].

Tatarska heljda je značajno bogatija antioksidantima u poređenju sa običnom heljom, o čemu svedoči raz-

**Tabela 5.** Sastav tokoferola u frakcijama mlevenja heljde ( $\mu\text{g/g}$ ) [10]; rezultati su prikazani kao srednja vrednost ± standardna devijacija ( $n = 3$ ); vrednosti obeležene različitim slovima u istoj koloni su signifikantno različite ( $P < 0,05$ )

**Table 5.** Tocopherols in buckwheat milling fractions ( $\mu\text{g/g}$ ) [10]; values are expressed as means ± standard deviation ( $n = 3$ ). Values with the different letters in the same column are statistically different ( $P < 0.05$ )

Uzorak	$\alpha$ -Tokoferol	$\gamma$ -Tokoferol	$\delta$ -Tokoferol	Ukupno
Celo zrno	9,44 ± 0,92 <sup>c</sup>	10,1 ± 0,89 <sup>d</sup>	0,37 ± 0,05 <sup>ab</sup>	19,8
Ljuska	8,13 ± 1,07 <sup>b</sup>	1,36 ± 0,11 <sup>c</sup>	0,46 ± 0,01 <sup>ab</sup>	9,95
Oluštено zrno	3,32 ± 0,04 <sup>a</sup>	22,1 ± 0,2 <sup>ab</sup>	0,42 ± 0,03 <sup>ab</sup>	25,8
Integralno heljdino brašno	8,62 ± 0,97 <sup>b</sup>	25,3 ± 0,97 <sup>b</sup>	0,52 ± 0,06 <sup>b</sup>	34,4
Belo heljdino brašno	2,73 ± 0,05 <sup>a</sup>	17,2 ± 0,76 <sup>a</sup>	0,08 ± 0,11 <sup>a</sup>	20,0

lika u antioksidativnoj delotvornosti etanolnih ekstrakata zrna obične i tatarske heljde, uslovljena razlikama u sadržaju rutina i kvercetina, koju su registrovali Yao i sar. [95], primenjujući DPPH test. Stoga ekstrakti pojedinih sorti tatarske heljde ispoljavaju izrazitu antioksidativnu aktivnost, pre svega antiradikalnu aktivnost na DPPH<sup>•</sup> i ABTS<sup>•+</sup>, kao i sposobnost supresije oksidativne degradacije  $\beta$ -karotena u emulziji  $\beta$ -karoten-linolna kiselina [2]. Najizraženiju antioksidativnu aktivnost (antiradikalnu aktivnost na DPPH<sup>•</sup> i ABTS<sup>•+</sup> i redukcionu aktivnost na Fe<sup>2+</sup>) ispoljavaju ljska i grube mekinje tatarske heljde, dok su slabije antioksidativno delotvorne fine mekinje i belo brašno [96]. Antioksidativni potencijal tatarske heljde potvrđuju i Liu i sar. [8] i Li i sar. [61].

S obzirom da je obična heljda daleko zastupljenija u proizvodnji heljdinog brašna u poređenju sa tatarskom heljom, poznavanje njenog antioksidativnog potencijala predstavlja osnovu za unapređenje pekarskih, testeničarskih i brašneno-konditorskih proizvoda u kategoriju funkcionalne hrane.

Antioksidativna aktivnost frakcija mlevenja heljde, koja potvrđuje delotvornost identifikovanih polifenola i tokoferola u heljadi, određuje se, između ostalog, shodno uobičajenom protokolu za *in vitro* ispitivanje antioksidativne aktivnosti biljnih materijala, primenom indirektnih spektrofotometrijskih testova (antiradikalna aktivnost na DPPH<sup>•</sup>, redukciona aktivnost, antioksidativna aktivnost (AOA) u sistemu  $\beta$ -karoten-linolna kiselina i helataciona aktivnost na Fe<sup>2+</sup>).

Ispitivanje antioksidativne aktivnosti etanolnih ekstrakata frakcija mlevenja heljde primenom navedene serije testova rezultiralo je registrovanjem upečatljive antioksidativne potentnosti, pre svega ljske i frakcije koja sadrži delove ljske (integralno heljino brašno) (tabela 6) [48–51,97,98], što je u saglasnosti sa distribucijom polifenola u zrnu heljde [46].

Sagledavajući sve izračunate IC<sub>50</sub> vrednosti iz tabele 6, koje predstavljaju koncentraciju ekstrakta koja ostvaruje 50% inhibicije u ispitivanom modelu sistemu pojedinačnog testa, a u obrnutoj su srazmeri sa antioksidativnim potencijalom ekstrakta, može se uspostaviti sledeći redosled potentnosti: ljska >> celo zrno  $\geq$  integralno heljino brašno > oljušteno zrno > belo heljino

brašno [48,49,51,94]. IC<sub>50</sub> vrednosti pojedinačnih testova za frakcije mlevenja heljde znatno su niže od adekvatnih za frakcije mlevenja pšenice, što ukazuje na značajno viši antioksidativni kapacitet brašna od heljde u poređenju sa pšeničnim brašnima [48,49,51,99].

Visoku antiradikalnu aktivnost ekstrakata frakcija mlevenja heljde na DPPH<sup>•</sup> moguće je objasniti prisutstvom rutina, čija je visoka antiradikalna aktivnost na DPPH<sup>•</sup> ustanovljena od strane Afanas'ev i sar. [100] i Hsu i sar. [67]. Yang i sar. [66] su čak utvrdili veću efikasnost rutina u eliminisanju DPPH<sup>•</sup> u poređenju sa komercijalno dostupnim antioksidantom butilhidroksitoluolom (BHT). Izrazita antiradikalna potentnost rutina objašnjava najveću ustanovljenu antiradikalnu aktivnost na DPPH<sup>•</sup> ekstrakta heljde u poređenju sa ekstraktima pšenice, ječma i raži [101].

Značajno niže IC<sub>50</sub> vrednosti redukcionih aktivnosti ekstrakata frakcija mlevenja heljde (5–12 puta) u poređenju sa istim za frakcije mlevenja pšenice [34,48–51,98], koje ukazuju na znatno viši redukcionu kapacitet heljde, potvrđuju i rezultati Alvarez-Jubete i sar. [52], koji svedoče da je ekstrakt heljde mnogo potentniji redukcioni agens od ekstrakta pšenice.

Test u lipidnom sistemu  $\beta$ -karoten-linolna kiselina rezultira, takođe, značajno većom antioksidativnom aktivnošću registrovanom za ekstrakte frakcija mlevenja heljde u odnosu na ekstrakte frakcija mlevenja pšenice [48–50]. Prisustvo fenolnih kiselina u ekstraktima frakcija mlevenja pšenice, uključujući ferulnu, vanilinsku, *p*-kumarinsku, kafenu i hlorogensku kiselinu, odgovorno je za ostvarenje njihove antioksidativne aktivnosti [31,102,103], dok prisustvo rutina, kvercetina i drugih flavonoida, karakterističnih za zrno heljde, doprinosi antioksidatinoj aktivnosti ekstrakata frakcija mlevenja heljde, odnosno heljdnih brašna [39,67]. Tokoferoli, kvantifikovani u svim ispitanim frakcijama mlevenja heljde (tabela 5) [10,48–51], mogu, takođe, biti odgovorni za ustanovljene antioksidativne aktivnosti.

Helirajuće supstance mogu znatno inhibirati oksidaciju lipida u biološkim sistemima [104], te se, stoga, smatra da je helatacija jedan od vrlo značajnih mehanizama antioksidativne aktivnosti [105]. Ekstrakti frakcija mlevenja heljde ispoljavaju helatacionu aktivnost

Tabela 6. Antioksidativna aktivnost (IC<sub>50</sub> / mg mL<sup>-1</sup>) frakcija mlevenja heljde [10,48–51]; rezultati su prikazani kao srednja vrednost  $\pm$  standardna devijacija (n = 3); vrednosti obeležene različitim slovima u istom redu su signifikantno različite (P < 0,05)

Table 6. Antioxidant activity (IC<sub>50</sub> / mg mL<sup>-1</sup>) of buckwheat milling fractions [10,48–51]; values are expressed as means  $\pm$  standard deviation (n = 3). Values with the different letters in the same row are statistically different (P < 0.05)

Uzorak	DPPH <sup>•</sup>	Redukciona aktivnost	AOA	Helataciona aktivnost na Fe <sup>2+</sup>	•OH	O <sub>2</sub> <sup>•-</sup>
Celo zrno	1,37 $\pm$ 0,02 <sup>b</sup>	1,95 $\pm$ 0,05 <sup>e</sup>	3,78 $\pm$ 0,78 <sup>a</sup>	1,29 $\pm$ 0,00 <sup>a</sup>	0,64	0,16
Ljska	0,39 $\pm$ 0,02 <sup>a</sup>	0,69 $\pm$ 0,01 <sup>b</sup>	1,95 $\pm$ 0,02 <sup>b</sup>	0,92 $\pm$ 0,01 <sup>b</sup>	0,08	0,01
Oljušteno zrno	1,66 $\pm$ 0,01 <sup>d</sup>	2,52 $\pm$ 0,02 <sup>d</sup>	4,75 $\pm$ 0,11 <sup>a</sup>	1,37 $\pm$ 0,01 <sup>d</sup>	0,11	0,05
Integralno heljino brašno	1,49 $\pm$ 0,02 <sup>c</sup>	2,57 $\pm$ 0,01 <sup>a</sup>	6,42 $\pm$ 0,94 <sup>c</sup>	1,13 $\pm$ 0,02 <sup>c</sup>	0,20	0,17
Belo heljino brašno	1,87 $\pm$ 0,02 <sup>e</sup>	2,58 $\pm$ 0,01 <sup>a</sup>	7,56 $\pm$ 0,06 <sup>d</sup>	1,28 $\pm$ 0,01 <sup>a</sup>	0,29	0,06

na  $\text{Fe}^{2+}$  (tabela 6), ali je ona niža od helatacionog potencijala frakcija mlevenja pšenice [49,50,106–108], što je u saglasnosti sa rezultatima Gawlik-Dziki i sar. [9], koji su ustanovili da je obogaćivanje formulacije za pšenični hleb ekstraktom zelenih delova heljde praćeno porastom redupcionog kapaciteta i ukupne antioksidativne aktivnosti ispitivanih hlebova, dok je helatacionalna aktivnost na  $\text{Fe}^{2+}$  tih hlebova smanjena.

Reaktivne kiseonikove vrste, kao, na primer, hidroksil ( $\cdot\text{OH}$ ) i superoksid anjon ( $\text{O}_2^{\cdot-}$ ) radikali, predstavljaju inicijatore oksidativnog stresa, odnosno stanja organizma u kome je na ćelijskom nivou ravnoteža između oksidanata i antioksidanata narušena, te ono rezultira oštećenjem biomolekula, poput DNK, proteina, lipida i ugljenih hidrata [109]. Oksidativni stres se danas prepoznaje kao važan faktor u nizu ozbiljnih patoloških promena organizma i oboljenja, pre svega aterosklerozi, reumatoidnom artritisu, amiotrofnoj lateralnoj sklerozi, katarakti, neurodegenerativnim bolestima, kao što su Parkinsonova ili Alchajmerova demencija, u moždanom i srčanom infarktu, kardiompatijama, kanceru, kao i u samoj osnovi procesa stareњa. Stoga je izrazita antiradikalna aktivnost na  $\cdot\text{OH}$  i  $\text{O}_2^{\cdot-}$  heljdnih brašna, kao i ostalih frakcija mlevenja heljde (tabela 6), značajno viša od frakcija mlevenja pšenice (10 puta viša u slučaju  $\cdot\text{OH}$  i 2 puta viša u slučaju  $\text{O}_2^{\cdot-}$ ) [49–51], jedan od mogućih razloga za prolongiranje simptoma oksidativnog stresa kod potrošača koji se opredeljuju na ishranu bogatu proizvodima od heljde.

Efikasnost ekstrakata frakcija mlevenja heljde u slučaju stvaranja i transformacije  $\cdot\text{OH}$  (tabela 6) [49–51] u saglasnosti je sa delotvornošću ekstrakata integralnog heljdnog brašna koju beleže Zhang i sar. [110], dok je u slučaju stvaranja i transformacije  $\text{O}_2^{\cdot-}$  potentnost ekstrakta heljde, takođe, potvrđena [111].

Viši antioksidativni kapacitet frakcija mlevenja heljde u poređenju sa frakcijama mlevenja pšenice predstavlja osnovu za korišćenje heljdnog brašna kao supstituenta pšeničnog brašna u formulacijama za pekarske, testeničarske i brašneno-konditorske proizvode. Integralno heljokino brašno poseduje viši antioksidativni kapacitet u odnosu na belo heljokino brašno [49,93,108,111], jer su polifenolne komponente, pretežno zastupljene u slobodnoj formi, primarno locirane u omotaču i ljusci heljde [46]. Stoga se za postizanje viših nivoa funkcionalnosti proizvoda preporučuje upotreba integralnog heljdnog brašna pre nego belog ukoliko se takvo opredeljenje značajno ne odražava na narušavanje senzorske slike finalnog proizvoda [112–114].

#### **Proizvodi od heljde sa povećanim antioksidativnim kapacitetom**

Danas se sve češće u formulacijama za proizvodnju pekarskih, testeničarskih i brašneno-konditorskih proiz-

voda koriste brašna dobijena mlevenjem pseudocerealija (amarantus, heljda, kvinoa i sirak) ili nekog drugog biljnog materijala sa ciljem da se upotrebom alternativnih sirovina proizvede funkcionalan proizvod.

Jedan od razloga za korišćenje brašna od heljde kao supstituenta u formulacijama navedenih proizvoda je njegov izražen antioksidativni potencijal, značajno viši od antioksidativnog kapaciteta cerealija [22,115–118]. Korišćenjem heljdinog brašna postiže se razvoj grupe funkcionalnih proizvoda sa dodatom vrednošću, ali i razvoj bezglutenskih proizvoda kao specijalne kategorije funkcionalne hrane.

U tabeli 7 dat je prikaz nekih pekarskih, testeničarskih i brašneno-konditorskih proizvoda iz kategorije proizvoda sa dodatom vrednošću koji su proizvedeni upotrebom heljdinog brašna i za koje u navedenim literaturnim navodima postoje dokazi o povišenoj funkcionalnosti.

Heljrina brašna korišćena su i za oplemenjivanje, odnosno kreiranje funkcionalnih bezglutenskih pekarskih, testeničarskih i brašneno-konditorskih proizvoda (tabela 8).

#### **Uticaj termičkih tretmana na antioksidativni potencijal proizvoda**

Visok antioksidativni kapacitet, odnosno visok sadržaj polifenola i drugih antioksidativno delotvornih jedinjenja u heljdinom brašnu, obezbeđuje funkcionalnost proizvoda koji u svojoj formulaciji sadrže heljino brašno. Međutim, neka dosadašnja istraživanja ukazuju da uslovi tehnološkog postupka proizvodnje pekarskih, testeničarskih i brašneno-konditorskih proizvoda mogu da utiču na sastav i sadržaj polifenola na različite načine [143,144]. Najučestaliji su navodi o redukciji sadržaja polifenola tokom termičkih tretmana koji se primenjuju u proizvodnji ili pripremi navedenih grupa prehrabnenih proizvoda [39]. Stoga je važno imati saznanje o stepenu redukcije polifenola tokom postupka proizvodnje nekog proizvoda, kako bi se mogla proceniti njegova konačna funkcionalnost.

Sakač i sar. [99] navode da u procesu proizvodnje bezgluteneskog hleba na bazi pirinčanog i heljdinog brašna pečenje značajno utiče na profil polifenola, s obzirom da se sadržaj određenog rutina snižava u odnosu na njegovu kalkulativnu vrednost u slučajevima hleba sa 20 i 30% belog ili integralnog heljdnog brašna (do 40%), dok se sadržaj kvercetina višestruko uvećava. Ovakvo zapažanje može se smatrati primerom redukcije sadržaja polifenola izazvane termičkim tretmanom, koja je praćena registrovanim padom antioksidativne aktivnosti proizvedenih hlebova, određene primenom serije testova (antiradikalna aktivnost na DPPH $^{\bullet}$ , redukciona aktivnost, AOA u sistemu  $\beta$ -karoten/linolna kiselina i helatacionalna aktivnost na  $\text{Fe}^{2+}$ ) [99]. Ustanovljeno sniženje sadržaja rutina u bezglutenkim hlebovima praćeno je porastom sadržaja kvercetina, koji se,

*Tabela 7. Pregled nekih pekarskih, testeničarskih i brašneno-konditorskih proizvoda iz kategorije proizvoda sa dodatom vrednošću koji su proizvedeni upotrebom heljdinog brašna*

*Table 7. Bakery, pasta and confectionary products from the category of buckwheat-containing added value products*

Formulacija	Proizvod	Literaturni podatak
50% pšeničnog brašna supstituisano belim/integralnim heljdinim brašnom	Hleb	[115]
10–50% pšeničnog brašna supstituisano integralnim heljdinim brašnom	Hleb	[119]
15% pšeničnog brašna supstituisano belim/integralnim heljdinim brašnom	Hleb	[116]
15–50% pšeničnog brašna supstituisano heljdinim brašnom	Hleb	[120]
30–100% pšeničnog brašna supstituisano brašnom od tatarske heljde	Hleb	[121]
20–100% pšeničnog brašna supstituisano integralnim heljdinim brašnom	Tarana	[122]
70% pšeničnog brašna supstituisano integralnim heljdinim brašnom	Rezanci	[123]
40% pšeničnog brašna supstituisano heljdinim brašnom (8 različitih frakcija)	Rezanci	[124]
10–40% pšeničnog brašna supstituisano integralnim heljdinim brašnom	Rezanci	[125]
30–50% pšeničnog brašna supstituisano belim heljdinim brašnom	Testenina	[126]
50% pšeničnog brašna supstituisano heljdinim brašnom	Testenina	[120]
5–40% pšeničnog brašna supstituisano integralnim heljdinim brašnom	Eriste (turska testenina)	[127]
5–30% pšeničnog brašna supstituisano belim/integralnim heljdinim brašnom	Špageti	[128]
10–30% pšeničnog brašna supstituisano integralnim heljdinom brašnom	Špageti	[129]
20% integralnog pšeničnog brašna supstituisano integralnim heljdinom brašnom	Taljatele	[113]
100% pirinčanog brašna supstituisano ekspandiranim heljdinim grizom	Kolači	[130]
10–50% pšeničnog brašna supstituisano belim/integralnim heljdinim brašnom	Keks	[126]
30–50% pšeničnog brašna supstituisano belim/integralnim heljdinim brašnom	Medenjaci	[112]

*Tabela 8. Pregled nekih bezglutenskih pekarskih, testeničarskih i brašneno-konditorskih proizvoda koji su proizvedeni upotrebom heljdinog brašna*

*Table 8. Gluten-free bakery, pasta and confectionary buckwheat-containing products*

Formulacija	Proizvod	Literaturni podatak
Kukuruzni skrob 100 g, krompirov skrob 200 g, heljdino brašno 200 g	Bezglutenska smeša	[131]
Kukuruzni skrob 54%, tamno pirinčano brašno 25%, sojino brašno 12,5%, heljdino brašno 8,5%	Bezglutenska smeša za proizvodnju hleba	[117]
Pirinčano brašno 50%, heljdino brašno 50%	Bezglutenska smeša za proizvodnju hleba	[7,52,132]
Pirinčano brašno 70–90%, integralno/belo heljdino brašno 10–30%	Bezglutenska smeša za proizvodnju hleba	[99,133]
Komercijalna bezglutenska smeša za hleb 90–60%, heljdino brašno 10–40%	Bezglutenska smeša za proizvodnju hleba	[134]
Heljdino brašno 40%, pirinčano brašno 26,8%	Bezglutenka smeša za proizvodnju testenine	[135]
Komercijalna bezglutenska smeša za hleb 60%, heljdino brašno 40%	Bezglutenska smeša za proizvodnju hleba	[136]
Heljdino brašno 20–60%, brašno od amarantusa 15–25%, brašno od kinoe 15–50%	Bezglutenska smeša za proizvodnju testenine	[137]
Integralno heljdino brašno 100%	Špagete	[138]
Integralno heljdino brašno 40%, kukuruzni skrob 30% i pirinčano brašno 30% ili Integralno heljdino brašno 60%, kukuruzni skrob 20% i pirinčano brašno 20%	Tarana	[139]
Tamno pirinčano brašno 50%, kukuruzni skrob 30%, heljdino brašno 10%, pahuljice od prosa 10%	Bezglutenska smeša za proizvodnju biskvita	[140]
Heljdino brašno 25–75%, brašno od pasulja 25–75%	Bezglutenska smeša za proizvodnju biskvita	[141]
Heljdino brašno 130 g, krompirov skrob 70 g, kukuruzno brašno 60 g	Bezglutenska smeša za proizvodnju biskvita	[142]
Heljdino brašno 70%, kukuruzno brašno 30%	Bezglutenska smeša za krekere	[114]

najverovatnije, može pripisati hidrolizi rutina u kvercetin. Im i sar. [130] navode da vreme termičkog tretiranja najviše utiče na promenu sadržaja rutina u proiz-

vodima koji sadrže heljdino brašno, dok temperatura tretmana i sadržaj vlage ispoljavaju nešto slabiji uticaj.

Redukciju sadržaja polifenola tokom različitih termičkih tretmana registrovali su i drugi autori. Tako su, na primer, Zhang i sar. [110], ispitujući uticaj pečenja na brašno od tatarske heljde, registrovali pad u sadržaju polifenola, ali tek pri višoj temperaturi i dužem tretmanu ( $120^{\circ}\text{C}$ , 120 min), dok je tretman značajnije uticao na sadržaj flavonoida, i posledično, na ustavljene antiradikalne aktivnosti na  $\cdot\text{OH}$  i  $\text{O}_2^-$ , snižavajući ih. Slabije izražen pad u sadržaju ukupnih fenola u poređenju sa padom sadržaja flavonoidima autori pripisuju mogućem nastajanju proizvoda Maillard-ove reakcije, koji daju pozitivnu reakciju sa reagensom za određivanje ukupnih fenola i poseduju dokazano antioksidativno dejstvo [145]. Isti autori su zabeležili i pad u sadržaju polifenola i flavonoida, praćen padom antiradikalne aktivnosti na  $\cdot\text{OH}$  i  $\text{O}_2^-$ , u slučaju primene hidrotermičkog i mikrotalasnog tretmana na brašno od tatarske heljde. Poredeći ispitivane tretmane, autori navode da je najupečetljiviji pad u sadržaju polifenola ostvaren hidrotermičkim tretmanom.

Iako u literaturi dominiraju navodi o redukciji polifenola tokom termičkih tretmana, postoje i kontradiktorni rezultati. Tako su, na primer, Sensoy i sar. [146] došli do zaključka da pečenje belog i integralnog heljдиног brašna na  $200^{\circ}\text{C}$  tokom 10 min blago snižava sadržaj ukupnih fenola i antiradikalnu aktivnost na DPPH $^\bullet$ , dok ekstrudiranje na  $200^{\circ}\text{C}$  ne izaziva promene u sadržaju polifenola ovih brašna.

Interesantno je, takođe, da je zabeležen i gubitak rutina tokom mešenja testa, odnosno dodavanja vode heljđinom brašnu [147], kada se pod dejstvom rutin-degradirajućih enzima rutin konvertuje u kvercetin [148], koji je odgovoran za gorak ukus finalnog proizvoda. Usled ovakve konverzije zabeležen je dramatičan pad u sadržaju rutina (oko 69%) tokom proizvodnje heljđinih rezanaca [123]. Minimiziranje konverzije rutina u kvercetin u prisustvu vode postiže se prethodom hidrotermičkom obradom heljдинog brašna tokom koje dolazi do denaturacije rutin-degradirajućih enzima [149].

Proizvodnju špageta od integralnog heljдинog brašna karakteriše gubitak od 45,9% ukupnih slobodnih fenola, prouzrokovani njihovom degradacijom tokom mešenja testa, ekstruzije i sušenja špageta, pri čemu su stepeni redukcije za pojedinačne polifenole različiti (17,1–49,8%) [138]. Sadržaj vezanih formi polifenola špageta se, takođe, snižava za 59,4% tokom proizvodnje špageta. Priprema, odnosno kuvanje špageta, vodi dodatnim gubicima slobodnih (52,9%) i vezanih (53%) formi polifenola koji dospevaju u vodu od kuvanja.

## Zahvalnica

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## SUMMARY

### ANTIOXIDANT PROPERTIES OF BUCKWHEAT FLOURS AND THEIR CONTRIBUTION TO FUNCTIONALITY OF BAKERY, PASTA AND CONFECTIONARY PRODUCTS

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(Review paper)

Buckwheat is grown primarily due to its grain which, after undergoing the processes of dehulling, grinding and sieving, is used to produce buckwheat flour that is characterized by a considerable content of antioxidants, especially polyphenols and tocopherols. Buckwheat polyphenols are represented by phenolic acids and flavonoids, mainly rutin, a proven potent antioxidant. The content of polyphenols and tocopherols in buckwheat grain primarily depends on the buckwheat species, growing area, climate and growing conditions. Common buckwheat (*Fagopyrum esculentum* Möench), which is often used for the production of light and wholegrain buckwheat flour, contains less polyphenols than tartary buckwheat. The content of polyphenols in common buckwheat grain varies depending on the grain part. As their the largest amount is contained in the hull and the outer layers of the grain, the wholegrain buckwheat flour is superior in polyphenols than the light buckwheat flour. Therefore, the wholegrain buckwheat flour is characterized by a higher antioxidant capacity. Polyphenols in buckwheat flour exist in free and bound forms, where the contribution of free polyphenols ranges between 48–64%. Due to a relatively high content of antioxidants in light and wholegrain buckwheat flour, they are used for substitution of wheat or other cereal flours in bakery, pasta and confectionary formulations in order to create either added value or gluten-free products. The aim of a long-term consumption of buckwheat flours is to achieve health benefits and protect from many chronic diseases. Technological procedures and some treatments used during the food preparation influence polyphenol composition and content and consequently the functionality of food. Therefore, in order to minimize polyphenol losses and preserve the antioxidant capacity of the final products it is necessary to understand the thermal treatments and their mechanisms.

**Keywords:** Light and wholegrain buckwheat flour • Antioxidants • Polyphenols • Tocopherols • Cereal-based products



# Karboksilacija natrijum-2-naftoksida. Preispitivanje mehanizma pomoću meta-hibridnog funkcionala gustine

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## Izvod

Mehanizam reakcije karboksilacije natrijum-naftoksida (NaONaph) pomoću CO<sub>2</sub> u položajima 1, 3 i 6 ispitana je na nivou teorije M062X/LanL2DZ. Dobijeni rezultati se značajno razlikuju od rezultata dobijenih pomoću B3LYP metode. Nađeno je da reaktanti mogu da nagrade dva NaONaph-CO<sub>2</sub> kompleksa, od kojih se samo jedan transformiše do proizvoda. Svaki reakcioni put (1, 3 ili 6) odvija se preko dva prelazna stanja i jednog intermedijera. Put 6 je i kinetički i termodinamički nepovoljan, dok su putevi 1 i 3 kompetitivni: put 1 zahteva niže energije aktivacije, a put 3 vodi do najstabilnijeg proizvoda. Naši rezultati su u odličnoj saglasnosti sa eksperimentalnim rezultatima.

**Ključne reči:** Kolbe–Schmitt reakcija, funkcional M062X, reakcioni mehanizam, hidroksi-naftoeve kiseline.

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Kolbe–Schmitt reakcija je reakcija karboksilacije fenoksida i naftoksida alkalnih i zemnoalkalnih metala, u kojoj nastaju aromatične hidroksi kiseline [1–20]. Proizvodi Kolbe–Schmitt reakcije imaju značajnu industrijsku ulogu u sintezi brojnih jedinjenja kao što su farmaceutski preparati i aktivne supstance, antiseptici, fungicidi, insekticidi i razvijači boja, zatim u tekstilnoj industriji, proizvodnji poliestara i visoko polimerizovanih tečnih kristala, itd. [1,6,7].

Iako je Kolbe–Schmitt reakcija poznata više od 150 godina, sam mehanizam nije u potpunosti razjašnjen. Brojni istraživači su smatrali da reakcija karboksilacije započinje tako što se formira intermedijerni kompleks sa fenoksidom ili naftoksidom (MOPh-CO<sub>2</sub> ili MONaph-CO<sub>2</sub>, M označava metal). Prisustvo NaOPh-CO<sub>2</sub> kompleksa potvrđeno je pomoću IR apsorpcionih spektara [5], kao i pomoću FT-IR spektara i DTA analize [8].

Teorijska istraživanja mehanizma Kolbe–Schmitt reakcije fenoksida alkalnih metala potvrdila su nastanjanje intermedijernog MOPhCO<sub>2</sub> kompleksa [12–17]. Dokazano je da prinos para supstituisanog proizvoda raste sa povećanjem jonskog radiusa prisutnog alkalnog metala [13]. Ispitan je i uticaj rastvarača na kinetiku Kolbe–Schmitt-ove reakcije kod natrijum-fenoksida [14,15]. Svi ovi rezultati odnose se na monomolekulske sisteme, tj. u njima učestvuje kao reaktant samo jedan molekul fenoksida. Novija istraživanja su pokazala da reakcija može biti i bimolekulska sa dva molekula

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fenoskida, i to simetrična i asimetrična, pri čemu je pokazano da je asimetrični put povoljniji [20].

Natrijum-2-naftoksid (NaONaph) je još jedan zanimljiv reaktant u Kolbe–Schmitt reakciji, zato što pokazuje reaktivnost u položajima 1, 3 i 6 koji su međusobno konkurentni. Opšti pregled reakcije karboksilacije natrijum-2-naftoksida prikazan je na slici 1.

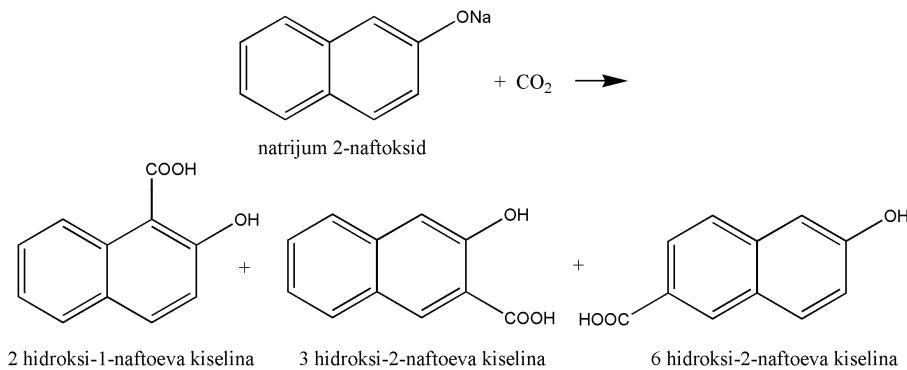
Između nekoliko ranih postulata o mehanizmu reakcije karboksilacije NaONaph, koje je Lindsey prikazao u revijalnom radu [6], ističemo rad u kome Hales smatra da reakcija CO<sub>2</sub> sa metalnim ariloksidom započinje slabim helatiranjem gasa sa metalom, pri čemu nastaje nestabilni kompleks, slično kao u reakciji karboksilacije fenoksida [5]. U novijim radovima je ispitana i uticaj različitih alkalnih metala, pritiska i temperature na distribuciju proizvoda karboksilacije naftoksida, pri čemu je nađeno da vrsta alkalnog metala bitno utiče na odnos dobijenih proizvoda [10]. Pokazalo se da je NaONaph potrebno zagrejati iznad 200 °C, pri čemu nastaje smesa 2-hidroksi-1-naftoeve kiseline, kao i 3- i 6- hidroksi-2-naftoevih kiselina (slika 1). Nedavna teorijska istraživanja reakcionog mehanizma karboksilacije NaONaph u položajima 1, 3 i 6 ponovo su potvrdila da reakcija teče preko intermedijernog NaONaph-CO<sub>2</sub> kompleksa [18,19]. Prema ovim istraživanjima ugljenik iz CO<sub>2</sub> vrši elektrofilni napad na naftalenski prsten u položaju 1, što vodi do obrazovanja natrijum-2-hidroksi-1-naftoata (E1). Iznenadujući je podatak da prelazna stanja za elektrofilne napade u položajima 3 i 6 nisu pronađena. Obrazovanje natrijum-3-hidroksi-2-naftoata (E3) je objašnjeno 1,3-premeštanjem CO<sub>2</sub>Na grupe, a obrazovanje natrijum-6-hidroksi-2-naftoata (E6) započinje elektrofilnim napadom na prsten u položaju 8, što je praćeno nizom uzastopnih premeštanja.

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Slika 1. Opšta šema reakcije karboksilacije natrijum-2-naftoksid-a.

Figure 1. General scheme for the carboxylation reaction of sodium 2-naphthoxide.

Sa druge strane, Rahim i sar. predložili su mehanizam direktnе karboksilacije koji podržavaju brojni eksperimenti [9–11]. Oni su potvrdili nastajanje intermedijnog kompleksa, koji, međutim, ne reaguje dalje do aromatičnih hidroksi kiselina, već se raspada do fenoksida ili naftoksida. Po njima, nastajanje MOPh-CO<sub>2</sub> ili MONaph-CO<sub>2</sub> kompleksa se takođe odvija, i kompetitivno je reakciji karboksilacije. Eksperimenti vršeni sa kompleksom KONaph-<sup>13</sup>CO<sub>2</sub>, koji je pripremljen korišćenjem ugljen-dioksida sa obeleženim <sup>13</sup>C, potvrdili su mehanizam direktnе karboksilacije. GC/MS analiza, kao i CP-MAS NMR spektar KOPh-<sup>13</sup>CO<sub>2</sub> kompleksa u saglasnosti su sa mehanizmom direktnе karboksilacije, jer su pokazali da se upotreboom kompleksa sa obeleženim <sup>13</sup>C atomom ne dobijaju hidroksikarboksilne kiseline sa obeleženim C-13, već nastaju one sa "normalnim" ugljen-dioksidom.

Dakle, do današnjih dana ostala je nerazjašnjena kontradiktornost između mehanizma direktne karboksilacije i mehanizma koji podrazumeva da reakcija započinje formiranjem MOPh-CO<sub>2</sub> ili MONaph-CO<sub>2</sub> kompleksa. Uz to, u teorijskim radovima koji podržavaju mehanizam zasnovan na MOPh-CO<sub>2</sub> ili MONaph-CO<sub>2</sub> kompleksu [12–19], pojavljuju se reakcioni koraci sa veoma visokom energijom aktivacije. Konačno, mehanizam karboksilacije NaONaph u položajima 3 [18] i 6 [19] koji se ne zasniva na elektrofilnom napadu ugljenika na C3 i C6, danas više nije ubedljiv. Važno je istaći da su svi teorijski radovi o Kolbe-Schmitt-ovoј reakciji izvedeni pomoću metode B3LYP [12–20]. Danas postoje mnogo sofisticiraniji funkcionali, kao što su meta-hibridni funkcionali, koji su tako parametrizovani da se uspešno primenjuju u ispitivanju termohemije i kinetike hemijskih reakcija [21,22]. Sve ove činjenice navele su nas na ponovno ispitivanje mehanizma reakcije karboksilacije NaONaph.

#### Metode B3LYP i M062X

Hibridni funkcionali izmene/korelacije konstruišu se kao linearna kombinacija egzaktnog funkcionala izmene definisanog u okviru Hartree-Fock-ove teorije i funkcio-

nala izmene i korelacije definisanih u okviru teorije funkcionala gustine (DFT). Hibridna energija izmene/korelacije može se predstaviti jednačinom (1):

$$E_{XC}^{\text{hibrid}} = c_{HF} E_X^{\text{HF}} + c_{DFT} E_{XC}^{\text{DFT}} \quad (1)$$

gde  $E_X^{\text{HF}}$  i  $E_{XC}^{\text{DFT}}$  predstavljaju nelokalnu Hartree-Fock energiju izmene i lokalnu DFT energiju izmene/korelacije, dok su  $c_{HF}$  i  $c_{DFT}$  konstante. Veoma često korišćena metoda B3LYP kombinuje Hartree-Fock funkcional izmene sa funkcionalima izmene  $E_X^{\text{LDA}}$  i  $E_C^{\text{B88}}$ , funkcionalima korelacije  $E_C^{\text{LYP}}$  i  $E_C^{\text{VWN}}$ , i funkcionalom izmene/korelacije  $E_{XC}^{\text{LDA}}$  [23]. Ovaj funkcional je uspešno primenjen u rešavanju mnogobrojnih hemijskih problema, ali je pokazao i neke nedostatke, kao što su potcenjivanje aktivacionih energija, nemogućnost da se opišu Van der Waals kompleksi, nepouzdanost rezultata vezanih za hemiju prelaznih metala, itd.

U teoriji meta-hibridnih funkcionala se pretpostavlja da lokalna energija zavisi ne samo od gustine elektrona i gradijenta redukovane gustine, već i od gustine kinetičke energije. Hibridna energija se može predstaviti jednačinom (2) [21]:

$$E_{XC}^{\text{hibrid}} = \frac{X}{100} E_X^{\text{HF}} + \left(1 - \frac{X}{100}\right) E_X^{\text{DFT}} + E_C^{\text{DFT}} \quad (2)$$

gde X predstavlja procenat Hartree-Fock-ove izmene u hibridnom funkcionalu, a  $E_X^{\text{DFT}}$  i  $E_C^{\text{DFT}}$  su lokalne DFT energije izmene i korelacije. Funkcional korelacije obuhvata samo dinamičku korelacionu energiju, dok je statička korelaciona energija uključena u funkcional izmene, pri čemu je Hartree-Fock-ova izmene promenljiva. Metoda M062X sadrži 54 % Hartree-Fock izmene. Funkcionali izmene i korelacije zasnivaju se na odgovarajućim funkcionalima M05 i VSXC. M062X ne sadrži članove za modeliranje nekovalentnih interakcija, ali je tako parametrizovan da dobro opisuje disperzione efekte na kratkim i srednjim međuatomskim rastojanjima ( $\leq 500$  pm). Ova osobina funkcionala M062X (kao i drugih metoda iz klase M06) ublažava jednu od najvećih mana tradicionalnih metoda funkcionala gustine.

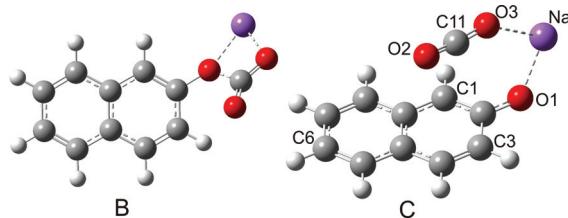
M062X se preporučuje za istraživanja hemijskih problema vezanih za sve elemente izuzev prelaznih metala, naročito onih slučajeva gde su od značaja i hemijska termodinamika i kinetika, kao i nevezivne interakcije [21,22].

## METODOLOGIJA IZRAČUNAVANJA

Sva izračunavanja su izvedena pomoću programskog paketa Gaussian 09 [24] koristeći metodu M062X. Imajući u vidu obimnost izračunavanja primjenjen je bazinski skup LanL2DZ, koji je i ranije korišćen u istraživanjima Kolbe–Schmitt-ove reakcije [12,13,17–19]. LanL2DZ je dvostruki zeta bazinski skup koji se u slučaju elemenata od vodonika do neon-a ne kombinuje sa pseudopotencijalima [25], a za elemente od natrijuma do lantana i od hafnijuma do bizmuta razvijeni su odgovarajući ECP (*effective core potential*) parametri [26–28]. Struktura u gasovitom stanju na 298 K svih učesnika u reakciji dobijena je optimizacijom geometrija bez ikakvih ograničenja. Priroda stacionarnih tačaka je određena izvođenjem frekvencionog računa: ravnotežne geometrije nemaju ni jednu imaginarnu vibraciju, dok prelazna stanja imaju tačno jednu imaginarnu vibraciju. Prelazna stanja su dodatno potvrđena izvođenjem IRC (*intrinsic reaction coordinates*) računa, da bi se potvrdilo da određeno prelazno stanje zaista povezuje dva odgovarajuća intermedijera.

## REZULTATI I DISKUSIJA

Naša istraživanja pomoću M062X metode pokazala su da NaONaph i CO<sub>2</sub> mogu da nagrade dva NaONaph-CO<sub>2</sub> intermedijera: B i C (slika 2). Formiranje oba intermedijera odigrava se spontano, uz stabilizaciju sistema od 56,8 kJ/mol u slučaju intermedijera B, odnosno 10,8 kJ/mol u slučaju intermedijera C.



Slika 2. Optimizovane geometrije dva moguća NaONaph-CO<sub>2</sub> intermedijera. Struktura C prikazuje način obeležavanja atoma.

Figure 2. Optimized geometries of two possible NaONaph-CO<sub>2</sub> intermediates. The atom labeling scheme is presented in the structure C.

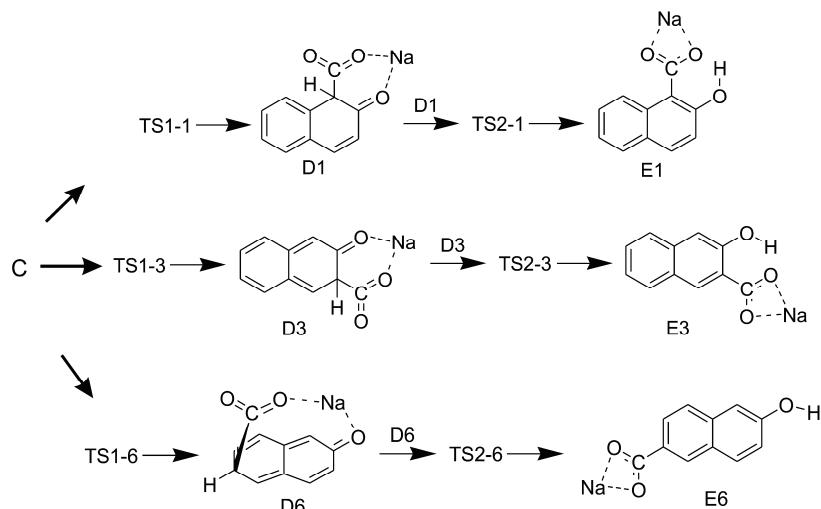
Intermedijer B se pojavljuje u starijim teorijskim radovima posvećenim mehanizmu Kolbe–Schmitt reakcije [12–19], dok kod intermedijera C ugljen-dioksidni C11 zauzima idealan položaj za elektrofilne napade u sva tri položaja (1, 3 i 6). ATP (atomic polar tensors)

parcijalna naelektrisanja na C1, C3, C6 i C11 iznose -0,452, -0,188, -0,210 i 1,330, i potvrđuju elektrofilnost ugljen-dioksidnog ugljenikovog atoma i nukleofilnost ugljenikovih atoma iz naftalenskog prstena. Osim toga ova struktura rezultira iz IRC računa za prelazno stanje TS1 u sva tri položaja. Ove činjenice ukazuju da C prikazuje strukturu polaznog NaONaph-CO<sub>2</sub> kompleksa koji se dalje transformiše do proizvoda karboksilacije. Ovakav zaključak je u saglasnosti sa rezultatima Rahim i sar. koji su pokazali da je obrazovanje intermedijera B kompetitivno reakciji karboksilacije [8,9]. Pošto se Kolbe–Schmitt reakcija odvija na povišenim temperaturama, nagrađeni kompleks B se raspada na polazna jedinjenja koja dalje podležu reakciji karboksilacije.

Mehanizam karboksilacije NaONaph, koji se zasniva na polaznom intermedijeru C, prikazan je na slici 3, dok su strukture prelaznih stanja date na slici 4. Međatombska rastojanja koja prikazuju evoluciju veza tokom reakcije data su u tabeli 1. Energetske promene tokom tri reakciona puta prikazane su u tabeli 2, i ilustrovane slikom 5. Relativne slobodne energije na slici 5 izračunate su u odnosu na ukupnu slobodnu energiju razdvojenih reaktanata.

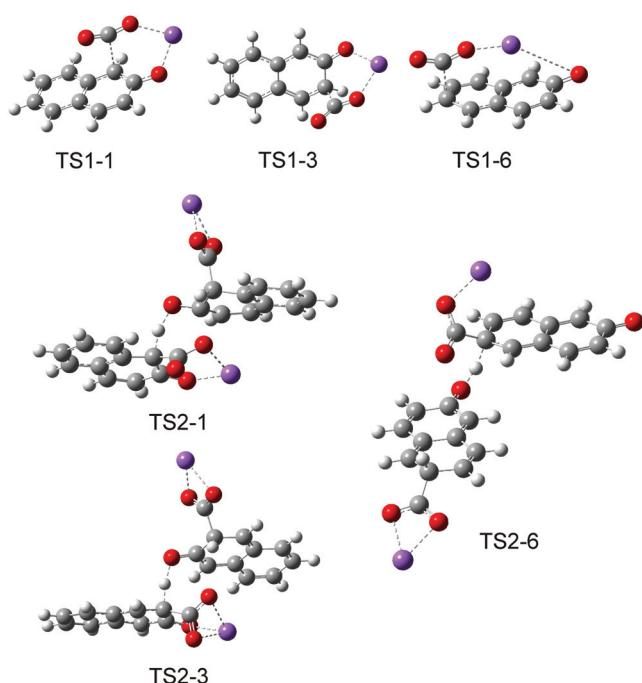
Prvi korak reakcije je elektrofilni napad C11 na C1, C3 ili C6. Pomoću metode M062X otkrivena su sva tri prelazna stanja: TS1-1, TS1-3 i TS1-6 (slika 4). Kod sva tri prelazna stanja stvara se nova C-C veza: C1–C11, C3–C11, odnosno C6–C11, što u daljem toku reakcije vodi do obrazovanja odgovarajućih intermedijera D1, D3 i D6 (tabela 1). Premda smo intermedijer C optimizovali i na nivou teorije B3LYP/LanL2DZ, naši brojni pokušaji da otkrijemo TS1-3 i TS1-6 ostali su, kao i u ranijim radovima [18,19], bezuspešni. Ovaj korak reakcije odlično prikazuje različitost u funkcionalisanju i superiornost metode M062X u odnosu na B3LYP. Obrazovanjem intermedijera D3 i D6 preko jednog prelaznog stanja izbegnut je niz premeštanja [18,19], što opis reakcionog mehanizma čini realističnim. I sama struktura intermedijera C optimizovnog na nivou teorije B3LYP/LanL2DZ ilustruje manjkavost metode B3LYP u opisu intermolekulskih interakcija, u ovom slučaju privlačenja između elektrofilnog C11 i ugljenikovih atoma u prstenu. Naime, rastojanja C1–C11, C3–C11 i C6–C11 značajno su duža (318,9, 440,8 i 610,1 pm, redom) od odgovarajućih rastojanja dobijenih pomoću modela M062X/LanL2DZ (tabela 1).

Što se tiče drugog koraka Kolbe–Schmitt reakcije ispitane su dve elementarne reakcije: intramolekulsko premeštanje protona susednog CO<sub>2</sub>Na grupi na O1, i intermolekulska razmena protona susednih CO<sub>2</sub>Na grupama (slika 3). Pronađena su prelazna stanja za intramolekulsko premeštanje protona sa C1 i C3 na O1. Ova prelazna stanja vrlo su slična već objavljenim prelaznim stanjima [18,19], i zahtevaju dosta visoke energije akti-



*Slika 3. Predloženi mehanizam za obrazovanje natrijum-2-hidroksi-1-naftoata (E1), natrijum-3-hidroksi-2-naftoata (E3) i natrijum-6-hidroksi-2-naftoata (E6). D1, D3 i D6 predstavljaju intermedijere a TS1 i TS2 prelazna stanja u položajima 1,3 i 6.*

*Figure 3. The proposed mechanism for the formation of sodium 2-hydroxy-1-naphthoate (E1), sodium 3-hydroxy-2-naphthoate (E3), and sodium 6-hydroxy-2-naphthoate (E6). D1, D3 and D6 represent intermediates, and TS1 and TS2 stand for transition states in the positions 1, 3 and 6.*



*Slika 4. Optimizovane geometrije prelaznih stanja u reakciji karboksilacije natrijum-2-naftoksidu u položajima 1, 3 i 6.*

*Figure 4. Optimized geometries of transition states in the carboxylation reaction of sodium 2-naphthoxide states in the positions 1, 3 and 6.*

vacije od 258,0 i 231,6 kJ/mol. Odgovarajuće prelazno stanje u položaju 6 nije pronađeno zbog veoma velike udaljenosti H6 od O1. Bimolekulska elementarna reakcija podrazumeva da dva D intermedijera prvo zauzmu pogodan položaj gradeći dimere 2D1, 2D3 i 2D6 (tabela 1) i zatim razmene protone susedne CO<sub>2</sub>Na grupama. Bimolekulska razmena protona odigrava se na sledeći način. Prvo dolazi do prelaza protona sa C1, C3 ili C6 na O1' preko prelaznih stanja TS2-1, TS2-3 ili TS2-6, pri

čemu nastaje protonovani i deprotonovani oblik intermedijera D. Zatim H1', H3' ili H6' protonovanog intermedijera D spontano prelazi na O1 deprotonovanog oblika. Na ovaj način nagrade se po dva molekula E1, E3 ili E6, koji su međusobno povezani elektrostatičkim i Van der Wals-ovim interakcijama.

Naša istraživanja su pokazala da svaki par intermedijera D može da razmeni protone. Na slici 6 prikazana su prelazna stanja i odgovarajući prethodni intermedi-

**Tabela 1.** Krucijalna međuatomska rastojanja (pm) u reakciji karboksilacije natrijum-2-naftoksidu u položajima 1, 3 i 6  
**Table 1.** Crucial interatomic distances (pm) in the carboxylation reactions in the positions 1, 3 and 6

Veza	C	TS1-1	D1	2D1	TS2-1	2E1	E1
C1–C11	291,0	245,1	165,3	153,8	152,2	148,9	148,5
C1'–C11'				153,7	153,8	149,4	
C1–H1	108,9	109,0	109,8	110,6	129,0		
H1–O1'				328,4	132,4		
O1–Na	208,0	210,2	221,5	242,9	220,0		
O2–Na				241,1	376,5	228,9	225,2
O3–Na	230,3	224,9	210,9	238,4	220,4	229,4	224,1
O1–H1						108,6	102,0
O1'–H1'						103,1	
O2'–Na'				226,0	225,5	231,0	
O3'–Na'				225,3	225,0	228,5	
	C	TS1-3	D3	2D3	TS2-3	2E3	E3
C3–C11	347,8	190,1	168,5	155,7	153,5	149,9	148,8
C3'–C11'				154,5	154,9	149,2	
C3–H3	108,6	109,0	110,0	111,0	129,3		
H3–O1'				226,8	135,3		
O1–Na	208,0	215,2	217,8	235,0	226,2		
O2–Na				242,7	248,4	233,4	226,3
O3–Na	230,3	217,3	212,6	245,7	235,8	231,6	225,0
O1–H1						102,1	100,4
O1'–H1'						106,4	
O2'–Na'				226,6	227,5	232,7	
O3'–Na'				225,5	226,2	230,0	
	C	TS1-6	D6	2D6	TS2-6	2E6	E6
C6–C11	540,9	201,5	167,1	163,6	154,4	148,6	149,6
C6'–C11'				154,1	154,6	148,6	
C6–H6	108,5	108,4	108,9	109,1	140,9		
H6–O1'				295,9	119,3		
O1–Na	208,0	381,2	419,8	480,4	701,9		
O2–Na						224,8	224,7
O3–Na	230,3	214,3	207,3	209,2	214,8	226,1	224,7
O1–H1						101,5	97,6
O1'–H1'						101,4	
O2'–Na'				226,5	227,5	226,5	
O3'–Na'				226,5	227,9	224,9	

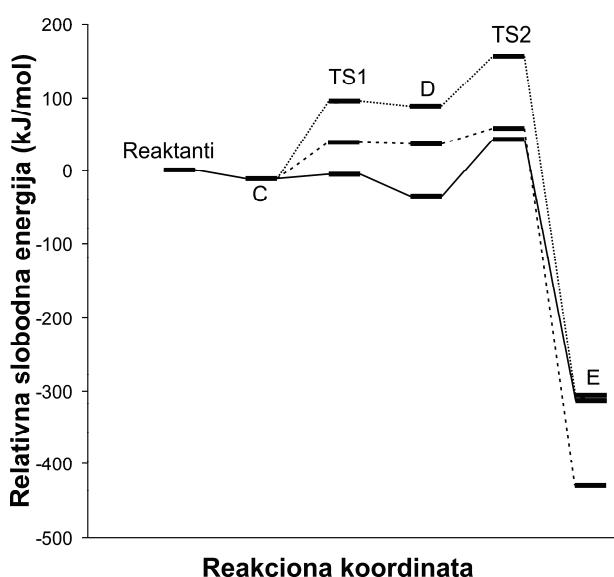
jeri za razmenu protona između neistoimenih intermedijera D. Izračunate slobodne energije aktivacije za reakciju između D1 i D3, D3 i D6, i D1 i D6 iznose 41,5, 24,7 i 67,8 kJ/mol, redom. Ove energije aktivacije po vrednosti su slične energetskim barijerama za razmenu protona između dva istoimena intermedijera D (tabela 2). Jasno je da ovakve reakcije vode do dva različita naftoata kao proizvoda reakcije. Važno je istaći da je bimolekulska reakcija kod sličnih sistema uspešno ispitana i pomoću metode B3LYP [20].

**Tabela 2.** Slobodne energije aktivacije ( $\Delta G_a^\ddagger$ ) i energije reakcije ( $\Delta G_r$ ) ispitanih reakcionih puteva. Energije su date u kJ/mol

**Table 2.** Free activation energies ( $\Delta G_a^\ddagger$ ) and reaction energies ( $\Delta G_r$ ) of the examined reaction pathways. The energies are given in kJ/mol

Položaj	$\Delta G_a^\ddagger$ (TS1)	$\Delta G_a^\ddagger$ (TS2)	$\Delta G_r$
1	6,1	76,5	-311,0
3	51,4	21,2	-429,3
6	105,0	67,3	-306,8

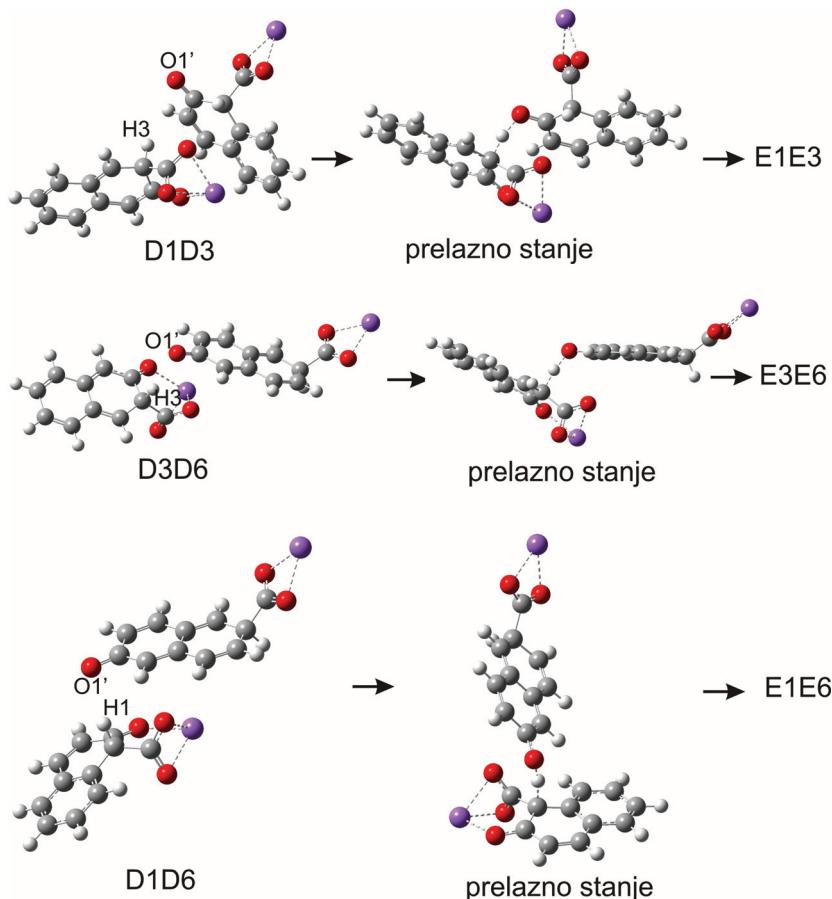
Tabela 2 i slika 5 pokazuju da je reakcija u položaju 6 i kinetički i termodinamički nepovoljna. Ovaj naš

**Reakcionala koordinata**

*Slika 5. Energetski profili tri ispitana reakciona puta. Puna, isprekidana i tačkasta linija predstavljaju reakcije karboksilacije u položajima 1, 3 i 6.*

*Figure 5. Energy profiles for three examined reaction pathways. Solid, dashed, and dotted lines represent the carboxylation reactions in the positions 1, 3 and 6.*

rezultat je u saglasnosti sa eksperimentima koji pokazuju da se natrijum-6-hidroksi-2-naftoat dobija u veoma malom prinosu. Sa druge strane, reakcije u položaju 1 i 3 su kompetitivne. Korak koji određuje brzinu reakcije u položaju 1 je obrazovanje TS2-1, a u položaju 3 to je TS1-3. Ako posmatramo samo vrednosti u tabeli 2 zaključujemo da je energija prelaznog stanja TS2-1 veća od energije prelaznog stanja TS1-3, međutim, grafik na slici 5 razjašnjava stvarno stanje. Naime, TS1-1 je rano prelazno stanje, a TS1-3 je kasno prelazno stanje. To se vidi iz tabele 1 po međuatomskim rastojanjima C1–C11 u TS1-1 i C3–C11 u TS1-3. Zato TS1-1 zahteva veoma malu energiju aktivacije, a energija intermedijera D3 samo malo je niža od energije prelaznog stanja. Iz ovoga proizilazi da se prelazno stanje TS2-3 nalazi na višem energetskom nivou u odnosu na prelazno stanje TS2-1. Može se, dakle, zaključiti da reakcija u položaju 1 zahteva niže energetske barijere. Sa druge strane, natrijum-3-hidroksi-2-naftoat je značajno stabilniji od proizvoda reakcije u položajima 6 i 1. I ovi rezultati su u saglasnosti sa eksperimentalnim podacima. Na veoma niskoj temperaturi od 293 K obrazuje se jedino natrijum-2-hidroksi-1-naftoat u veoma malom prinosu, dok prinos natri-

**Slika 6. Bimolekulska razmena protona između raznoimenih intermedijera D.****Figure 6. Bimolecular proton exchange between D intermediates of different types.**

jam-3-hidroksi-2-naftoata i natrijum-6-hidroksi-2-naftoata raste sa porastom temperature reakcije. Pošto se reakcija karboksilacije izvodi na relativno visokim temperaturama (oko 500 K) glavni proizvod reakcije je termodinamički najstabilniji natrijum-3-hidroksi-2-naftoat.

## ZAKLJUČAK

Na osnovu eksperimentalnih i teorijskih rezultata dašlo se do stanovišta da Kolbe–Schmitt reakcija započinje obrazovanjem MOPh-CO<sub>2</sub> ili MONaph-CO<sub>2</sub> kompleksa. Nasuprot ovakvog stanovišta, koje je postalo opšteprihvaćeno, je pretpostavka Rahim-a i Kosugi-ja sa saradnicima o direktnoj karboksilaciji. Naše istraživanje Kolbe–Schmitt-ove reakcije NaONaph izvedeno pomoću metode M062X pokazalo je da NaONaph i CO<sub>2</sub> mogu da nagrade dva NaONaph-CO<sub>2</sub> kompleksa: B i C, pri čemu se C dalje transformiše do reakcionih proizvoda preko tri reakciona puta: 1, 3 i 6, a B se na povišenoj temperaturi raspada. Sva tri reakciona puta odvijaju se preko dva prelazna stanja i jednog intermedijera. Podsetimo se da metoda B3LYP nije mogla da locira prvo prelazno stanje kod puteva 3 i 6, što je iziskivalo veliki broj premeštanja. Zahvaljujući sofisticiranoj parametrizaciji a i samom funkcionalnom obliku, metoda M062X je pokazala mnogo bolje performanse u modeliranju intermolekulskih interakcija. Reakcioni put 6 je i kinetički i termodinamički nepovoljan. Putevi 1 i 3 su kompetitivni, pri čemu put 3 zahteva više energije aktivacije ali vodi do stabilnijeg proizvoda. Dobijeni rezultati su u odličnoj saglasnosti sa eksperimentima Rahim-a i Kosugi-ja sa saradnicima.

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## SUMMARY

### CARBOXYLATION OF SODIUM 2-NAPHTHOXIDE. REINVESTIGATION OF THE MECHANISM BY MEANS OF A HYBRID META DENSITY FUNCTIONAL THEORY METHOD

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(Scientific paper)

Aromatic hydroxy acids, the compounds of large industrial importance, can be prepared in the Kolbe–Schmitt reaction, *i.e.*, a carboxylation reaction of alkali metal phenoxides (MOPh) and naphthoxides (MONaph). On the basis of the experimental results the two contradictory reaction mechanisms have been proposed: the one of direct carboxylation, and the other involving initial formation of the MOPh-CO<sub>2</sub> or MONaph-CO<sub>2</sub> complex. Previous theoretical investigations of the carboxylation reaction of sodium 2-naphthoxide, performed by means of the B3LYP method, confirmed the initial formation of the NaONaph-CO<sub>2</sub> complex, and showed that the carbon of the CO<sub>2</sub> moiety performs an electrophilic attack at C1 of the ring, leading to the formation of sodium 2-hydroxy-1-naphthoate (E1). Surprisingly, transition states for possible electrophilic attacks at C3 and C6 were not revealed, and the formation of other two products (E3 and E6) was explained by a number of consecutive rearrangements. In addition, this mechanism includes a reaction step with rather high activation energy. Since more sophisticated functionals are today available, the aim of this work is to reinvestigate the mechanism of the Kolbe–Schmitt reaction of NaONaph in all three positions (1, 3 and 6). Our investigations with the M062X method demonstrated that CO<sub>2</sub> and NaONaph can spontaneously build two complexes: B (the one previously reported) and C. While B cannot be further transformed to yield the reaction products, the CO<sub>2</sub> moiety in C takes perfect position for electrophilic attacks at all three sites of the ring. These attacks are realized *via* the transition states TS1, which lead to the formation of the new C-C bonds, and corresponding intermediates D. In the next, bimolecular reaction step two D intermediates exchange the protons adjacent to the CO<sub>2</sub> groups. These intermolecular reaction steps require significantly lower activation energies in comparison to the intramolecular proton shift from C to O. The carboxylation reaction in the position 6 is both kinetically and thermodynamically unfavourable, whereas the pathways in the positions 1 and 3 are competitive. Pathway 1 requires the lowest activation energies, but E3 is significantly more stable than other two products. In accordance with these findings are the experimental results which show that, at very low temperature (293 K) only E1 is formed at low yield, whereas the yields of E3 and E6 increase with the increasing temperature. Since the Kolbe–Schmitt reaction is experimentally performed at relatively high temperatures (around 500 K), the main product is thermodynamically most stable E6.

**Keywords:** Kolbe–Schmitt reaction • M062X functional • Reaction mechanism • Hydroxynaphthoic acids

# Razdvajanje industrijskog rasta od uticaja na životnu sredinu: Studija slučaja za sliv Južne Morave

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## Izvod

Predmet istraživanja u ovom radu je prikaz koncepta razdvajanja industrijskog rasta od uticaja na životnu sredinu kreiranjem i izračunavanjem odgovarajućih indikatora na primeru aglomeracija sliva Južne Morave za period koji obuhvata tri prethodne dekade. Vrednosti indikatora razdvajanja identifikuju najmanje razdvajanje u prvoj dekadi (1981–1990) koju karakteriše najveći porast fizičkog obima industrijske proizvodnje (*indexIND*) i najslabiji kvalitet vodotokova sliva ( $s_{SWQI_{rb}}$ ). Istraživanje potvrđuje značaj primene koncepta razdvajanja ekonomskog rasta od uticaja na životnu sredinu. Uzimajući u obzir veoma nizak nivo prečišćenih u odnosu na ukupne količine otpadnih voda, indikatori razdvajanja identifikuju izazove sa kojima ćemo se suočavati u daljem periodu. Indikatori razdvajanja služe donosiocima političkih odluka i stručnoj javnosti kao ključni alat za proveru uspešnosti politike zaštite vodnih resursa.

**Ključne reči:** razdvajanje, uticaj na životnu sredinu, industrijski rast, rečni sliv.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

U okviru domena održivog razvoja rasprava je osamdesetih godina prošlog veka započela pitanjem definicije i proširila se na određivanje indikatora kvalitativnih promena koje dovode do degradacije životne sredine, a rezultat su ekstenzivnog privrednog razvoja i neracionalne upotrebe prirodnih resursa. Završnim dokumentima sa konferencija o životnoj sredini i razvoju, Svetski samit o održivom razvoju RIO, RIO+10, RIO+20 održanih 1992, 2002 i 2012. godine, savremeni svet se opredelio za koncept održivog razvoja koji je usmeren ka očuvanju ekosistema i korišćenju prirodnih resursa u skladu sa ograničenjima prirode. Na osnovu dokumenta sa prve konferencije tokom devedesetih godina XX veka pojavili su se brojni programi koji su imali za cilj izradu kvalitativnih indikatora održivog razvoja. Opšte prihvaćeni pojam održivi razvoj je poseban razvojni koncept i zahteva kompozitne indikatore koji pokazuju uzročno-posledične veze između ekonomije, ekologije i društva. Ujedinjene nacije od 1996. godine objavljaju periodično vodič i metodologiju za izradu socioekonomskih i ekoloških indikatora pod nazivom *CSD Indicators of Sustainable Development* [1]. Očigledno je koncept ljudskog razvoja mnogo složeniji nego što se to može shvatiti iz bilo kog zbirnog ekonomskog indeksa ili iz detaljnih prikaza skupova socioekonomskih statističkih i ekoloških pokazatelja. Nova UNEP inicijativa ima za cilj da ukaže na značaj koncepta kojim se razdvajaju tendencije indikatora ekonomskog

rasta od indikatora upotrebe resursa i degradacije životne sredine [2]. Predmet istraživanja u ovom radu je koncept razdvajanja tendencija indikatora industrijskog rasta i indikatora kvaliteta životne sredine na primeru aglomeracija sliva Južne Morave za period koji obuhvata tri prethodne dekade. Cilj istraživanja je kreiranje indikatora razdvajanja koji će služiti donosiocima političkih odluka i stručnoj javnosti kao ključni alat za proveru uspešnosti politike zaštite vodnih resursa.

## Osnova koncepta razdvajanja u oblasti životne sredine

Korišćenje resursa ima više različitih potencijalnih uticaja na životnu sredinu. Sposobnost prirodnog okruženja da apsorbuje ostatke od upotrebe prirodnih resursa (emisija i otpad) je ograničena ekološkim kapacitetom. Do zagađenja životne sredine dolazi ako porast količine ostataka prevazilazi ekološki kapacitet. Trenutno ne postoji opšte prihvaćena standardizovana metodologija za upoređivanje različitih međusobnih uticaja. Štaviše, još uvek svi uticaji na životnu sredinu u potpunosti nisu istraženi a neke posledice eksploracije i upotrebe resursa uključuju mnoge složene interakcije u ekosistemu koje je teško kvantifikovati i modelirati. Životni ciklus materije (sirovine) podrazumeva nekoliko faz, od eksploracije preko tehnoloških procesa proizvodnje, korišćenja i odlaganja otpada od kojih sve faze imaju različite uticaje na životnu sredinu. Opšta iskustva govore da povećanje korišćenja resursa dovodi do povećanja negativnih uticaja na životnu sredinu, osim ako se odgovarajućim tehnološkim merama ne smanje uticaji, odnosno smanje uzročno-posledične veze između upotrebe resursa i uticaja na životnu sredinu. Jedan od ključnih ciljeva politike koja se odnosi na upravljanje i korišćenje prirodnih resursa je razdvajanje

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tendenčija indikatora ekonomskog rasta od indikatora kvaliteta životne sredine i indikatora korišćenja resursa (Slika 1) [3].

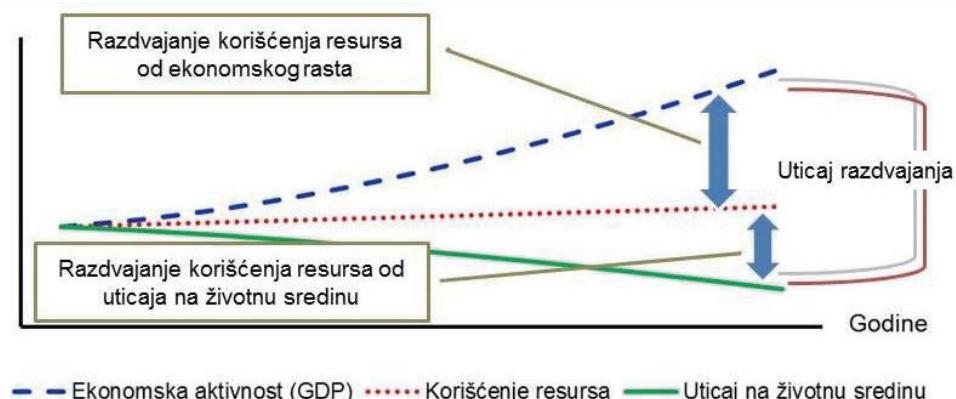
Gornja isprekidana linija dijagrama predstavlja ekonomski rast izražen kao povećanje BDP, srednja isprekidana linija predstavlja korišćenje resursa, dok donja puna linija pokazuje smanjenje uticaja na životnu sredinu tokom vremena. Kako strategija održivog razvoja ima za cilj smanjenje negativnih uticaja na životnu sredinu, koncept razdvajanja tendencija indikatora ekonomskog rasta od indikatora korišćenja resursa i uticaja na životnu sredinu postaje instrument za praćenje ostvarenja ciljeva strategije. Imajući u vidu sadašnji nivo ekonomskog rasta u razvijenom svetu verovatno je da će biti potrebno kombinovati dva različita procesa razdvajanja, tendencije indikatora korišćenja resursa od indikatora ekonomskog rasta i tendencija indikatora korišćenja resursa od indikatora uticaja na životnu sredinu, da bi se postiglo smanjenje uticaja na životnu sredinu. Grafički prikaz ilustruje kako se indikatori koji vrednuju ove procese mogu kombinovati sa ciljem da se prikažu uticaji. Izazov ovog koncepta nije teorijski pristup, već kreiranje indikatora koji vrednuju odgovore društva obuhvaćenih merama na zaštitu životne sredine.

Koncept razdvajanja otvara mnoga pitanja na temu da li postoji mogućnost dostizanja održivog stepena ekonomskog rasta uz istovremeno očuvanje visokih standarda kvaliteta životne sredine. Da li će svet moći da izdrži ekonomski razvoj bez iscrpljivanja resursa ili će doći do nepopravljive degradacije životne sredine? Koje su metode kvantifikacije odgovarajuće za razne tipove izvora zagađenja? Dosadašnja istraživanja u oblasti kontrole zagađenja, uglavnom, su bila usmerena na tačkaste izvore zagađenja, čija je lokacija poznata i lako uočljiva, a njihovo zagađenje se kvantifikuje rutinskim laboratorijskim postupcima. Tačkasti tipovi zagađenja, kao što su efluenti iz javnih kanalizacionih sistema i industrije, i iz uređaja za prečišćavanje komunalnih i industrijskih otpadnih voda, kao i drugi izvori koji direktno ispustaju zagađene otpadne vode u vodoprijemnike

predstavljaju izvore zagađenja koji su zakonski pokriveni obvezama kontrole i izveštavanja o uticaju svojih delatnosti na kvalitet životne sredine, ali i o ekonomskim pokazateljima poslovanja. Sa druge strane, difuzno zagađenje nastaje iz difuznih (netačkastih, rasutih) izvora koji ne potiču iz jedne tačke, već su prostorno široko rasprostranjeni. Difuzno zagađenje, može poticati iz istaloženih zagađujućih materija u sedimentu jezera, reka, močvara, i iz atmosferskih depozicija. Javlja se, takođe, u oticaju sa urbanim površinama (ulica, parking prostora, krovova, travnjaka, kućnih vrtova i bašti i sl.), u oticaju i procednim vodama sa gradilišta i deponija, poljoprivrednih površina, seoskih dvorišta, stočnih farmi, sa lokaliteta rudnika, i sa površinama gde se vrši rasad i odvija seča šuma. Difuzno zagađenje je po svojoj prirodi složeno i kvantifikacija izvora količine zagađenja koje prođe u određenom vremenskom intervalu kao posledica oticaja sa definisanog prostorno ograničenog područja podrazumeva kontinualno mereњe protoka i koncentracije zagađenja u vodotoku. Postoje različita ograničenja za ovakav pristup, a pre svih finansijska, ali i nedostatak ostalih informacija na ruralnom području. Zato se u savremenoj praksi pristupa modeliraju difuznog zagađenja kao načinu procenjivanja masenog protoka [4].

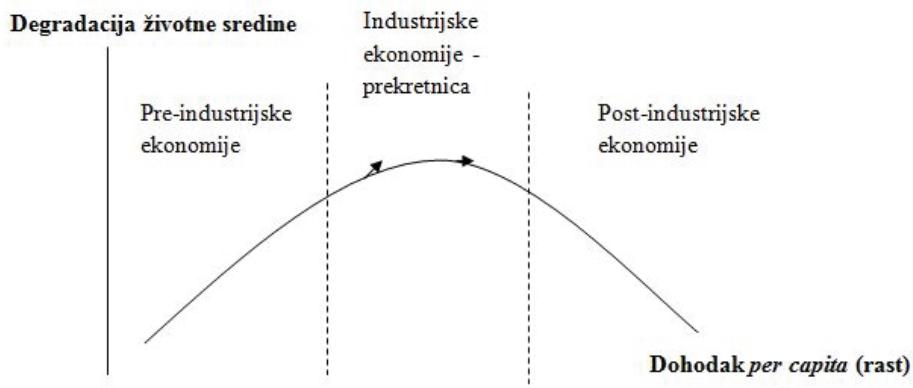
U setu otvorenih pitanja sledeće je, verovatno, najvažnije: Koji je odnos između zahteva stalnog povećanja dohotka i kvaliteta životne sredine? U tom smislu se istorija savremenog razvoja ljudskog društva, prema stepenu ekonomskog razvoja zemalja, može podeliti na pre-industrijske ekonomije, industrijske ekonomije i post-industrijske ekonomije (Slika 2).

Da li se degradacija životne sredine povećava ujednačeno, smanjuje ujednačeno ili prvo povećava pa onda smanjuje u toku razvoja jedne ili više zemalja, može se posmatrati na tzv. Kuznetsovovoj krivoj životne sredine (*Environmental Kuznets Curve*, EKC), koja se poklapa sa smerom krive industrijskog razvoja (Slika 2) [5]. Prema Kuznetsu, ekonomije u tranziciji nalaze se na levoj strani prekretnice EKC, to jest na uzlaznom delu



Slika 1. Razdvajanje ekonomskog rasta od korišćenja resursa i uticaja na životnu sredinu.

Figure 1. Decoupling of environmental impact of resource use from economic growth.



Slika 2. Kuznecova kriva životne sredine – odnos razvoja i životne sredine.

Figure 2. The Environmental Kuznets Curve - a development and environment relationship.

krive industrijskog rasta, gde se rast ostvaruje narušavanjem kvaliteta životne sredine. Suprotno tome, tržišno razvijene ekonomije se nalaze desno od prekretnice, na silaznom delu krive EKC. Međutim, moguće je i da zemlje koje ostvaruju niski dohodak smanje negativan uticaj na životnu sredinu uz ekonomski rast, ako uspeju da smanje zagađenje životne sredine istovremeno uz ekonomski rast. To može da bude ostvareno strukturalnim i tehnološkim promenama i promenom politike ili kombinacijom istih.

U klasičnoj ekonomskoj teoriji rasta, industrija se smatra vodećom oblašću privređivanja sa dominantnom ulogom u formiranju bruto domaćeg proizvoda (BDP) i rasta, učešću u proizvodnim fondovima, zaposlenosti i investicionim ulaganjima. Ocena dostignutog nivoa industrijske razvijenosti neke zemlje se tako bazira na komparativnoj analizi sintetskih pokazatelja koji ukazuju na stepen razvijenosti industrije, a definišu ih pokazatelji uspešnosti, kao što su: stepen industrijske zaposlenosti (nivo industrijalizacije), produktivnost rada, fizički obim proizvodnje, tehnička opremljenost rada, kao i niz proizvodnih faktora koji indirektno ukazuju na nivo razvijenosti. Jedna zemlja može da ima visok BDP koji se bazira na proizvodnji i uslugama koje se ne zasnivaju na prljavim tehnologijama i ne produkuju zagađenje, a sa druge strane zemlja sa nižim BDP može imati dominantnu industrijsku proizvodnju sa zastarem i „prljavom tehnologijom“. Kao grana privrede savremena industrijska proizvodnja je uglavnom materijalno-intenzivnog karaktera sa velikim utroškom sirovina, energije, vode i istovremeno najčešće prekomerne nekontrolisane emisije zagađujućih materija u vazduh, površinske i podzemne vode. Porast industrijske aktivnosti ne mora nužno da utiče na kvalitet vodoprijemnika ukoliko se primenjuju mere prečišćavanja izlivenih otpadnih voda, odnosno ukoliko je „odvojen“ uticaj na kvalitet vodotokova. Predmet istraživanja u ovom radu je prikaz koncepta razdvajanja tendencija industrijskog rasta i uticaja na kvalitet vodo-

tokova kreiranjem i izračunavanjem odgovarajućih indikatora. Primjenjen metodološki pristup treba da na najbolji način identificuje da li je industrijska aktivnost „odvojena“ od uticaja na kvalitet vodotokova kao prijemnika otpadnih voda iz aglomeracija sliva Južne Morave.

#### RAZDVAJANJE INDUSTRIJSKOG RASTA OD UTICAJA NA KVALITET VODOTOKOVA SLIVA JUŽNE MORAVE

##### Osnovne karakteristike industrijskog razvoja aglomeracija sliva Južne Morave

Industrijska struktura Srbije je uglavnom formirana u dolinama reka Dunava, Morave i Save, što predstavlja dunavsko-moravski razvojni pojas. Aglomeracije sliva Južne Morave obuhvataju tri glavna industrijska centra na osovini Niš–Leskovac–Vranje. Ovi industrijski centri su komunikaciono i infrastrukturno dobro povezani sa ostala dva manja centra Pirotom i Proklipljem, čineći razvojni „kostur“ privrednih delatnosti područja sliva Južne Morave. Industrijska struktura je koncentrisana oko razvoja nekoliko grana sa jasno izraženom podelom na funkcionalna područja koja su bliska administrativnim okruzima (Pčinjski, Jablanički, Nišavski, Toplički i Pirotski). U južnom delu prostora aglomeracija dominira pomoćna, tradicionalna i delom propulzivna industrija: tekstilna, metaloprerađivačka, drvna i prehrabrena industrija sa centrima Leskovcem i Vranjem. Severni deo je sa propulzivnom i pomoćnom industrijom i manjim brojem tradicionalnih industrija: elektronском, mašinogradnjom, tekstilnom, metaloprerađivačkom i proizvodnjom gume sa centrom u Nišu i dva manja centra u Pirotu i Prokliplu.

Prema pojedinim pokazateljima razvoja industrije, južni deo aglomeracije je u periodu od 1976. do 1990. godine bio industrijski najslabije razvijen u Srbiji sa stopom industrijskog rasta od 4,5%. U kraćem periodu analize od 1976. do 1985. godine industrijski rast je pokazivao 10,5% zadovoljavajućih razvojnih perfor-

mansi. Industrijski razvoj severnog dela sa centrom u Nišu, u periodu od 1976. do 1990. godine zasnivao se na niskim stopama ekonomske aktivnosti, tada merene rastom tzv. društvenog proizvoda od 5,2%. U periodu od 1976. do 1985. godine dinamika rasta industrijskog proizvodnje od 6,9% je bila ispod proseka industrije Srbije [6]. Tendencije industrijskog razvoja administrativnog područja sliva Južne Morave u prethodnim decenijama pokazuju da su rad i kapital kao proizvodni činioci tada ostvarili dominantan uticaj, dok je tehnički progres u velikoj meri izostao. To je delom posledica opadanja opšte produktivnosti proizvodnih faktora usled delovanja ekonomske recesije započete 1986. godine.

Na administrativnom području sliva Južne Morave industrija je uglavnom locirana u okviru planski određenih industrijskih zona ili na granici sa stambenim zonama i najčešće je povezana na komunalnu vodovodnu i kanalizacionu infrastrukturu. Uticaj industrijskih otpadnih voda je na taj način usmeren preko zajedničkog ispusta u najbliži vodoprijemnik predstavljajući najznačajnije zagađivače površinskih voda u kvalitativnom i kvantitativnom smislu. Prema stepenu doprinosa u zagađenju otpadnih voda stanovništvo je u ovom slučaju konstantna promenljiva u odnosu na industriju, jer promena broja stanovnika priključenih na opšti kanalizacioni sistem dovodi do neuporedivo manjeg uticaja na kvalitet ispuštenih otpadnih voda. Uz opšte karakteristike industrijske proizvodnje značajno je da je stepen prečišćavanja-predtretmana industrijskih otpadnih voda toliko minoran, da se može reći da faktički i ne postoji, i da se može samo govoriti o prečišćavanju komunalnih otpadnih voda. Raspored komunalnih postrojenja za prečišćavanje otpadnih voda (KPPOV) u slivu Južne Morave sa brojem stanovnika priključenih na njihove kanalizacione sisteme i ostvareni efekti prečišćavanja izraženi u ekvivalentnim stanovnicima (ES) daju sliku mera koje su preduzete na zaštitu voda. U slivu Južne Morave je u funkciji 6 KPPOV, i to: Bela Palanka (8.195 stan., 11.200 ES), Vlasotince (11.349 stan., 4.200 ES), Dimitrovgrad (6.759 stan., 5.320 ES), Medveđa (2.529 stan., 1.680 ES), Sokobanja (7.375 stan., 2.100 ES) i Surdulica (11.241 stan., 5.250 ES) [7].

#### Ocena kvaliteta vodotokova sliva Južne Morave

Za ocenu kvaliteta vodotokova sliva Južne Morave korišćen je indikator *Serbian Water Quality Index (SWQI)* koji je namenjen izveštavanju javnosti, stručnjaka i donosioca političkih odluka (lokalna samouprava, državni organi). Dosadašnja istraživanja i objav-

ljeni rezultati pokazuju da se primenom ove metode može dobiti sveobuhvatna predstava stanja kvaliteta površinskih voda sa analizom trenda [8]. Metodom *SWQI* deset odabranih parametara (zasićenost kiseonikom,  $BPK_5$ , amonijum ion, pH vrednost, oksidi azota, ortofosfati, suspendovne materije, temperatura, elektroprovodljivost i koliformne bakterije) svojim kvalitetom ( $q_i$ ) reprezentuju osobine površinskih voda svodeći ih na jedan indeksni broj. Udeo svakog od deset odabranih parametara na ukupni kvalitet vode nema isti relativni značaj, zato je svaki od njih dobio svoju težinu ( $w_i$ ) i broj bodova prema udelu u ugrožavanju kvaliteta. Sumiranjem proizvoda ( $q_i w_i$ ) dobija se indeks 100 kao idealan zbir udela kvaliteta svih parametara [9]. Kvalitet voda je na osnovu ove skale prema nameni i stepenu kvaliteta razvrstan u pet opisnih indikatora (Tabela 1).

Tabela 1. Numerički i opisni indikator kvaliteta površinskih voda Serbian Water Quality Index

Table 1. Numerical and descriptive indicator of surface water quality Serbian Water Quality Index

Numerički indikator	Opisni indikator	Boja
100–90	Odličan	
84–89	Veoma dobar	
72–83	Dobar	
39–71	Loš	
0–38	Veoma loš	

Kvalitet vode na pojedinačnim mernim stanicama reka u sливу je pod antropogenim uticajem, a zavisi i od hidroloških uslova. Kod izračunavanja srednje vrednosti indikatora *SWQI* rečnog sliva, za hidrološki ciklus (sezonski), potrebno je uzeti u obzir i uticaj proticaja na mernim stanicama sliva sa kojih se osrednjjava kvalitet (Slika 3).

Reka sa većim proticajem ima veći uticaj na opšti nivo kvaliteta celog sliva. Zbog toga je metodološkim pristupom usvojena metoda ponderisanja indikatora  $SWQI_{1-k}$  sa svake stanice, uzimajući u obzir proticaje sa svake pripadajuće merne stanice. Indikator  $SWQI_{1-k}$  za svaku mernu stanicu se ponderiše (vaga) ponderacionim faktorom proticaja, koji se dobija iz odnosa proticaja date merne stanice i odgovarajućeg proticaja na izlaznom profilu sliva. Na ovaj način se izračunavanjem srednje vrednosti kvaliteta vode na nivou sliva, metodom ponderisanja, kvalitetu vode dodeljuje proticaj kao odgovarajući faktor važnosti [10]. Formula za proračun indikatora  $SEASONAL SWQI_{RIVER BASIN (rb)}$  postupkom ponderisane aritmetičke sredine biće:

$$SEASONAL SWQI_{RIVER BASIN (rb)} = \frac{SWQI_1 \times \alpha_1 + SWQI_2 \times \alpha_2 + \dots + SWQI_k \times \alpha_k}{\alpha_1 + \alpha_2 + \dots + \alpha_k} = \frac{\sum_{i=1}^k SWQI_i \times \alpha_i}{\sum_{i=1}^k \alpha_i}$$



Slika 3. Aglomeracije sliva Južne Morave sa mernim stanicama za kontrolu površinskih voda.

Figure 3. The agglomeration of the Južna Morava basin with the measuring stations of surface water control.

gde je:  $sSWQI_{RB}$  sezonski indikator kvaliteta vode rečnog sliva,  $SWQI_{1-k}$  indikator kvaliteta vodotoka na mernim stanicama sliva, a  $\alpha_{1-k}$  ponderacioni faktor proticaja.

Ovako sračunat  $sSWQI_{RB}$  za ceo rečni sliv uzima u obzir odgovarajuće važnosti pojedinih proticaja na mernim stanicama, tako što proticaj kao relativni ponder pokazuje odgovarajući ideo pojedinačnog proticaja sa te merne stanice na kvalitet rečnog sliva kao celine. Za određivanje mogućeg trenda promene kvaliteta (rastući, opadajući ili beznačajan) korišćen je neparametrijski Mann-Kendall test. Ova metoda omogućuje testiranje hipoteze o postojanju trenda zajedno sa neparametrijskom Sen'S metodom za ocenu nagiba (intenziteta) trenda. Sen'S metodom za neparametrijsku ocenu nagiba izračunavaju se nagibi svih parova vremenskih tačaka, a zatim se prosek (medijana) ovih nagiba koristi kao ocena ukupnog nagiba. [11,12].

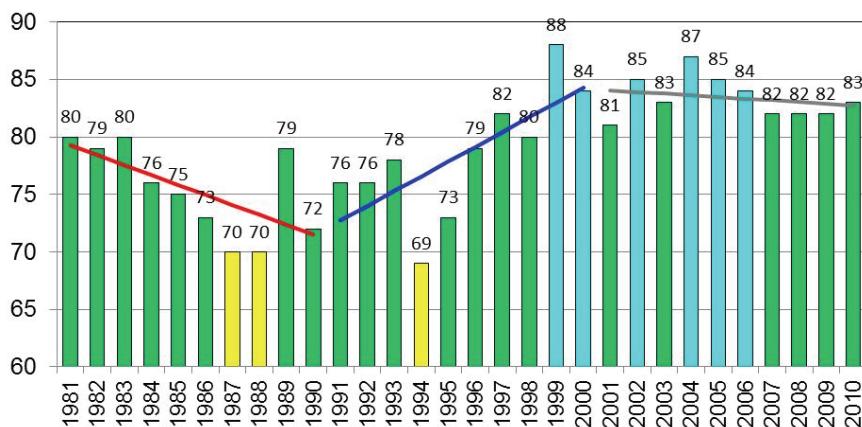
Istraživanje obuhvata analizu kvaliteta vodotokova sliva prema odgovarajućim parametrima metodom *Serbian Water Quality Index* izračunavanjem indikatora  $SWQI_{1-k}$  za svako merno mesto na mesečnom nivou [13]. Postupkom ponderisane aritmetičke sredine sračunat je sezonski indikator kvaliteta vode rečnog sliva

$sSWQI_{RB}$  za malovodni (jun-decembar), viševodni (januar-maj) i godišnji (januar-decembar) period. Rezultati istraživanja za godišnji period su prezentovani na histogramu (Slika 4).

Sezonski indikator  $sSWQI_{RB}$  za sliv Južne Morave pokazuje jasno izraženu promenu trenda kvaliteta vode po dekadama (Mann-Kendall test): osamdesete – opadajući, devedesete – rastući, prva dekada novog veka – beznačajan (Slika 4, Tabela 2).

## IDENTIFIKACIJA RAZDVAJANJA – REZULTATI I DISKUSIJA

Da bi se sagledao doprinos industrije razvoju privrede i njenom uticaju na kvalitet vodotokova u slivu, kao posledica izlivenih otpadnih voda, potrebno je izabrat ključni indikator efikasnosti razvoja industrije. Pojam efikasnosti razvoja industrije je kompleksan pojam koji definiše uspešnost u realizovanju niza zadataka: brzine i dinamike razvoja industrije, dinamike izmene njene strukture (od tradicionalne do napredne industrije), doprinosa industrije porastu nacionalnog dohotka, povećanje broja zaposlenih i promeni kvalifikacione strukture, dinamike porasta spoljno-trgovinske

Slika 4. Vrednosti  $sSWQI_{RB}$  – sezonskog indikatora kvaliteta slica Južne Morave.Figure 4. Values of  $sSWQI_{RB}$  – seasonal quality indicator of the Južna Morava basin.Tabela 2. Srednje vrednosti  $sSWQI_{RB}$  sezonskog indikatora kvaliteta vodotokova slica Južne MoraveTable 2. Mean values of  $sSWQI_{RB}$  seasonal quality indicator of the Južna Morava basin

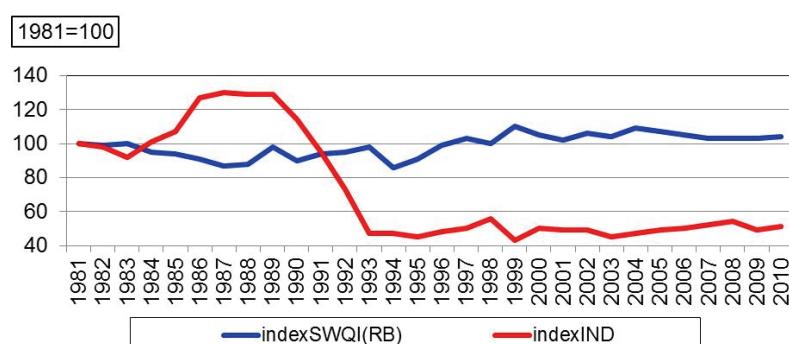
Period	Kvalitet vode			Trend kvaliteta (Mann-Kendall test)		
	Januar–maj	Jun–decembar	Januar–decembar	Januar–maj	Jun–decembar	Januar–decembar
	Viševodni	Malovodni	Godina	Viševodni	Malovodni	Godina
1981–1990	79	73	75	Opadajući	Opadajući	Opadajući
1991–2000	81	77	78	Rastući	Rastući	Rastući
2001–2010	86	82	83	Opadajući	Beznačajan	Beznačajan

razmene. Odgovarajući indikator uticaja industrijske aktivnosti na kvalitet vodoprijemnika je fizički obim industrijske proizvodnje jer u metodologiji izračunavanja objedinjuje fizičke jedinice proizvoda (težine, komade i dužine) ne uzimajući u obzir nekvalitet proizvoda i kasnije zalihu [14]. Za identifikovanje razdvajanja uticaja industrijskog rasta na stanje kvaliteta voda u slicu Južne Morave korišćen je metodološki postupak kreiranja i izračunavanja odgovarajućeg indikatora razdvajanja. Indikator razdvajanja je prema konceptu DPSIR framework kreiran iz odnosa dva indikatora, kvalitet površinskih voda  $sSWQI_{RB}$  je usvojen kao indikator stanja i fizički obim industrijske proizvodnje  $indexIND$  kao indikator pokretačkog faktora [15,16] (Slika 5). Indikatori su dati kao indeksi sa stalnom bazom

(1981=100). Zamena originalnih podataka indeksima sa stalnom bazom ne menja njihov pravac i meru i istovremeno daje jasnije poređenje nivoa pojave u različitim vremenskim intervalima, kao i lakše poređenje razvojnih tendencija posmatranih pojava naročito kad njihove merne jedinice nisu iste.

U našem slučaju indikator razdvajanja je sračunat iz odnosa indikatora stanja koji je dat indikatorom  $sSWQI_{RB}$  i indikatora pokretačkog faktora koji je dat indeksom fizičkog obima industrijske proizvodnje ( $indexIND$ ). Korišćenjem metodološkog pristupa numeričkih vrednosti indikatora mogu se sračunati dva parametra.

Prvi parametar je:



Slika 5. Vrednosti istraživanih indikatora za identifikaciju razdvajanja na slicu Južne Morave.

Figure 5. Values of indicators used for identifying separation in the Južna Morava basin

$$\text{Stepen razdvajanja (SR)} = \frac{sSWQI_{RB} \text{ kraj perioda} / indexIND \text{ kraj perioda}}{sSWQI_{RB} \text{ početak perioda} / indexIND \text{ početak perioda}}$$

koji pokazuje da li postoji razdvajanje u određenom intervalu vremena koji se odnose na početak i kraj perioda istraživanja. Ako je dobijena vrednost manja od 1 tada se odnos  $sSWQI_{RB}/indexIND$  smanjio tokom vremena i postoji razdvajanje. U slučaju da je odnos  $sSWQI_{RB}/indexIND \geq 1$  ne postoji razdvajanje.

Drugi parametar je:

$$\text{Faktor razdvajanja (FR)} = 1 - \text{Stepen razdvajanja (SR)}$$

koji razumljivije odražava intezitet razdvajanja. Naime kada je faktor razdvajanja  $\leq 0$  nema razdvajanja, a u intervalu (0,1) pokazuje intezitet razdvajanja koji je maksimalan kada je Faktor razdvajanja (FR) = 1. Na taj način ovaj parametar kvantitativno definiše uticaj preduzetih mera na zaštitu životne sredine.

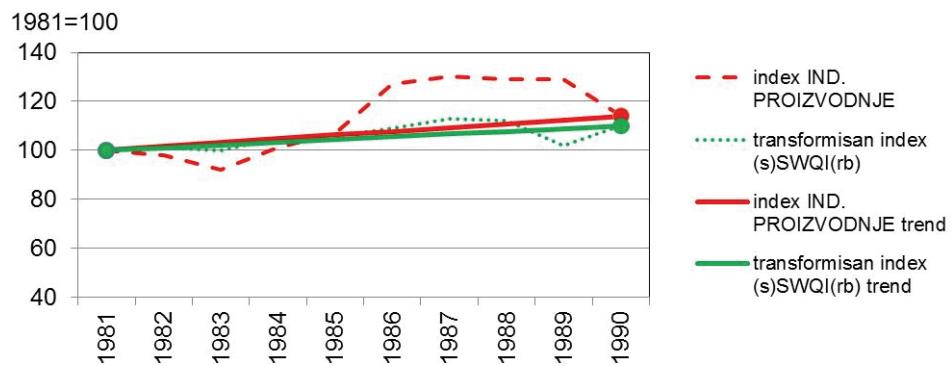
U našem primeru istraživanja razdvajanja industrijskog rasta od uticaja na kvalitet vodoprijemnika u slivu Juže Morave, odgovarajući indikator stanja kvaliteta je *Serbian Water Quality Index* čija se vrednost kreće u intervalu od 0 do 100 u smjeru poboljšanja kvaliteta koji prati veću vrednost indeksa. Ovo praktično znači da se, povećanjem emisije otpadnih voda u vodoanke, indikator pritiska (maseni protok zagađujućih materija u vodoanke) povećava, a vrednost indikatora

kvaliteta izražena  $sSWQI_{RB}$  indeksom se smanjuje što je u suprotnosti sa logikom formule razdvajanja. Da bi se u skladu sa konceptom razdvajanja korektno identificovalo postojanje razdvajanja indikator  $sSWQI_{RB}$  je transformisan suprotnim trendom i istim intezitetom tako što je promenjena orientacija, a odstojanje je ostalo isto u odnosu na vremensku osu. Struktura formule za stepen razdvajanja pokazuje da ovaj parametar zavisi samo od vrednosti na početku i na kraju analiziranog perioda i zato se u identifikaciji razdvajanja prikazane vrednosti indikatora na granicama dekada (slike 6–8, Tabela 3). U periodu 1981–1990 indeks industrijske proizvodnje je rastao intenzivnije nego indeks  $sSWQI_{(rb)}$  (Slika 6).

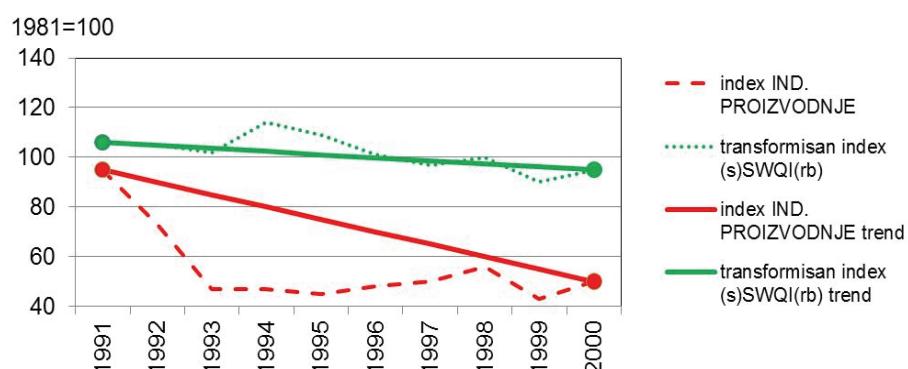
U periodu 1991–2000 indeks industrijske proizvodnje je opadao intenzivnije nego index  $sSWQI_{RB}$  (Slika 7).

U periodu 2001–2010 i indeks industrijske proizvodnje i index  $sSWQI_{RB}$  su neznatno rasli, ali je intezitet rasta industrijske proizvodnje bio nešto veći (Slika 8).

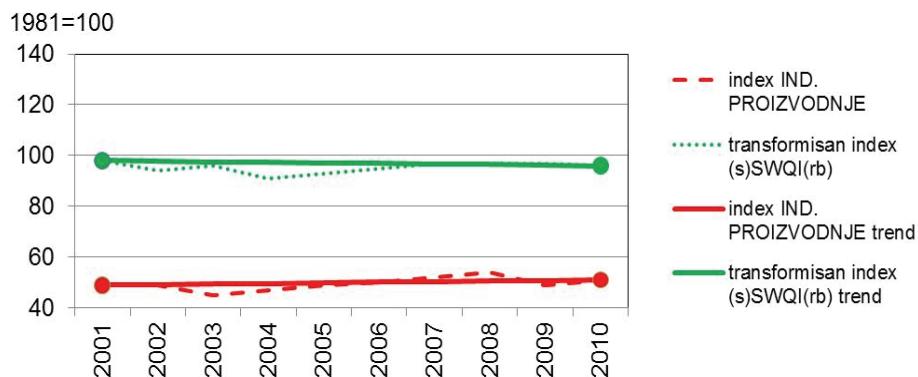
Analiza vrednosti parametara *stepena razdvajanja* i *faktora razdvajanja* za aglomeracije sliva Južne Morave pokazuju jasnu karakteristiku ekonomskih prilika koju identificuje pokretni faktor indeks industrijske proizvodnje *indexIND* i indikator stanja kvaliteta životne sre-



Slika 6. Identifikacija razdvajanja za period 1981–1990 na slivu Južne Morave  
Figure 6. Identification of separation for 1981–1990 in the Južna Morava basin.



Slika 7. Identifikacija razdvajanja za period 1991–2000 na slivu Južne Morave.  
Figure 7. Identification of separation for 1991–2000 in the Južna Morava basin.



Slika 8. Identifikacija razdvajanja za period 2001–2010 na slivu Južne Morave.  
Figure 8. Identification of separation for 2001–2010 in the Južna Morava basin.

dine koju reprezentuje  $s\text{SWQI}_{\text{RB}}$ . Industrijsku proizvodnju u prethodne tri dekade karakteriše razvojni trend koji pokazuje rast u prvoj dekadi (1981–1990) tako da je u poslednjoj godini serije fizički obim industrijske proizvodnje bio za 14% veći nego na početku dekade. Već početkom druge dekade započinje drastičan pad, tako da je fizički obim industrijske proizvodnje 1991. iznosio 95%, 1992. 73%, a 1993. svega 47% proizvodnje iz 1981. godine. U poslednjoj dekadi 2001–2010 fizički obim industrijske proizvodnje beleži blagi porast, ali generalno privredna aktivnost na području aglomeracija sliva Južne Morave je skromna, tako da je industrijska proizvodnja u granicama oko 50% obima iz 1981. godine (Slika 8). Uzimajući u obzir nizak nivo prečišćavanja otpadnih voda za aglomeracije sliva Južne Morave od oko 10% u odnosu na ukupne količine, koji se nije povećao u prethodne tri dekade, uticaj industrijske proizvodnje na kvalitet vodotokova je jasno definisan identifikacijom razdvajanja [17] (Tabela 3).

Tabela 3. Rezultati identifikacije razdvajanja po dekadama na slivu Južne Morave  
Table 3. Results of identification of separation, broken down by decades in the Južna Morava basin

Parametar	Period		
	1981–1990	1991–2000	2001–2010
Stepen razdvajanja	0.965	1.703	0.941
Faktor razdvajanja	0.035	-0.703	0.059

U dekadi 1981–1990 faktor razdvajanja i pored povećanog obima industrijske proizvodnje identificuje malo razdvajanje uticaja industrijskog rasta na kvalitet vodotokova sliva, jer je bio izražen uticaj emisija otpadnih voda. U dekadi 1991–2000 gde je drastično smanjen obim industrijske proizvodnje razdvajanje ne postoji jer je parametar faktor razdvajanja manji od 1 (-0,703). Kvalitet vode sliva se u tom periodu poboljšao ali mnogo manje nego što je opao obim industrijske proizvodnje. Blagi porast fizičkog obima industrijske proizvodnje i malo poboljšanje kvaliteta vode sliva tokom dekade 2001–2010 uzrokuju nešto veće razdv-

janje nego u dekadi 1981–1990 što ukazuje na poželjan trend uticaja industrijskog rasta na kvalitet vodotokova, ali je to samo posledica sporog rasta industrijske proizvodnje, a ne primenjenih mera na zaštitu vodotokova.

## ZAKLJUČAK

Istraživanje i analiza vrednosti indikatora razdvajanja za aglomeracije sliva Južne Morave jasno pokazuju karakteristike uticaja industrijskog rasta na kvalitet vodotokova sliva u prethodne tri decenije. Vrednosti parametara stepen razdvajanja i faktor razdvajanja identificuju najmanje razdvajanje u prvoj dekadi 1981–1990 koju karakteriše najveći porast fizičkog obima industrijske proizvodnje (*indexIND*) i najslabiji kvalitet vodotokova sliva ( $s\text{SWQI}_{\text{RB}}$ ). Poboljšanje kvaliteta vodotokova sliva u poslednjoj dekadi 2001–2010, koju karakteriše viša vrednost faktora razdvajanja, je posledica sporog rasta industrijske proizvodnje i velikog pozitivnog uticaja drastičkog pada ukupne ekonomске aktivnosti još iz druge dekade 1991–2000. Istraživanje potvrđuje značaj primene koncepta razdvajanja ekonomskog rasta od uticaja na životnu sredinu. Uzimajući u obzir veoma nizak nivo prečišćenih u odnosu na ukupne količine otpadnih voda u Srbiji, indikatori razdvajanja upućuju na izazove sa kojima ćemo se suočavati u daljem periodu. Indikatori razdvajanja mogli bi da posluže donosiocima političkih odluka i stručnoj javnosti kao ključni alat za proveru uspešnosti politike zaštite vodnih resursa.

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## SUMMARY

### DECOUPLING ENVIRONMENTAL IMPACTS FROM INDUSTRIAL GROWTH: CASE STUDY FOR SOUTH MORAVA RIVER BASIN

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(Professional paper)

The widely accepted term "sustainable development" is a comprehensive concept that requires multi-dimensional indicators showing links between economy, ecology and society. The concept of human development is obviously more complex than it could be understood from any aggregate economic index or from detailed sets of socio-economic statistical and ecological indicators. The research and analysis of the values of separation indicators for the Južna Morava basin agglomerations clearly show the impacts of industrial growth on the quality of the basin water bodies over the last three decades. Separation indicators have been derived from the statistical relationship between the situation indicators and drive indicators. The situation indicator  $sSWQI_{RB}$  was derived as a composite index from ten chosen parameters represented by their quality, the characteristics of surface water, by reducing it to one index number weighed from the interrelation between the discharge at a given measurement station and the discharge at the exit profile of the basin. The index of the physical volume of industrial production ( $indexIND$ ) has been accepted as the drive indicator. The indicators were calculated as a series of index numbers with 1981 as the base year. The values of separation indicators, *i.e.*, degree of separation and factor of separation show the least separation in the first decade (1981–1990) when the volume of industrial production ( $index IND$ ) increased the most and the quality of the basin water bodies was the poorest  $sSWQI_{RB}$ . The improvement of the quality of basin water bodies in the last decade (2001–2010), marked by a higher value of the separation factor is a result of a slow growth of industrial production and positive impacts of an abrupt fall of total economic activity occurring already in the second decade (1991–2000). The research has confirmed the importance of applying the concepts of separation of economic growth from environmental impacts. Taking into consideration very low quantities of treated waters as opposed to the total amount of waste waters in Serbia, the separation indicators pose challenges to be faced in the near future. Separation indicators will serve to decision makers and the expert community as a key toolkit for verifying the results of the water resources protection policy.

**Keywords:** Decoupling • Environmental impact • Industrial growth • R basin

# Evaluation of formulation and effects of process parameters on drug release and mechanical properties of tramadol hydrochloride sustained release matrix tablets

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## Abstract

This study investigates the use of high molecular weight polyethylene oxide (PEO WSR coagulant) for the preparation of sustained release matrix tablets containing high dose, highly water soluble drug, tramadol HCl. Proportion of PEO polymer, type of insoluble filler, proportion of tramadol HCl, amount of drug in tablet, tablet diameter and compression pressure were recognized as critical formulation and process parameters and their influence on drug release and tablet mechanical properties was evaluated. Percentages of tramadol HCl released after 30 and 240 min were selected for evaluation of drug release, while tensile strength was used as indicator of tablet mechanical properties. Only proportion of tramadol HCl exhibits statistically significant effect on percentages of tramadol HCl released after 30 and 240 min, with higher, wherein increasing of the tramadol HCl proportion increased its release rate among the evaluated variables in selected ranges. All of the investigated factors exhibit statistically significant effect on tablets tensile strength, with the largest influence of filler type. Tablets prepared with highly compressible filler (microcrystalline cellulose) exhibit higher tensile strength and therefore better mechanical properties to those prepared with partially pregelatinized starch (starch 1500).

**Keywords:** tramadol HCl, matrix tablets, PEO WSR coagulant, drug release, mechanical properties of tablets.

Available online at the Journal website: <http://www.ache.org.rs/HI/>

The usage of hydrophilic polymers, as the release rate control agents in matrix sustained release formulations, has been considered as the simplest and most cost-effective method for preparation of extended release solid dosage forms [1]. Numerous types of synthetic and natural polymers have been established for formulating hydrophilic matrix tablets, such as hypromellose, polyethylene oxide, hydroxypropylcellulose, etc. [2]. Polyethylene oxides (PEOs) are class of hydrophilic nonionic linear polymers, commercially available in variable grades, with average molecular weight in range from 100000 to 8000000. They were introduced as alternative to cellulose derivatives, the most commonly used polymers in the formulation of extended release matrix tablets [3,4]. There are several important features of PEO polymers that favor their wide application in the formulation of hydrophilic extended release matrix tablets, such as their physical and chemical stability, non-toxicity, insensitivity to the pH of the physiological fluids, good compressibility and high swelling ability [5]. In the previous studies, the high

molecular weight PEOs have been successfully used in the preparation of sustained release matrix tablets by direct compression, containing either water soluble or insoluble drug [6,7]. Once in contact with water, the PEO matrix starts to swell due to formation of hydrogen bonds between polymeric chains and water. Formation of the hydrophilic gel layer is responsible for prolonged drug release [8]. Basic processes, which determine drug release from hydrophilic matrix tablets, are drug diffusion through the gel layer as well as erosion of the swollen gel layer [9]. Solubility of the drug has profound influence on the mechanism that determined drug release from matrix and should be considered in selecting proper type and grade of matrix polymer. Release of highly water soluble drug from hydrophilic matrix system is mainly controlled by the diffusion through the swollen gel layer, whereas release of poorly soluble drug is dominantly controlled by the polymer erosion (relaxation) [10]. Tablet geometry has also profound influence on the drug release from diffusion-controlled systems and this has been studied in details by Siepmann *et al.* [11]. Thus, the effect of a tablet surface/tablet volume ratio (SA/Vol) on the drug release from hydrophilic matrices was investigated for hypromellose matrix tablets [12] and for hydroxypropylcellulose matrix tablets [13].

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In formulation of hydrophilic matrix tablets with high-dose, highly soluble drug, the two important issues, regarding selecting of proper matrix polymer, should be considered: the capability of polymer to prevent premature drug release, as well as ability to sustain drug release with predictable kinetics. Another important characteristic of matrix tablets should be acceptable mechanical properties in order to maintain tablet integrity during further processing, such as packaging and coating and application by the patient. Mechanical properties of powder mixtures and compacts can be assessed through evaluation of compressibility (solid fraction vs. compaction pressure), compactibility (tensile strength vs. solid fraction) and tabletability (tensile strength vs. compaction pressure). Yang *et al.* have proved that under applied compression pressure the consolidation mechanism of PEO follows plastic deformation. However, characteristic viscoelastic behavior of PEOs with large axial expansion after compression results in production of low tensile strength tablets. According to these results it was suggested that PEO polymers should be used with highly compactible excipients [14]. Comparison of the drug release, as well as mechanical characteristics between hypromellose 100000 mPa s and different high viscosity PEOs (Polyox<sup>TM</sup> WSR 1105, 301 and 303), was performed on matrix tablets containing metformin HCl as a model drug. The results of this study proved lower tabletability of PEO in respect to HPMC [15]. Most of the studies that investigate mechanical characteristics of hydrophilic matrix tablets were conducted on either excenter tablet press or instrumented small rotary press. There are numerous advantages of using compaction simulator in comparison to conventional tablet presses. It offers studying of basic compaction mechanisms, evaluation of influence of process variables on tablet properties, determination of scale-up parameters, creating compaction data bank, and fingerprinting of new active pharmaceutical ingredients (APIs) or excipients [16]. Using of compaction simulator enables evaluation and comparison of powder mechanical properties in simulated production conditions. Mathematical equations can be used to describe compaction events and density-pressure relationships that predict the pressures required for achieving an optimum compact density. This understanding has found active application in solving the analytical problems related to tableting, such as capping, lamination, picking, sticking, etc. Mathematical models, such as force-time, force-distance, and die-wall force parameters are used to describe work of compaction, elasticity, plasticity, and time dependent deformation behavior of pharmaceuticals. Parameters such as the bonding index, brittle fracture index and strain index can be used to predict compaction related problems [16].

This study investigates using of high molecular weight PEO (Polyox WSR coagulant) for the preparation of sustained release matrix tablets with high dose, highly soluble model drug, tramadol HCl. Both drug release and tablets mechanical properties were recognized as critical quality attributes of hydrophilic matrix tablets. Critical formulation (proportion of PEO polymer, type of insoluble filler, proportion of tramadol HCl, amount of drug in tablet and tablet diameter) and process parameters (compression pressure) were selected in the study and the influence of these parameters on drug release and tablet mechanical properties was evaluated. Aim of this study was preliminary evaluation of selected variables on tablet quality attributes, since all variables were varied on two levels through screening experimental design.

## EXPERIMENTAL

### Materials

The following materials were used: polyethylene oxide (Polyox WSR coagulant, Dow Chemical Company, Midland, TX, USA), microcrystalline cellulose (Avicel PH 102, FMC Biopolymer, USA), partially pregelatinized maize starch (Starch 1500, Colorcon, Dartford, Kent, UK), colloidal silicon dioxide (Aerosil 200 Pharma, Degussa), magnesium stearate (Mallinckrodt, St. Louis, MO, USA). Tramadol HCl (Hemofarm A.D., Vršac, Serbia) was used as model of high dose, highly water soluble drug.

### Experimental design

In the first part, the experiments were conducted to evaluate the influence of formulation (proportion of PEO polymer, type of insoluble filler, proportion of tramadol HCl, amount of drug in tablet and tablet diameter) and process parameter (compression pressure) on tramadol release. Percentage of drug release from matrix tablets after 30 and 240 min were selected as response variables. Experiments were performed according to  $2^{5-2}$  fractional factorial design, as presented in the Table 1.

In order to eliminate influence of matrix tablets geometry on drug release, normalization of the percentage of drug released after 30 and 240 min, was performed by dividing obtained drug released percentage with surface area per volume ratio ( $SA/Vol$ ) of tablets.

In the second part of the study  $2^3$  full factorial design was used to evaluate the influence of the matrix polymer proportion, type of filler and drug proportion on the tablet mechanical properties. Levels of investigated variables are presented in Table 1. Tensile strength ( $\sigma_t$ ) was used as an indicator of tablets mechanical properties, enabling comparison of mechanical

**Table 1.** Experimental matrix according to  $2^{5-2}$  and  $2^3$  experimental design

Formulation	Proportion of PEO polymer, %	Filler type <sup>a</sup>	Proportion of tramadol HCl, %	Compression pressure, MPa	Tramadol HCl per tablet, mg	Tablet diameter, mm
F1	25	Starch 1500	55.6	300	200	10
F2	35	Starch 1500	55.6	150	100	7
F3	25	Avicel PH 102	55.6	150	200	13
F4	35	Avicel PH 102	55.6	300	100	7
F5	25	Starch 1500	27.8	300	100	10
F6	35	Starch 1500	27.8	150	200	13
F7	25	Avicel PH 102	27.8	150	100	10
F8	35	Avicel PH 102	27.8	300	200	13

<sup>a</sup>Starch 1500 – partially pregelatinized maize starch; Avicel PH 102 – microcrystalline cellulose

properties of tablets with different dimensions. Tensile strength was calculated according to the following equation:

$$\sigma_t = \frac{2F}{\pi d h} \quad (1)$$

where  $F$  is the crushing force,  $d$  is the tablet diameter and  $h$  is the tablet thickness.

### Tablets preparation

Powder mixtures for compression were prepared by using Turbula® shaker-mixer (Glen Mills Inc., Clifton, NJ, USA). Tablets were compressed with direct compression method by using of Presster™ single station compacting simulator (Metropolitan Computing Corporation, East Hanover, NJ, USA). Simulation of the rotary tablet press Korsch PH336 was used, with simulated die table speed of 30 rpm which conforms to 65000 tablets per hour and dwell time of 20 ms. Tablets were prepared using punches with diameters of 7, 10 and 13 mm, while tablet masses were 180, 360 and 720 mg, respectively, according to the experimental design. Compression pressure was calculated from the measured compaction force per cross-sectional area of tablets. Tablets were compressed on different compaction pressures in the range of 100–500 MPa with simulation of the compaction profile of the Korsch PH336 rotary tablet press. Tensile strength was calculated from measured hardness and tablet dimensions. Influence of the selected input variables on the tablet tensile strength was analyzed.

### Drug release testing

Drug release test was performed using rotating basket apparatus (Erweka, Hausenmann, Germany) during 4 h (rotational speed 75 rpm, medium volume 600 ml). Samples were taken after predefined time intervals: 30, 120, 180, 240, 360 and 480 min and the amount of dissolved tramadol HCl was determined spectrophotometrically at  $\lambda = 271$  nm. The data collected up to 240 min were selected for modeling of

drug release. During the drug release testing, pH value of the medium was changed by adding buffer solutions as follows: in the first 30 min, pH 1.2; from 30–120 min, pH 2.3; from 120–180 min, pH 6.8; from 180–240 min, pH 7.2. Artificial gastric juice pH 1.2 was used as well as two buffer solutions for changing pH value during dissolution test:

Buffer 1:  $K_2HPO_4$ , 63 g/100 ml (6 ml at 31 min).

Buffer 2:  $NaOH$  15 g/100 ml (6 ml at 121 min and 3 ml at 181 min).

### Mechanical characterization of tablets

Tablet hardness was measured using the 8M tablet hardness tester (Dr. Schleuniger Pharmatron, Thun, Switzerland) and tablet dimensions were measured with digital caliper. Tensile strength was calculated from dimensions of tablets and measured hardness, according to the Eq. (1).

## RESULTS AND DISCUSSION

### Evaluation of drug release from PEO matrices

Tramadol HCl release profiles from PEO matrices (formulations F1–F8) are presented in Figure 1.

Based on assumption that in hydrophilic matrices with high viscosity polymers and high water-soluble drug diffusion release mechanism is dominating in respect to erosion, drug release process follows Higuchi's model. There is linear relationship between drug release and square-root-of-time (Figure 2) in the certain time intervals and it is related to geometrical characteristics of matrix tablets. Calculated determination coefficient values ( $R^2$ ) for all formulations were above 0.96, indicating good fitting to Higuchi model.

Percentages of tramadol HCl released after 30 and 240 min were selected as indicator of polymer ability to prevent premature drug release and enable sustained release of drug. Furthermore, both time points are within the interval in which drug release follows Higuchi's model. Normalized values of drug released percentage were used in order to eliminate influence

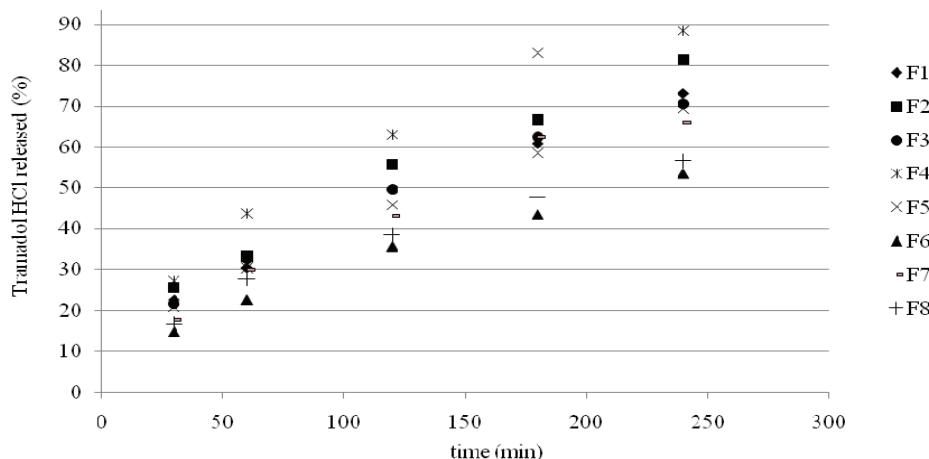


Figure 1. Tramadol HCl release from PEO matrix tablets (formulation F1–F8).

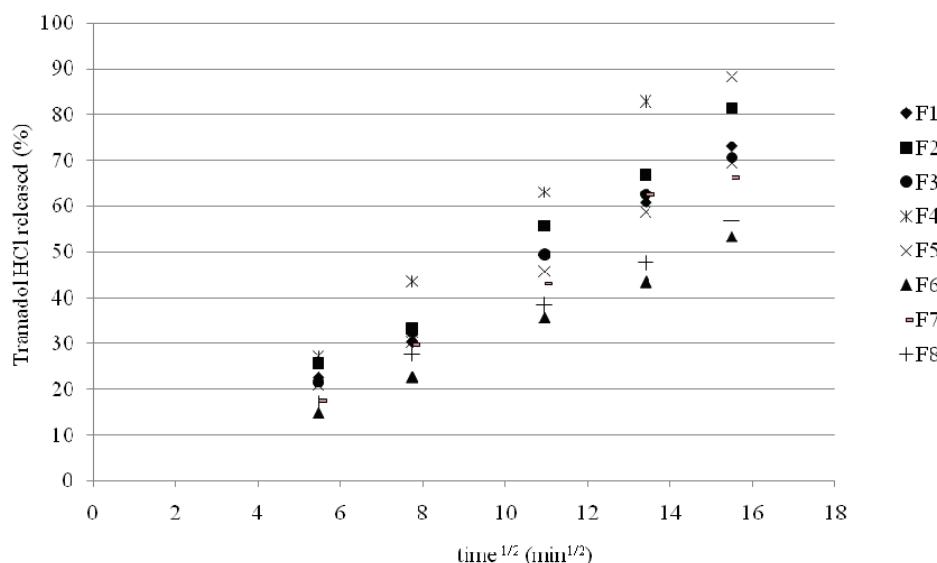


Figure 2. Tramadol HCl released from formulation F1–F8 per square root of time.

of geometrical characteristics on drug release rate. Normalization was performed by dividing percentage of drug released with surface area per volume ratio (*SA/Vol*) of matrix tablets (Table 2).

The influence of selected formulation and process parameters (proportion of PEO polymer, type of insol-

uble filler, proportion of tramadol HCl, amount of drug in tablet, tablet diameter and compression pressure) on normalized percentage of drug released at 30 and 240 min was analyzed and presented on Figure 3. Proportion of drug in tablet exhibits statistically significant effect on drug release after 30 min ( $p < 0.05$ ). With Inc-

Table 2. Normalized values of Tramadol HCl released after 30 and 240 min

Formulation	Drug released after 30 min, %	Drug released after 240 min, %	SA/Vol	Normalized values of drug released after 30 min, %	Normalized values of drug released after 240 min, %
F1	22.6	73.0	0.893	25.3	81.8
F2	25.7	81.3	1.047	24.5	77.6
F3	21.5	70.6	0.890	24.2	79.3
F4	27.3	88.3	1.057	25.8	83.6
F5	21.0	69.3	0.904	23.2	76.7
F6	14.9	53.3	0.716	20.9	74.5
F7	17.6	66.1	0.916	19.3	72.2
F8	16.8	56.7	0.749	22.4	75.8

reasing proportion of drug in tablet, drug release increased.

Influence of other formulation and process variables in selected range have no statistical significant influence on tramadol HCl release rate. For the drug release at later time point, 240 min, proportion of tramadol HCl in the formulation has statistical significant influence on drug release, too.

#### Mechanical characterization of matrices with polyethylene oxide

Full factorial design was performed in order to evaluate influence of input variables (proportion of poly-

mer, type of filler and proportion of tramadol HCl per tablet) on mechanical characteristics of matrix tablets. Due to different dimensions of tablets the tensile strength was used for evaluation of mechanical characteristics of matrix tablets.

Profiles of tensile strength versus compaction pressure are presented in the Figure 4.

Compression of tablet mixture was performed with compression force up to 40 kN, since the punch face diameter of 13 mm and compression pressure of about 300 MPa compression force is near to maximum possible compression force which could be performed on compaction simulator. Tensile strengths of matrix tab-

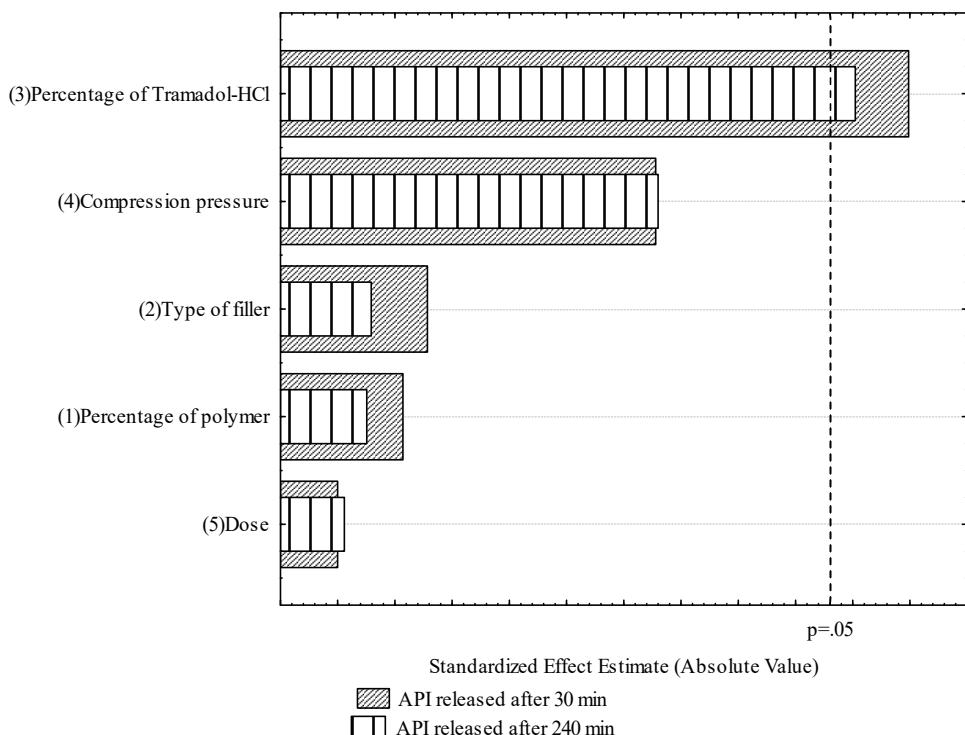


Figure 3. Pareto chart of formulation and process parameters effects on drug released at 30 and 240 min from PEO matrix tablets.

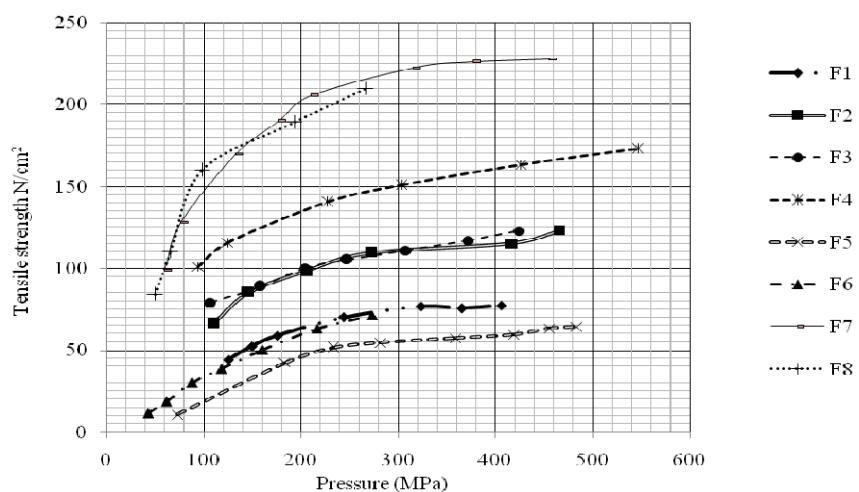


Figure 4. Profiles of tensile strength versus compaction pressure for powder mixtures with PEO WSR coagulant.

lets on compaction pressures of 120 and 250 MPa were analyzed and compared as output variables (Table 3).

Those values of tensile strength were extracted from trend lines of tabletability profiles (Figure 4). Value for compaction pressure of 120 MPa corresponds to values of tensile strength in ascending part of the tabletability profiles diagrams in all trials, while compression pressure of 250 MPa is in the part where the profile reaches plateau. The higher values of tensile strength were obtained when Avicel PH 102 was used as filler comparing to the Starch 1500. Increase of tramadol HCl in tablet mixture negatively influences on tensile strength, while increase of PEO polymer in tablet mixture has positive impact on tensile strength.

Pareto diagram of the influence of input variables on tensile strength (for compression pressure of approximately 120 and 250 MPa respectively) is presented in Figure 5.

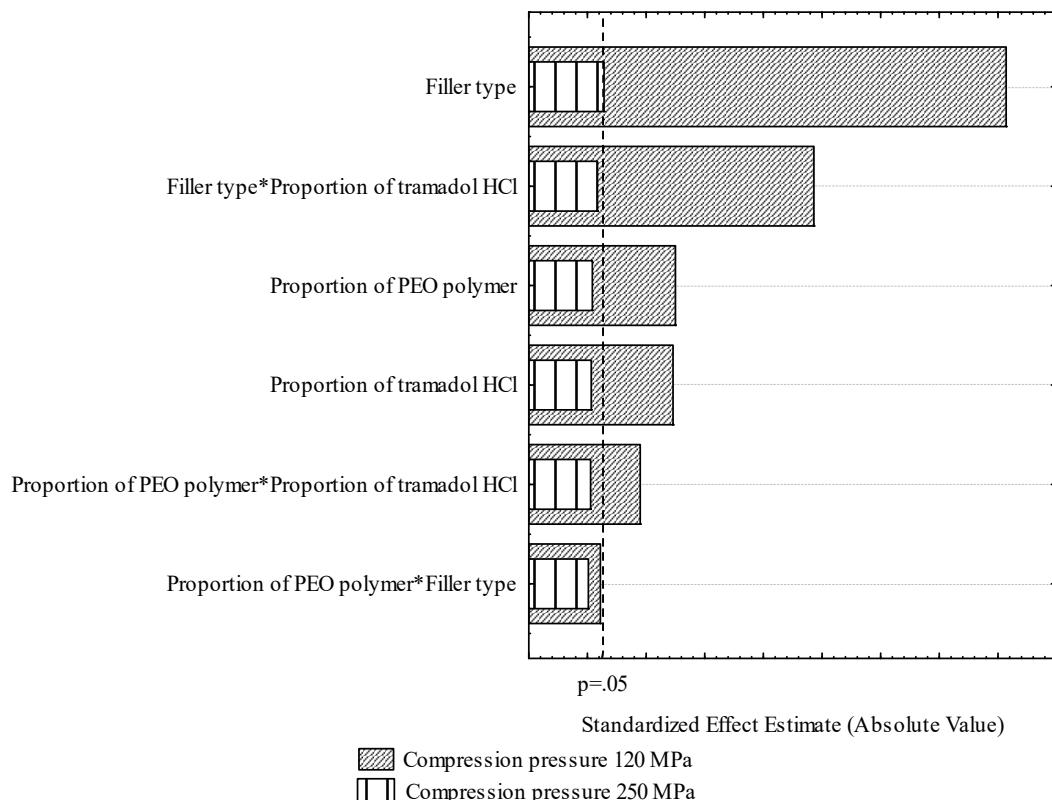
For PEO matrix tablets almost all input variables exhibit statistically significant effect on tablet tensile strength, with the largest influence of filler type. Mechanical properties of the matrix tablets with highly compressible MCC as filler are significantly better comparing to Starch 1500.

Influence of proportions of polymer, filler and drug (comprising 100% of tablet) on tensile strength is presented in Figure 6a and b for Avicel PH 102 and Starch 1500, respectively.

*Table 3. Tensile strengths of PEO matrix tablets*

Formulation	Proportion of PEO polymer, %	Filler type <sup>a</sup>	Proportion of tramadol HCl, %	Tensile strength 120 MPa, N/cm <sup>2</sup>	Tensile strength 250 MPa, N/cm <sup>2</sup>
F1	25	Starch 1500	55.6	44	71
F2	35	Starch 1500	55.6	71	107
F3	25	Avicel PH 102	55.6	82	105
F4	35	Avicel PH 102	55.6	115	145
F5	25	Starch 1500	27.8	25	53
F6	35	Starch 1500	27.8	30	68
F7	25	Avicel PH 102	27.8	160	212
F8	35	Avicel PH 102	27.8	170	205

<sup>a</sup>Starch 1500 – partially pregelatinized maize starch; Avicel PH 102 – microcrystalline cellulose



*Figure 5. Pareto chart of selected variables effects on tensile strength for PEO matrices for compression pressure of 120 and 250 MPa.*

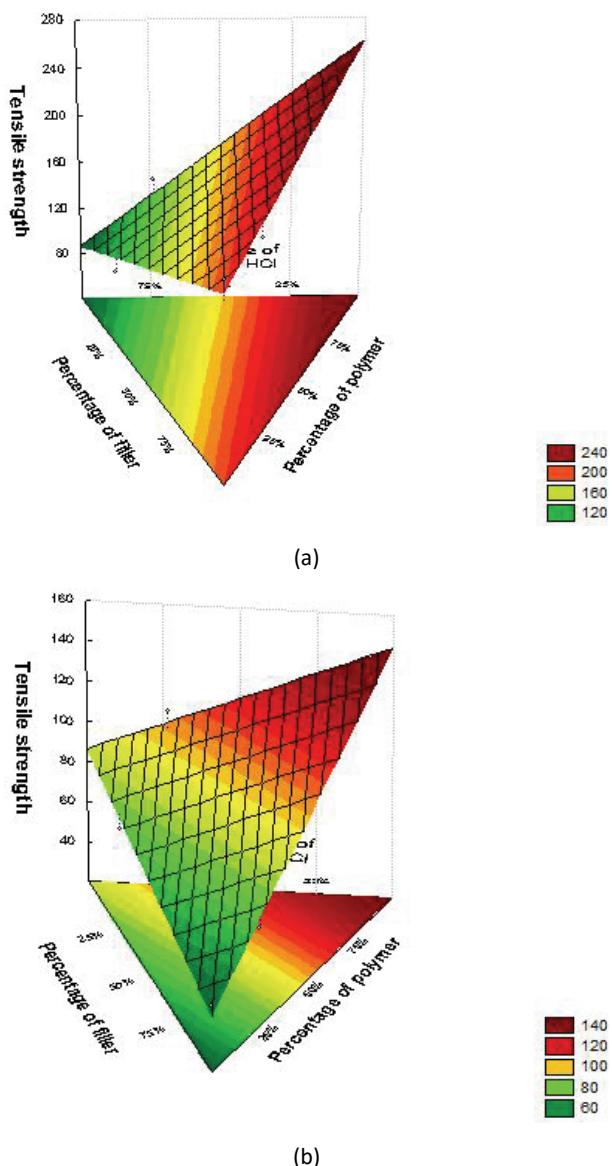


Figure 6. Influence of proportions of polymer, filler and drug (comprising 100% of tablet) on tensile strength: a) Avicel PH 102 and b) Starch 1500.

## CONCLUSION

In this study, the influence of critical formulation and process parameters on tramadol HCl release from PEO matrix tablets and tablet mechanical properties was evaluated using screening experimental design. Proportion of drug in tablet exhibits statistically significant effect on drug release in the evaluated range. By increasing the proportion of drug in tablet, the drug release will be increased. Other formulation and process variables in selected ranges have no statistical influence on tramadol HCl released rate.

Regarding mechanical properties of tablets, type of filler has the most critical effect on the powder mixture tabletability. Mechanical properties of matrix tablets

are significantly better with microcrystalline cellulose compared to partially pregelatinized starch. The highest tensile strength was obtained with Avicel PH 102 as filler and with higher percentage of polymer. Obtained results present preliminary evaluation of investigated variables on tablet quality attributes. Since all results were obtained from experiments where variables were varied on two levels, additional experimental work is needed in the future in order to establish mathematical model which will define influence of investigated variables on drug release and tablet mechanical properties.

These findings could be useful in optimization of PEO sustained release matrix tablets with tramadol HCl, as high dose, highly soluble model drug.

## Acknowledgements

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## IZVOD

### ISPITIVANJE UTICAJA FAKTORA FORMULACIJE I PROCESA NA OSLOBAĐANJE TRAMADOL HIDROHLORIDA I MEHANIČKE KARAKTERISTIKE Matriks TABLETA SA PRODUŽENIM OSLOBAĐANJEM

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(Naučni rad)

U radu je ispitivan uticaj formulacijskih i procesnih promenljivih na brzinu oslobođanja i mehaničke karakteristike matriks tableta izrađenih sa polietilen oksidom velike molekulske mase (PEO WSR koagulant), kao matriks formirajućim materijalom i visoko rastvorljivom lekovitom supstancicom prisutnoj u velikoj dozi, tramadol hidrohloridom. Kao formulacijske promenljive varirane su: ideo polietilen oksidnog polimera (25 ili 35%), vrsta nerastvornog sredstva za dopunjavanje (mikrokristalna celuloza i parcijalno pregelirani skrob), ideo tramadol hidrohlrida (27,8 i 55,6%), količina leka u tabletu (100 ili 200 mg). Pritisak kompresije je variran kao procesna promenljiva. Procenat tramadol hidrohlrida rastvoren nakon 30 i 240 min ispitivanja je izabran kao zavisno promenljiva za ispitivanje oslobođanja lekovite supstance, dok je zatezna čvrstoća izabrana kao zavisno promenljiva koja je indikator mehaničkih karakteristika tableta. Izvedena su dva seta eksperimenata, koji odgovaraju  $2^{5-2}$ , odnosno  $2^3$  eksperimentalnom dizajnu. Tablete su izrađene na simulatoru kompakcije Prester. Simuliran je rad rotacione tablet prese Korch PH336, sa brzinom rotacije 30 rpm, što odgovara kapacitetu od 65000 tableta na sat. Ispitivanje uticaja faktora formulacije i procesa na oslobođanje tramadol hidrohlrida pokazalo je da se iz svih formulacija tramadol hidrohlrid oslobođa usporeno, linearnom kinetikom. Najveći uticaj na procenat oslobođenog leka imao je ideo leka u tabletu. Sa povećanjem udela leka u tabletu, povećavao se i procenat oslobođenog leka u navedenim vremenskim intervalima. Ostali ispitivani faktori nisu imali značajan uticaj na brzinu oslobođanja. Ispitivanje mehaničkih karakteristika tableta pokazalo je da na zateznu čvrstoću izrađenih tableta najveći uticaj ima vrsta sredstva za dopunjavanje. Najveće vrednosti zatezne čvrstoće su dobijene u slučaju kada je mikrokristalna celuloza korišćena kao sredstvo za dopunjavanje, kao i kada je procenat polimera u tabletu bio na višem nivou. Analiza dobijenih rezultata omogućava pravilan izbor vrste i koncentracije pomoćnih materija u formulaciji matriks tableta sa produženim oslobođanjem izrađenih sa polietilen oksidnim polimerom i visoko rastvorljivom lekovitom supstancicom prisutnoj u velikoj dozi.

**Ključne reči:** Tramadol HCl • Matriks tablete • PEO WSR koagulant • Oslobođanje lekovite supstance • Mehaničke karakteristike tableta

# Effects of pH on the stability of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside in aqueous solution

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## Abstract

The colour variation, colour intensity and stability at various pH values (2.0, 4.0, 7.0 and 9.0) of cyanidin 3-O- $\beta$ -glucopyranoside (Cy3Glc) and its aglycone cyanidin were investigated during a period of 8 hours storage at 25 °C. Our data showed that pH of aqueous solution had impact on spectroscopic profile of cyanidin and Cy3Glc. Beginning with the most acidic solutions, increasing the pH induce bathochromic shifts of absorbance maximum in the visible range for all examined pH values (with the exception pH 4.0 for cyanidin), while the presence of the 3-glucosidic substitution induce hypsochromic shift. Compared to cyanidin, Cy3Glc has higher colour intensity and higher stability in the whole pH range, except at pH 7.0. The 3-glucosidic substitution influences on the colour intensity of Cy3Glc in the alkaline region. After 8-hour incubation of Cy3Glc and cyanidin at pH 2.0 and 25 °C, 99% of Cy3Glc and only 27% of cyanidin remained unchanged.

**Keywords:** anthocyanins, anthocyanidins, cyanidin, cyanidin 3-glucopyranoside, colour variation, colour intensity, stability, brown index, UV–Vis absorption spectra.

Available online at the Journal website: <http://www.ache.org.rs/HI/>

The anthocyanins are the large water-soluble group of natural pigments responsible for the attractive colours - ranging from strawberry red to blue – of most fruits, flowers, leaves, and some vegetables. More than 225 individual compounds are known. Chemically, they are glycosides of 2-phenylbenzopyrylium or flavylium salts. Anthocyanin sugars comprise monosides (glucose, galactose, rhamnose and arabinose), biosides, and triosides (linear and branched-chain). Additionally, the sugars can be acylated, notably with phenolic acids such as para-coumaric and caffeic acids which impart stability on the molecule by intra-molecular interactions [1].

Anthocyanins are commercially used in acid solutions such as soft drinks (usually within the pH range 2.5–3.8) where they are red (due to the flavylium cation). At higher pH values (6 and upwards) they turn blue, due to formation of quinonoidal bases [1]. Since anthocyanins form the red and blue colours of most fruits and vegetables they provide the attractive colours of many fruit juices, wines, jams and preserves [2,3].

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## SCIENTIFIC PAPER

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Commercial applications of anthocyanins include sugar confectionery, jams, and bakery toppings as well as soft drinks [1]. There is worldwide interest in additional use of anthocyanins as a consequence of perceived consumer preferences as well as legislative action, which has continued the delisting of approved artificial dyes [2]. Today, there is considerable interest in the development of food colourants from natural sources to replace synthetic food colourants [4,5]. The reason behind this is to develop safe, economical, and efficient food colourants to replace the banned coal tar and azo dyes [4,6]. Many of the products so coloured are exported to countries where regulations do not permit the use of artificial colours or where there is consumer resistance to artificial additives [1]. Here, coloured anthocyanins have some advantages: they are safe, coloured especially in the red region, and relatively soluble, which simplifies their incorporation into aqueous food systems [4,7].

However, there are some limitations to the use of anthocyanins as food colourants, which include their chemical instability, their need for purification, and their tinctorial power, which is nearly 100-fold lower than that of the coal tar dyes. In food products, a number of reactions can occur, although the major problem associated with the use of anthocyanins as food colourants is their temperature, oxygen, light and enzymatic instability [4,7–12]. A particular problem is the pH

influence on their behavior [3,7]. Based on observation of a few relatively simple anthocyanins *in vitro*, the following scheme is generally accepted [7,13,14]: at a pH ≤ 3, the orange, red or purple flavylium cation predominates. As the pH increases, kinetic and thermodynamic competition occurs between the hydration reaction on position 2 of the flavylium cation and the proton transfer reactions related to the acidic hydroxyl groups of the aglycone. While the first reaction gives a colourless hemiacetal form, which can undergo ring opening to a yellow chalcone, the latter reactions give rise to more violet quinonoidal bases. Further deprotonation of the quinonoidal bases can take place at pH values between 6 and 7 with the formation of purplish, resonance-stabilised quinonoid anions. It is generally accepted that anthocyanins exhibit their most intense colour when they are in their flavylium cation form [7]. At the pH values typical for fresh and processed fruits and vegetables, each anthocyanin will thus most probably be represented by a mixture of equilibrium forms [8].

The anthocyanins has great importance due to their demonstrated pharmacological activities [15,16]. Numerous studies have reported the beneficial health effects of consuming dietary fruits and vegetables containing anthocyanins [17–19]. They have attracted much attention in relation to their physiological activities, and their role has become an important issue in the relationship between health and human diet. In particular, the potential positive effects associated with consumption of fruit-derived foods are attributed to the presence of such natural compounds [20]. The anthocyanins have several biological activities, including antioxidant, antihepatocarcinogenic, anti-inflammatory, anti-tumor, neuroprotective, antihemolytic, anti-diabetic, hypolipidemic and cancer chemopreventive [21–35]. Epidemiological studies have suggested that anthocyanins have cardioprotective functions in human [36], and other studies have suggested that anthocyanins inhibit tumor-cell growth *in vitro* and suppress tumor growth *in vivo* [37].

An intensive research has been done to identify the content of potential anthocyanin sources [9], and the content of the principal commercial available colourant

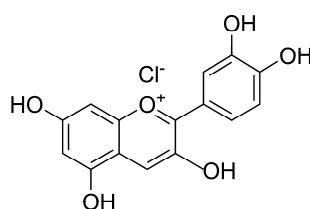
sources covers a variety of different anthocyanins: grape (*Vitis vinifera*), red cabbage (*Brassica oleraceae*), elderberry (*Sambucus nigra*, *S. canadensis*), purple carrot (*Daucus carota*), red radish (*Raphanus sativus*), blackcurrant (*Ribes nigrum*), roselle (*Hibiscus sabdariffa*), black chokeberry (*Aronia melanocarpa*) [1,38]. All these products may be characterized as crude or partially purified extracts containing a mixture of anthocyanins in addition to other components. The information regarding characteristics and the stability of these extracts has increased in recent years. However, there remain few data in the literature related to the properties and stability of pure anthocyanins, and especially of the anthocyanidins during storage. The major reason for this is that most anthocyanins are difficult to purify and have limited commercial accessibility, especially in large quantities [3]. It is important to know how the structural transformations according to pH and the structural modifications, such as glucosidation, influence the colour and stability of the anthocyanins. These facts are important from the viewpoint of the possibility to use these compounds as natural food colourants.

Cyanidin 3-O- $\beta$ -glucopyranoside (Cy3Glc) is a typical representative for the simple type of anthocyanins found in elderberry, blueberry, cowberry, whortleberry, blackcurrant, roselle, black chokeberry, etc. [3]. In this paper the colour and stability of Cy3Glc and his aglycone moiety of cyanidin (Figure 1), in aqueous solutions, were examined at four pH values between 2.0 and 9.0. The colour and stability changes were measured during incubation at different pH values in the period of 8 h at 25 °C. Thus, it has been possible to compare under various pH conditions and times impacts of 3-glucosidic substitution, on colour and stability. The results vary tremendously, and this emphasizes the importance of structure on anthocyanin and anthocyanidin properties.

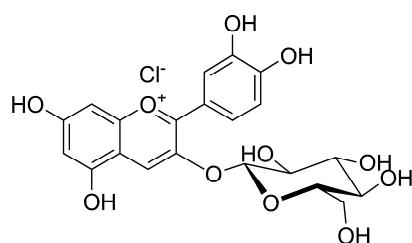
## EXPERIMENTAL

### Chemicals and reagents

The chloride salts of cyanidin (2-(3,4-dihydroxyphenyl)chromenylum-3,5,7-triol chloride, CAS number:



cyanidin



cyanidin 3-glucoside

Figure 1. Structures of the cyanidin and cyanidin 3- glucopyranoside.

528-58-5,  $C_{15}H_{11}O_6Cl$ , molecular weight 322.7 g/mol) and cyanidin 3-O- $\beta$ -glucopyranoside ((2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxochromenyl-ium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol chloride, CAS number: 7084-24-4,  $C_{21}H_{21}O_{11}Cl$ , molecular weight 484.8 g/mol) were from Polyphenols Laboratories AS (Sandnes, Norway). Acetic acid, ammonium acetate, citric acid monohydrate, formic acid, sodium citrate and methanol were obtained from Merck (Darmstadt, Germany). Ammonium formate and ammonium hydroxide were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity >18 M $\Omega$  cm) (Millipore, Bedford, MA, USA).

### Measurements of colour and stability

To determine the colour and stability the spectrophotometric analysis of cyanidin and Cy3Glc kept at different pH values was performed in specified time intervals in the period of 8 h. Buffer solutions of four different pH values with  $2.5 \times 10^{-2}$  mol dm $^{-3}$  concentration were prepared for dilution of cyanidin and Cy3Glc. The following buffers were used: ammonium formate/formic acid (pH 2.0), sodium acetate/acetic acid (pH 4.0), sodium citrate/citric acid (pH 7.0) and ammonium acetate/ammonium hydroxide (pH 9.0). The pH values of the various samples did not change during storage. The colour and stability of cyanidin and Cy3Glc were determined at  $25.0 \pm 0.1$  °C. The chloride salts of the cyanidin and Cy3Glc were dissolved in each buffer to a final concentration of  $5 \times 10^{-5}$  mol dm $^{-3}$ . The visible absorption spectra (380–800 nm) of the cyanidin and Cy3Glc solutions were recorded at specified pH values at  $25.0 \pm 0.1$  °C. Pure buffers were used as blank. Spectrophotometric measurements were made immediately after dissolution and then after specified time intervals in the period of 8 h. The absorbencies in the visible range of the freshly made samples were between 0.01 and 0.96 absorbance units, and none sample were diluted before measurements. The spectral behavior of anthocyanins is dependent on solvent [2], and substances present in the solution may influence the colour and stability. The impacts of atmospheric oxygen and other factors, such as the composition of the buffer solutions, were not examined in this study.

### Instruments

The accurate pH values for each buffer solution were measured by Mettler Toledo S20 Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland) equipped with Mettler Toledo InLab electrode (Mettler Toledo, Schwerzenbach, Switzerland).

The spectrophotometric measurements were performed using a Cary 100 Bio UV–Visible spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length quartz cell.

## RESULTS AND DISCUSSION

According to Cabrita *et al.* [2] and Fossen *et al.* [3], the colour variations of cyanidin and Cy3Glc were expressed as the changes in the positions of the absorbance maximum in the visible range ( $\lambda_{\text{max-vis}}$ ), colour intensities were measured as absorbance values at visible absorbance maximum  $\lambda_{\text{max-vis}}$  immediately after dissolution ( $t_0$ ) and after a certain time interval and expressed as molar absorptivities ( $a$  in  $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ ), and the stability was expressed as the percentage of the absorbance remained after a certain time interval, measured at initial  $\lambda_{\text{max-vis}}$ . Brown index ( $BI$ ) was expressed as the absorbance ratio at 430 nm by that at 520 nm according to the Malien-Aubert *et al.* [38,39]. The pH values of the dissolution have a large influence on the spectroscopic profiles of the cyanidin and Cy3Glc obtained after dissolution in the selected buffers prior to the analysis. The self-association of cyanidin and Cy3Glc occur at high concentrations ( $>10^{-3}$  mol dm $^{-3}$ ) [40]. In our investigations, cyanidin and Cy3Glc were dissolved to final concentration of  $5 \times 10^{-5}$  mol dm $^{-3}$ . At this low concentrations cyanidin and Cy3Glc exist as monomers in the studied solutions [41].

### Colour variation of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The most common way to indicate anthocyanin colours is based on presentation of visible  $\lambda_{\text{vis-max}}$  values from UV/Vis absorption spectra. By plotting the  $\lambda_{\text{vis-max}}$  values (Table 1) obtained for the cyanidin and Cy3Glc immediately after dissolution in aqueous solutions at different pH values, a similar buffered pattern was achieved (Figure 2). The following tendency was established for Cy3Glc: beginning with the most acidic solution, increase in pH produced bathochromic shifts (Figure 2). This pattern correlates well with earlier reports for Cy3Glc [2,3,8]. At the pH 4.0, cyanidin showed no spectral band in the visible spectrum. At the other pH values, special higher pH values, maxima of absorbance in the visible range for cyanidin resulted in

Table 1. Visible absorbance maxima ( $\lambda_{\text{vis-max}}$  nm) and molar absorptivities of spectral bands in the visible range ( $a$ ,  $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ ) for the cyanidin and Cy3Glc ( $5 \times 10^{-5}$  mol dm $^{-3}$ ) immediately after dissolution in buffered aqueous solutions at 25 °C

pH	Cyanidin		Cy3Glc	
	$\lambda_{\text{vis-max}}$	$a$	$\lambda_{\text{vis-max}}$	$a$
2.0	517	5034.42	508	19297.16
4.0	0	0	513	2429.10
7.0	570	1467.17	549	441
			7732.31	7904.24
9.0	591	1503.02	569	12349.24

bathochromic shift (Figure 2). The visible absorbance maxima for cyanidin were in all instances (except pH 4.0) higher than the corresponding  $\lambda_{vis\text{-max}}$  values for Cy3Glc (Table 1). This reveals the impact of the 3-glycosylation in Cy3Glc on the position of the visible absorption maximum: the presence of the 3-glucosidic substitution causes hypsochromic shift.

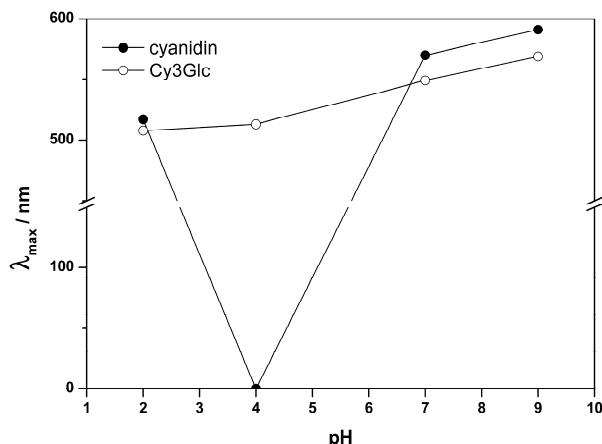


Figure 2. Visible absorbance maxima ( $\lambda_{vis\text{-max}} / \text{nm}$ ) at different pH values, for the cyanidin and Cy3Glc ( $5 \times 10^{-5} \text{ mol dm}^{-3}$ ) immediately after dissolution in buffered aqueous solutions at 25 °C.

### Colour intensity of cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside

The pH variation affects the colour intensities of the cyanidin and Cy3Glc (Table 1). Comparing the molar absorptivities of spectral bands in the visible range for cyanidin and Cy3Glc (Figures 3 and 4) it can be seen that 3-glucosidic substitution strongly increases the molar absorptivity of the aglycone moiety. The molar absorptivities were highest at pH 2.0 for the both pigments and strongly decreased toward pH 4.0, where local minimum for Cy3Glc are achieved, while cyanidin shows no spectral band in the visible spectrum. According to the previously published data simple anthocyanins, like Cy3Glc, are unstable and are quickly decolorized by hydration at the 2-position of the anthocyanidin skeleton in the pH region 5–7 [3,13], which is in good agreement with our results here. Further pH increase cause increase in molar absorptivities for the both pigments. The similarity between the curves indicates that the both pigments have the same type and distribution of equilibrium forms (Figures 5 and 6): the colourful favylum form dominates at pH 2.0, and the occurrence of colourless hemiacetal forms increases toward pH 4.0 [2,3,7,13,41,42]. At pH values above 7.0, cyanidin and Cy3Glc shows a hyperchromic effect until pH 9.0, when anthocyanins are expected to occur mainly in their quinonoidal and quinonoidal anion forms [2,7,13,41,42]. It was noticed that Cy3Glc

showed relatively high  $\alpha$  values in alkaline solutions. The ratio between the absorbance at the local maximum in the alkaline region at pH 9.0 and at pH 2.0 for cyanidin was 0.32, while the ratio for Cy3Glc was 0.65 indicating that 3-glucosidic substitution is favorable for colour intensity in the alkaline region. Obtained results for molar absorptivities of Cy3Glc (Table 1) is in good accordance with previously published data [2,3].

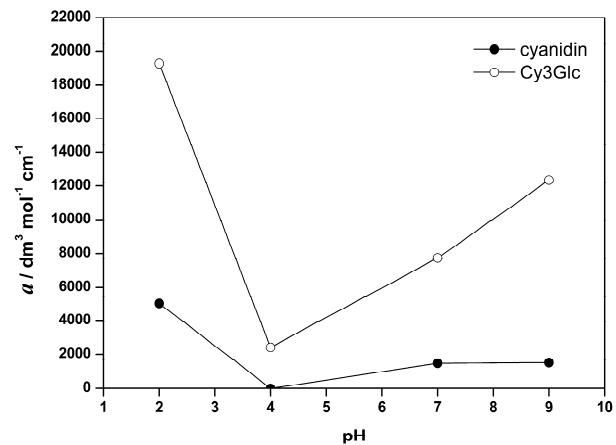


Figure 3. Molar absorptivities ( $\alpha / \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) of spectral bands in the visible range for the cyanidin and Cy3Glc ( $5 \times 10^{-5} \text{ mol dm}^{-3}$ ) immediately after dissolution in buffered aqueous solutions at 25 °C.

Molar absorptivities of Cy3Glc solution during storage at pH 2.0 and 25 °C were high and constant, according to the favylum cationic structure [7,13,41,42] (Figure 4A). Surprisingly, molar absorptivities of cyanidin solution during storage at pH 2.0 and 25 °C decrease, although it is in the form of favylum cation [7,13] (Figure 4A). This indicated the shift of the coloured favylum cation into the other structures. Molar absorptivities of Cy3Glc solution at pH 4.0 and 25 °C were significantly lower compared to the pH 2.0, but were constant during storage (Figure 4B). At pH 4.0 cyanidin does not show the spectral band in the visible spectrum (Figure 4B). At pH 7.0 Cy3Glc showed two bands with visible absorbance maximum at 441 and 549 nm (Figure 4C). The Cy3Glc showed great decrease in molar absorptivities with time at 25 °C. At the same conditions cyanidin shows low and almost constant molar absorptivity values (Figure 4C). At pH 9.0 the initial, relatively high value of Cy3Glc molar absorptivity decreases during storage at 25 °C. The molar absorptivity of cyanidin at pH 9.0 was low and slightly decreases during storage. This reveals the impact of the 3-glycosylation in Cy3Glc on the molar absorptivities: the presence of the 3-glucosidic substitution strongly increased molar absorptivities at all examined pH values and improved stability at pH 2.0 and 4.0.

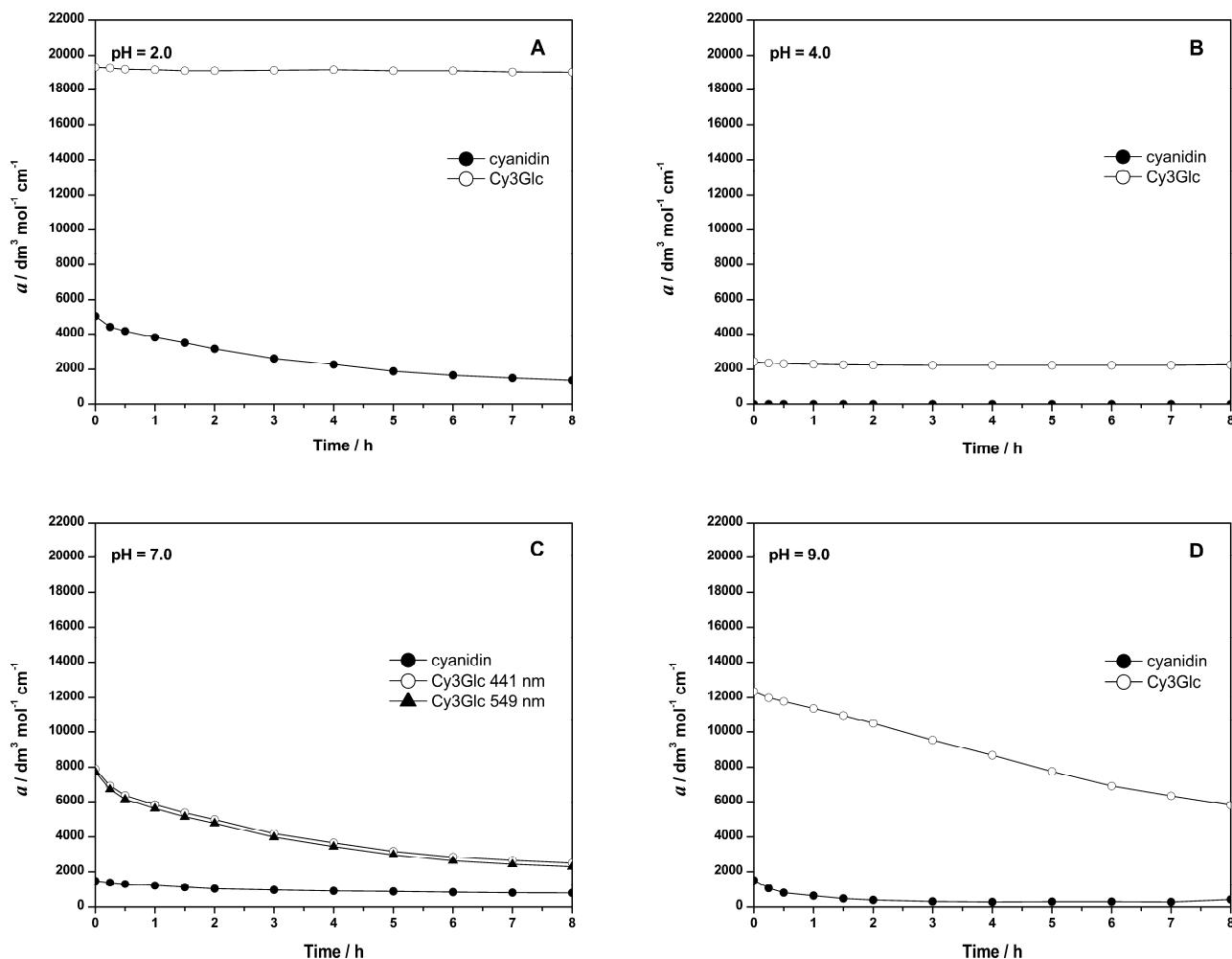


Figure 4. Molar absorptivities ( $a$ ) of spectral bands in the visible range (measured at  $\lambda_{\text{vis}-\text{max}}$ ) for the cyanidin (●) and Cy3Glc (○, ▲) ( $5 \times 10^{-5} \text{ mol dm}^{-3}$ ) solutions after different time of storage at  $25^\circ\text{C}$  in buffered aqueous solutions at different pH values.

#### Cyanidin and cyanidin 3-O- $\beta$ -glucopyranoside stability on storage at $25^\circ\text{C}$

The stabilities of cyanidin and Cy3Glc highly depend on pH and structure. The cyanidin and Cy3Glc were incubated for 8 h in buffered solutions at 4 different pH values at  $25^\circ\text{C}$ , and their visible absorption spectra were registered at determined time intervals. According to Cabrita *et al.* [2] stability was described on the basis of absorbance changes measured at the cyanidin and Cy3Glc  $\lambda_{\text{vis}-\text{max}}$  for each pH value. During incubation at  $25^\circ\text{C}$  and at pH 2.0, Cy3Glc showed significantly higher visible absorption values in comparison with those of cyanidin (Table 2, Figures 5A and 6A). According to the Brouillard [7], in water, for ordinary anthocyanins, the only stable coloured species is the flavylium cation, which is generally obtained for pH values lower than 3. The Cy3Glc, in accordance with this, showed stability above 98% after 8 h at  $25^\circ\text{C}$  and at pH 2.0 (Figures 6A and 7A). At pH 4.0 after 8 h Cy3Glc showed stability above 90%, although the corresponding colour intensities are modest (Table 2, Figures 6B

and 7B). During incubation Cy3Glc at pH 7.0 and  $25^\circ\text{C}$  a strong decrease in visible absorbance took place (Table 2, Figures 6C and 7C). After 8 h of incubation it was found that stability decreased rapidly as pH increased toward pH 7.0, with stability values around 30% (Figure 7C). The stability of Cy3Glc slightly increased as pH increased into the alkaline region, and at pH 9.0 displayed around 50% stability after 8 h at this pH value (Figures 6D and 7D). Spectrophotometric analysis at pH 2.0 during 8 h revealed strong decrease of the absorption band in visible range for cyanidin solution. At this pH cyanidin exhibited low stability, despite being findings in the form of flavylium cation [7,13]. Cyanidin kept only 27% of their initial absorbance after 8 h incubation at  $25^\circ\text{C}$  (Figure 7A). Figure 7A and Table 2 clearly show that the Cy3Glc is much more stable than his aglycone cyanidin. According to the Brouillard [7], since anthocyanidins have been shown to be unstable in water and much less soluble than anthocyanins, glycosylation is assumed to confer solubility and stability to the pigment. At pH 4.0 cyanidin shows no spec-

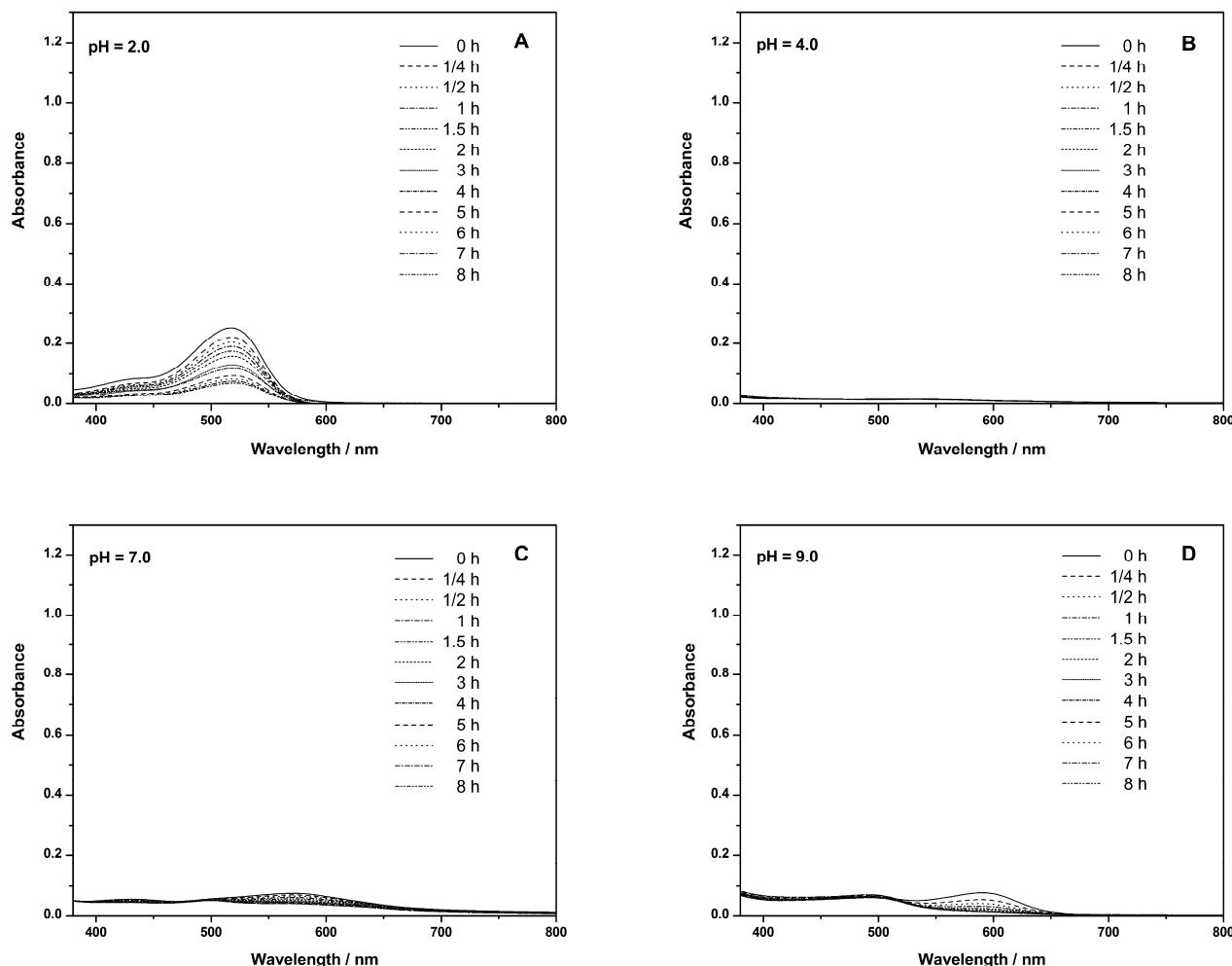


Figure 5. Visible spectra of cyanidin during 8-hour incubation at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0 during 8 h of storage at 25 °C. The concentration of cyanidin was  $5 \times 10^{-5}$  mol dm $^{-3}$ , and the temperature was 25 °C.

tral band in the visible spectrum (Figure 5B). However, the stability of cyanidin improved as pH increased toward pH 7.0 (Figures 5C and 7C). In fact, cyanidin showed some degree of stability only at this pH value, although the corresponding colour intensities are low. Cyanidin displayed around 50% stability after 8 h at this pH value. On the other hand, cyanidin was very unstable at alkaline values, and kept only around 17% of their initial absorbance at pH 9.0 (Figures 5D and 7D). Only at pH 7.0 the stability of cyanidin became higher than that of Cy3Glc, while at all the other pH values Cy3Glc showed higher stability (Figure 7). From a structural point of view, it seems that the presence of the 3-glucosidic substitution strongly increase stability of aglycone moiety, possibly by protecting the flavylium nucleus from nucleophilic attack of water molecule at C-2 that, which leads to the colourless forms, hemiacetals and chalcones (Table 2, Figures 5–7).

#### Brown index of cyanidin and Cy3Glc on storage at 25 °C

The initial brown index (*BI*, absorbance ratio, at 430 nm divided by that at 520 nm), for cyanidin and Cy3Glc

at lowest pH were similar and amounted around 0.3 (Figure 8). At pH 2.0 cyanidin and Cy3Glc exist predominately as red-orange flavylium cations ( $\lambda_{\text{vis}-\text{max}}$  for cyanidin was at 517 nm and  $\lambda_{\text{vis}-\text{max}}$  for Cy3Glc was at 508 nm, Table 1) [7,41,42]. The cyanidin and Cy3Glc displayed very low BI values at pH 2.0 during 8 h of storage at 25 °C. The BI for Cy3Glc remained the same during experiment (Figure 8A). The cyanidin showed very gradually increase of BI, which was accompanied with decrease in absorbance at  $\lambda_{\text{vis}-\text{max}}$  (Figure 5A and 8A). At pH 4.0 Cy3Glc was stable (Figure 7B) with initial BI below 0.48 (Figure 8B). At this pH value BI for Cy3Glc remained almost constant during 8 hours of storage at 25 °C. At the pH 4.0 cyanidin showed no spectral bands in the visible spectrum (Figure 5B), and displayed BI values >1. The cyanidin solution at pH 4.0 thus was colourless with yellowish shades. At pH 7.0 Cy3Glc showed two spectral bands in the visible region with absorbance maximum at 441 and 549 nm (Figure 6C). Accordingly to the high absorbance values at 441 nm the *BI* (absorbance ratio, that at 430 nm divided by that

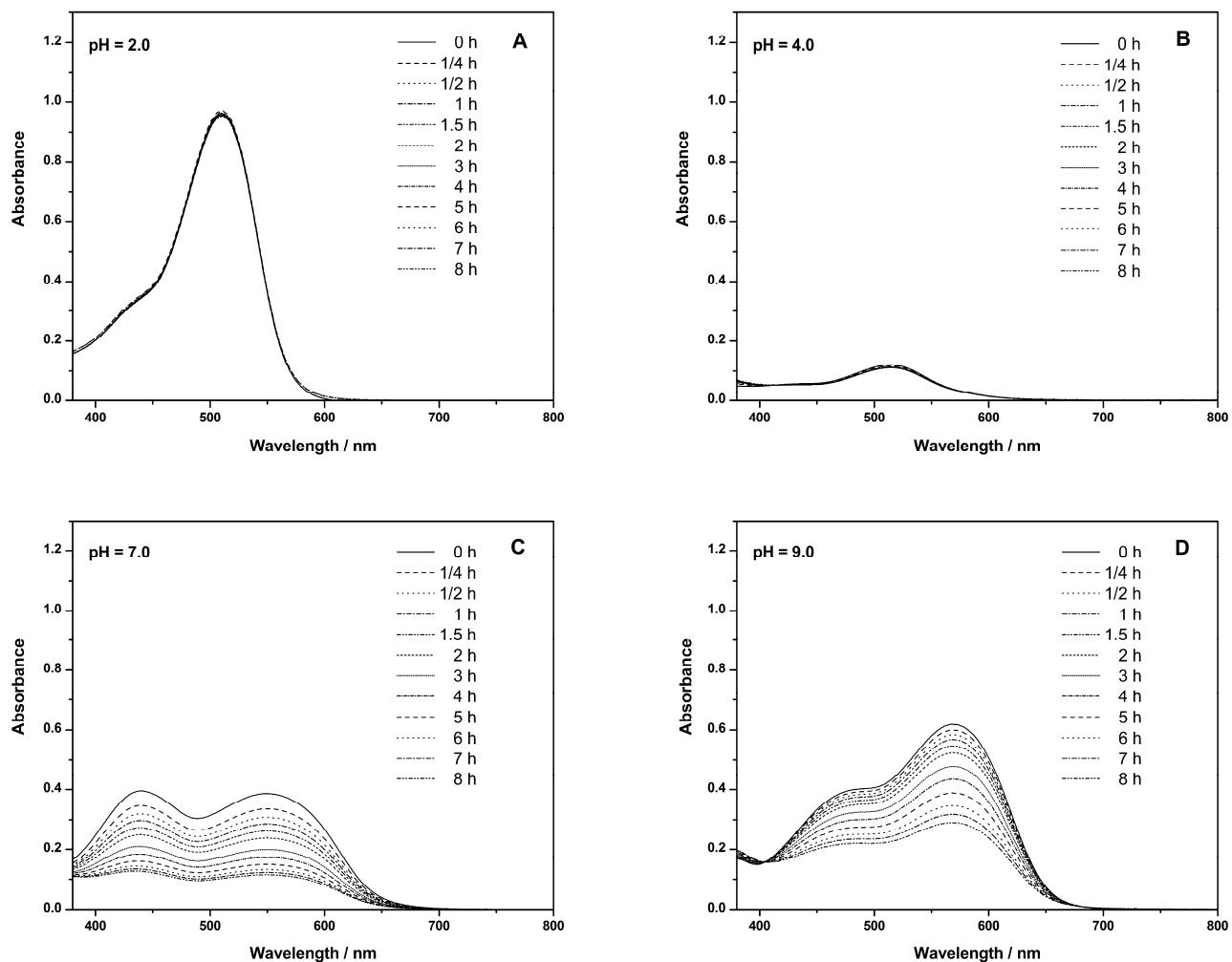


Figure 6. Visible spectra of Cy3Glc during 8-hour incubation at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0. The concentration of Cy3Glc was  $5 \times 10^{-5}$  mol dm $^{-3}$ , and the temperature was 25 °C.

Table 2. Absorbance of cyanidin and Cy3Glc ( $5 \times 10^{-5}$  mol dm $^{-3}$ ) solutions, (measured at  $\lambda_{vis-max}$ ) at pH 2–9 during 8 h of storage at 25 °C, in the dark using air atmosphere; the upper and lower value in each interval correspond to cyanidin and Cy3Glc, respectively

pH	Time, h											
	0	0.25	0.5	1	1.5	2	3	4	5	6	7	8
2.00	0.25	0.22	0.20	0.19	0.17	0.16	0.13	0.12	0.09	0.08	0.08	0.07
	0.96	0.96	0.96	0.96	0.96	0.95	0.96	0.95	0.95	0.95	0.95	0.95
4.00	0	0	0	0	0	0	0	0	0	0	0	0
	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
7.00	0.07	0.07	0.06	0.06	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04
	0.441 nm	0.40	0.35	0.32	0.30	0.27	0.25	0.21	0.18	0.16	0.14	0.13
449 nm	0.39	0.34	0.31	0.29	0.26	0.24	0.20	0.17	0.15	0.13	0.12	0.11
	9.00	0.08	0.05	0.04	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01
		0.62	0.60	0.58	0.57	0.55	0.52	0.48	0.43	0.39	0.35	0.32
												0.29

at 520 nm) for Cy3Glc was 1.09, thus giving yellowish shades [38]. The gradually increase in BI values with time (Figure 8C) was accompanied with decrease in visible absorbance at these two absorbance maxima and change in the absorbance ratio at these two wave-

lengths (Figure 6C). The initial BI value for cyanidin at pH 7.0 was 0.89 and gradually increases with the time (Figure 8C). The increase in BI values with time was accompanied with very gradual loss of colour in the visible range (decrease in absorbance, Figure 5C). How-

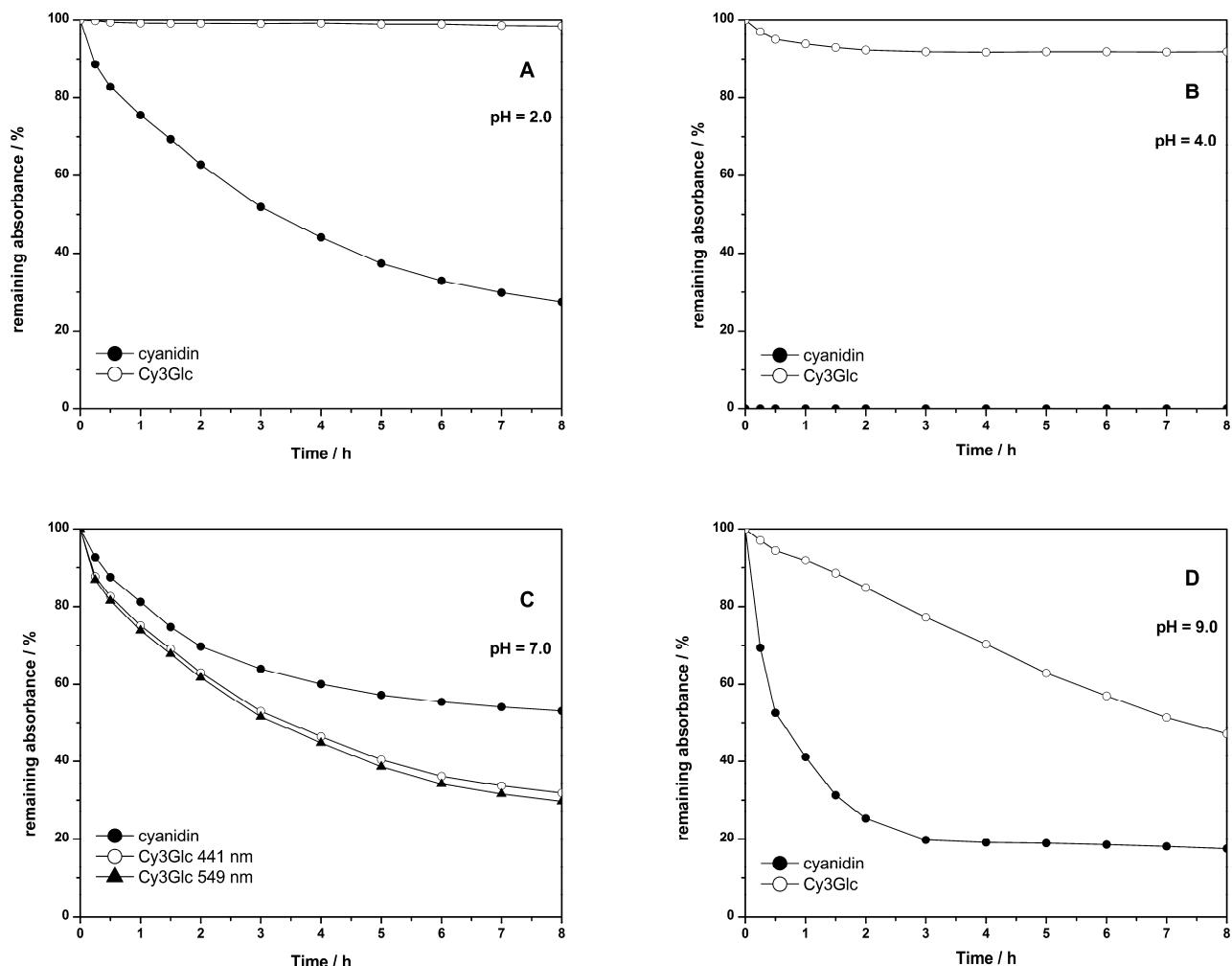


Figure 7. Percentages of remaining absorbance (measured at  $\lambda_{vis-max}$ ) after different time of incubation at 25 °C in buffered aqueous solutions at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0, for the cyanidin (●) and Cy3Glc (○, ▲) ( $5 \times 10^{-5}$  mol dm $^{-3}$ ) solutions.

ever, cyanidin displayed BI values <1 during experiment (Figure 8C). The initial BI for Cy3Glc at pH 9.0 was 0.55 and gradually increase during 8 h (Figure 8D) accompanied by decrease in visible absorbance (Figure 6D). However, BI values were low and remained <1 all the time. At pH 9.0 cyanidin displayed very low absorbance values (Figure 5D) and initial BI values 0.98. The BI values after 15 min became >1, and remained higher than 1 during experiment. The gradual increase in BI values with time (Figure 8D) was accompanied with gradual decrease in visible absorption bands and changes in their position. The BI appears to be sensitive indicator of the stability of cyanidin and Cy3Glc at different pH values (Figure 5D). By comparing Figures 5–8 it can be seen that at pH 2.0 and 4.0 Cy3Glc is stable and it has very low and constant BI values, while at pH 7.0 and 9.0 was unstable and BI values gradually increased all the time. The cyanidin was unstable at all examined pH values, which was accompanied by gradual increase in BI values during storage. After a

while, the Cy3Glc turned on yellow at pH 7.0, while some effect was seen for cyanidin at pH 4.0 and 9.0.

## CONCLUSION

The cyanidin and Cy3Glc display great differences in colour variation, colour intensity, stability and BI. At an obtained pH values, such differences mainly result from the structure (presence of the sugar moieties at aglycone). Increasing the pH induces bathochromic shifts of absorbance maxima in the visible range for the both. The visible absorbance maxima for cyanidin were in all instances (with the exception pH value 4.0) higher than the corresponding visible absorbance maxima for Cy3Glc, indicating that 3-glucosidic substitution of aglycone caused hypsochromic shift. Comparing the molar absorptivities for cyanidin and Cy3Glc there is an evident high impact of the 3-glucosidic substitution on the molar absorptivity of the aglycone moiety: the 3-glucosidic substitution strongly increases molar absorptivity of the aglycone moiety, and it is favorable for colour

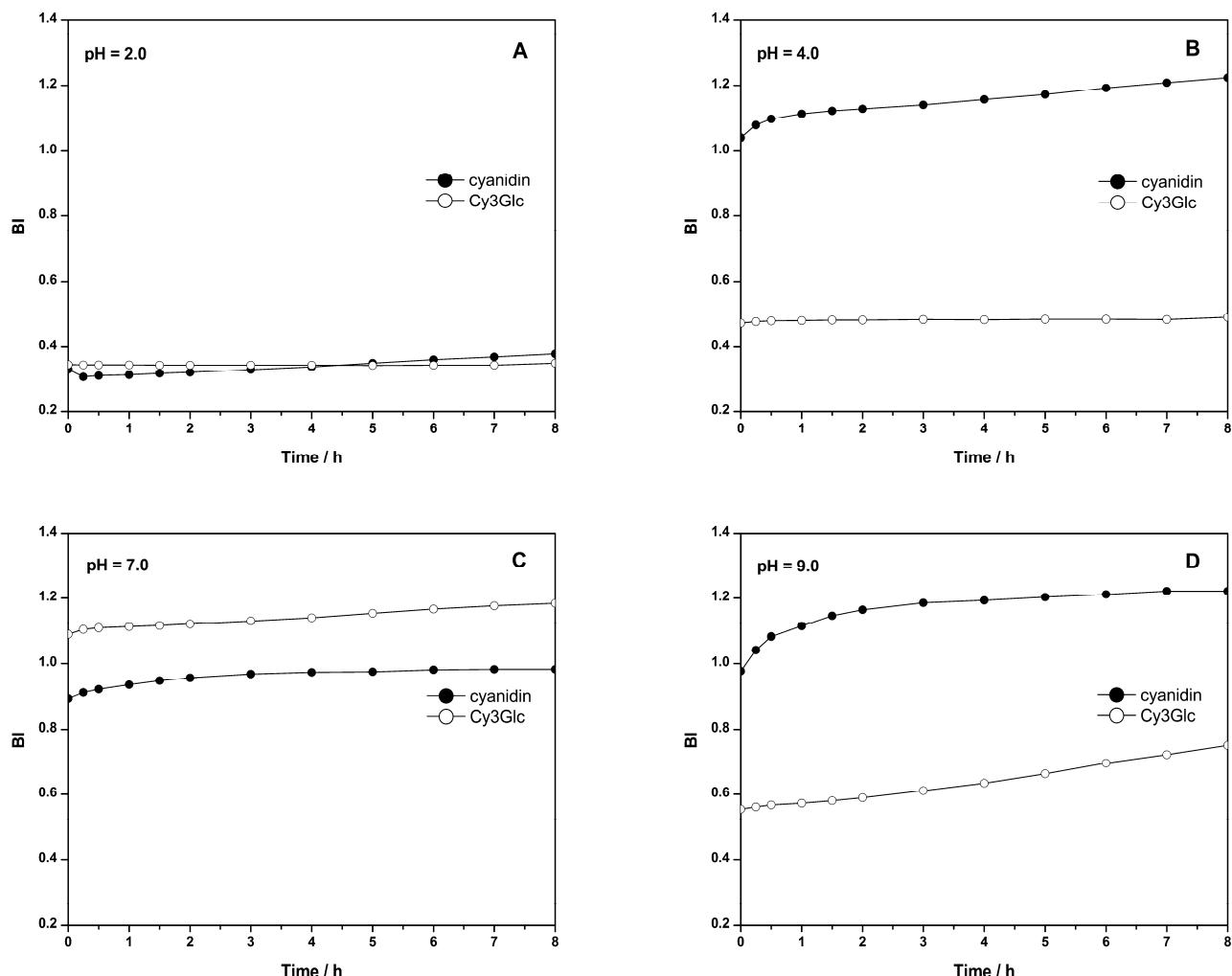


Figure 8. The brown index (BI) immediately after dissolution and after different time of incubation at 25 °C in buffered aqueous solutions at different pH values: A) 2.0; B) 4.0; C) 7.0; D) 9.0, for the cyanidin (●) and Cy3Glc (○) ( $5 \times 10^{-5}$  mol dm $^{-3}$ ) solutions.

intensity in the alkaline region. During storage the presence of the 3-glucosidic substitution strongly increased molar absorptivities at all examined pH values and improved stability at pH 2.0 and 4.0. Surprisingly, spectrometric analysis revealed low stability for aglycone at pH 2.0 regardless of whether it was found in the form of flavylium cation. Only at pH 7.0 the stability of cyanidin became higher than that of Cy3Glc, while at all the other pH values Cy3Glc showed higher stability. From a structural point of view, it seems that the presence of the 3-glucosidic substitution strongly increases the stability of aglycone moiety, possibly by protecting the flavylium nucleus from nucleophilic attack of water molecule at C-2 that leads to the colourless forms. The BI appears to be sensitive indicator of the stability cyanidin and Cy3Glc at different pH values. At pH 2.0 and 4.0 Cy3Glc is stable and it has very low and constant BI values, while at pH 7.0 and 9.0 was unstable and BI values gradually increased all the time. The cyanidin was unstable at all examined pH values which was been

accompanied by gradual increase in BI values during incubation. During some time period, the Cy3Glc turned yellow at pH 7.0, while cyanidin at pH 4.0 and 9.0.

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**IZVOD****UTICAJ pH VREDNOSTI SREDINE NA STABILNOST CIJANIDINA I CIJANIDIN 3-O- $\beta$ -GLUKOPIRANOZIDA U VODENOM RASTVORU**

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(Naučni rad)

U ovom radu ispitivani su varijacija i intenzitet boje i stabilnost cijanidina i cijanidin 3-O- $\beta$ -glukopiranozida (Cy3Glc) pri različitim pH vrednostima (2,0; 4,0; 7,0 i 9,0) tokom inkubacije na temperaturi od 25 °C, u periodu od 8 sati. Dobijeni rezultati pokazuju da pH vrednost vodenog rastvora ima uticaj na spektroskopski profil cijanidina i Cy3Glc. Cijanidin i Cy3Glc su pokazali velike razlike u varijaciji i intenzitetu boje, stabilnosti i BI. Pri određenoj pH vrednosti, te razlike uglavnom rezultiraju iz strukture (prisustva šećera na aglikonu). Porast pH vrednosti izaziva batochromno pomeranje apsorpcionih maksimuma u vidljivoj oblasti spektra za sve ispitivane pH vrednosti (osim pH 4,0 za cijanidin), dok je prisustvo 3-glukozidne supstitucije dovodi do hipsochromnog pomeranja. Poređenjem molarnih apsorptivnosti cijanidina i Cy3Glc, uočen je veliki uticaj 3-glukozidne supstitucije: 3-glukozidna supstitucija jako povećava apsorptivnost aglikonskog dela i utiče na povećanje intenziteta boje u alkalnom regionu. Tokom inkubacije, prisustvo 3-glukozidne supstitucije jako je uticalo na povećanje molarne apsorptivnosti na svim ispitivanim pH vrednostima kao i na povećanje stabilnosti na pH 2,0 i 4,0. Spektroskopska analiza je pokazala nisku stabilnost aglikona na pH 2,0, bez obzira na činjenicu da se nalazi u obliku flavilijum katjona. Samo na pH 7,0 stabilnost cijanidina bila je veća od stabilnosti Cy3Glc, dok je na svim ostalim pH vrednostima Cy3Glc pokazivao veću stabilnost. Sa strukturne tačke gledišta, može se predpostaviti da 3-glukozidna supstitucija jako povećava stabilnost aglikonskog dela prema nukleofilnom napadu molekula vode na C-2 položaj aglikona, koji dovodi do formiranja bezbojnih oblika. Na osnovu dobijenih rezultata smatramo da je BI osetljivi pokazatelj stabilnosti cijanidina i Cy3Glc na različitim pH vrednostima. Na pH 2,0 i 4,0 Cy3Glc je bio stabilan i imao je veoma niske i konstantne BI vrednosti. Na pH 7,0 i 9,0 Cy3Glc je bio nestabilan, dok su BI vrednosti postepeno rasle tokom eksperimenta. Cijanidin je bio nestabilan pri svim ispitivanim pH vrednostima, što je bilo praćeno postepenim porastom BI vrednosti tokom stajanja. Tokom eksperimenta, Cy3Glc je dobio žućkastu nijansu na pH 7,0, dok je cijanidin dobio žućkastu nijansu na pH vrednostima 4,0 i 9,0.

**Ključne reči:** Antocijanini • Antocijanidini • Cijanidin • Cijanidin 3-glukopiranozid • Varijacija boje • Intenzitet boje • Stabilnost • Braon indeks • UV-Vis apsorpcioni spektar

# Glutarimidi: Biološka aktivnost, opšti postupci za sintezu i fizičko–hemiske karakteristike

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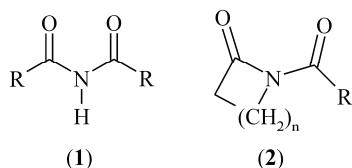
## Izvod

U ovom radu dat je prikaz metoda za sintezu šestočlanih cikličnih imida. Glutarimidi, 2,6-dioksopiperidini, su značajna biološka jedinjenja i deluju kao antagonisti adrenogenih receptora, antiinflamatorni agensi, anksiolitici, antivirotici, antibiotici i agensi koji sprečavaju rast pojedinih vrsta tumora. Prikazana je i njihova spektralna analiza (FT-IR i NMR), zbog potvrde strukture, kao i analiza graničnih molekulskih orbitala koja daje prikaz elektronskih svojstava ovih molekula, što je važno zbog njihove biološke aktivnosti. Da bi se predstavila hemijska reaktivnost glutarimida predstavljen je molekulski elektronski potencijal (MEP) iz prethodno optimizovane geometrije reprezentativnog primera *terc*-pentil-1-benzil-4-metil-glutarimid-3-karboksilata (skraćenica PBMG). Elektronska svojstva su objašnjena na primeru talidomida.

**Ključne reči:** glutarimidi, biološka aktivnost, sinteza glutarimidnih derivata, spektralna analiza, elektronska svojstva.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Imidi su diacilovani derivati amonijaka ili primarnog amina i po svojoj strukturi mogu biti aciklični (1), poluciklični (2) i ciklični (3,4), slika 1. Nakon svog otkrića posmatrani su kao derivati kiselina i nisu svrstavani u posebnu klasu jedinjenja. Pouzdani podaci o njihovim fizičkim svojstvima datiraju tek iz pedesetih godina prošlog veka, kada je počelo intenzivno ispitivanje ovih jedinjenja kao biološki aktivnih molekula i njihova primena u farmaceutskoj i drugim granama industrije [1].



Slika 1. Struktura imida.

Figure 1. Imide structure.

Aciklični imidi su, za razliku od cikličnih imida, veoma retka jedinjenja. S obzirom da poseduju značajnu reaktivnost, veoma su korisni kao sintetički intermedijeri. Ipak, poznato je samo nekoliko metoda za njihovu sintezu. Jedna od metoda za dobijanje *N*-alkil acikličnih imida opisana je u našem prethodnom radu [2].

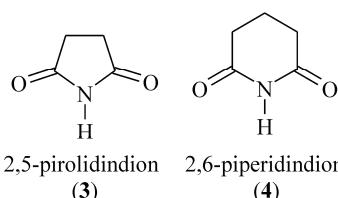
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Monociklični karboksilni imidi se smatraju proizvodima koji nastaju u reakciji odgovarajuće dikarboksilne kiseline i amonijaka, uz eliminaciju vode. U trivijalnoj nomenklaturi, ciklični imidi se imenuju prema imenu odgovarajuće alifatične dikarboksilne kiseline. Sistemski imeni izvode se iz odgovarajućih cikličnih amina (pirolidina i piperidina). Primeri su 2,5-pirolidindion (sukcinimid, 3) i 2,6-piperidindion (glutarimid, 4), Slika 1. Imidi prstenovi koji sadrže 4–7 članova (3–6



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ugljenikovih atoma) su opisani u literaturi, dok imidi sa 3 ili više od 7 članova u prstenu nisu poznati. Sukcinimid (3) je prvi ciklični imid koji je dobijen sintetičkim putem. Njegova sinteza izvedena je davne 1835. godine, nedugo pošto je Wöhler, prvi put sintetisao organsko jedinjenje iz neorganskog molekula [1].

## Biološka aktivnost glutarimida

U literaturi je opisan veliki broj sintetičkih imida od kojih su mnogi farmakološki aktivni. Šestočlani ciklični imidi su značajna bioaktivna jedinjenja, jer deluju kao antagonisti adrenogenih receptora, imunomodulatori, antiinflamatorni agensi, anksiolitici, antivirotici, antibio-

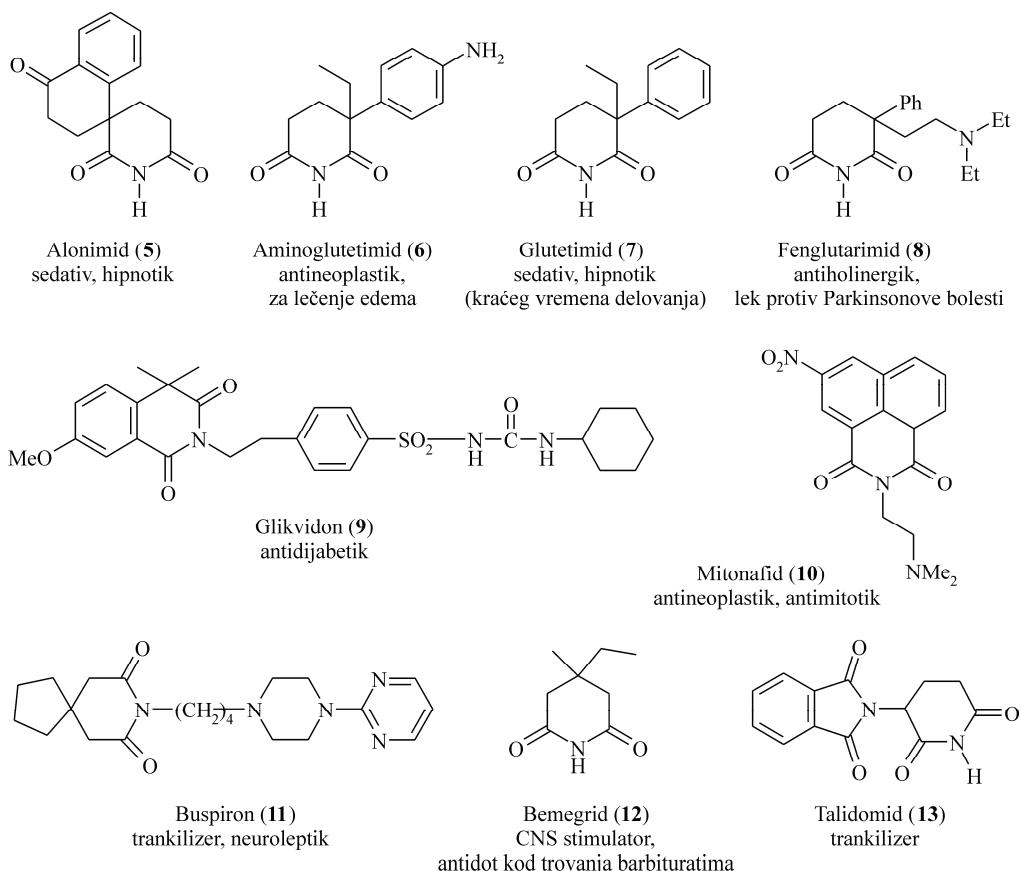
tici i agensi koji sprečavaju rast pojedinih vrsta tumora [3]. Na Slici 2 prikazani su odabrani glutarimidni derivati (**5–13**) i farmakološka aktivnost koju ispoljavaju [4].

Supstituisani glutarimidi sreću se i kod nekih farmakološki aktivnih prirodnih proizvoda. Od prirodnih glutarimidnih derivata najviše su proučavani sesbanimid, cikloheksimid, streptimidon, izomigrastatin, migrastatin i laktimidomicin. Sesbanimid A i B, koji su izolovani iz semena biljke *Sesbania drumondi*, su alkaloidi sa jakim citostatskim dejstvom i ispoljavaju značajnu inhibitornu aktivnost prema P388 ćelijama murin leukemije *in vivo* [5]. Cikloheksimid je prvobitno izolovan iz gljivice *Streptomyces griseus*. Ispoljava snažno antibiotsko dejstvo tako što inhibira biosintezu proteina u eukariotskim organizmima. Novija istraživanja u humanoj medicini pokazala su da cikloheksimid značajno pojačava citotoksični efekat rekombinantnog faktora- $\alpha$  nekroze humanog tumora (recombinant human tumor necrosis factor- $\alpha$  (rHuTNF- $\alpha$ ) na ćelije nasofaringealnog karcinoma (NPC) [6,7]. Streptimidon ispoljava antifungicidno dejstvo tako što sprečava razvoj nekih bolesti kod određenih biljaka i pri tom ne ispoljava toksičnost prema samoj biljci, dok novoizolovani derivati streptimidona (9-MS i S632A3) ispoljavaju antiinflamatorno i antitumorno dejstvo [8–10].

Makrolidni antibiotici izomigrastatin (izo-MGS) [11], migrastatin (MGS) i laktimidomicin (LTM), poseduju glutarimidni prsten u bočnom nizu, koji se retko sreće kod prirodnih proizvoda. Migrastatin je metabolit izomigrastatina, izolovan iz vrste *Streptomyces MK929-43F1*, koji ispoljava [12] inhibitorno dejstvo na migraciju tumornih ćelija i antimetastatsko dejstvo [13]. Iz fermenta bakterijske vrste *Streptomyces amphibiosporus* ATCC53964 izolovan je laktimidomicin (LTM). Ovaj glutarimidni derivat ispoljava snažno citotoksično dejstvo prema velikom broju ćelijskih linija humanih tumora ( $IC_{50}$  3–65 nM) u *in vitro* testovima, prema tumorima miševa u *in vivo* testovima i deluje inhibitorno na razvoj gljivica [11,14].

Sintetizovani su brojni preparati sa šestočlanim imidnim prstenom koji ispoljavaju raznovrsnu farmakološku aktivnost. Buspiron (Buspar®) se kao lek za tretiranje anksioznosti primenjuje još od osamdesetih godina prošlog veka. Ovo jedinjenje pripada klasi aza-spirodekandiona i deluje kao selektivan parcijalni agonist serotonin 5-HT<sub>1A</sub> receptora [15]. U Kini i Japanu, kao anksiolitik i antidepresiv, u širokoj upotrebi je njegov strukturalni analog tandospiron (Sediel®) [16,17].

Talidomid je uveden u upotrebu kasnih pedesetih godina prošlog veka kao sedativ, ali je već 1961. godine



Slika 2. Derivati glutarimida (5–13).

Figure 2. Glutarimide derivatives (5–13).

povučen sa tržišta zbog izražene teratogenosti i prouzrokovana neuropatijske. Nakon otkrića da ispoljava anti-inflamatorno i anti-angiogeno dejstvo, nakadno je odobren od strane FDA (*Food and Drug Administration*) 1998. godine, kao veoma efikasan lek u terapiji nekih vrsta kancera (multiple myeloma) i bolnih kožnih komplikacija prouzrokovanih leprom (*erythema nodosum leprosum* – ENL) [18]. Od nedavno su na tržištu dva nova leka bazirana na strukturi talidomida, lenalidomid (Revlimid®) [19,20] i pomalidomid (Pomalyst®) [21].

Takođe su poznata mnoga policiklična aromatična jedinjenja sa glutarimidnim prstenom koja imaju specifična optička i fluorescentna svojstva, pa su našla primenu kao biološki markeri i različiti, kao i u industriji optičkih i elektronskih uređaja [22–25]. Široka primena cikličnih imida nedvosmisleno ukazuje na značaj ove klase jedinjenja i aktuelnost razvoja novih metoda za njihovu sintezu.

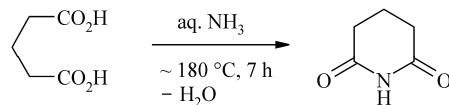
#### POSTUPCI ZA SINTEZU GLUTARIMIDA

U ovom tekstu biće prikazani opšti postupci za zatvaranje glutarimidnog prstena. Metode za sintezu šestočlanih cikličnih imida obuhvataju: 1) reakciju odgovarajućih dikarboksilnih kiselina sa amonijakom (ili derivatima amonijaka); 2) ciklizaciju amido-kiselina ili diamida; 3) ciklizaciju dinitrila, cijanokiselina i amido-nitrila; 4) ciklizaciju amido-estara; 5) ciklizaciju amido-acil-hlorida ili diacil-hlorida; 6) adiciju ugljen-monoksida na  $\alpha,\beta$ -nezasićene amide; 7) oksidaciju laktama ili cikličnih amina; 8) Michael adiciju aktivnih metilenskih jedinjenja na metilakrilamid; 9) tandem Michael adiciju aktivnih metilenskih jedinjenja na sekundarne konjugovane amide. Neki od prikazanih postupaka, primenjeni su u sintezama različitih biološki aktivnih glutarimidnih derivata.

#### Reakcija odgovarajućih dikarboksilnih kiselina sa amonijakom ili derivatima amonijaka

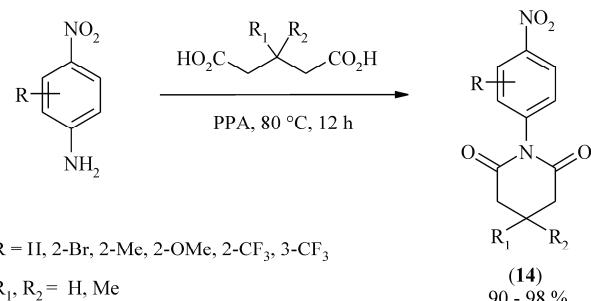
Reakcija predstavlja standardni sintetički postupak za dobijanje glutarimida jednostavne strukture. Ciklizacija se odvija na visokoj temperaturi u prisustvu ekvimolarne količine amonijaka (ili jedinjenja iz kog se pod reakcionim uslovima dobija amonijak) ili supstituisanih derivata amonijaka kao što su urea, tiourea, formamid, dicijandiamid, nitrili, dicijanidietanolamin, sulfaminska kiselina, natrijum-azid ili amonijum-karbonat. Prinosi cikličnih imida i njihovih *N*-supstituisanih derivata, koji se mogu dobiti ovim postupkom, su 60–80%. Reakcija se može uspešno primeniti i za ciklizaciju alifatičnih kiselina sa alkil ili aril supstituentima na ugljenikovom atomu i različito supstituisanih aromatičnih kiselina (alkil,  $\text{NO}_2$ ,  $\text{NH}_2$  i halogen). U modifikovanom postupku, koji podrazumeava upotrebu amonijumovih soli odgovarajućih kiselina, reakcija se izvodi

na nižim temperaturama [1]. Dobijanje glutarimida iz glutarne kiseline i amonijaka prikazano je na šemi 1 [26].



Šema 1. Dobijanje glutarimida iz glutarne kiseline i amonijaka.  
Scheme 1. Synthesis of glutarimide from glutaric acid and ammonia.

4-Nitro-*N*-aril supstituisani glutarimidi, strukture **14** mogu se dobiti iz odgovarajućih dikarboksilnih kiselina i supstituisanih anilina u prisustvu polifosforne kiseline (PPA). Ciklokondenzacija se odvija u jednom reakcionom stupnju, šema 2 [27]. *N*-supstituisani ciklični imidi su ključni strukturni delovi molekula prirodnih alkaloida koji ispoljavaju analgetska svojstva i sprečavaju grčeve.



R = H, 2-Br, 2-Me, 2-OMe, 2-CF<sub>3</sub>, 3-CF<sub>3</sub>

R<sub>1</sub>, R<sub>2</sub> = H, Me

(14)  
90 - 98 %

Šema 2. Reakcija supstituisanih anilina sa dikarboksilnim kiselinama u prisustvu polifosforne kiseline.

Scheme 2. The reaction of substituted anilines with dicarboxylic acids in the presence of polyphosphoric acid.

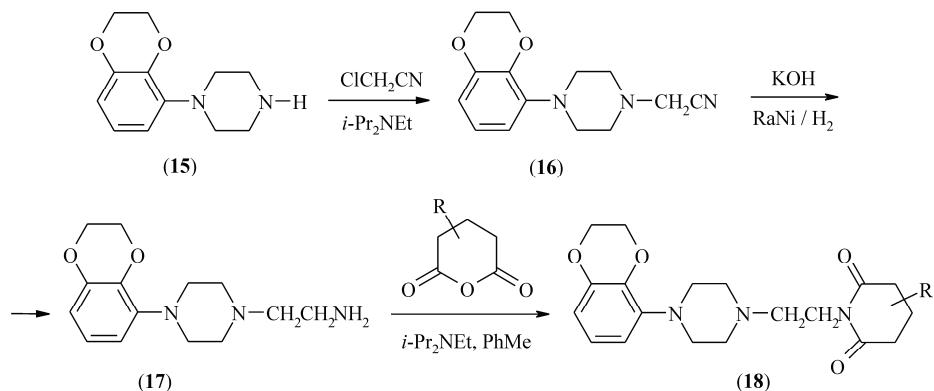
#### Ciklizacija amido-kiselina ili diamida

Dobijanje glutarimida iz amido-kiselina primenjeno u sintezama derivata buspirona i sesbanimida, prikazano je u šemama 3–5.

Sintetski postupak primenjen za sintezu analoga buspirona polazeći od glutaranhidrida i supstituisanih analoga glutaranhidrida prikazan je u šemi 3 [28]. Mono *N*-arilpiperazin (**15**) se *N*-alkiluje hlor-acetonitrilom, a postali nitrilni intermedijer (**16**) hidrogenizuje pod baznim uslovima pri čemu nastaje primarni amin (**17**). Direktnom kondenzacijom ovog amina sa odgovarajućim anhidridom, u prisustvu (i-Pr)<sub>2</sub>NEt kao baze, nastaju glutarimidi strukture (**18**), šema 3.

Zbog značajnih bioloških svojstava koju ispoljavaju alkaloidi sesbanimid A i B, kao i male rasprostranjenosti ovih jedinjenja u prirodi, postojalo je veliko interesovanje za iznalaženje efikasnih postupaka za njihovu sintezu. U postupcima opisanim u literaturi primenjeni su različiti načini zatvaranja glutarimidnog prstena sesbanimida, što je ilustrovano šemama 4, 5, 7 i 9.

Konstrukcija imidnog prstena u totalnoj sintezi sesbanimida A i B iz D-(+)-ksiloze započinje Michael-ovom



Šema 3. Opšti postupak za sintezu derivata bispirona.

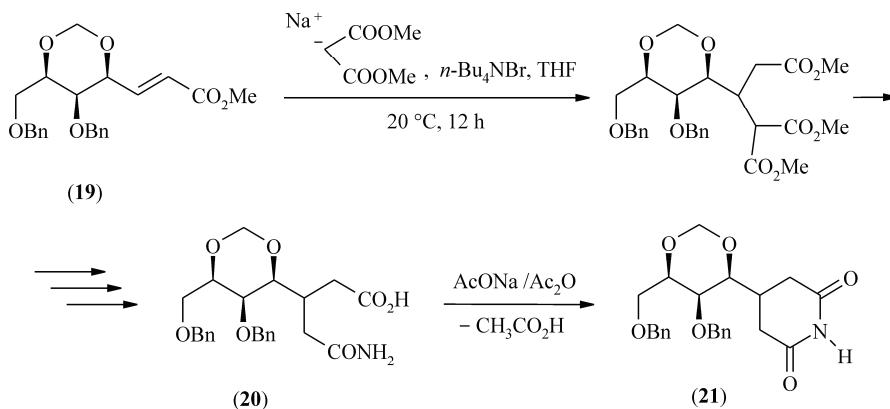
Scheme 3. General method for the synthesis of buspirone derivatives.

1,4-adicijom natrijumove soli dimetil-malonata na konjugovani estar (19). U nekoliko reakcionih faza iz estra (19) dobija se amido-kiselina (20) koja tretiranjem s mесом  $\text{AcONa}/\text{Ac}_2\text{O}$  ciklizuje do glutarimida (21), šema 4 [29].

Deo totalne sinteze (–)-sesbanimida A, optičkog antipoda citotoksičnog prirodnog proizvoda (+)-sesbanimida A, koji se odnosi na zatvaranje glutarimidnog prstena, prikazan je na šemi 5. Na konjugovani estar

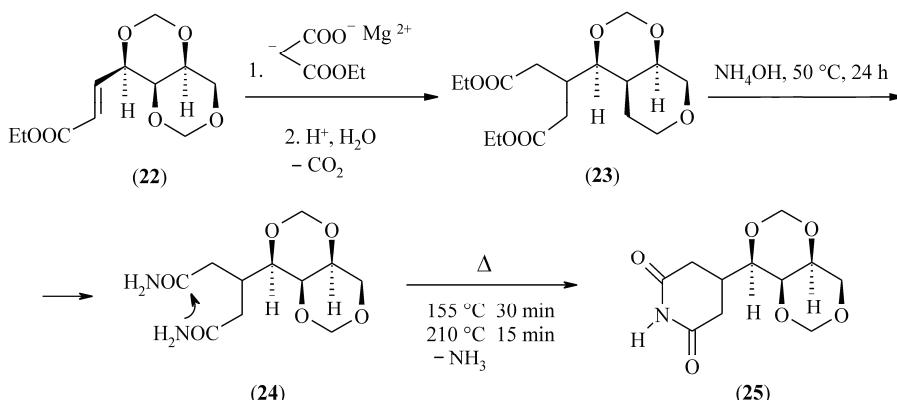
(22), adira se magnezijum-monoetyl-malonatni karbanjon pri čemu nastaje međuproizvod koji posle obrade razblaženom kiselinom i dekarboksilacije daje diestar (23). Glutarimidni prsten (25) dobijen je u dve reakcione faze: reakcijom diestra (23) sa  $\text{NH}_4\text{OH}$  na povišenoj temperaturi pri čemu nastaje diamid (24), a zatim sledi termoliza diamida do glutarimida (25) [30].

Zatvaranje glutarimidnog prstena ovom metodom primenjeno je u totalnoj sintezi ( $\pm$ )-sesbanimida A i B [31].



Šema 4. Nastajanje glutarimidnog prstena u totalnoj sintezi sesbanimida A i B.

Scheme 4. The formation of glutarimide ring in total synthesis of sesbanimide A and B.



Šema 5. Nastajanje glutarimidnog prstena termolizom diamida.

Scheme 5. The formation of glutarimide ring by thermolysis of diamide.

### Ciklizacija dinitriла, cijanokiselina i amidonitrila do glutarimida

$\alpha,\omega$ -Dinitrići, derivati cílibarne, glutarne i adipinske kiseline ciklizuju pod kiselim i anhidrovanim uslovima pri čemu postaje adukt (26) koji je stabilan u reakcionej sredini. Parcijalnom hidrolizom (pH 8–9) daju imino-imide strukture (27) čijom hidrolizom nastaju odgovarajući imidi (sukcinimid, glutarimid i adipimid). Pod sličnim, ali energičnijim uslovima,  $\alpha,\omega$ -cijanokiseline ciklizuju do odgovarajućih imida preko intermedijernog katjona (28), šema 6A i B. Prema ovom opštem postupku Tagmann i sar. sintetizovali su 3,3-disupstituisane sukciniimide, glutarimide i adipimide [32,33].

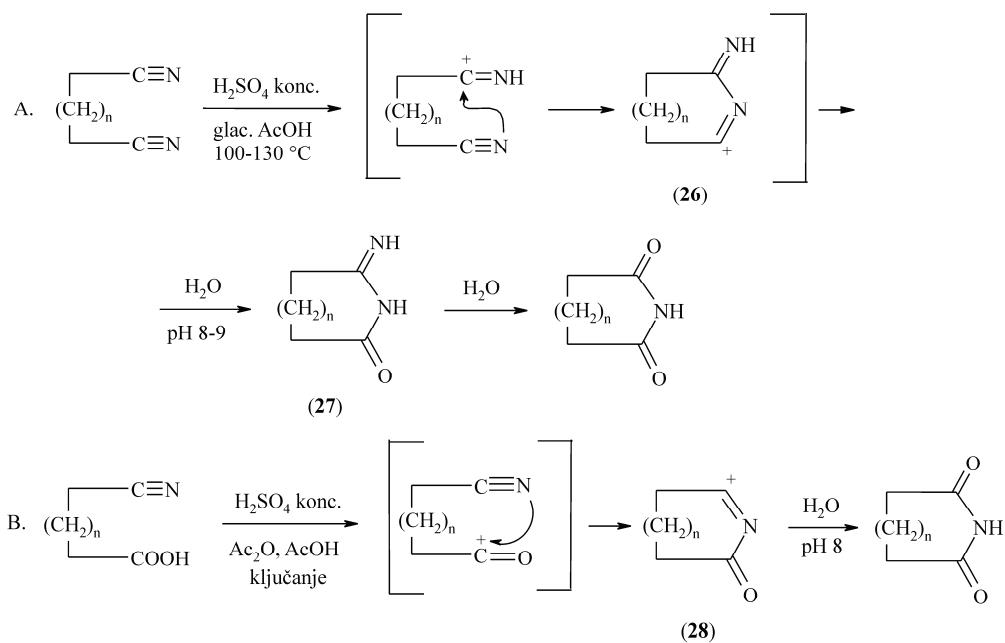
U još jednom sintetskom postupku za dobijanje sesbanimida, opisanom u literaturi, glutarimidni prsten je

dobijen ciklizacijom cijano-amida, šema 7 [34]. Iz konjugovanog estra (29), u nekoliko reakcionalih faza, nastaje cijano-amid (30) koji podleže ciklizaciji pod baznim uslovima, a intermedijerni imido-imin zatim hidrolizuje do glutarimida (31) pomoću mravlje kiseline.

### Ciklizacija amido-estara

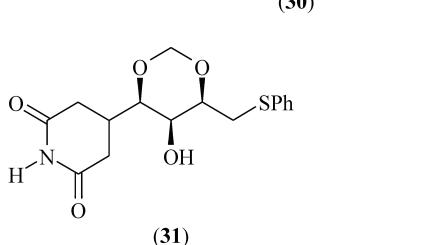
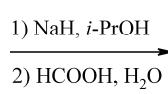
U literaturi su opisani primeri dobijanja šetočlanih cikličnih imida, ciklizacijom odgovarajućih amido-estara. Prema ovoj metodi prsten se zatvara nukleofilnom adicijom *N*-amidnog anjona na estarsku grupu. Uz pažljivo odabrane reakcione uslove, prinosi mogu biti i do 90%. Sledеći primer ilustruje primenu metode u sintezi šestočlanog imidnog derivata (32), šema 8 [35].

Šemama 9 i 10 ilustrovano je zatvaranje glutarimidnog prstena ciklizacijom odgovarajućih amido-es-

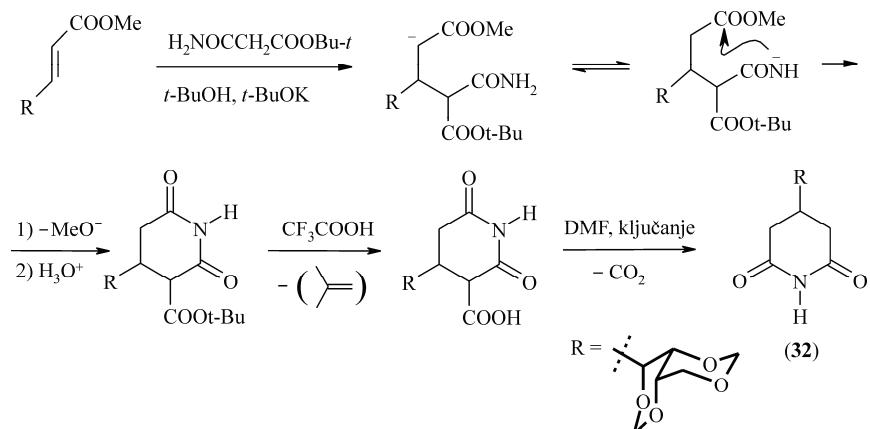


Šema 6. Ciklizacija  $\alpha,\omega$ -dinitriла и  $\alpha,\omega$ -cijanokiselina до одговарајућих glutarimida.  
Scheme 6. Cyclization of  $\alpha,\omega$ -dinitriles and  $\alpha,\omega$ -cyanoacids to corresponding glutarimides.

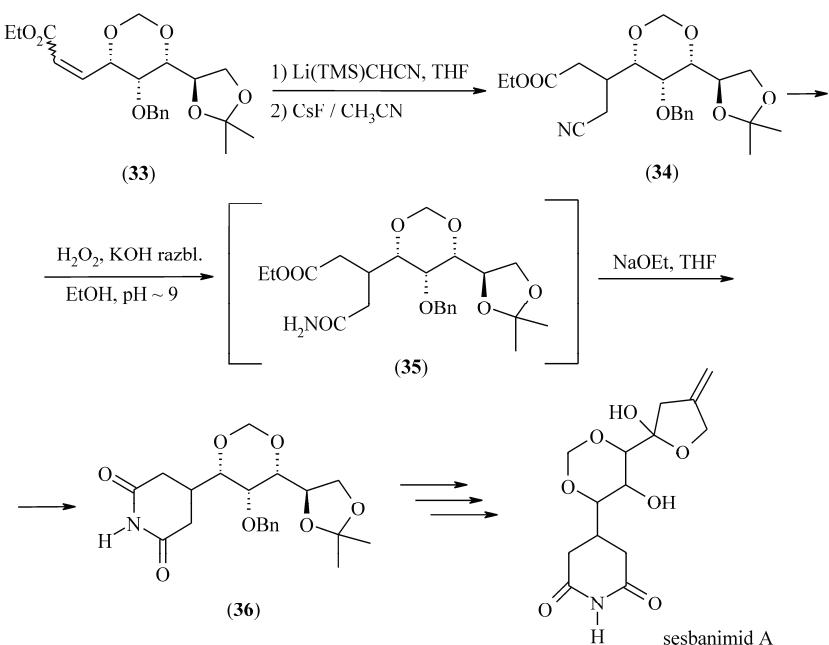
MOM = metoksimetil



Šema 7. Nastajanje glutarimidnog prstena ciklizацијом cijano-amida.  
Scheme 7. The formation of glutarimide ring by cyclization of cyanamide.



Šema 8. Nastajanje glutarimidnog prstena ciklizacijom amido-estara.  
Scheme 8. The formation of glutarimide ring by cyclization of amidoesters.



Šema 9. Formiranje glutarimidnog prstena u sintezi sesbanimida.  
Scheme 9. The glutarimide ring closure in the synthesis of sesbanimide.

tara. Ova metoda primenjena je u sintezama biološki aktivnih jedinjenja sesbanimida i inhibitora enzima aldo reduktaza (ARI).

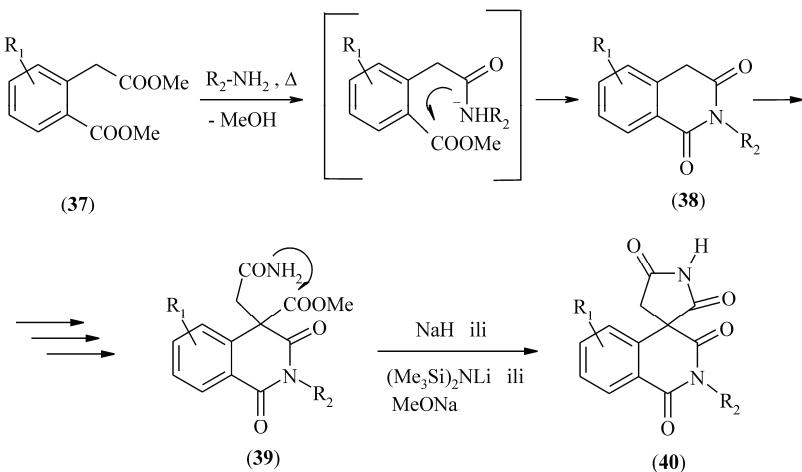
U totalnoj sintezi (+)-sesbanimida A iz D-glukoze primenjena je reakciona sekvenca prikazana u šemi 9. Na konjugovani estar (33) izvršena je 1,4-adicija  $\alpha$ -(trime-tilsilik)acetonitrilnog karbanjona pri čemu je posle desilanovanja postao cijano-estar (34). Selektivnom hidrolizom (hidratacijom nitrilne grupe pomoću vodonik-peroksida) dobijen je amido-estar (35) koji zatim, pomoću natrijum-etoksida kao baze, ciklizuje do glutarimida (36) [36].

U stereospecifičnoj sintezi (-)-sesbanimida A i (+)-sesbanimida A, iz derivata L- i D-ksiloze, Warner i sar. su takođe koristili ovu metodu za dobijanje glutarimidnog prstena [37].

Spiro-bisimid (40) je jedan od važnih intermedijera u sintezi novih inhibitora enzima aldo reduktaza (ARI) [38]. Farmakološki značaj ovih inhibitora je u tome što sprečavaju pojavu i razvoj neuropatskih oboljenja kod pacijenata koji boluju od dijabetesa. Polazno jedinjenje za spiro-bisimid može biti diestar homoftalne kiseline (37) čijom amonolizom postaje amido-estar (38) koji pod reakcionim uslovima ciklizuje do homoftalimida (39). Daljom transformacijom nastalog imida u nekoliko reakcionih faza dobija se spiro-bisimid (40), šema 10.

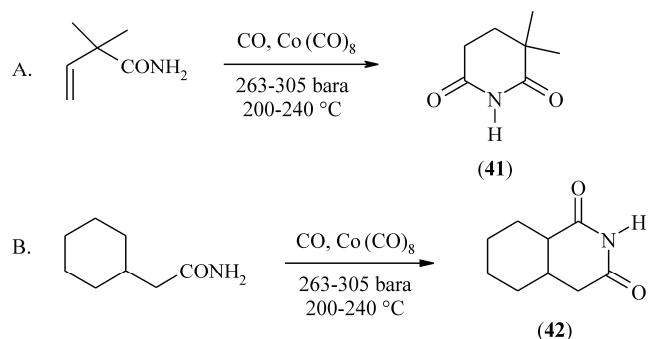
#### Adicija ugljen-monoksida na nezasićeneamide

Nezasićeni amidi, konjugovani i nekonjugovani, mogu se prevesti u petočlane i šestočlane ciklične imide pomoću ugljen-monoksida kao agensa za karbonilovanje. Reakcioni uslovi su ekstremni ( $300^\circ\text{C}$ ,  $>190$  bar,



Šema 10. Nastajanje intermedijernog spiro-bisimida u sintezi inhibitora enzima aldo reduktaza.

Scheme 10. The formation of the intermediate spiro-bisimide in the synthesis of aldose reductase inhibitors.



Šema 11. Nastajanje glutarimida adicijom ugljen-monoksida na nezasićene amide.

Scheme 11. The formation of glutarimide by addition of carbon monoxide on unsaturated amides.

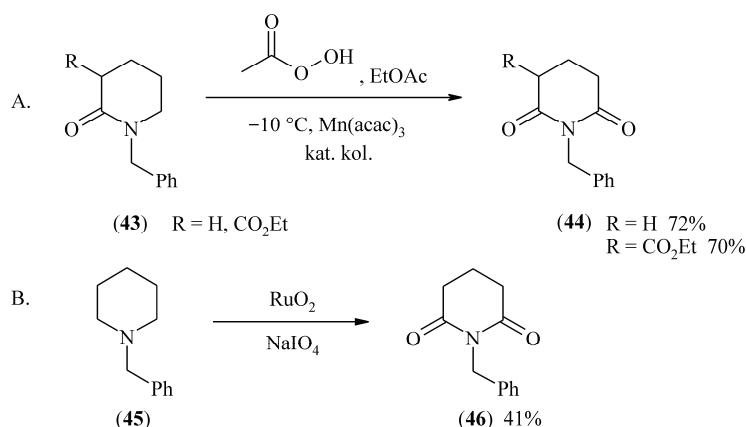
specijalan katalizator), ali su prinosi reakcije dobri za jednostavnije sisteme (**41** i **42**), šema 11 [39].

#### Oksidacija laktama ili cikličnih amina

Metilenska grupa u  $\alpha$ -položaju prema azotu može se oksidovati u nekim slučajevima, do karbonilne grupe. Šemom 12A prikazana je oksidacija laktama (**43**) do

odgovarajućeg imida (**44**). Pored laktama koji se lakše oksiduju i daje bolje prinose imida, takođe se mogu oksidovati i ciklični amini. Postoji veliki broj oksidacionih agenasa koji se mogu upotrebiti, ali perkiseline u prisustvu metalnih katalizatora (mangan-acetylacetonat) daju najbolje rezultate [40].

*N*-supstituisani piperidini (**45**) su oksidovani do

Šema 12. Oksidacija laktama i *N*-supstituisanog piperidina do odgovarajućih imida.Scheme 12. Oxidation of lactams and *N*-substituted piperidines to the corresponding imides.

odgovarajućih imida (**46**), a kao najuspešniji reagens za ova jedinjenja pokazao se rutenijum-dioksid u prisustvu natrijum-metaperjodata, šema 12B [41].

#### Michael adicija aktivnih metilenskih jedinjenja na metilakrilamid

Glutarimidi se mogu sintetisati Michael adicijom jedinjenja koja sadrže kiseli vodonikom atom (etilčjano-acetat i etilmalonat, **47** i **48**) na metilakrilamid u prisusutu aktivirajućeg sistema  $\text{CsF/Si(OMe)}_4$ . Reakcija se odvija u heterogenom sistemu, bez prisustva rastvarača. Kao proizvod reakcije nastaju glutarimidi **49** i **50**, supstituisani u položaju 3, šema 13 [42].

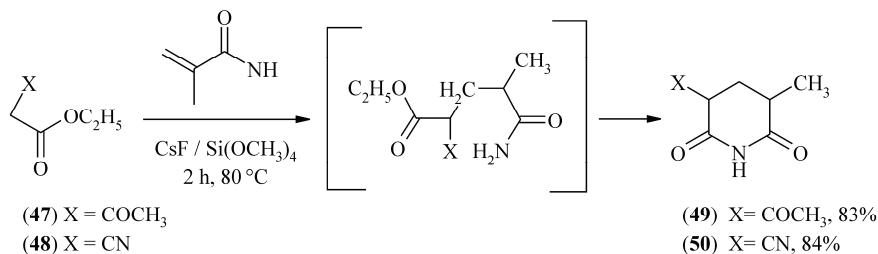
#### Tandem Michael adicija aktivnih metilenskih jedinjenja na sekundarne konjugovane amide

Novi tandem proces za sintezu glutarimida supstituisanih u položaju 3 kao i spirobikliklinskih imida, opisan

je u našem prethodnom radu [43]. Postupak obuhvata bazno-katalizovanu reakciju Michael adicije acikličnih i cikličnih  $\beta$ -keto-estara (kao aktivnih metilenskih jedinjenja) na sekundarne akrilamide i krotonamide (Michael akceptor), praćenu intramolekulskim *N*-acilovanjem karboksamido grupe. Metoda predstavlja jednostavan način za dobijanje korisnih sintetičkih intermedijera i potencijalno aktivnih farmakoloških jedinjenja.

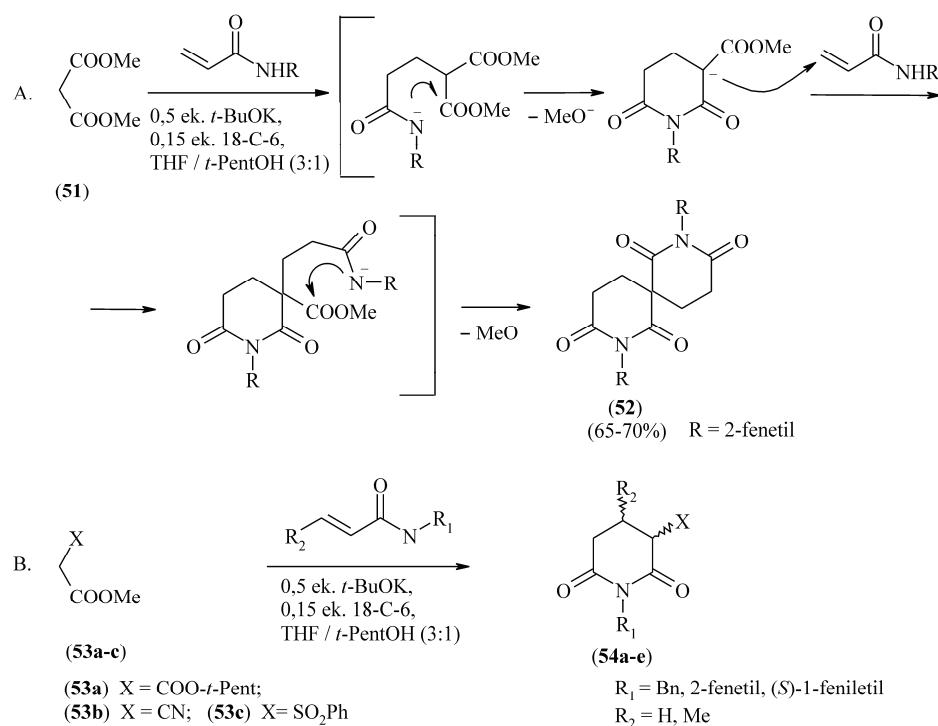
U reakciji dimetil-malonata (**51**) sa *N*-fenetilakrilamidom pod reakcionim uslovima prikazanim šemom 14A nastaje spiro-bisglutarimid (**52**). Pod istim reakcionim uslovima, akrilamid i *N*-fenetilakrilamid sa drugim aktivnim metilenskim jedinjenjima, (metil-*t*-pentilmalonat, etil-2-cijanoacetat i etil-2-(fenilsulfonil)-acetat) (**53a–c**) daju 3-supstituisane glutarimide (**54a–e**) u pri-nosu od 35–72%, šema 14B, Slika 3.

Reakcijom cikličnih  $\beta$ -ketoestara (**55a–f**) sa *N*-benzil-akrilamidom nastaju 3-supstituisani ili spiroglutarimidi



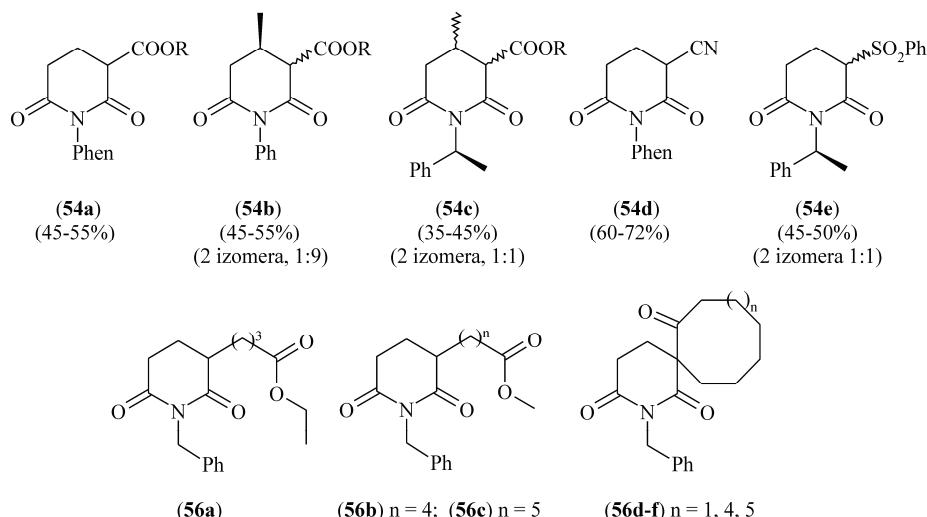
Šema 13. Michael-ova adicija aktivnih metilenskih jedinjenja na metilakrilamid.

Scheme 13. Michael addition of active methylen compounds on the methacrylamide.



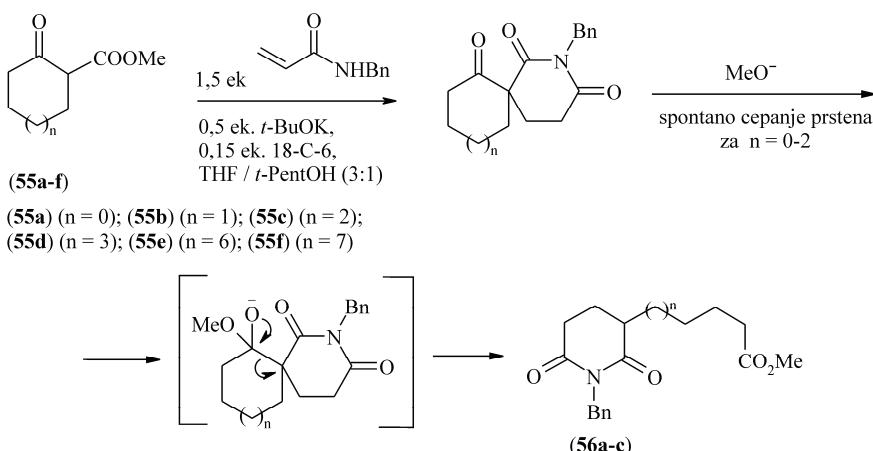
Šema 14. Tandem Michael adicija aktivnih metilenskih jedinjenja na sekundarne konjugovane amide.

Scheme 14. Tandem Michael addition of active methylene compounds to secondary conjugated amides.



Slika 3. Strukture imida (54a-e) i (56a-f).

Figure 3. Structures of imides (54a-e) and (56a-f).

Šema 15. Reakcija cikličnih  $\beta$ -ketoestara sa N-benzilakrilamidom.Scheme 15. The reaction of cyclic  $\beta$ -keto esters with N-benzylacrylamide.

(56a-f), šema 15. Ishod reakcije u najvećoj meri zavisi od veličine prstena polaznog  $\beta$ -ketoestra. Opisanim postupkom dobijena je serija novih spirobicikličnih imidnih sistema (56d-f), Slika 3.

U našem prethodnom radu [44], na osnovu literaturnih podataka o antiproliferativnoj aktivnosti pojedini glutarimidnih derivata, napravljeni su modeli i određen je odnos strukture i antiproliferativne aktivnosti seta tivne aktivnosti jedinjenja (3D QSAR) prema sledećim ćelijskim linijama: K562 (leukemija), ATCC-A549 (tumor pluća), Malme 3M (melanom), COLO205 (tumor debelog creva), UO31 (tumor slezine), U251 (tumor centralnog nervnog sistema), IGROV1 (tumor jajnika) i MFC7 (tumor dojke). Modeli su jasno pokazali strukturne elemente jedinjenja koji se pozitivno ili negativno korelišu sa biološkom aktivnošću. Rezultati dobijeni iz ove studije mogu poslužiti kao smernica za dizajniranje i sintezu novih glutarimidnih derivata za koje se očekuje da će pokazati antiproliferativnu aktivnost.

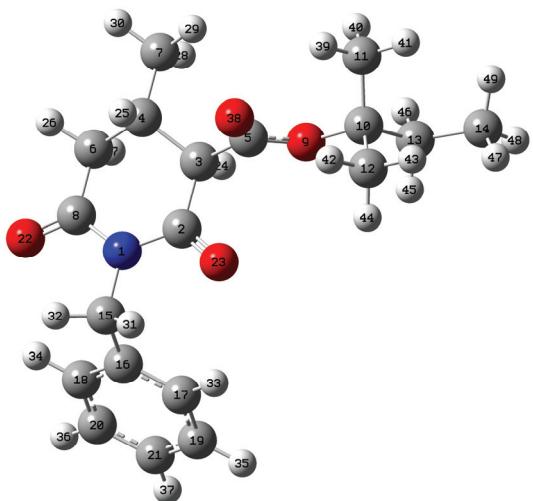
glutarimidnih derivata prema osam reprezentativnih ćelijskih linija humanih tumora. Podaci o strukturi molekula i njihovoj antiproliferativnoj aktivnosti su preuzeti iz baze podataka Nacionalnog Instituta za rak SAD (*National Cancer Institute-Developmental Therapeutics Program*, NCI-DTP) [45]. Napravljeni su modeli koji opisuju odnos trodimenzionalne strukture i antiprolifera-

## FIZIČKO-HEMIJSKE KARAKTERISTIKE

### Molekulska geometrija

Da bi se potvrdila struktura nekog jedinjenja i odredio najstabilniji izomer često se vrše kvantno-mehanički proračuni i dobijeni parametri (dužine veza i uglovi između njih) porede se sa podacima dostupnim iz literature. Ukoliko je jedinjenje nepoznato poređenje se vrši sa podacima analize pomoću X-zraka sličnog jedinjenja ili osnovnog skeleta kao što je u ovom slučaju

glutarimid. Petersen je kristalografskim ispitivanjem strukture glutarimida ustanovio da ovaj molekul nije planaran [46]. Utvrđeno je da je metilenska grupa u položaju 4 u odnosu na azotov atom za 0,59 Å iznad ravni molekula glutarimida. Konformacija molekula može se opisati kao polustoličasta. U kristalnoj rešetci postoje intermolekulske vodonične veze kojima se formira lanac od molekula glutarimida u pravcu b-ose. Teorijskim ispitivanjem geometrijske strukture glutarimida [47] potvrđeno je da se podaci dobijeni *ab initio* i DFT metodama odlično slažu sa eksperimentalnim vrednostima. U našem prethodnom radu prikazana je struktura novosintetisanog PBMG jedinjenja [48]. Geometrijska struktura optimizovana je B3LYP/6-311++G(d,p) metodom. Izračunate dužine veza C-C, C-N i C-H su 1,52, 1,4 i 1,09 Å u glutarimidnom prstenu i odlično se slažu sa eksperimentalnim vrednostima 1,5, 1,39 i 0,9–1,1 Å. Uglovi C-C-C i C-N-C su reda veličine 111 i 127° u glutarimidu i dobro se slažu sa izračunatim uglovima 108 i 124,7° u molekulu PBMG. Torzioni ugao N1-C2-C3-C4 je 0,38° što ukazuje da postoji određeno odstupanje od planarnosti glavnog prstena u molekulu PBMG. Molekul PBMG ima dva izomera *trans* i *cis*. DFT proračunom koristeći B3LYP/6-311++G(d,p) metod dokazano je da se molekul nalazi u najstabilnijoj konformaciji prikazanoj na slici 4. Iz slike 4 može se videti da je ovo *trans* oblik molekula u kome je –CH<sub>2</sub>Ph grupa ispod ravni glutarimidnog prstena.



Slika 4. Optimizovana geometrija najstabilnijeg *trans* izomera PBMG.

Figure 4. Geometry optimized structure of the most stable *trans* isomer of PBMG.

### NMR i IR analiza

Za karakterizaciju aktivnih bioloških jedinjenja NMR analiza je korisna jer analizom hemijskih pomeranja određenih grupa u molekulu mogu se pored strukture odrediti i odnosi izomera. Hemijska pomeranja su važan deo informacija koje se nalaze u NMR spektrima. Ova

pomeranja zavisna su od različitih konformacija molekula te su zbog toga veoma bitna za karakterizaciju istih. U <sup>13</sup>C-NMR analizi glutarimida potvrda glutarimidne grupe nalazi se u hemijskim pomeranjima u intervalu 160–170 ppm za dva ugljenika karbonilnih grupa. Tako na primer u različitim 3,4-dimetilenglutarimidima pomeranja karbonilnih ugljenika su u regionu 165,22–171,26 ppm [49]. Za molekul PBMG teorijski su izračunata hemijska pomeranja koristeći B3LYP/6-311++G(d,p) metod, a za izračunavanja NMR pomaka primenjen je GIAO (*Gauge-Independent Atomic Orbital*) metod. U molekulu PBMG eksperimentalno su određena pomeranja (u rastvoru CDCl<sub>3</sub>) za ugljenike C2 i C8 su 168,54 i 170,5 što se odlično slaže sa izračunatim vrednostima hemijskih pomeranja na 168,79 i 171,29 ppm. Slične vrednosti 174,78 i 176,32 ppm su dobijene i za ugljenike karbonilnih grupa petocikličnog imidnog prstena, sukcinimida [50]. Na primer alifatični ugljenici C13 i C15 su na 38,24, i 57,55 ppm i slažu se veoma dobro sa izračunatim vrednostima na 36,62 i 55,04 ppm.

Iz <sup>1</sup>H-NMR spektara može se odrediti i odnos *trans*/*cis* izomera. U molekulu PBMG ovaj odnos određen je iz odnosa dva dubleta na 3,08 i 3,45 ppm (dubleti koji potiču od atoma H24 na C3 atomu). Takođe isti odnos *trans/cis* diastereoizomera (5:1) utvrđen je i iz odnosa dva singleta metil grupa na C11 i C12 atomu kao i iz odnosa metil grupa na C7 i C14 atomu. Treba istaći da se i u izračunatim <sup>1</sup>H-NMR spektrima hemijska pomeranja odlično slažu sa eksperimentalnim.

U infracrvenoj spektroskopiji (IR) potvrdu strukture glutarimidnog prstena moguće je izvesti iz vibracija N-H veze kao i vibracija karbonilnih grupa. Analizom IR spektara glutarimida vibracioni normalni mod simetričnog istezanja N-H grupe, koja je povezana u kristalu vodoničnom vezom tipa N-H· ·O, nalazi se na 3189 (na 25 °C) i 3163 cm<sup>-1</sup> (na -180 °C) [51]. U rastvoru CCl<sub>4</sub>, gde ne postoji intermolekulska vodonična veza, ova vibracija se nalazi na 3383 cm<sup>-1</sup>. Vibracije simetričnih istezanja karbonilne grupe za molekul glutarimida su na 1722 (na 25 °C), 1722 cm<sup>-1</sup> (na -180 °C) i 1734 cm<sup>-1</sup> (u rastvoru CCl<sub>4</sub>), a za asimetrična istezanja 1703, 1703 i 1720 cm<sup>-1</sup>, redom.

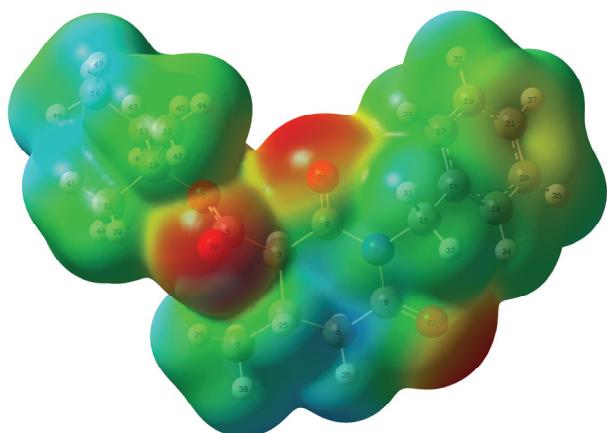
Vibracije simetričnih istezanja karbonilne grupe za molekul PBMG su na 1738 cm<sup>-1</sup> a za asimetrična istezanja 1785 cm<sup>-1</sup> izračunata B3LYP/6-311++G(d,p) metodom i odlično se slažu sa eksperimentalnim vrednostima na 1680 i 1720 cm<sup>-1</sup>. IR spektroskopija omogućava identifikaciju različitih tautomera nekog molekula. Potvrdu postojanja hidrazonskog tautomera novosintetisane azo-boje, 5-(4-metoksifenilazo)-3-cijano-1-etil-6-hidroksi-4-metil-2-piridona [52], koja ima imidnu grupu, daju vibracije istezanja na 1627 i 1672 cm<sup>-1</sup> koje su karakteristične za dve karbonilne grupe. Takođe, široka traka na 3433 cm<sup>-1</sup> u FT-IR spektru ove azo-boje

odgovara vibraciji istezanja N–H hidrazo grupe. Teorijski izračunate vrednosti (B3LYP/6-311++G(d,p) metod) ovih grupa su 1612, 1676 i 3160 cm<sup>-1</sup>, redom i dobro se slažu sa eksperimentalnim vrednostima.

### Molekulski elektrostatički potencijal (MEP)

MEP mapa pokazuje elektronsku gustinu molekula i daje slikovit prikaz potencijala molekula i na taj način pomaže predikciji hemijske reaktivnosti kako u elektrofilnim tako i u nukleofilnim reakcijama. Region sa negativnim potencijalom na MEP povezan je sa reaktivnošću molekula u elektrofilnim reakcijama i predstavljen je žutom i crvenom bojom. Region sa pozitivnim potencijalom na MEP povezan je sa reaktivnošću u nukleofilnim reakcijama i predstavljen je plavom bojom (za boje pregledati elektronsku verziju rada).

MEP je za molekul PBMG izračunat uz pomoć B3LYP/6-311++G(d,p) metoda i prikazan je na slici 5. Kao što se može videti sa slike 5, negativan potencijal molekula PBMG nalazi se na kiseonikovim atomima karbonilnih grupa a pozitivan potencijal oko C4 atoma glutarimidnog prstena. Iz svega izложенog može se zaključiti da će molekul PBMG učestvovati karbonilnim grupama tj. kiseonikovim atomima ovih grupa u elektrofilnim reakcijama a grupom na C4 u nukleofilnim reakcijama.



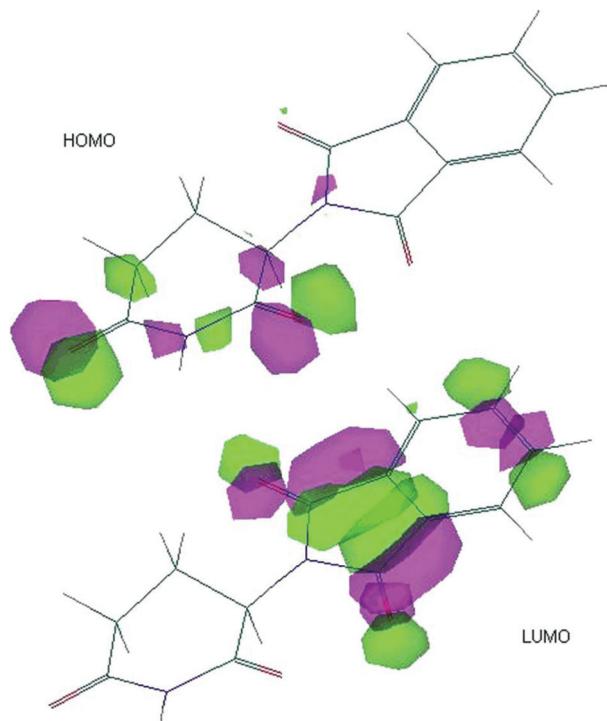
Slika 5. Mapa molekulskog elektrostatičkog potencijala (MEP) trans izomera PBMG.

Figure 5. Molecular Electrostatic Potential (MEP) map of trans isomer of PBMG.

### Elektronska svojstva

Granične molekulske orbitalne (Frontier Molecular Orbital – FMO) određuju elektronske i optičke svojstva molekula. One objašnjavaju način na koji molekul interaguje tj. kako se povezuje sa drugim molekulima i prikazuju njegovu reaktivnost i kinetičku stabilnost. FMO čine najviše popunjena molekulska orbitala HOMO (highest occupied molecular orbital) i najniža nepopunjena molekulska orbitala LUMO (lowest unoccupied molecular orbital). Razlika u energiji ovih orbitala nazi-

va se energetski jaz (*gap*) i predstavlja jednu od veoma bitnih karakteristika molekula. Molekuli sa velikim energetskim jazom imaju veliku energiju ekscitacije i kinetički su stabilni. Za molekul talidomida (2-(2,6-diokso-piperidin-3-il)-isoindol-1,3-dion), poznatog derivata glutarimida sa sedativnim svojstvima, HOMO-LUMO orbitale kao i energetski jaz izračunati su B3LYP/6-31G metodom i prikazani su na slici 6 [53]. Molekulska orbitala LUMO ima energiju -2,31 eV, a molekulska orbitala HOMO -6,72 eV. Energetski jaz je 4,41 eV što ukazuje na malu kinetičku stabilnost i veliku hemijsku reaktivnost ovog molekula. Sa slike 6 može se videti da se HOMO orbitala talidomida prostire u delu molekula glutarimidnog prstena a LUMO orbitala talidomida oko petocikličnog prstena izoindola.



Slika 6. Prikaz HOMO i LUMO talidomida.

Figure 6. The HOMO and LUMO plots of thalidomide.

### ZAKLJUČAK

U radu je prikazana biološka aktivnost i primena glutarimidnih derivata i dat je detaljan prikaz metoda za sintezu glutarimidnog prstena. Takođe je analizirana struktura molekula glutarimida, talidomida i novosintetisanog jedinjenja PBMG. Računski je određena geometrija najstabilnijeg izomera PBMG koja se dobro slaže sa podacima iz literature za molekul glutarimida. Za ovaj izomer predstavljene su IR i NMR analize. MEP analiza molekula pokazuje da kiseonikovi atomi karbonilnih grupa imaju negativan potencijal a C4 atom pozitivan potencijal. Elektronska svojstva derivata glutarimida prikazana su na primeru talidomida.

## Zahvalnica

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## SUMMARY

### GLUTARIMIDES: BIOLOGICAL ACTIVITY, GENERAL SYNTHETIC METHODS AND PHYSICOCHEMICAL PROPERTIES

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(Review paper)

Glutarimides, 2,6-dioxopiperidines, are compounds that rarely occur in natural sources, but so far isolated ones exert widespread pharmacological activities, which makes them valuable as potential pharmacotherapeutics. Glutarimides act as androgen receptor antagonists, anti-inflammatory, anxiolytics, antibacterials, and tumor suppressing agents. Some synthetic glutarimide derivatives are already in use as immunosuppressive and sedative (e.g., thalidomide) or anxiolytics (buspirone) drugs. The wide applicability of this class of compounds, justify the interest of scientists to explore new pathways for its syntheses. General methods for synthesis of six-membered imide ring are presented in this paper. These methods include: a) reaction of dicarboxylic acids with ammonia or primary amine, b) reactions of cyclization: amido-acids, diamides, dinitriles, nitrilo-acids, amido-nitriles, amido-esters, amidoacyl-chlorides or diacyl-chlorides, c) addition of carbon-monoxide on  $\alpha,\beta$ -unsaturated amides, d) oxidation reactions, e) Michael addition of active methylen compounds on methacrylamide or conjugated amides. Some of the described methods are used for closing glutarimide ring in syntheses of pharmacological active compounds sesbanimide and aldose reductase inhibitors (ARI). Analyses of the geometry, as well as, the spectroscopic analyses (NMR and FT-IR) of some glutarimides are presented due to their broad spectrum of pharmacological activity. To elucidate structures of glutarimides, geometrical parameters of newly synthesized *tert*-pentyl-1-benzyl-4-methyl-glutarimide-3-carboxylate (PBMG) are analyzed and compared with the experimental data from X-ray analysis for glutarimide. Moreover, molecular electrostatic potential (MEP) surface which is plotted over the optimized geometry to elucidate the reactivity of PBMG molecule is analyzed. The electronic properties of glutarimide derivatives are explained on the example of thalidomide. The Frontier Molecular Orbital (FMO) and their energies are presented, as well as the energy gap between them.

**Keywords:** Glutarimides • Biological activity • Syntheses of glutarimide derivatives • Spectral analysis • Electronic properties

# **Chemical composition of the thermomineral waters of Jošanička Banja Spa as an origin indicator, balneological valorization and geothermal potential**

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## **Abstract**

The chemical composition of the groundwater is directly dependent on the geological structure, hydrogeological and hydrochemical characteristics and as such it represents an output result of all the factors and processes which take place in the environment within which they were formed. The chemical composition of thermomineral waters often represents a crucial factor in determining the origin, balneological valorization and geothermal potential of the resources. This paper presents the analysis of origin, belneological valorization and geothermal potential of Jošanička Banja Spa, on the basis of the results gained through making the analysis of chemical contents of the thermomineral waters which occur in the area. The ratio of concentrations of specific chemical components in the thermomineral waters of Jošanička Banja has served as the basic tool for ascertaining the origin of these waters. On the basis of the analysis of the main anion–cation and gas compositions as well as the contents of specific micro-components, a balneological valorization of these resources has been carried out. Apart from that, this paper also presents the calculation of the expected temperatures in the primary geothermal reservoir, which was carried out on the basis of the results of chemical analysis of thermomineral waters that occur in the area. Geothermal potential of about 4 MW<sub>t</sub> and significant contents of balneologically active components of the chemical composition of these waters, open up a possibility for their multi-purpose use, which is also presented in the paper.

**Keywords:** thermomineral waters, geothermal potential, balneology, massif of Kopaonik, Jošanička Banja Spa, multipurpose use.

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The adequate comprehension and analysis of the groundwater chemical contents is the essential tool for determining almost all value characteristics of any water. The starting point in the development and valorization of water resources is reflected in studying the three main components: correct origin ascertaining, multi-parameter valorization and defining the energy potential. A special significance in studying these components is ascribed to spas, in other words to thermomineral waters. A fastened development of both wellness and spa concepts, as basic goals of the spa tourism development, has led to establishing an entire range of new methods which are deployed to define spa potentials in a qualitative way [1,2]. Studying the above-mentioned parameters is also significant for the development of chemical industry when it comes to extraction and industrial use of micro-components from thermomineral waters [3].

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Individual analyses regarding the origin ascertaining, balneological valorization as well as defining geothermal potential are analysed as separate cases by many scientists out of a simple reason: there are not so many occurrences on the Earth to which they can apply an integrated approach. Such researches can be found in the following papers [4–8].

Jošanička Banja Spa is a unique case where high-temperature waters can be used in cascades as both an energy resource and spa and sports-recreation wellness factor. Remaining similar cases in Europe can be found in Moravske Toplice (Slovenia) [9], Karlovy Vary (Czech Republic) [10], Baile Herculane (Romania) [11]. The data which describe the methodology and results of multi-criteria analysis are also presented in the mentioned papers.

This paper represents the methods which can be applied as the future general rule of chemical research for the water types similar to that in Jošanička Banja spa. On the basis of the gained results, once again it was confirmed that an adequate analysis and interpretation of chemical analyses cannot be suitably replaced in the contemporary geological, hydrogeological, geothermal and chemical sciences.

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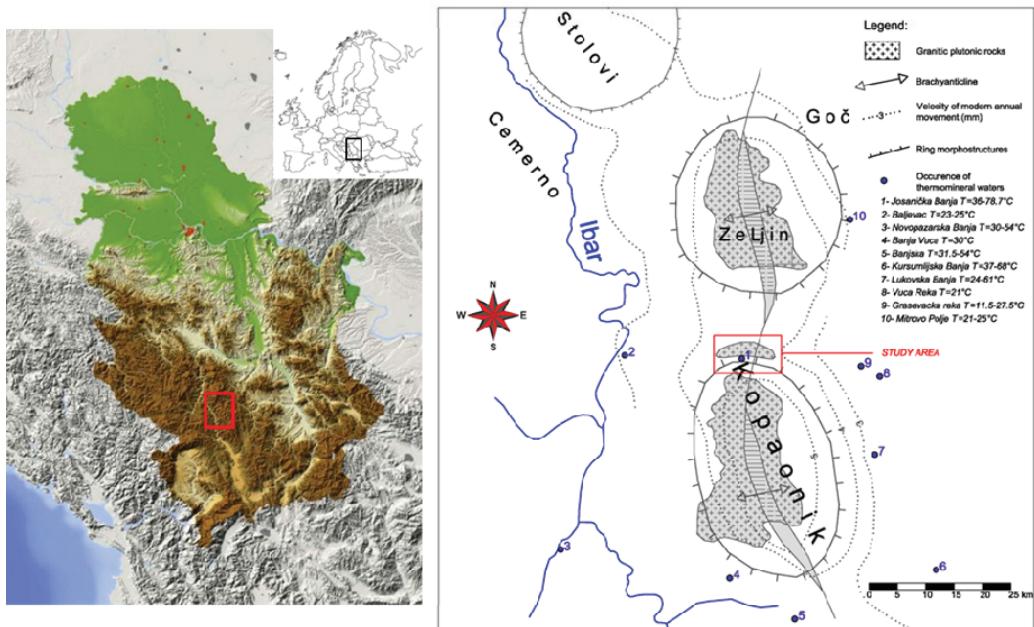


Figure 1. Geographical position of study area (right: modified after Marović and Đoković, 1991 [30]).

### Basic characteristic of the study area

Jošanička Banja Spa is located on the northern slope of the mountain massif of Kopaonik (Figure 1) in the valley of the Jošanica river, at the altitude of about 550 m.

Natural discharges of the thermomineral water in the area of Jošanička Banja Spa may be found in two zones. The first one is in "Banjski park", where a wide discharge zone was formed, with the yield of 14.1–15.4 l/s and temperature ranges from 70.1 to 78.7 °C. The other discharge zone is found on the site "Slanište" which is located in the valley of the Jošanica river, at the distance of 2 km to the west of "Banjski park". The total yield of a wide discharge zone is approximately 2 l/s, while the groundwater temperature at this source ranges from 36 to 37 °C. Apart from carrying out hydrogeological and hydrogeothermal researches at the two natural thermomineral water sources in the area of Jošanica Banja Spa, 9 study hydrogeological boreholes were also made, out of which the two: B-3 and B-6 are in exploitation.

Besides its significant potential, the groundwater in the area of Jošanička Banja Spa has not still been applied to the adequate purpose. In addition to the balneological valorization and geothermal potential estimates, recommendations for the future sustainable use of this significant resource are also given in this study.

### THE APPLIED CONCEPT AND METHODOLOGY OF RESEARCH

The ways of analyzing and researching the potentiality of a site or phenomenon can be multiple and multi-disciplinary. Depending on the needs and pur-

pose, when analyzing the potential of a hydrogeothermal phenomenon, we may analyze geographical factors, geological-hydrological characteristics, hydrochemical and geothermal features, as well as urban factors and development or economic factors. Each of these factors has its own unique features and is unique in its methodology and output results. The analyses of hydrogeothermal phenomena from the aspect of chemical composition as the main indicator of origin, balneological valorization and geothermal potential, represent possibly the most complex of all the enlisted principles, first of all due to their complexity, bulkiness and number of applicable methods.

The concept of researches which were carried out in the area of Jošanička Banja Spa, is methodologically split into three inter-connected units, whose aim was to evaluate the resources of thermomineral groundwater and afterwards the balneological valorization and geothermal potential of Jošanička Banja Spa thermomineral water (Figure 2).

### Geological and hydrogeological features of the area of Jošanička Banja Spa

The area of Jošanička Banja Spa is characterized by a heterogeneous geological structure. It is made of igneous, sedimentary and metamorphic rocks of different age, ranging from the youngest Quaternary alluvial sediments of Jošanica to the oldest Paleozoic schists (Figure 3). The granitoid complex of the rocks in the Kopaonik area, during its embedment performed the metamorphism of the aureole zone which is nowadays represented by marble and a series of fillits and seri-

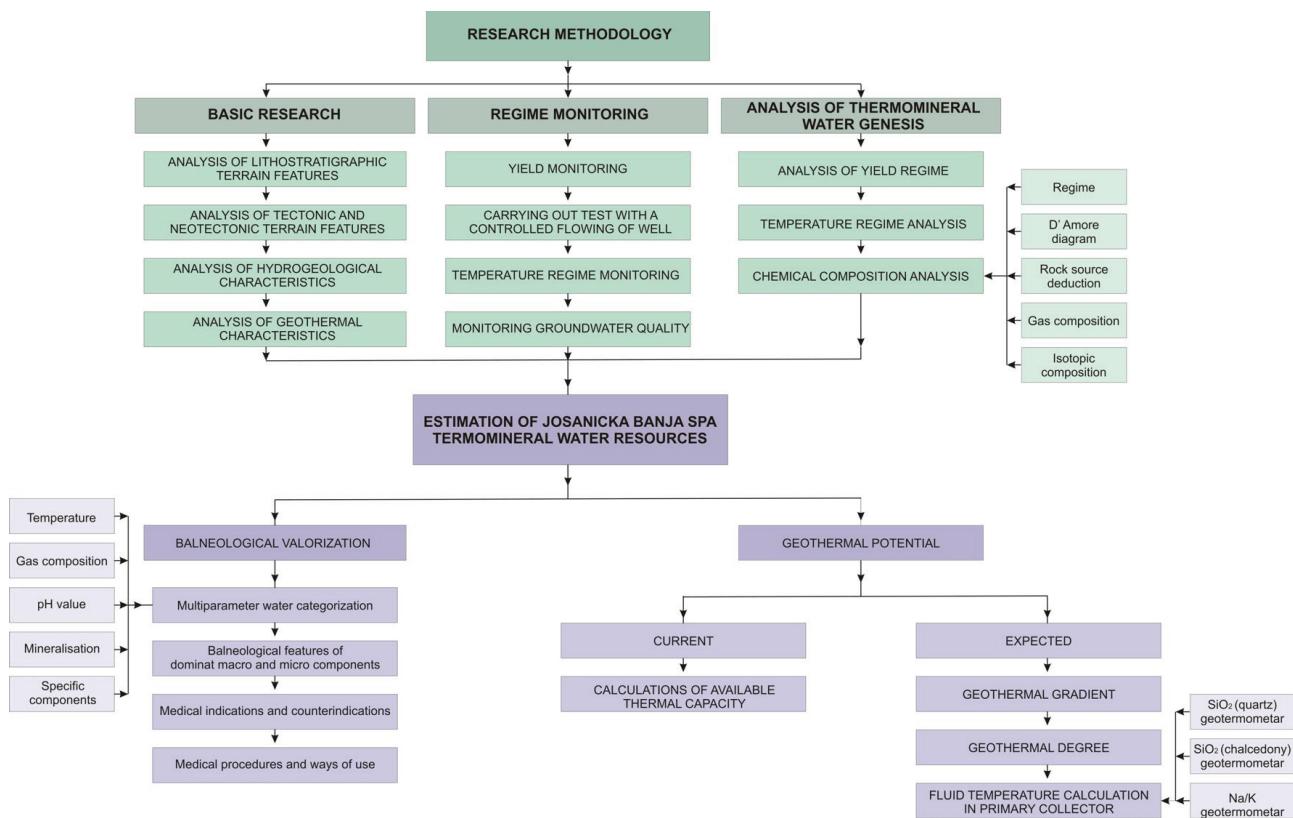


Figure 2. The applied concept and methodology of research.

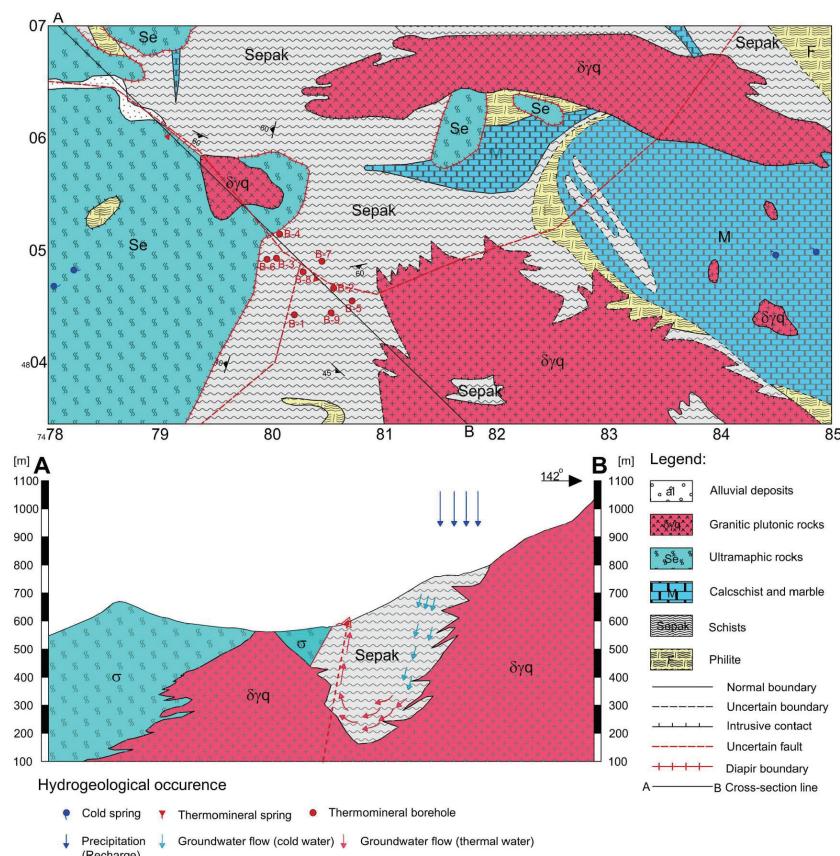


Figure 3. Geological and hydrogeological map with a hydrogeological profile.

cite–chlorite schists which are distributed in the central area of Jošanička Banja Spa and are super positionally found “trapped” between granitoid rocks.

Tectonic and neotectonic movements which are occurred in the area of Jošanička Banja Spa provided a good predisposition for the formation, circulation and discharges of thermomineral waters in the area of Jošanička Banja Spa.

From the aspect of the formation of thermomineral waters of Jošanička Banja Spa, the most significant development is that of the fractured aquifer type, which, according to the lithostratigraphic units, can be subdivided into the following subtypes:

- Fractured aquifer type formed within serpentinites and harzburgites;
- Fractured aquifer type formed within schists;
- Fractured aquifer type formed within granodiorites and quartz diorites;

Apart from the fractured aquifer type in the area of Jošanička Banja Spa, we can also classify the intergranular aquifer type formed in the alluvial sediments of the Jošanica river and karst-fractured aquifer type formed within calcite and marble. The origin of the thermomineral waters is related to the fractured aquifer type within schists, granodiorite and quartz diorite.

The fractured aquifer type formed within the schists is distributed in massive tectonic fractures and cracks on the Earth crust. This aquifer is located underneath the local erosion basis which is in the study area represented by the Jošanica river. Generally speaking, Paleozoic schists in this terrain belong to the weakly water-permeable rocks, and here the groundwater can only be formed within some segments of limited dimensions. Generally, the fractures are of small dimensions, but they are not fully filled which enables a better circulation of the groundwater. With the transfer to hornfels and skarns water-permeability of the schists complex is on the increase. Analyzing the results gained by carrying out some tests with the controlled artesian pressure at the borehole B-6, the coefficient of transmissivity,  $T$ , was calculated:  $5.49 \times 10^{-5}$ – $3.30 \times 10^{-4} \text{ m}^2/\text{s}$ . This is confined aquifer. The value of hydrostatic pressure at the head of the B-6 borehole is 0.6 bar. Thermomineral water as its path to the terrain surface uses the cracks in the schists rock complex. In the area of Jošanička Banja Spa the discharge of the fractured aquifer type formed within the schists rock complex, takes place by a natural and artificial way. The natural way means that the aquifer is discharged via thermomineral springs with the temperature from 70.1 to 78.7 °C and they are located in the vicinity of the basin of The Jošanica river in the spa park and via thermomineral springs with a lower temperature (36–37 °C) on the site “Slanište” which is located downstream on the left bank of the Jošanica river. The artificial way

means that the thermomineral water in the area Jošanička Banja Spa is drained at exploitative boreholes B-3 and B-6 (water temperature is around 52 °C at B-3 and 56 °C at B-6).

Fractured aquifer type formed within granodiorites and quartz diorites spreads over a significant area in Jošanička Banja Spa. These rock masses are mostly distributed in north-eastern, south-eastern and central parts of the study area, and here they are characterized by an expressed anisotropy of effective porosity scaling from 1.6 to 4% [12]. This aquifer type plays an important role from the aspect of formation, movement and discharge of the thermomineral groundwater in the area of Jošanička Banja Spa. The heat source of the hydro-geothermal system of Jošanička Banja Spa is represented by granodiorites and quartz diorites which are at certain depths cut by ruptures along which thermomineral groundwater circulates. In some parts of the terrain these rock masses are compact and here they stand as a barrier to the water movement. The thermomineral groundwater of Jošanička Banja Spa is probably partly formed within this aquifer type in granodiorites and quartz diorites, and this is made clear by the calculations gained via the geothermometer method.

#### **Hydrochemical characteristics of the thermomineral water occurrences in the area of Jošanička Banja Spa**

The results gained by carrying out the analysis of the qualitative features of thermomineral waters (Table 1) in the area of Jošanička Banja Spa indicate that this is a single body of thermomineral waters. The chemical composition of the groundwater from the analyzed occurrences and objects mutually differs according to some parameters of its quality, and this is comprehensible, bearing in mind the complexity of petrological and mineralogical, tectonic, hydrogeological, hydrogeothermal, hydrochemical and other features of the study area. The thermomineral water of Jošanička Banja Spa is characterized by a low mineralization with the predominant sodium ion (Figure 4), high contents of silicon acid and an expressed alkaline reaction.

Regarding its organoleptic features, this water is translucent, without colour and with the smell of hydrogen sulfide ( $\text{HS}^-$ ). Regarding some specific components, the thermomineral waters of Jošanička Banja Spa are characterized by the presence of fluoride, lithium and boron.

#### *The origin of the thermomineral waters in the area of Jošanička Banja Spa*

In order to define structural-geological and hydrogeological conditions of the formation, movement and discharges of the thermomineral waters in Jošanička Banja Spa, the following methods were applied:

- Lithostratigraphic and tectonic analysis of the inner area of Jošanička Banja Spa;

*Table 1. Preview of the results of examining quantitative and qualitative features of thermomineral water in springs and boreholes in Jošanička Banja Spa [13,16,19]*

Sampling site	Depth m	Q l/s	t °C	pH	M	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	CO <sub>3</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	F <sup>-</sup>	Li	B	SiO <sub>2</sub>	HS <sup>-</sup>
																mg/l	
Banjski park	–	14.1–15.4	70.1–78.7	8.4	239	74.6	1.4	1.2	0.4	66	24	17.4	5	0.18	0.68	90	0.41
„Slaniste“	–	2	36–37	>8.4	256.4	98.0	0.4	0.5	0.1	60	70	28.4	5.6	–	–	90	0.12
B-1	155	–	72	7.5	411.7	70	0.8	0.6	0.3	70	43	28.4	0.25	–	–	26	–
B-2	121	–	73	8.4	249.3	83	1.4	0.7	0.3	60	62	10.6	4.8	–	–	90	1.27
B-3	235	1.5	52.5	>8.4	173.8	71.5	1.1	0.8	0.2	66.0	15.0	21.3	4.4	–	–	32	1.02
B-4	170.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B-5	395	–	82	7.6	432.7	12	0.6	0.4	0.3	70	62	10.6	0.24	–	–	18	0.26
B-6	294	3	56	9.7	185.0	63.4	1.5	0.8	0.4	54.6	32.4	10.1	3.75	0.14	0.65	79.5	2.1
B-7	356	–	78	7.3	–	118.2	1.6	0.8	0.7	40.5	36	21.3	–	–	–	22	0.08
B-8	?	–	–	9.2	340.6	65.8	0.9	0.8	0.7	48	25	14	4.3	–	–	99	–
B-9	596	–	81	8.1	–	86.2	0.9	0.8	0.6	30	48	7.1	0.4	–	–	24	0.14

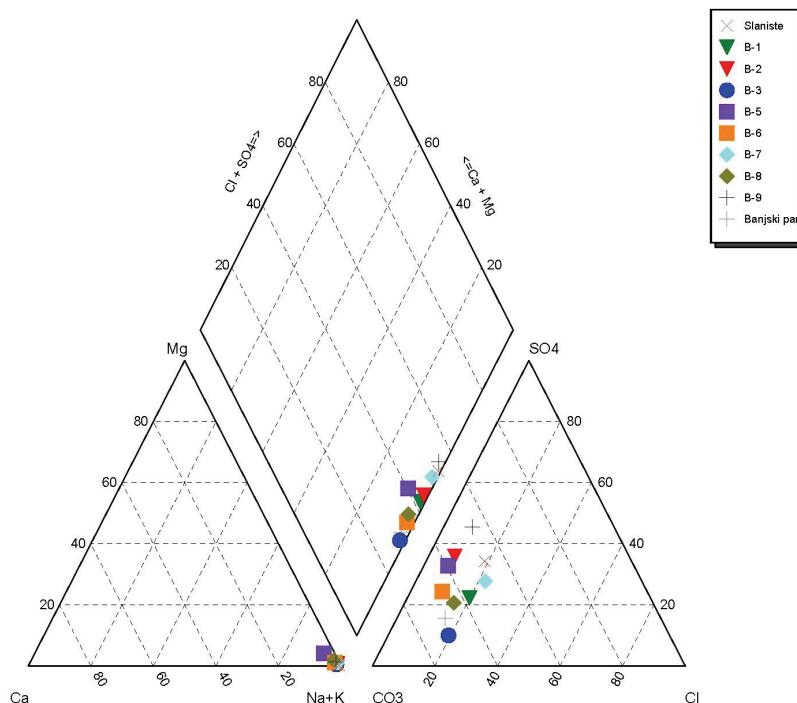
- Analysis of the thermomineral water discharge hydrographs;
- Analysis of the thermomineral waters temperature regime;
- Analysis of the thermomineral waters qualitative characteristics;
- Analysis of geothermal characteristics.

On the basis of the hydrogeological research carried up to now, within the body formed in the fractured aquifer it is possible to determine the origin of thermomineral waters in the area of Jošanička Banja Spa. A greater part of the analysis of the thermomineral water origin in Jošanička Banja Spa was performed on the

basis of the research carried out at the spring “Banjski park”, which is characterized by the biggest yield, highest temperatures and the highest degree of exploration.

#### Lithostratigraphic and tectonic analysis of the conditions for the formation of thermomineral waters in Jošanička Banja Spa

Thanks to the tectonic and neotectonic activity which took place in the area of Kopaonik region, a considerable number of faults were created. From the hydrogeological, that is hydrogeothermal aspect, in the area of Jošanička Banja Spa there are two most sig-



*Figure 4. Piper's diagram of the thermomineral water in Jošanička Banja Spa.*

nificant faults. The first one stretches along the Jošanica river basin, generally, in the east–west direction, while the other runs right to it and goes along the basin of the Velestica river in the direction southwest–northeast (Figure 5).

The thermomineral occurrences in the area of Jošanička Banja Spa are related to the overlapping zones of these faults, where a seepage spring of thermomineral groundwater is formed ("Banjski park") and it is characterized by temperatures from 70.1 to 78.7 °C. Going from the above mentioned spring to the west, the thermomineral temperature falls down to 37 °C (Figure 5).

#### Analysis of the temperature regime of thermomineral waters of Jošanička Banja Spa

Groundwater monitoring at the site "Banjski park" was carried out at 6 monitoring spots (Figure 6). The above mentioned monitoring has shown that the water temperature at the spring of "Banjski park" ranges from 70.1 to 78.7 °C (Figure 7). The highest amplitude of temperature fluctuations was registered at monitoring spot 7 and it equalled 1.7 °C, and this indicates a stable temperature regime of the groundwater (Figure 7) and the absence of mixing up thermomineral waters with "cold" surface water.

From the balneological aspect, the thermomineral waters in Jošanička Banja Spa belong to the group of hypertherms, that is the group of waters whose temperature is above the human body temperature. The exception to this is the thermomineral water from the spring of "Slanište" which belongs to the group of homeotherms, that is the group of waters whose temperature is within the human body temperature limits. From the geothermal aspect, this water belongs to hydrogeothermal resources of low enthalpy (*t* of the fluid is <100 °C).

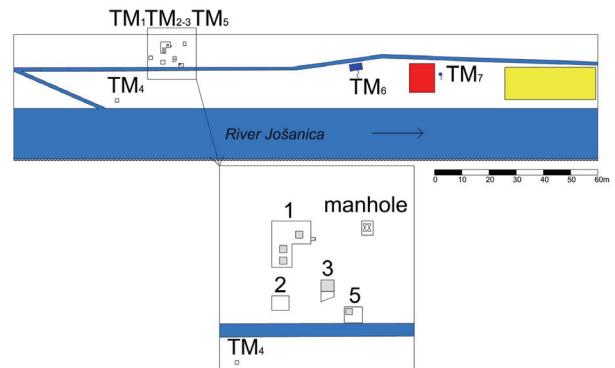


Figure 6. Monitoring network situational plan on the location "Banjski park" in Jošanička Banja Spa.

#### Hydrochemical determination of the origin of thermomineral waters of Jošanička Banja Spa

The formation of physical-chemical and gas composition of the thermomineral water in the area of Jošanička Banja Spa is related to the younger deep igneous rocks and thermo-metamorphic processes caused by them and they took place in the Kopaonik region in the course of Paleogene and Neogene. On the basis of the collected data on the qualitative features of thermomineral waters in Jošanička Banja Spa (Table 1), the analysis of the thermomineral water origin was carried out from different hydrochemical aspects.

#### Quality regime

The regime of the groundwater at spring "Banjski park" is characterized by a significant degree of stability of the basic anion–cation composition and this is indicated by the results of monitoring the basic anion–cation composition in the past 30 years. In the diagram presented in Figure 8, we may see that in the course of the period the basic anion-cation composition did not significantly change.

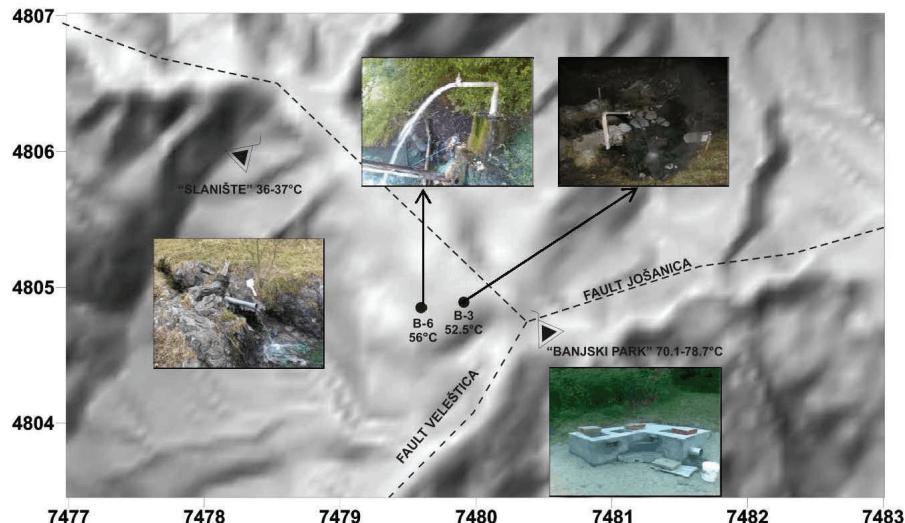


Figure 5. Map with thermomineral water occurrences and presentation of the most significant faults in the area of Jošanička Banja Spa.

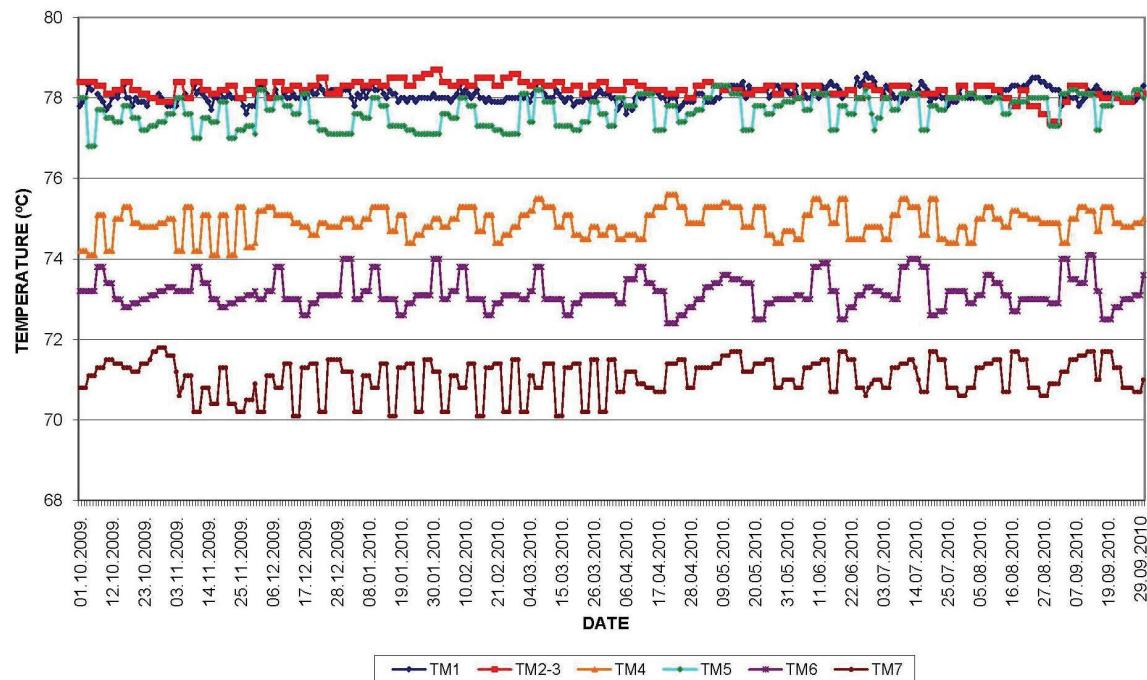


Figure 7. Temperature regime of thermomineral waters at the spring "Banjski park" (period from 1 October, 2009 to 30 September, 2010).

#### D'Amore diagram

The six new hydrochemical parameters for determining water groups on the basis of geological characteristics of the main reservoirs are defined by using the basic anions and cations [14]. Applying these hydrochemical parameters, we may come to the answers related to the groundwater origin. Hydrochemical parameters are marked with letters A to F and with the range of +100 to -100 meq/l.

In Figure 9 there is D'Amore diagram of the thermo-mineral water of Jošanička Banja Spa, whereas Figure 10 is showing the type D'Amore diagram for different water types.

Figures 9 and 10 clearly show that the thermomineral waters of Jošanička Banja Spa, according to their characteristics, are found in between the waters characterized by a metamorphic type and those characterized by a clastic type. Taking into account the geological structure of the terrain and hydrogeological character-

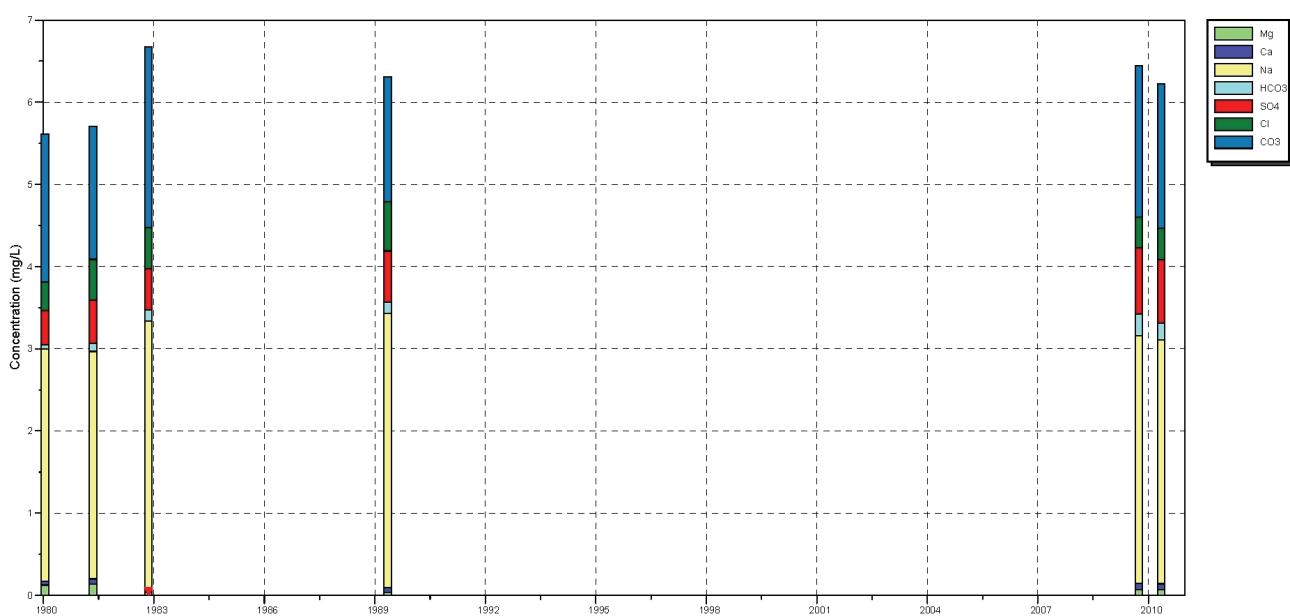


Figure 8. Histogram of the anion and cation concentrations change in the period 1980–2010.

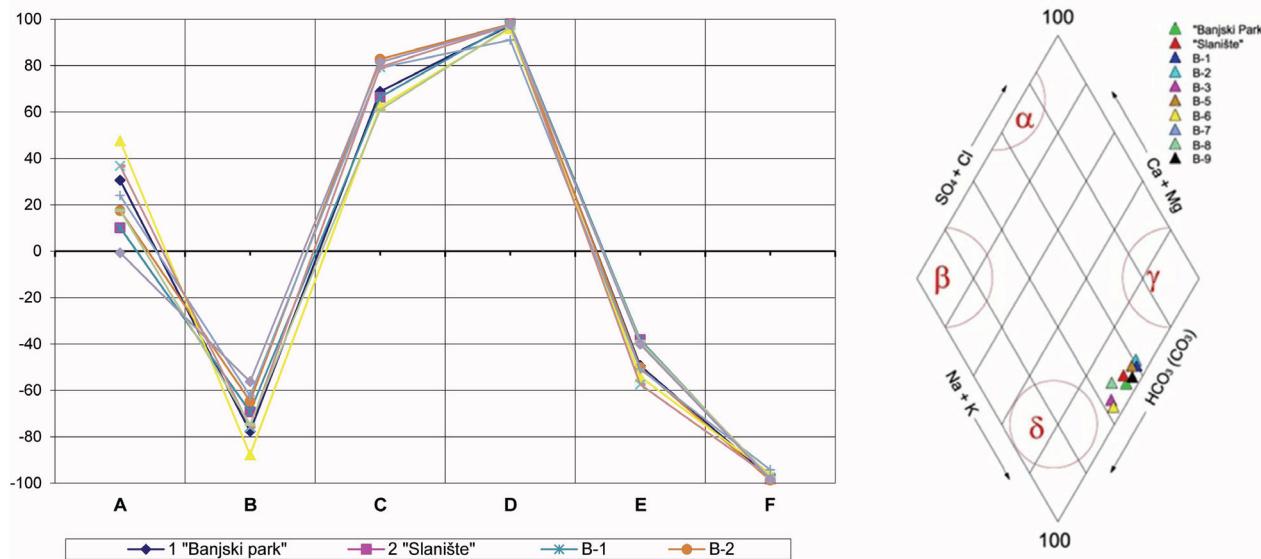


Figure 9. D'Amore diagram of the thermomineral waters in the area of Jošanička Banja Spa.

istics, it is more likely that the origin of these waters is related to metamorphic, that is thermometamorphic processes which took place in the area throughout its geological past.

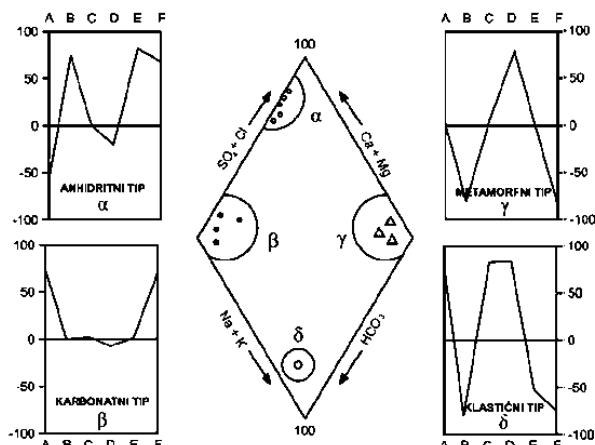


Figure 10. Preview of the type D'Amore diagrams for different water type [14].

#### Gas composition

Previous researches which were related to the gas composition of these waters [15,16] showed that the predominant gas in the thermomineral waters of Jošanička Banja Spa is nitrogen with 91% of the volume, then comes O<sub>2</sub> with 5% of the volume, CO<sub>2</sub> with 2.4% of the volume and H<sub>2</sub>S with 1.6% of the volume and this points to the fact that the thermomineral waters of Jošanička Banja Spa have a vadose origin and this is also indicated by the presence of nitrogen in the water [15].

When carrying out the terrain researches at thermomineral occurrences and objects of Jošanička Banja Spa, the presence of gases was registered. The smell of

the thermomineral water pointed to the presence of hydrogen-sulfide (HS<sup>-</sup>), which is almost non-existent in the air, so it acidifies with the formation of the water and SO<sub>2</sub>. At larger depths of the Earth's crust, hydrogen sulfide is related to thermocatalytic processes of the dissolution of sulphur compounds and reduction of sulfates under the conditions of high temperatures.

#### Analysis of the contents of genetically relevant chemical components in the thermomineral waters of Jošanička Banja Spa

On the basis of the performed analyses of qualitative features of the thermomineral groundwater from the springs in the Jošanička Banja Spa area, the following conclusions were drawn:

- Silicon dioxide amount (SiO<sub>2</sub>) of 1.621 mmol/l is typical for thermomineral water. With the temperature increase, the solvent quality of the water also changes. Under ordinary conditions the solubility of silicon acid is quite low. In high temperatures quite a frequent occurrence is that of silicate-carbonate-sodium waters, and this is exactly the case of Jošanička Banja Spa.
- HCO<sub>3</sub>/SiO<sub>2</sub> < 5 (0.161) ratio indicates that the chemical composition of thermomineral waters is related to the silicate dissolution.
- On the grounds of the SiO<sub>2</sub>/(Na+K-Cl) < 1 ratio, we may conclude that at larger depths of the aquifer there is an ionic change (Ca<sup>2+</sup> and Mg<sup>2+</sup> into Na<sup>+</sup>).
- (Na/Na+Cl) ratio which is bigger than 0.5 (0.889) also points to the ionic (cationic) change.
- (Mg/Ca+Mg) ratio points to the presence of ferro-magnesium minerals (actinolite Ca<sub>2</sub>(Mg,Fe)<sub>5</sub>Si<sub>8</sub>O<sub>22</sub>(OH,F)<sub>2</sub> which occurs within the chlorite-epidote-actinolite slates). The basic chemical process which takes place in these slates is the process of albitization. The

formation of the albite mineral is related to a retrograde metamorphism of basic plagioclases or recrystallization in the course of metamorphosis, when the ionic change occurs ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  into  $\text{Na}^+$ ). In metamorphites, the change-causing cations can be found in huge amounts and their character depends on the character of their inner plagioclase. In albite molecules, the plagioclase has about 10% of anorthite component, that are mostly sodium compounds, which as the product when they decompose leaves behind the change-causing sodium which makes up over 90% ekv. in all the analyzed samples of thermomineral waters.

- The dry residues value which is less than 500 mg/l, as well as the  $\text{Cl}^-/\text{anion}$  sum  $< 0.8$  (0.119) concentration indicate that the chemical composition of thermomineral waters has to do with the silicate dissolution.

### BALNEOLOGICAL VALORIZATION OF THERMO-MINERAL WATERS OF JOŠANIČKA BANJA SPA

The chemical composition of thermomineral waters besides the temperature represents the most important balneological component of these resources. The chemical composition of the groundwater may quite often serve as a significant tool for defining geothermal potential of a specific geothermal system.

The thermomineral waters of Jošanička Banja Spa were formed within a single groundwater body, which caused a similar chemical composition of thermomineral waters of all the analysed occurrences and objects. According to other analyzed parameters (gas composition, pH value, mineralization and specific components contents) all the waters were classified within the same category (Table 2).

The thermomineral waters of Jošanička Banja Spa according to the basic anion-cation composition belong to the group of carbonate-sulphate-sodium waters, while according to the pH value parameter these waters can be classified as the waters characterized by an alkali reaction (Table 2). Out of some specific components, the thermomineral waters of Jošanička Banja Spa are characterized by the presence of silicates, fluorides, lithium, boron and hydrogen sulfide.

Balneological valorization of the thermomineral waters of Jošanička Banja Spa based on temperature, basic anion composition, basic cation composition, gas composition and micro components contents.

#### Temperature

The temperature parameter is one of the basic determinants of the thermomineral waters of Jošanička Banja Spa. Hot groundwaters are regarded as useful in the process of treating some rheumatism diseases, sports injuries and post-surgical processes.

*Table 2. Multi-parameter balneological classification of thermomineral waters in Jošanička Banja Spa*

Parameter	Banjski park	Slanište	B-3	B-6
Temperature				
Hypotherms $t < 36^\circ\text{C}$				
Homeotherms $t: 36\text{--}37^\circ\text{C}$		+		
Hypertherms $t > 37^\circ\text{C}$	+	+	+	+
Gas composition				
Carbonate				
Hydrogen sulfide carbonate				
Hydrogen sulfide	+	+	+	+
Nitrogen	+	+	+	+
Nitrogen methane				
Methane				
pH value				
Strongly acid $\text{pH} < 3.5$				
Acid $\text{pH}: 3.5\text{--}5.5$				
Weakly acid $\text{pH}: 5.5\text{--}6.8$				
Neutral $\text{pH}: 6.8\text{--}7.2$				
Weakly alkaline, $\text{pH}: 7.2\text{--}8.5$				
Weakly alkaline $\text{pH}: 7.2\text{--}8.5$				
Alkaline $\text{pH}$ above 8.5	+	+	+	+
Mineralization				
Low mineralization waters $< 1 \text{ g/l}$	+	+	+	+
Waters of increased mineralization $1\text{--}5 \text{ g/l}$				
Medium-mineralization waters $5\text{--}15 \text{ g/l}$				
High-mineralization waters $15\text{--}35 \text{ g/l}$				
Brine waters $35\text{--}150 \text{ g/l}$				
Specific components contents				
Therapeutic mineral waters with no specific components or features				
Carbonated mineral waters				
Sulphide mineral waters	+	+	+	+
Iron, arsenic waters and waters with the increased contents of Mn, Al, Cu and Zn				
Bromine and iodine waters				
Waters with high contents of organic matter				
Silica waters	+	+	+	+

#### The basic anion composition

The presence of carbonate ions which are dominant in the thermomineral waters of Jošanička Banja Spa is caused by some increased pH values. From the balneological aspect, a more significant presence is that of sulphate ions which can be used for treating a huge range of diseases, from skin infections to respiratory

problems and skin inflammations. Sulphate waters may be also used for treating liver diseases or gastro-intestinal tract issues, as well as for respiratory problems when the inhalation therapy is applied.

### The basic cation composition

Sodium ions are dominant in the thermomineral waters of Jošanička Banja Spa. The magnesium and calcium ion contents are much lower than the sodium ions amount. Sodium waters are used as an adjuvant tool for treating arthritis diseases and they can also stimulate the lymph system in the body when used for bathing.

### Balneologically relevant gases

The most significant of all the gases in the thermomineral waters of Jošanička Banja Spa is hydrogen sulphide. Its healing amount is caused by the contents of free hydrogen-sulphide and hydrosulphide ion. These waters are applied in treating skin diseases, rheumatism, mental disorders and vaginal dryness issues and inhalations.

### Balneologically relevant micro components

**Fluorides.** An increased amount of fluorine can be found in alkali waters which contain lower concentrations of the calcium ion  $\text{Ca}^{2+}$ , which is the case of thermomineral waters in Jošanička Banja Spa (0.24–5.6 mg/l). Generally, the fluorine in thermomineral waters originates from the minerals which contain fluorine (fluorites and apatites). From the balneological aspect fluorides are significant in the prevention of tooth decay.

**Lithium.** The lithium concentrations in the thermomineral waters from the spring in Jošanička Banja Spa ranged from 0.14 to 0.18 mg/l. The lithium in these waters originates from igneous and metamorphic processes which occur in the area. In the igneous rocks formation  $\text{Li}^+$  is gathered in acid rocks, especially at the last stage of the magma crystallization, and then lithium minerals are formed: spodumene and lepidolite, etc. From the aspect of balneological valorization, lithium is significant for the mental disorders treatment.

**Boron.** This micro component belongs to the group of elements which are found in waters in traces only. In the thermomineral waters of Jošanička Banja Spa, boron was found in concentrations 0.65 to 0.68 mg/l. Its origin is related to some volcanic processes which took place in the area of Kopaonik. Balneological valorization of boron is manifested in the skin disease treatment.

## GEOTHERMAL POTENTIAL OF THE THERMOMINERAL WATERS OF JOŠANIČKA BANJA SPA

The area of Jošanička Banja Spa has always attracted researchers due to its geothermal potential and they

have tackled the issue of the thermomineral waters of Jošanička Banja Spa from different perspectives [13,15–19].

The heat sources of the hydrogeothermal system of Jošanička Banja Spa are granodiorites and quartz diorites of the Paleogene and Neogene ages. The manifestations of geothermal energy at the surface of the terrain, as it has been mentioned before, occur in the form of springs created along the basic fault structures. The temperatures of geothermal springs fall are moving in the direction southeast to northwest (Figure 11). Besides the geothermal springs as the main indicators of an area's geothermal potential, there are also paleo-hydrogeothermal occurrences located 10 km to the south of Jošanička Banja Spa. The existence of these phenomena points to the fact that the original outlet of the thermomineral waters was carried out at another location in the altitude range from 600 to 1000 m [20]. After the Jošanica River's basin full formation and neotectonic upraise of the terrain, the outlet of the thermomineral waters "moved" from the original location to the today's site of Jošanička Banja Spa. According to the same author, the total area of this bank is approximately 45 km<sup>2</sup>, and its thickness is about 1000 m.

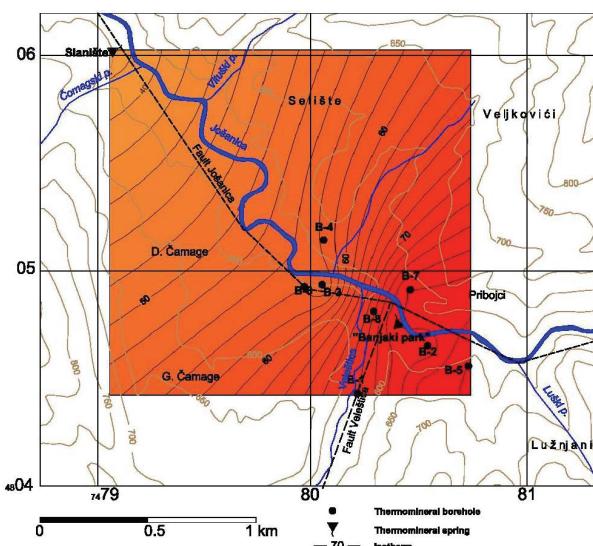


Figure 11. The map of hydroisotherms in the area of Jošanička Banja Spa.

In the area which is today regarded as Jošanička Banja Spa, apart from the natural outlet of the geothermal water, there are also two active boreholes. Borehole B-3, 235 m deep, has the artesian pressure with the yield of 1.5 l/s and temperature of 52.2 °C, whereas borehole B-6, which is 294 m deep, owns the artesian pressure of 3.0 l/s and the temperature of 56 °C.

The possibility of finding even higher temperatures of the geothermal waters has not been fully examined still. In the area of Banja there have been made 9 study

boreholes, out of which borehole labelled B-9 is the deepest (596 m). Borehole B-9 is negative, including the other boreholes apart from B-3 and B-6. The measured temperature of the dry rocks at the bottom of B-9 borehole is 81 °C, whereas borehole B-5 (395 m) has the temperature higher by a degree, which is 82 °C. The results of the drilling did not match the expected ones, that is the drilling did not get through to the primary geothermal reservoir (drilling was terminated due to the disaster and the fact that the drilling tools got stuck), so the real (maximum) temperature of geothermal fluids in the primary reservoir was not confirmed.

Due to the impossibility of the direct temperature measuring of the geothermal fluids in the primary reservoir, the method which was applied was that of the chemical geothermometers with a view to providing an empirical definition of the expected maximum temperatures. In practice, up to now, the geothermometer method has played a significant role in researching and managing the geothermal reservoirs of an area. The method of chemical geothermometers is based on the concentration and ratio of certain chemical elements in the groundwater. The most applicable geothermometers are based on the silicon solutions (quartz and chalcedony), then sodium, potassium, calcium, magnesium, lithium, etc. In the course of making calculations, one must bear in mind the limits which each of the aforementioned geothermometers possess, and their mutual comparison leads to a higher degree of the data reliability.

Taking into account the way in which geothermal phenomena appear on the terrain surface as well as their physical manifestations, in order to calculate the expected temperatures in the Jošanička Banja Spa reservoir, silicon geothermometers were used and as a control geothermometer they used the Na/K ratio geothermometer (Table 3). The calculation of the expected temperature in the primary reservoir of geothermal energy in Jošanička Banja Spa was executed on the basis of the chemical composition of the waters in the "Banjski park" spring, since it is a natural outlet and it is the highest ever measured temperature of the groundwater on the terrain surface.

On the basis of the applied method of chemical geothermometers, the expected temperatures in the reservoir move within the range 102–130 °C. The tem-

perature of 130 °C was gained by means of a quartz geothermometer under the conditions of conductive heat transfer without the loss of vapour. If by means of this geothermometer they gain the temperatures 120–180 °C, there is a great possibility that chalcedony could regulate the silicon solubility and it is necessary to use the chalcedony geothermometer as the control one. If the temperature range goes from 100 to 120 °C, when the chalcedony is applied, there is a huge possibility that this is exactly the representative temperature value in the reservoir [22].

The Na-K geothermometer was used as the control geothermometer, and it provides the most reliable results when the temperatures in the reservoir are high (180–200 °C). Apart from the temperature, the geothermometer's reliability is also affected by the low contents of Ca (which is the case of the waters of Jošanička Banja Spa), then the negative log value  $(Ca^{1/2}/Na)+2.06$  (which is not the case of the waters of Jošanička Banja Spa), neutral pH value and belonging to the chloride waters (which is also not the case of the Jošanička Banja Spa waters spa). The application of other geothermometers, such as K-Mg, Na-Ca and K-Ca, in the area of Jošanička Banja Spa is somewhat restricted, since they are applied only if the K and Mg concentrations in the groundwaters are high and if by applying Na/K exceptionally high temperatures are gained.

Taking into account all the factors which have an effect on the temperature calculations by the geothermometer method, it may be concluded that the expected temperature of geothermal fluids in the primary reservoir of Jošanička Banja Spa is somewhere about 100 °C, and this result was obtained by means of the  $SiO_2$  (chalcedony) geothermometer.

Knowing the temperatures and yield of geothermal occurrences and objects in the area of Jošanička Banja Spa, it is possible to predict the thermal capacity of geothermal waters. When defining the thermal capacity which is based on the currently available temperatures and yields, the following equation was applied [21]:

$$\text{Therm. Cap. } [MW_t] = \text{Flow rate } [l/s] \times (\text{Inlet temp.} - \text{Outlet temp. } [^\circ C]) \times 0.004184 [MW]$$

The overall available thermal capacity of the geothermal waters of Jošanička Banja Spa is about 4 MW<sub>t</sub>,

*Table 3. Calculation of the expected temperature in the primary geothermal reservoir of Josanicka Banja spa via the chemical geothermometers' method; measured groundwaters temeprature on surface: 70–78 °C*

Applied geothermometers	Estimated groundwaters temeprature, °C
$SiO_2$ (quartz) [21]	≈130
$SiO_2$ (chalcedony) [22]	≈102
Na/K [23]	≈107
Na/K [24]	≈126

and Table 4 is presenting the thermal capacity of each spring.

*Table 4. The calculation of the thermal capacity of the geothermal waters from the existent springs in Jošanička Banja Spa*

Occurrence	Parameter			
	$Q_{sr} / l s^{-1}$	$t_{sr} / ^\circ C$	$t / ^\circ C$	$E / MW_t$
„Banjski Park“	≈15	≈75	50	3.15
„Slanište“	2	≈36	20	0.17
B-3	1.5	≈53	30	0.19
B-6	3	≈56	30	0.38
Total			3.89 ≈ 4	

Taking into account the total available thermal capacity of the geothermal waters, which is about 4 MW<sub>t</sub>, in the area of Jošanička Banja Spa, it is possible to heat about 830 households with the average surface of 60 m<sup>2</sup> and average consumption of 80 W/m<sup>2</sup>. Currently, the Jošanička Banja Spa groundwaters are used for the sake of heating a few public facilities (health centre, school, post office) and for the sake of some outdoor swimming pools in the summertime, and use as such is definitely below the available capacity.

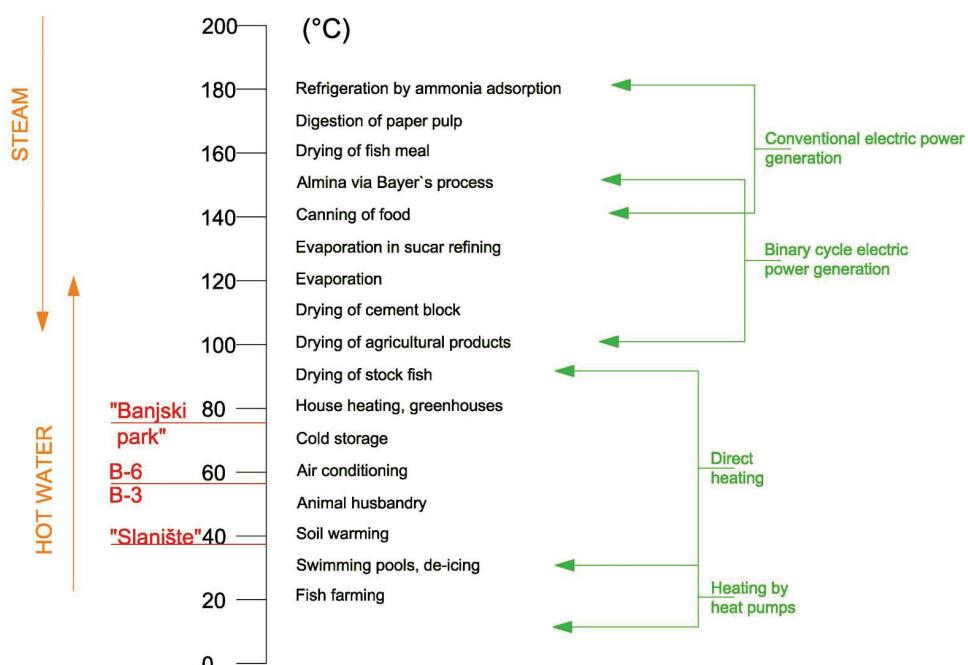
Apart from the heating, geothermal resources of Jošanička Banja Spa have a great perspective within the greenhouse vegetable production. The average energy needed to heat 1ha per year is about 20 TJ [20]. The energy used in the Jošanička Banja Spa area is about 112 TJ/year [27], and this is enough to heat almost 6 ha of the greenhouse. If one knows that the annual pep-

per yield is 20 t/ha, tomato yield is 30 t/ha, cucumber yield is 72 t/ha [28] out of the greenhouse production in the area of Jošanička Banja Spa, it would be possible to produce annually 120 t of peppers, 180 t of tomatoes and about 430 t of cucumbers.

For the sake of using geothermal waters for spa purposes, the scope of optimum temperatures goes from 25 to 37 °C. The conditions for the direct use for the aforementioned purposes are fulfilled by the waters of the “Slanište” spring ( $t = 36 ^\circ C$ ), whereas the waters of B-3 and B-6 boreholes and of the “Banjski park” spring need to go through the temperature lowering process, which actually means that they can firstly be used for the objects’ heating. Likewise, the groundwater temperatures of the scope 25–27 °C are suitable to be used for sports and recreational purposes (outdoor and indoor swimming pools intended for the unlimited use).

#### POSSIBILITIES OF MULTI-PURPOSE USE OF THERMOMINERAL WATERS OF JOŠANIČKA BANJA SPA

The thermomineral waters of Jošanička Banja Spa are a significant resource which has not still been adequately deployed. Regarding their yield of about 20 l/s and a wide temperature scope (36 to 78 °C), these waters can be used for different purposes. The main advantage of using these waters resides in the possibility to apply a cascade model of use, which would gain a significant energy efficiency and saving. From Lindal's diagram [29] (Figure 12), we may see that the possibility of using the thermomineral waters from the



*Figure 12. Possible uses of thermomineral waters, depending on the temperature – a modified Lindal's diagram.*

springs in Jošanička Banja Spa is multiple, starting with fish farming which requires the lowest temperatures ( $20\text{ }^{\circ}\text{C}$ ) up to cereal drying ( $100\text{ }^{\circ}\text{C}$ , the estimated temperature in the primary reservoir of geothermal fluids). If we add to this the healing component of the groundwaters of Jošanička Banja Spa, the possibilities of their applications expand to the usage for spa purposes.

## CONCLUSION

On the basis of the performed analysis of the chemical composition of the thermomineral waters in Jošanička Banja Spa, the origin of the thermomineral waters was ascertained, that is the fact that these waters are genetically related to the massifs of crystalline rocks, so the formation of physical and chemical composition as well as the gas contents of the thermomineral waters are connected to the younger deep igneous rocks and thermometamorphic processes caused by them.

The thermomineral waters of Jošanička Banja Spa belong to the group of nitrogen, lowly-mineralized silica thermomineral waters of atmospheric origin. The silica concentration in the groundwater of Josanicka Banja is typical of thermomineral waters and the ratio of silica and other elements indicates that the chemical composition of thermomineral waters is related to the silicates breakup and the ratio of  $\text{Cl}^-$  concentration and the anions sum, that is the dry residue value lower than  $500\text{ mg/l}$ .

The results of the analysis of hydrogeothermal phenomena of Jošanička Banja Spa from the aspect of chemical composition as the indicator of balneological valorization, point to significant curative properties of the groundwater. The thermomineral waters of Jošanička Banja Spa according to the basic anion–cation composition belong to the group of carbonate–sulfate–sodium waters. The presence of sulfate ions helps to treat skin infections and respiratory issues, as well as diseases connected to the problems with the liver and gastrointestinal tract. Sodium in the waters is used to treat arthritic diseases and to stimulate the lymphatic system in the body. Out of some specific components, the Jošanička Banja Spa thermomineral waters are characterized by the presence of silicates, fluorides, lithium, boron and hydrogen-sulfides. Lithium is significant for treating mental disorders, whereas the balneological valorization of boron is reflected in its application for treating skin diseases.

Apart from considering the balneological potential, the chemical composition of Jošanička Banja Spa waters was analysed with the aim of indicating the geothermal potential of the area. According to the currently available quantities and temperatures of geothermal waters, the heat capacity equals about  $4\text{ MW}_t$ . Applying the geothermometer method, the expected temperature of geothermal fluids in the primary reservoir

of Jošanička Banja Spa is about  $100\text{ }^{\circ}\text{C}$ , which significantly increases the geothermal potential and opens up the possibility for a potential use of these waters in the binary cycle for the electricity production.

With the research carried out for the sake of this work, a qualitative valorization of the Jošanička Banja Spa thermomineral waters was carried out and the instructions for their sensible use were given. In the forthcoming period, people should take up the research directed towards: proving the geothermal potential indicated by the application of geothermometer method, developing the methodology of multi-purpose use of thermomineral waters in cascades as well as analysing the effects of using these waters for balneological needs.

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## IZVOD

### HEMIJSKI SASTAV TERMOMINERALNIH VODA JOŠANIČKE BANJE KAO INDIKATOR POREKLA, BALNEOLOŠKE VALORIZACIJE I GEOTERMALNOG POTENCIJALA

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(Naučni rad)

Na osnovu izvedenih analiza hemijskog sastava termomineralnih voda Jošaničke Banje utvrđeno je da su iste genetski povezane sa masivima kristalastih stena, te da je formiranje fizičko-hemijskog i gasnog sastava termomineralnih voda vezano za mlađe dubinske magmatske i njima izazvane termometamorfne procese. Termomineralne vode Jošaničke Banje pripadaju grupi azotnih malomineralizovanih silicijumskih termomineralnih voda atmosferskog porekla. Koncentracija silicije u podzemnim vodama Jošaničke Banje karakteristična je za termomineralne vode, a odnos silicije sa ostalim elementima ukazuje da je hemizam termomineralnih voda u vezi sa raspadanjem silikata i da u dubljim delovima ležišta dolazi do jonske izmene. Vezu između hemizma i raspadanja silikata potvrđuje i odnos odnos koncentracije  $\text{Cl}^-$  i sume anjona, odnosno vrednost suvog ostatka nižeg od 500 mg/l. Rezultati analize hidrogeermalnih pojava Jošaničke Banje sa aspekta hemijskog sastava kao indikatora balneološke valorizacije ukazuju na značajnu lekovitost podzemnih voda. Termomineralne vode Jošaničke Banje prema osnovnom anjonsko-katjonskom sastavu pripadaju grupi karbonatno-sulfatno-natrijumskih voda. Prisustvo sulfatnih jona pomaže u lečenju kožnih infekcija i respiratornih problema, kao i oboljenja vezanih za probleme sa jetrom i gastrointestinalnim traktom. Natrijum u vodama koristi se za lečenje artritičnih bolesti i stimulaciju limfnog sistema u telu. Od specifičnih komponenti termomineralne vode Jošaničke Banje se odlikuju prisustvom silikata, fluorida, litijuma, bora i vodonik-sulfida. Litijum je značajan u lečenju psihičkih poremećaja, dok se balneološka valorizacija Bora ogleda se u primeni pri lečenju kožnih oboljenja. Osim za sagledavanje balneološke potencijalnosti, hemizam termomineralnih voda Jošaničke Banje analiziran je u funkciji indikatora geermalnog potencijala ovog područja. Prema trenutno raspoloživim količinama i temperaturama geermalnih voda termalni kapacitet iznosi oko  $4\text{MW}_t$ . Primenom metode geotermometara očekivana temperatura geermalnih fluida u primarnom rezervoaru Jošaničke Banje iznosi oko  $100^\circ\text{C}$ , što značajno uvećava geermalnu potencijalnost i utiče na višenamenske mogućnosti primene ovih voda.

*Ključne reči:* Termomineralne vode • Geermalni potencijal • Balneologija • Kopaonički masiv • Jošanička Banja • Višenamensko korišćenje



# Određivanje zapreminskog koeficijenta prenosa mase ozona u barbotažnoj koloni sa dvofluidnom mlaznicom

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## Izvod

U ovom radu prikazani su rezultati eksperimentalnih ispitivanja prenosa mase gas-tečnost pri ozonizaciji vode u barbotažnoj koloni sa dvofluidnom mlaznicom (BKDM) pod različitim operativnim uslovima. Cilj ovih ispitivanja bio je određivanje vrednosti zapreminskog koeficijenta prenosa mase ozona u vodi,  $k_L a$ , u mirnoj zoni kolone, pri različitim protocima gasne i tečne faze. U radu je dato i poređenje  $k_L a$  u BKDM sa vrednostima  $k_L a$  u klasičnoj protivstrujnoj barbotažnoj koloni sa poroznom pločom tj. fritom. Eksperimentalno je određena vrednost kritičnog protoka tečnosti, pri kojem sadržaj gasa u disperziji, u mirnoj zoni kolone, dostiže svoju maksimalnu vrednost. Rezultati su pokazali da se pri kritičnom protoku tečnosti dobija maksimalna vrednost zapreminskog koeficijenta prenosa mase ozona u vodi.

**Ključne reči:** zapreminski koeficijent prenosa mase, barbotažna kolona sa dvofluidnom mlaznicom, ozon.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Danas, po svom izboru, ne živimo na mestima gde je najbolja voda za piće, već u gradovima gde je čiste vode sve manje. Zbog toga gradska postrojenja za dobijanje pijaće vode moraju koristiti čitav niz različitih postupaka prečišćavanja sirove vode, uključujući predozonizaciju i glavnu ozonizaciju. Gasoviti ozon je jako oksidaciono sredstvo i veoma brzo reaguje sa većinom neorganskih i organskih jedinjenja, mikroorganizmima i virusima prisutnim u vodenoj sredini. Ozon je i efikasno dezinfekcino sredstvo u širokom opsegu pH vrednosti i temperature, pri čemu vodi ne daje nikakav miris ili ukus [1,2].

Dezinfekcija vode za piće ozonom je proces koji se uglavnom izvodi u barbotažnim kontaktorima [3–6]. Veliki broj autora je radio na usavršavanju i preciznijem i podrobnjem definisanju klasično korišćenih barbotažnih uređaja [3–11], kao i na istraživanju i razvoju novih tipova reaktora [2,12–15], a sve sa ciljem da se ne upotrebi mehaničko mešanje, a da se intenzitet mešanja i brzina prenosa mase unapredi u odnosu na klasične barbotažne kolone.

1979. godine ukazano je na mogućnost korišćenja različitih tipova dvofluidnih mlaznica pri kontaktu gase i tečnosti (ejektori, injektori, Venturi mlaznice, mlazni

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aeratori) [16]. Korišćenjem ovih mlaznica intezivira se mešanje i prenos mase u odnosu na klasične barbotažne kolone i to bez mehaničkog mešanja. S obzirom da se gas uvodi kroz jedan otvor nema potrebe za dispergovanjem gase kroz poroznu ploču. Zajedničko za sve pomenute mlaznice je da se za dispergovanje gase koristi kinetička energija tečnosti.

Ideja da se intenzivno mešanje, odnosno kinetička energija tečnog mlaza iskoristi za dispergovanje gasnih mehurova, realizovana je kod nas nekoliko godina kasnije, a prvi rezultati saopšteni su 1981. godine [17]. Iste godine tehničko rešenje BKDM je prijavljeno kao patent [18].

Istraživanja obavljena na BKDM specijalne konstrukcije [18], pokazala su da pri određenim operativnim uslovima (dovoljno velikom protoku tečnosti-iznad neke kritične vrednosti  $L_c$ , za definisan protok gase) u koloni obrazuju dve različite zone [19]. Donja, turbulentna zona formira se u blizini dvofluidne mlaznice i okarakterisana je intenzivnim mešanjem i prekidanjem gasnih mehurova, dok se iznad nje pa do vrha kolone nalazi mirna zona. U mirnoj zoni prisutni su sitni mehurovi gase u disperziji, koji su približno uniformne veličine i vizuelna slika toka u njoj podseća na strujnu sliku u klasičnim barbotažnim kolonama sa poroznom pločom kao raspodeljivačem gase. Konstatovano je da se mirna zona BKDM u svemu ponaša slično istostrujnoj barbotažnoj koloni sa poroznom pločom kao raspodeljivačem gase. Nasuprot tome, hidrodinamičko ponašanje turbu-

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lentne zone BKDM slično je ponašanju u aerotoru sa mehaničkim mešanjem [17].

Iako su do sada publikovana brojna istraživanja apsorpcije ozona u vodi, najveći broj rezultata se odnosi na ispitivanja u dva osnovna-klasična tipa barbotažnih kontaktora, barbotažnoj koloni i reaktoru sa mehaničkim mešanjem. Apsorpcija ozona u vodi i vodenim rastvorima u BKDM do sada nije opisana u literaturi.

Cilj eksperimenata u ovom radu je da se utvrdi mogućnost primene BKDM za izvođenje procesa gas-tečnost, umesto klasično korišćenih uređaja za ovu svrhu, ukoliko se pokaže da se u njoj mogu ostvariti slične ili veće vrednosti zapreminskih koeficijenata prenosa mase.

### Teorijski pristup

Da bi se odredila brzina apsorpcije gasa potrebno je poznavati vrednost zapreminskog koeficijenta prenosa mase  $k_L a$  ili odvojeno vrednosti koeficijenta prenosa mase i specifične površine kontakta faza. S obzirom na strujne uslove u koloni na koeficijent prenosa mase se ne može bitno uticati, tako da je veličina koja dominantno određuje brzinu prenosa mase upravo specifična međufazna površina.

Specifična površina kontakta u laminarnom toku u mirnoj zoni kolone funkcija je sadržaja gasa u disperziji,  $\varepsilon_G$ , i srednjeg prečnika mehura,  $d_{BS}$ :

$$\alpha = \frac{6\varepsilon_G}{d_{BS}} \quad (1)$$

prema kojoj je jasno zbog čega je potrebno ostvariti formiranje sitnijih mehurova i većeg sadržaja gasa u disperziji.

U literaturi je konstatovana zavisnost sadržaja gasa u disperziji od protoka tečne faze u mirnoj zoni BKDM, pri apsorpciji kiseonika iz vazduha u vodi [19]. Porast sadržaja gasa u disperziji sa povećanjem protoka tečnosti do kritične vrednosti protoka tečnosti  $L_c$  pripisuje se usitnjavanju gasnih mehurova zbog intenziviranja turbulentnosti u turbulentnoj zoni BKDM, pri povećanju protoka tečnosti. Sitniji mehurovi imaju manju brzinu dizanja, što dovodi do povećanja sadržaja gasa u disperziji prema izrazu [20]:

$$\varepsilon_G = \frac{W_G}{W_D} = \frac{W_G}{W_D^0 + \frac{U_L}{1 - \varepsilon_G}} \quad (2)$$

U literaturi je pokazano da mehurovi prečnika 2–5 mm imaju konstantnu brzinu dizanja,  $W_D$ , koja iznosi 26,5 cm/s, što se pripisuje „cik-cak“ kretanju mehurova kroz tečnost, a da porast veličine mehurova iznad 5 mm izaziva porast njihove brzine dizanja [21]. Na osnovu prethodnog zapažanja i zabeleženog ponašanja sadržaja gasa u disperziji pri promeni protoka tečne faze u

mirnoj zoni BKDM, može se zaključiti da su primarni mehurovi, koji se formiraju na izlazu iz dovodne cevi za gas, prečnika znatno većeg od 5 mm.

Povećanje protoka tečnosti izaziva njihovo prekidanje i usitnjavanje i smanjenje brzine dizanja, sve do protoka tečnosti koji obezbeđuje da su svi mehurovi prečnika većeg od 5 mm prekinuti. Taj protok odgovara kritičnom protoku tečnosti,  $L_c$ , pri kojem se dostiže maksimalna vrednost sadržaja gasa u disperziji. Porast protoka tečnosti iznad  $L_c$  nije svrshodan jer, iako se prekidanje mehurova nastavlja, ne dolazi do smanjenja brzine dizanja,  $W_D$ , a raste brzina tečnosti, što dovodi do smanjenja sadržaja gasa u disperziji. Specifična površina kontakta se praktično ne menja, a značajno raste pad pritiska na mlaznici, pa i potrebna snaga za obavljanje procesa.

Prenos mase iz gasne u tečnu fazu određen je vrednošću zapreminskog koeficijenta prenosa mase  $k_L a$ , što znači da je sem specifične međufazne površine kontakta bitna i vrednost koeficijenta prenosa mase,  $k_L$ . Predložena su dva izraza za određivanje  $k_L$  [22]:

$$k_L = 0,31(gV_L)^{1/3} \left( \frac{D_A}{V_L} \right)^{2/3} \quad (3)$$

$$k_L = 0,42(gV_L)^{1/3} \left( \frac{D_A}{V_L} \right)^{1/2} \quad (4)$$

Jednačina (3) se odnosi na jako sitne mehurove koji se hidrodinamički ponašaju kao krute sfere, a jednačina (4) na veće mehurove sa pokretnim površinama.

Zapreminska koeficijenta prenosa mase ozona iz smeša sa kiseonikom određuju se na osnovu opšte diferencijalne jednačine prenosa mase u aksijalnom z pravcu:

$$U_L \frac{\partial c_L}{\partial z} + \frac{\partial c_L}{\partial \tau} = D \frac{\partial^2 c_L}{\partial z^2} + k_L a(c_L^* - c_L) \quad (5)$$

Kod protočnog sistema bez recirkulacije tečnosti proticanje je stacionarno ( $\partial c / \partial \tau = 0$ ), a disperzionalni član:

$$D \frac{\partial^2 c_L}{\partial z^2} \ll U_L \frac{dc_L}{dz}$$

kod kolona čija je visina znatno veća od prečnika ( $Hc > 4Dc$ ), pa opšta diferencijalna jednačina prenosa mase dobija sledeći oblik:

$$U_L \frac{dc_L}{dz} = k_L a(c_L^* - c_L) \quad (6)$$

odakle je zapreminski koeficijent prenosa mase:

$$k_L a = \frac{U_L \frac{dc_L}{dz}}{(c_L^* - c_L)} \quad (7)$$

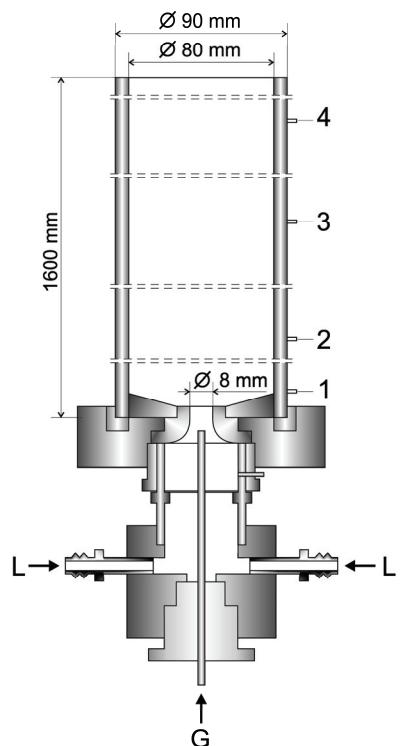
## EKSPERIMENTALNI DEO

Šema eksperimentalnog sistema korišćenog u ovom radu prikazana je na slici 1.

Kao ulazni gas korišćen je čist kiseonik tako da dodatna priprema (kompresija, hlađenje, filtracija, sušenje gasa) nije bila neophodna. Za generisanje ozona korišćen je OzoneLab OL100/DS ozonizator, koji radi na principu tihog (korona) električnog pražnjenja. Promenljiva frekvencija pražnjenja (0–10 kHz) omogućila je generisanje ozona u širokom opsegu koncentracija sa velikom preciznošću. Koncentracija nastalog ozona je nezavisno regulisana sa dva („R“ i „L“) desetpoložajna preklopnika, pomoću kojih se menjala frekvencija pražnjenja. Gas je dovođen kroz jedan otvor (cev) unutrašnjeg prečnika 3 mm.

Voda i ozon su uvođeni na dno kolone, pa se protok faza odvijao istostrujno naviše, dok je na vrhu kolone bio postavljen preliv koji je obezbeđivao da kolona radi kao stacionarni protočni sistem. Eksperimenti su vršeni u BKDM unutrušnjeg prečnika 80 mm i visine 1,6 m sa dvofluidnom mlaznicom prečnika 8 mm, u koju se uvođe i gasna i tečna faza. Šematski prikaz BKDM prikazan je na slici 2.

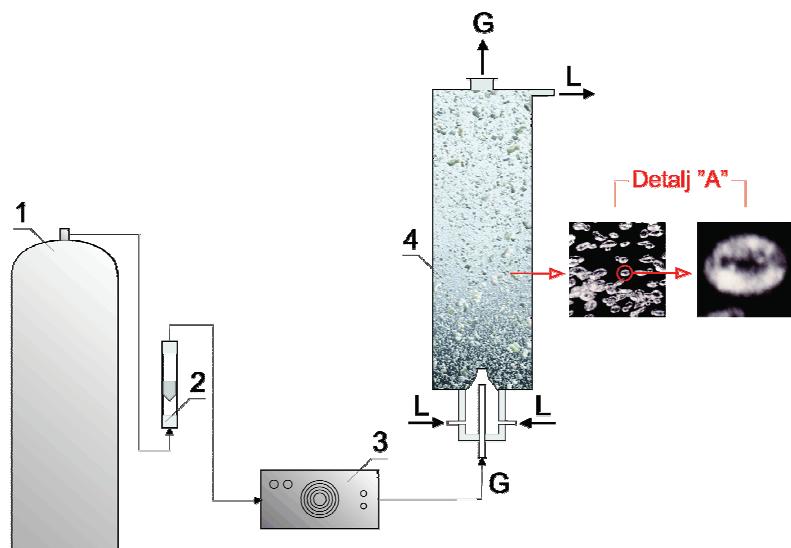
Uzorci vode sa ozonom su uzimani sa četiri merna mesta na visinama 30, 50, 90, 139 cm (slika 2 pozicije 1–4) od dna kolone. Za određivanje zapreminskog koeficijenta prenosa mase korišćena su dva protoka tečne faze i to 180 i 360 L/h. Prva vrednost je daleko ispod kritičnog protoka i pri njoj prekidanje i dispergovavanje primarnih mehurova gasa tek počinje, a druga je nešto iznad kritičnog protoka tečnosti, čime se obezbeđuje da su svi mehurovi prečnika većeg od 5 mm sigurno prekinuti. Izabrani protoci gasne faze iznosili su



Slika 2. Barbotažna kolona sa dvofluidnom mlaznicom (BKDM).

Figure 2. Bubble column with two-fluid nozzle gas distributor.

45, 60 i 120 L/h. Korišćenje većih protoka gasa nije bilo moguće zbog ograničenog opsega merenja instrumenta za merenje koncentracije rastvorenog ozona. Svaka od šest mogućih kombinacija protoka tečnosti i gasa urađena je pri dve vrednosti položaja preklopnika R i L na generatoru ozona, dakle pri dve različite vrednosti koncentracije ozona u ulaznom gasu. Sadržaj gasa u disper-



Slika 1. Šema eksperimentalnog sistema (1 – boca sa kiseonikom, 2 – rotametar, 3 – generator ozona i 4 – barbotažna kolona sa dvofluidnom mlaznicom).

Figure 1. Experimental apparatus (1 – oxygen bomb, 2 – rotameter, 3 – ozone generator and 4 – column).

ziji i srednji prečnik mehurova gasa određeni su samo pri protoku gase od 120 L/h. Eksperimenti su izvođeni na temperaturi vode od 15,5 °C kojoj odgovara vrednost koeficijenta rastvorljivosti ozona od 0,285 [7]. Sve vrednosti izmerenih protoka vazduha preračunate su na normalne uslove pritiska i temperature (101,32 kPa, 0 °C).

Sadržaj gase u disperziji određivan je manometarskom metodom. Na mernim mestima BKDM postavljene su piezometarske cevi, a sadržaj gase u disperziji u pojedinim segmentima kolone između mernih mesta određivana je preko razlike visine nivoa disperzije u koloni i visine nivoa vode u piezometarskim cevima ( $H_G$ ):

$$\epsilon_G = \frac{H_G}{z} \quad (8)$$

Sauterov srednji prečnik mehura određivan je fotografskom metodom. Disperzija gas-tečnost fotografsana je kroz providne zidove kolone pri različitim operativnim uslovima. Konstatovano je da su mehurovi oblika spljoštene sfere, a da je projekcija na fotografijama oblika elipse (slika 1, detalj A). Dobijeni snimci analizirani su merenjem osa projekcije mehura sa fotografije, za najmanje 50 mehurova. Iz izmerenih vrednosti određene su stvarne dužine velike i male poluose elipse,  $a$  i  $b$ , deljenjem izmerenih vrednosti osa sa faktorom uveličanja fotografije i sa dva jer su u pitanju poluose. Sauterov srednji prečnik mehura određivan je zatim preko izraza:

$$d_{BS} = \frac{\sum_{i=1}^n d_{B3i}^3}{\sum_{i=1}^n d_{B2i}^2} \quad (9)$$

pri čemu se  $d_{B3i}^3$  i  $d_{B2i}^2$  računaju po izrazima:

$$d_{B3i}^3 = 2b_i(3a_i^2 + b_i^2) \quad (10)$$

$$d_{B2i}^2 = 2(a_i^2 + b_i^2) \quad (11)$$

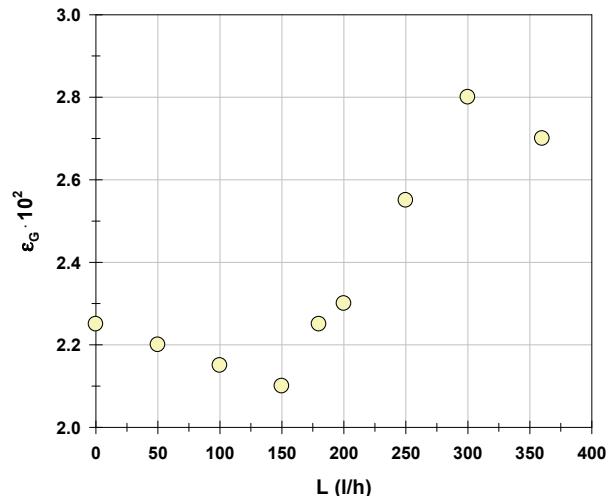
Specifična površina kontakta određivana je iz izraza (1).

Aktuelna koncentracija ozona u vodi merena je pomoću Pocket Colorimeter™ II, proizvodnje Hach kojišćenjem indigo metode [23,24], na mernim mestima po visini kolone. Fitovanjem dobijenih krivih polinomom drugog stepena, određeno je  $dc/dz$  u svakom od eksperimentalnih položaja u koloni.

Zapreminski koeficijent prenosa mase određivan je iz izraza (7).

## REZULTATI I DISKUSIJA

U mirnoj zoni BKDM, pri protoku gase od 120 L/h, sadržaj gase u disperziji se menja sa promenom protoka tečne faze, što je pokazano na slici 3.



Slika 3. Sadržaj gase u disperziji u funkciji protoka tečnosti, pri protoku gase od 120 L/h.

Figure 3. Gas hold up vs. liquid flow rate (gas flow rate 120 L/h).

Sa slike 3 se može zaključiti da pri malim protocima tečnosti (<150 l/h) dolazi do smanjenja sadržaja gase u disperziji, jer povećanje istostrujnog toka tečnosti u BKDM dovodi do porasta brzine kojom mehurovi napuštaju kolonu, a značajnijeg prekidanja gasnih mehurova još uvek nema. Dalje povećanje protoka tečnosti izaziva intenzivnu turbulenciju u turbulentnoj zoni kolone, koja utiče na prekidanje mehurova i smanjenje njihovog prečnika u mirnoj zoni kolone, smanjenje brzine dizanja mehurova, pa stoga i porast sadržaja gase u disperziji. Po dostizanju kritičnog protoka tečnosti od 300 l/h svi mehurovi prečnika većeg od 5 mm su prekinuti, ne menja im se više brzina dizanja, pa sadržaj gase u disperziji počinje da opada sa porastom protoka tečnosti zbog porasta brzine tečnosti.

Sauterov srednji prečnik mehura u mirnoj zoni kolone opada kontinualno pri porastu protoka tečnosti što je pokazano u tabeli 1.

Tabela 1. Sauterov srednji prečnik mehura u funkciji protoka tečnosti, pri protoku gase od 120 L/h

Table 1. Sauter mean bubble diameter vs liquid flow rate (gas flow rate 120 L/h)

$L / L h^{-1}$	180	360	700	1800
$d_{BS} / \text{mm}$	3,50	3,15	2,90	2,40

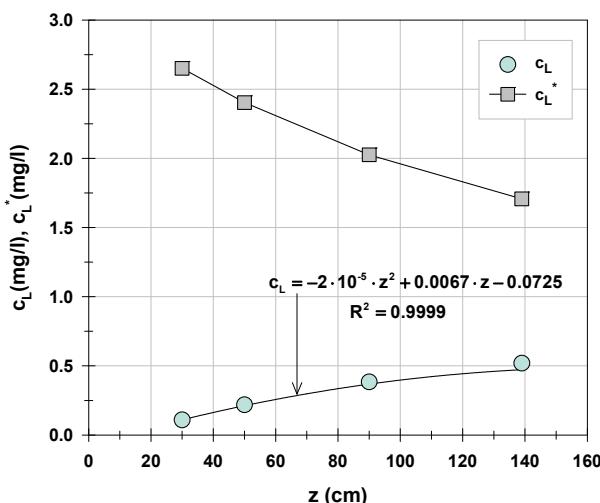
Sve do dostizanja kritičnog protoka tečnosti u sistemu dolazi do vrlo značajnog povećanja specifične međufazne površine kontakta, kao posledice stalnog

povećanja sadržaja gase u disperziji, u mirnoj zoni kolone, i istovremeno stalnog smanjenja Sauterovog srednjeg prečnika mehura, što se vidi iz tabele 2.

*Tabela 2. Specifična površina kontakta u funkciji protoka tečnosti, pri protoku gasa od 120 L/h  
Table 2. Intefacial area vs liquid flow rate (gas flow rate 120 L/h)*

$L / L h^{-1}$	180	360
$a / cm^{-1}$	0,386	0,514

Pri proračunu vrednosti zapreminskega koeficijenta prenosa mase prema izrazu 7, potrebno je poznavanje promene koncentracije rastvorenog ozona u vodi,  $c_L$ , po visini kolone, što se određuje merenjem pomoću kolorimetra. Po visini kolone dolazi i do stalnog opadanja ravnotežne koncentracije ozona u vodi, idući u smeru od dna ka vrhu, što je posledica apsorpcije ozona i smanjenja koncentracije ozona u gasnim mehurovima, u istom smeru, kao i smanjenja pritiska u mehurovima. Zbog obimnosti proračuna za određivanje zapreminskog koeficijenta prenosa mase, u daljem tekstu će biti prikazan samo primer proračuna za eksperiment pri protoku gasa od 60 L/h i tečnosti od 360 L/h i položaje preklopnika  $R = 0$  i  $L = 8$ . Promena izmerenih vrednosti koncentracija rastvorenog ozona i izračunatih vrednosti ravnotežnih koncentracija ozona, po visini kolone, za ovaj eksperiment prikazana je na slici 4.

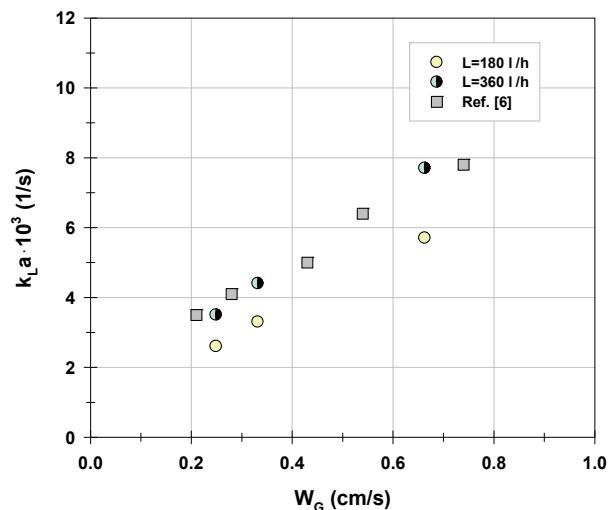


*Slika 4. Zavisnost aktuelne i ravnotežne koncentracije ozona u vodi od položaja u koloni pri  $G = 60 \text{ L/h}$ ,  $L = 360 \text{ L/h}$ , "R" = 0 i "L" = 8.*

*Figure 4. Dissolved and equilibrium ozone concentration vs. the distance from the nozzle ( $G = 60 \text{ L/h}$ ;  $L = 360 \text{ L/h}$ ; "R" = 0 and "L" = 8).*

Fitovanjem dobijene krive polinomom drugog stepena, određena je vrednost  $dc_L/dz$  na svim visinama gde je izvršeno merenje. Na taj način može se odrediti vrednost zapreminskega koeficijenta prenosa mase

ozona na različitim položajima po visini kolone. Rezultati su pokazali da se ova vrednost ne menja sa položajem u koloni, kao ni pri promeni koncentracije ozona u ulaznom gasu. Eksperimentalno određene vrednosti zapreminskega koeficijenta prenosa mase ozona, pri svim operativnim uslovima protoka gasa i tečnosti, prikazani su na slici 5.



*Slika 5. Zavisnost zapreminskega koeficijenta prenosa mase ozona od prividne brzine gasa i protoka tečnosti.*

*Figure 5. Ozone volumetric mass transfer coefficient vs. superficial gas velocity and liquid flow rate.*

Na istoj slici prikazane su, radi poređenja, i vrednosti zapreminskega koeficijenta prenosa mase ozona ostvarene u protivstrujnoj barbotažnoj koloni sa fritom kao raspodeljivačem gasa [6]. Rezultati pokazuju da povećanje prividne brzine gase, utiče na povećanje zapreminskega koeficijenta prenosa mase u mirnoj zoni BKDM, na sličan način kao i kod klasične protivstrujne barbotažne kolone. Za sistem ozon-voda u barbotažnoj koloni nedvosmisleno je pokazano da zapreminski koeficijent prenosa mase ozona u vodi raste sa porastom prividne brzine gase na eksponent čija se vrednost kreće od 0,54 do 1,02 [7,8]. Konstatovano je da je zapreminski koeficijent prenosa mase kiseonika iz vazduha u mirnoj zoni kolone proporcionalan promeni prividne brzine gase na eksponent 0,7 [10].

Pri promeni protoka tečnosti od 180 do 360 L/h dolazi do značajnog porasta vrednosti zapreminskega koeficijenta prenosa mase, nezavisno od protoka gasa. Pri protoku gasa od 120 L/h, ovaj porast iznosi 1,35 puta. S druge strane, pri istoj promeni protoka tečnosti, prema podacima iz tabele 2, specifična međufazna površina poraste 1,33 puta, što znači da pri porastu protoka tečnosti promena  $k_L a$  potiče samo od promene specifična međufazne površine, a da koeficijent prenosa mase ostaje praktično nepromenjen i iznosi  $1,5 \times 10^{-2} \text{ cm/s}$ .

Dobijene vrednosti  $k_L a$  u mirnoj zoni BKDM, pri protocima tečnosti oko  $L_c$  se neznatno razlikuju od vrednosti  $k_L a$  u klasičnoj protivstrujnoj barbotažnoj koloni sa fritom [6], što znači da se dvofluidna mlaznica može koristiti kao raspodeljivač gasa umesto frite. Ovo je posebno značajno kod tretmana otpadnih voda ozonom, jer je korišćenjem dvofluidne mlaznice isključena mogućnost začepljivanja suspendovanim česticama, što se može desiti korišćenjem frite.

## ZAKLJUČAK

U ovom radu je ispitivan uticaj protoka faza (gasne i tečne) na zapreminski koeficijent prenosa mase ozona u vodi,  $k_L a$  u mirnoj zoni BKDM.

Rezultati eksperimentalnih ispitivanja pokazuju da povećanje prividne brzine gasa, utiče na povećanje zapreminskog koeficijenta prenosa mase ozona, na sličan način kao i kod klasične protivstrujne barbotažne kolone.

Porast protoka tečnosti do njegove kritične vrednosti, utiče značajno na povećanje zapreminskog koeficijenta prenosa mase ozona, što je posledica povećanja specifične međufazne površine kontakta, dok se vrednost koeficijenta prenosa mase ozona praktično ne menja.

Dobijene vrednosti  $k_L a$  u mirnoj zoni BKDM, pri protocima tečnosti oko  $L_c$ , se neznatno razlikuju od vrednosti  $k_L a$  u klasičnoj protivstrujnoj barbotažnoj koloni sa fritom, što znači da se dvofluidna mlaznica može koristiti kao raspodeljivač gasa umesto frite.

## Simboli

- $k_L a$  zapreminski koeficijent prenosa mase,  $s^{-1}$
- $a$  specifična međufazna površina kontakta,  $cm^{-1}$
- $\varepsilon_G$  sadržaj gasa u disperziji
- $d_{BS}$  Sauterov srednji prečnik mehura, mm
- $L_c$  kritični protok tečnosti, L/h
- $W_G$  prividna brzina gasa,  $cm\ s^{-1}$
- $W_D$  brzina dizanja mehurova,  $cm\ s^{-1}$
- $W_D^0$  brzina dizanja mehurova kroz tečnost koja miruje,  $cm\ s^{-1}$
- $U_L$  prividna brzina tečnosti koja istostrujno protiče sa gasom,  $cm\ s^{-1}$
- $k_L$  koeficijent prenosa mase sa strane tečnosti,  $cm\ s^{-1}$
- $g$  gravitaciona konstanta,  $9,81\ m\ s^{-2}$
- $\nu_L$  kinematska viskoznost,  $m^2\ s^{-1}$
- $D_A$  koeficijent difuzije,  $cm^2\ s^{-1}$
- $c_L^*$  aktuelna koncentracija ozona u tečnoj fazi,  $g/m^3$
- $c_L$  ravnotežna koncentracija ozona u vodi,  $g/m^3$
- $\tau$  vreme, s
- $z$  rastojanje od ulaza u kolonu, cm
- $H_G$  razlika visine nivoa disperzije i visine nivoa vode u piezometarskoj cevi, cm
- $d_{B3}$  zapreminski ekvivalentni prečnik i-tog mehura, mm

- $d_{B2i}$  površinski ekvivalentni prečnik i-tog mehura, mm
- $a_i$  velika poluosa elipse kao projekcija i-tog mehura, mm
- $b_i$  mala poluosa elipse kao projekcija i-tog mehura, mm

## Zahvalnica

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## SUMMARY

### DETERMINATION OF THE OZONE VOLUMETRIC MASS TRANSFER COEFFICIENT IN BUBBLE COLUMN WITH TWO-FLUID NOZZLE GAS DISTRIBUTOR

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(Scientific paper)

Experimental investigation of gas-liquid mass transfer of ozone in water, in bubble column with two-fluid nozzle gas distributor (BKDM), under different operating conditions, is presented in this work. The main objective was to determine the ozone volumetric mass transfer coefficient,  $k_L a$ , in calm uniform section of the column, under different values of gas and liquid flow rates. Obtained values of these coefficients were compared with the values in countercurrent bubble column. The critical liquid flowrate, when gas hold up reaches its maximum, was experimentally determined. It was shown that the maximum value of the ozone volumetric mass transfer coefficient is obtained just when liquid flowrate is at its critical value.

**Keywords:** Ozone volumetric mass transfer coefficient • Bubble column with two-fluid nozzle gas distributor



# Impact of reaction vessel pressure on the synthesis of sliced activated carbon from date palm tree fronds

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## Abstract

The effects of the reaction vessel pressure on the BET surface area, pore volume and pore size of the synthesis of sliced activated carbons (SAC) at 850 °C starting from 0.10 to 0.40 bar were investigated. Other synthetic variables like dwell time, CO<sub>2</sub> flow rate and heating ramp rate were kept constant during the whole study. Methodology involves a single step procedure using the mixture of gases (N<sub>2</sub> and CO<sub>2</sub>). During activation of flow rate, the both gases are kept at 150 and 50 ml/min, respectively. The BET surface areas of the SAC prepared at 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40 bar after 30 min of activation time were 666, 745, 895, 1094, 835, 658 and 625 m<sup>2</sup>/g, respectively. Scanning electron microscopy (SEM) for surface morphology, energy dispersive spectroscopy (EDS), transmission electron microscopy (TEM) for nano particle size were also carried out, which confirmeds the same trend.

**Keywords:** activated carbon, physical activation, gaseous mixture, Saudi date fronds, agro waste.

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Any type of carbonaceous material [1] can be a good precursor for the synthesis of activated carbon for instance oil-palm stones [2], palm shell [3], olive stones [4], coconut shell [5], nut shell [6], agricultural wastes [7,8], etc. Date palm tree fronds in the Kingdom of Saudi Arabia are the waste material from the date trees during pruning process [9]. This locally available waste material is ideal for the synthesis. The synthesis steps of activated carbon from physical method normally involve two stages, in the first stage it involves the carbonization of the raw material in an inert atmosphere like nitrogen and in the second stage, the activation of the carbonized products with steam and/or CO<sub>2</sub>. During the first stage, *i.e.*, in the carbonization step, all the volatile matters are released, and the remaining solid carbon structure is generally called as char. In the second stage, *i.e.*, the activation step, the char produced in the first stage reacts with activating agents to form activated carbon (AC) with enhanced pore structure and surface properties. Nevertheless, well modified activated carbon for explicit application and having a specific surface area greater than 500 m<sup>2</sup> g<sup>-1</sup> cannot be easily obtained by simply carbonizing the carbonaceous materials or biomass above, and due to its ready availability and stability in production, much study has been done on coal for the industrial production of activated carbon [10–13].

## SCIENTIFIC PAPER

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During chemical activation, the raw material is first impregnated with certain chemicals like phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), potassium hydroxide (KOH), calcium chloride (CaCl<sub>2</sub>) and zinc chloride (ZnCl<sub>2</sub>) and then carbonization was carried out in a furnace.

Regardless of the benefits provided by the chemical activation process, the chemical consumption led to the secondary pollution problem during the disposal stage. For that reason, the physical activation consists of single step carbonization followed by activation using steam or carbon dioxide (CO<sub>2</sub>) as the activation agent is the most commonly used. Yet, since single stage CO<sub>2</sub> activation has received lack of scientific research and there is still limited publication data in the literature. In view of that, further research work on this area is deemed very momentous. Through recent published research [14], one step activation process on the coconut shell produces comparable results as the two step approaches. The benefits of utilizing the single step activation are due to lower operating time and the energy consumption. The effect of ramp rate and the CO<sub>2</sub> flow rate on the synthesis of sliced activated carbon (SAC) from date palm tree has already been published [15,16]. The objective of the present work was to study the effect of reaction vessel pressure on the synthesis of effective SAC from date palm tree fronds by physical activation.

## EXPERIMENTAL

The date palm tree fronds were dried at 105 °C for 8 h to reduce the moisture content followed by the slicing of the dried fronds with sophisticated wood

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cutter to a size range of 2–3 mm. 4.0 g of the sliced precursor was used for the study. Pyrolysis was carried out at a temperature of 850 °C by using Grade 5 (99.999%) nitrogen gas at a flow rate of 150 ml/min whereas the activation was done with a mixture of Grade 5 nitrogen and carbon dioxide gas at a flow rate of 150 and 50 ml/min, respectively. Carbolyte MTF 12/38/250 horizontal tube furnace was used both for pyrolysis and activation processes. The effects of the reaction vessel pressure were studied from 0.10 to 0.40 bars during the study and the prepared activated carbon was characterized by calculating BET surface area, pore size, pore volume, SEM, EDS and TEM. Digital pressure switch, solenoid valve from SMC Japan and the indigenously developed electronics facilitated the process to attain the desired pressure within a variation of ±0.02 bar. Fig. 1 shows the schematic of the process.

## RESULTS AND DISCUSSION

### BET surface area, pore size and pore volume

BET surface areas, pore size and pore volumes of the SAC prepared from date palm tree fronds were determined by N<sub>2</sub> gas adsorption at 77 K with an automated adsorption instrument (Gemini VII, 2390 Micromeritics). Prior to the determination, the sample

(about 0.05 g) was degassed for 45 min at 150 °C under nitrogen to remove moisture and other volatiles from the sample. The effects of the reaction vessel pressure on the, BET surface area, pore volume and pore size of the SAC at 850 °C starting from 0.10 to 0.40 bar are shown in Table 1.

Decreasing the system pressure from 0.40 to 0.25 bar increases the evolution of volatiles from raw materials by drawing off organic vapor and uncondensable gas formed during the activation process in the reactor, resulting in pore development thus increasing in the activated carbon. However, further decreasing the system pressure to 0.10 bar, results in significant decline in the BET surface area and pore volume of the activated carbon. This can be attributed to very fast volatilization of the volatiles generated during the activation process, which may affect the formation of activated carbon and decrease the number of pore and even destroy the pore structures.

From Table 1, it can be seen that the pore surface area and the pore volume of the activated carbon exhibit identical trends. At the system pressure of 0.25 bar, the pore surface area and the pore volume have maximum values of 1094 m<sup>2</sup>/g and 0.4382 cm<sup>3</sup>/g, respectively. The BET surface area, the total pore volume and the average pore diameter all reach the maximum at the system pressure of 0.25 bar, but the pore surface

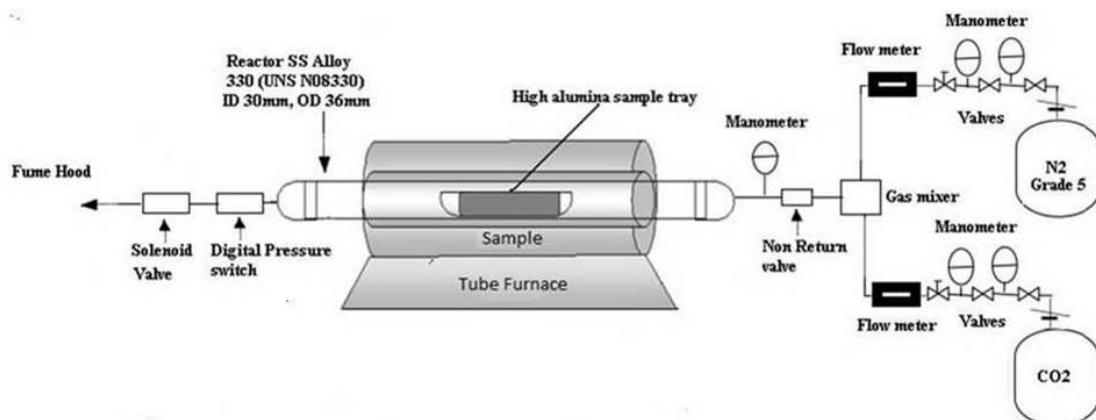


Figure 1. Schematic of process.

Table 1. Effect of reaction vessel pressure on surface area, pore volume and pore size of activated carbon; a-b-c-d-e-f denotes SAC-activation temperature (°C)-heating ramp rate (°C/min)-activation dwell time (min)-CO<sub>2</sub> flow rate (ml/min)-reaction vessel pressure (bar)

Sample No.	Sample name	Surface area, m <sup>2</sup> /g	Pore volume, cm <sup>3</sup> /g	Pore size, Å
1	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.40 <sup>f</sup>	666	0.2817	16.94
2	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.35 <sup>f</sup>	745	0.3207	17.21
3	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.30 <sup>f</sup>	895	0.3620	16.16
4	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.25 <sup>f</sup>	1094	0.4382	16.09
5	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.20 <sup>f</sup>	835	0.3540	16.96
6	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.15 <sup>f</sup>	658	0.2820	17.13
7	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.10 <sup>f</sup>	625	0.2697	17.24

area and pore volume are lower than those obtained at the system pressure of 0.25 bar. The result indicates that system pressure of 0.25 bar is beneficial to develop the pore structure of activated carbon during physical activation process. The effects of reaction vessel pressure on the surface area, pore volume and pore size of the activated carbons are shown in Fig. 2a and b.

### SEM Analysis

SEM analysis was carried out by using Jeol JSM-6380 LA instrument. Figure 3 shows the scanning electron microscope (SEM) graphs for: a) the raw date fronds. The image clearly shows the surface is curvy due to cellulose, hemicelluloses and lignin and with less slit like fractures or cracks. Pyrolysis and activation at reaction vessel pressure of 0.25 bar produced predominantly micro porous with a little presence of mesoporosity as shown in Fig. 3b.

### EDS Analysis

Table 2 shows the EDS results of date palm tree frond and SAC prepared at the optimum condition of reaction vessel pressure *i.e.*, 0.25 bar. The determined element content includes carbon (C), oxygen (O), and other elements. Among the elements, those that should be mainly focused on are the carbon and oxy-

gen content. An activated carbon will usually have higher carbon content than the starting material. In this case, the carbon content is 57.53% for raw date fronds, whereas the carbon content increased to 77.83% after the optimized reaction vessel condition for SAC<sup>a</sup>-850<sup>b</sup>-10<sup>c</sup>-30<sup>d</sup>-50<sup>e</sup>-0.25<sup>f</sup> (see Table 1 for definition). This phenomenon is due to the volatilization of H and O atom from the carbon matrix during the activation process at high temperature.

Table 2: EDS results (%) of date palm tree frond and SAC<sup>a</sup>-850<sup>b</sup>-10<sup>c</sup>-30<sup>d</sup>-50<sup>e</sup>-0.25<sup>f</sup> (see Table 1 for definition)

No.	Sample name	Carbon	Oxygen	Others
1	Date Palm Tree Frond	57.83	39.20	2.97
2	SAC <sup>a</sup> -850 <sup>b</sup> -10 <sup>c</sup> -30 <sup>d</sup> -50 <sup>e</sup> -0.25 <sup>f</sup>	77.83	16.16	6.01

### TEM Analysis

The pore size distribution observed in the TEM images was calculated using the program Image J and plotted in graph. Figure 4a shows the tem image of SAC<sup>a</sup>-850<sup>b</sup>-10<sup>c</sup>-30<sup>d</sup>-50<sup>e</sup>-0.25<sup>f</sup> and Figure 4b shows the graphical illustration of the particle size distribution. The TEM results clearly indicate that the product is fairly uniform and is present in the form of nanoparticles.

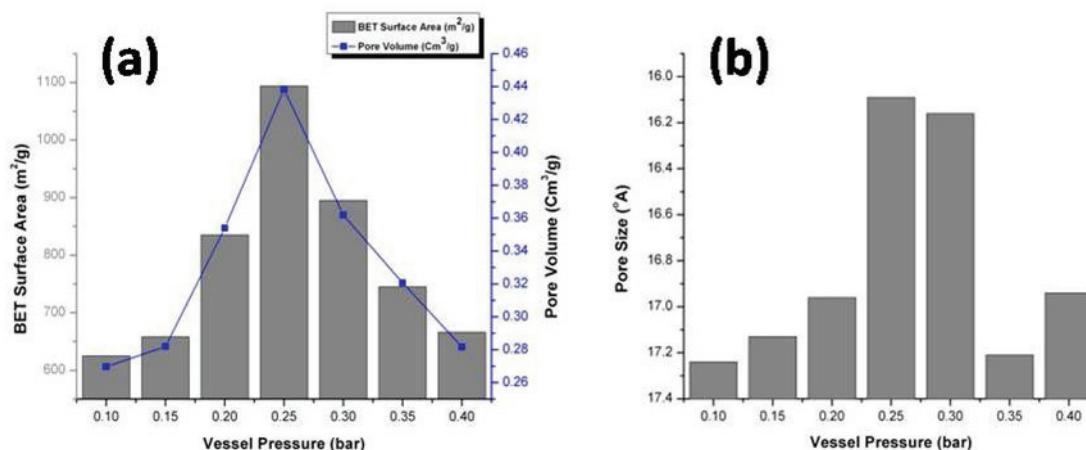


Figure 2. Effects of reaction vessel pressure on: a) surface area and pore volume and b) pore size.

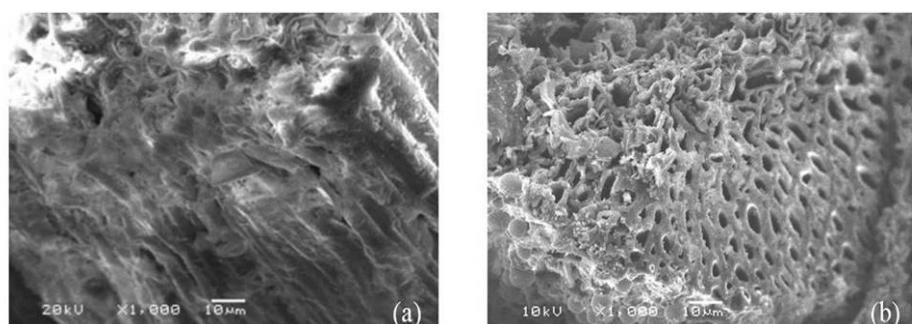


Figure 3: SEM micrographs showing the surface morphologies of: a) raw date frond and b) SAC<sup>a</sup>-850<sup>b</sup>-10<sup>c</sup>-30<sup>d</sup>-50<sup>e</sup>-0.25<sup>f</sup> (see Table 1 for definition).

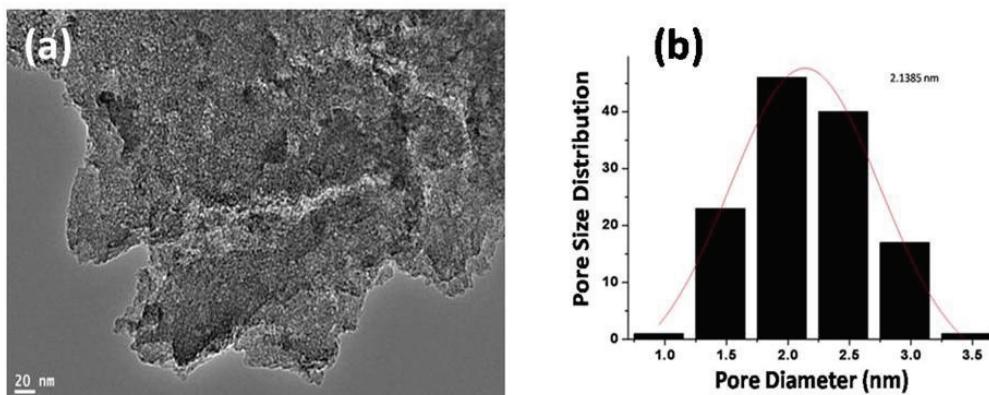


Figure 4. TEM image of: a) SAC<sup>a</sup>-850<sup>b</sup>-10<sup>c</sup>-30<sup>d</sup>-50<sup>e</sup>-0.25<sup>f</sup> and b) graphical illustration of the particle size distribution.

As per my knowledge there is no data available on the effect of vessel pressure on the synthesis of activated carbon from any agro waste material, the literature shows that the BET surface area of activated carbon from cherry stones, grape seeds, nut shells, pistachio-nut shells, almond shells, corn cob, oak, corn hull, corn stover, rice husk, and pecan shells reported in the review article [17] and they all have lower BET surface area and pore volume except the pistachio nut shell which also has a lower surface area, but has a bit higher pore volume as compared to the activated carbon prepared in this study.

## CONCLUSION

SAC prepared from date palm tree fronds at a reaction vessel pressure varying from 0.1 to 0.4 bar. The pressure of 0.25 bar was found to be optimum, as it produced SAC with larger surface area of 1094 m<sup>2</sup>/g and pore volume 0.4382 cm<sup>3</sup>/g as compared to the ones obtained under other pressure conditions studied. The pore size 16.09 Å at 0.25 bar verifies that the synthesized activated carbon is predominantly micro porous. This shows that the pressure of the reaction vessel plays an important role in obtaining high surface area. Synthesized SAC can be used as effective adsorbent for NO<sub>x</sub>/SO<sub>x</sub> emission adsorption from electricity generation plants, purification of gases, sewage treatment and many other applications, the further studies in this direction are being carried out by the group and shall be reported later.

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## IZVOD

### UTICAJ PRITISKA U REAKCIONOM SUDU NA SINTEZU REŽNJEVITOGL AKTIVNOG UGLJA IZ LIŠĆA DRVETA URME

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(Naučni rad)

Proučavan je uticaj pritiska u reakcionom sudu na specifičnu BET površinu, zapreminu i veličinu pora režnjevitog aktivnog uglja (SAC) na 850 °C, pri primeњenim pritiscima od 0,10 do 0,40 bar. Druge promenljive, kao što su reakcionalno vreme, protok CO<sub>2</sub> i brzina zagrevanja rampe su održavane konstantnim za vreme čitavog istraživanja. Metodologija uključuje jednostepeni proces upotreboom sмеši gasova (N<sub>2</sub> i CO<sub>2</sub>). Za vreme aktivacije protok oba gasa je održavan na 150 (N<sub>2</sub>) i 50 ml/min (CO<sub>2</sub>). BET površine pripremljenog SAC pri pritiscima od 0,10; 0,15; 0,20; 0,25; 0,30; 0,35 i 0,40 bar posle perioda aktivacije od 30 min su 666, 745, 895, 1094, 835, 658 i 625 m<sup>2</sup>/g, redom. Primjenjene metode, SEM za ispitivanje morfologije površine, EDS analiza i TEM za ispitivanje veličine nano čestica, takođe, pokazuju isti trend.

*Ključne reči:* Aktivni ugalj • Fizička aktivacija • Gasna sмеša • Lišće drveta saudijske urme • Poljoprivredni otpad



# Antioksidativna aktivnost etanolnog ekstrakta lista gajene jagode (*Fragariae folium*)

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## Izvod

Cilj rada bio je ispitivanje antioksidativnog potencijala etanolnog ekstrakta lista gajene jagode (*Fragariae folium*) sorte Zenga zengana primenom različitih antioksidativnih testova (DPPH, FRAP, FIC, H<sub>2</sub>O<sub>2</sub> i TBA-MDA). U ekstraktu je utvrđen visok sadržaj ukupnih fenola (metoda po Folin-Ciocalteu), dok je sadržaj ukupnih flavonoida znatno niži (metoda sa AlCl<sub>3</sub>). Koncentracija ekstrakta potrebna za neutralisanje 50% početne koncentracije DPPH radikala iznosi 7,91 µg cm<sup>-3</sup>. Maksimum heliranja jona gvožđa od 67,89% ostvaren je sa koncentracijom ekstrakta 2 mg cm<sup>-3</sup>; 70% inhibicije lipidne peroksidacije ostvareno je sa koncentracijom 0,03125 mg cm<sup>-3</sup>, dok se 30,47% H<sub>2</sub>O<sub>2</sub> neutrališe ekstraktom koncentracije 0,5 mg cm<sup>-3</sup>. FRAP vrednost ekstrakta iznosi 284,51 mg Fe<sup>2+</sup>/g suvog ekstrakta.

**Ključne reči:** list jagode (*Fragariae folium*), Zenga zengana, ukupni fenoli, ukupni flavonoidi, antioksidativna aktivnost.

Dostupno na Internetu sa adresu časopisa: <http://www.ache.org.rs/HI/>

Lekovito bilje se koristi u ishrani kao začin, za pripremu čajnih mešavina, kao i u medicinske svrhe za izradu fitopreparata. U ekstraktima lekovitog bilja utvrđeno je prisustvo fenolnih kiselina, flavonoida, katehina, tokoferola, tanina, terpena i drugih bioaktivnih jedinjenja koja pokazuju antimikrobnu, antiinflamatornu i antioksidativnu svojstva [1]. Bioaktivni proizvodi iz biljnih sirovina, poznati kao prirodni izolati ili prirodne biohemikalije, imaju sve veću primenu u proizvodnji lekova, kozmetičkih i prehrambenih proizvoda ili kao prekursori u sintezi novih proizvoda sa specifičnim bioaktivnim svojstvima [2]. Sagledavaju se novi aspekti primene tradicionalnih biljnih lekova i sprovode obimna fitohemijska ispitivanja u cilju pronalaženja novih prirodnih izvora i aktivnih principa.

Jagoda pripada familiji ruža (*Rosaceae*, podfamilija *Rosoideae*) iz roda *Fragaria*. Gajene (baštenske) sorte komercijalnih jagoda obično su označene kao *Fragaria ananassa* [3]. Zenga zengana je poreklom iz Nemačke, spada u srednje pozne sorte jagoda i privredno je najznačajnija u svetu [4]. Jagoda je bogata vitaminima B-kompleksa, vitaminom C, E, provitaminom A (β-karotenom), kalijumom, kalcijumom, gvožđem, fosforom i magnezijumom [5,6]. Takođe sadrži fenole, flavonoide, alkohole, terpene, aldehyde, ketone, estre, jedinjenja sumpora, furane, epokside, organske kiseline i dr. [3]. List jagode sadrži flavonoide (kvercetin i rutin), tanine, etarska ulja, vitamin C i fenolna jedinjenja, u većoj meri nego u plodu pa se zato upotrebljava za pravljenje čajeva [7,8].

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U literaturi ima podataka o različitim biološkim aktivnostima (pre svega antioksidativnoj i antimikrobojnoj) ekstrakata ploda i lista kako šumske, tako i gajenih sorti jagoda. Najveći broj ispitivanja odnosi se na ekstrakte iz ploda jagode, dok su ekstrakti iz lista gajenih jagoda, kao izvor bioaktivnih jedinjenja sa potencijalnim korisnim biološkim dejstvima manje ispitivani [8–10]. Cilj ovog rada je određivanje antioksidativne aktivnosti etanolnog ekstrakta lista gajene jagode sorte Zenga zengana, primenom različitih antioksidativnih testova.

## EKSPERIMENTALNI DEO

### Biljni materijal

U radu je korišćen suvi list gajene jagode (*Fragaria ananassa* Duch.), sorta: *Senga sengana*. Jagoda je gajena u plasteniku visine 2,5 m, pokrivenom polietilen-skrom folijom (0,15 mm debljine; Ginegar Plastic Products-Ltd.). Biljni materijal ubran je početkom maja 2013, u periodu cvetanja, sušen na promaji u hladovini i neposredno pre analize samleven u električnom mlinu (laboratorijski električni mlin Braun Aromatic KSM2) do granulacije 0,5 mm.

### Hemikalije i reagensi

2,4,6-Tris(2-piridil)-1,3,5-triazin (TPTZ reagens), Folin-Ciocalteu reagens, galna kiselina, 1,1-difenil-2-pikrilhidrazil (DPPH radikal), aluminijum(III)-hlorid heksahidrat, kalijum-acetat, natrijum-acetat trihidrat, gvožđe(III)-hlorid heksahidrat, gvožđe(II)-sulfat heptahidrat, ferozin (natrijumova so 3-(2-piridil)-5,6-difenil-difenil-1,2,4-triazin-4',4"-disulfonske kiseline), etilen-diamino-tetra-sirćetna kiselina (EDTA), butilovani hidroksi-toluen (BHT), butilovani hidroksi-anizol (BHA), tiobarbi-

turna kiselina (TBA), 2,2'-azobis(2-metilpropionamidin) dihidrohlorid (AAPH), kvercetin (Sigma Aldrich, St. Louis, USA); gvožđe (II)-hlorid tetrahidrat, trihlorisirčetna kiselina (TCA), L-askorbinska kiselina (J.T. Baker, VA Deventer, Netherlands); rutin (Merck, Darmstadt, Germany); etanol (70 vol.%) analitičkog stepena čistoće (p.a.). Fosfolipidi (Phospholipon® 90; PL90) su poklon firme Phospholipid GMBH (Köln, Germany). Prema deklaraciji smeša PL90 se sastoji iz: fosfatidilholina 94,6%, lizo-fosfatidilholina 1,3%; masnih kiselina: palmitinska kiselina 12±2%, stearinska kiselina 3±1%, oleinska kiselina 10±3%, linolna kiselina 66±5%, linoleenska kiselina 5±2%; peroksidni broj 1,4.

### **Ekstrakcija uz refluks**

Usitnjeni i homogenizovani biljni materijal (10 g) ekstrahovan je 70 vol.% etanolom u toku 120 min, pri solvomodulu (odnos biljni materijal/rastvarač) 1:20 m/V na temperaturi ključanja rastvarača uz refluks. Nakon ekstrakcije biljni materijal je odvojen od tečnog ekstrakta filtriranjem uz vakuum na Bichner-ovom levku. Rastvarač je uklonjen uparavanjem na rotacionom vakuum uparivaču na 50 °C. Dobijeni ekstrakt je sušen u vakuum sušnici na 40 °C do konstantne mase i čuvan u frižideru na 4 °C do analize. Prinos ekstrahovanih supstanci (suvi ekstrakt) je određen na Scaltec SMO 01 (Scaltec Instruments, Germany) aparatu. Alikvotni deo (2 cm<sup>3</sup>) ispitivanog ekstrakta je sušen do konstantne mase na 105 °C.

### **Određivanje sadržaja ukupnih fenola**

Sadržaj ukupnih fenola određen je po proceduri Folin–Ciocalteu [11] sa određenim modifikacijama [12–14]. Ukupni fenoli se izražavaju kao ekvivalenti galne kiseline po gramu biljnog materijala (mg GKE/g biljnog materijala) [14].

### **Određivanje sadržaja ukupnih flavonoida**

Sadržaj ukupnih flavonoida određen je spektrofotometrijskom metodom sa aluminijum(III)-hloridom [15–17] sa određenim modifikacijama [14]. Rezultati se izražavaju kao ekvivalenti rutina po gramu biljnog materijala (mg RE/g biljnog materijala) [14].

### **Antioksidativna aktivnost**

#### **DPPH-test**

Etanolni rastvor DPPH radikala (1 cm<sup>3</sup>, 300 µmol rastvor ( $3 \times 10^{-4}$  mol dm<sup>-3</sup>)) dodat je u 2,5 cm<sup>3</sup> ekstrakta različitih koncentracija. Postupak se radi u dve probe. Jednom uzorku odmah je merena absorbanca na 517 nm, drugi uzorak je inkubiran na sobnoj temperaturi, u mraku, 20 min i merena absorbanca na 517 nm. Apsorbanca na 517 nm određuje se i za čist etanolni rastvor DPPH radikala razblažen u navedenom odnosu (1 cm<sup>3</sup> DPPH radikala date koncentracije kome je dodato 2,5 cm<sup>3</sup> etanola, „kontrola“), i za ekstrakt pre tretiranja

DPPH radikalom (2,5 cm<sup>3</sup> ekstrakta kome je dodat 1 cm<sup>3</sup> etanola, „blank“). Kapacitet hvatanja slobodnih radikala se izračunava prema jednačini (1) [14,18,19]:

Stepen neutralisanja DPPH radikala (%) =

$$= 100 - \left[ \frac{100(A_U - A_B)}{A_K} \right] \quad (1)$$

gde je:  $A_U$  – apsorbancija „uzorka“,  $A_B$  – apsorbancija probe „blank“,  $A_K$  – apsorbancija „kontrole“.

DPPH test je urađen i za standardne komponente: rutin i L-askorbinsku kiselinu i sintetske antioksidanse BHA i BHT.

#### **FRAP metoda**

Antioksidativna aktivnost etanolnog ekstrakta lista jagode primenom FRAP metode određena je po metodi Benzie i Strain [20], sa izvesnim modifikacijama [14].

**Standardna kriva.** Priprema FRAP reagensa: (300 mmol dm<sup>-3</sup>, pH 3,6), TPTZ reagensa (10 mmol dm<sup>-3</sup> u 40 mmol dm<sup>-3</sup> HCl) i FeCl<sub>3</sub>·6H<sub>2</sub>O (20 mmol dm<sup>-3</sup>) u odnosu 10:1:1. U pet epruveta je odmereno po 3 cm<sup>3</sup> FRAP reagensa i njima dodato po 0,1 cm<sup>3</sup> standardnih rastvora FeSO<sub>4</sub>·7H<sub>2</sub>O (0,2–1 mmol dm<sup>-3</sup>). Apsorbanca je merena na 593 nm u odnosu na slepu probu (3 cm<sup>3</sup> FRAP reagensa + 0,1 cm<sup>3</sup> vode). Kalibraciona kriva je konstruisana na osnovu poznatih koncentracija dobijenih vrednosti apsorbanci.

**Određivanje antioksidativne aktivnosti ekstrakata.** U epruvetu je dodato po 0,1 cm<sup>3</sup> ekstrakta i 3 cm<sup>3</sup> FRAP reagensa i nakon inkubacije od 30 minuta na 37 °C izmerena je apsorbanca na 593 nm. Iz jednačine kalibracione krive određena je koncentracija (mmol dm<sup>-3</sup>) Fe<sup>2+</sup> u uzorku i preračunata na masu suvog ekstrakta (mg Fe<sup>2+</sup>/g suvog ekstrata, tj. FRAP vrednost).

#### **H<sub>2</sub>O<sub>2</sub> test**

Antioksidativna aktivnost ekstrakta na H<sub>2</sub>O<sub>2</sub> određena je po metodi koju su opisali Ruch i saradnici [21] sa izvesnim modifikacijama. Napravljena je serija različitih koncentracija (0,0078–0,5 mg cm<sup>-3</sup>) ekstrakata. Rastvoru H<sub>2</sub>O<sub>2</sub> (40 mmol dm<sup>-3</sup>) pripremljenom u 0,1 M fosfatnom puferu pH 7,4 određena je apsorbanca na 230 nm. Ekstraktima različitih koncentracija (1 cm<sup>3</sup>) dodato je 3,4 cm<sup>3</sup> fosfatnog pufera, rastvor H<sub>2</sub>O<sub>2</sub> (0,6 cm<sup>3</sup>) i nakon 10 min inkubacije u mraku izmerena apsorbanca na 230 nm.

Apsorbanca je određena i za rastvor H<sub>2</sub>O<sub>2</sub> razblažen u navedenom odnosu (0,6 cm<sup>3</sup> H<sub>2</sub>O<sub>2</sub> date koncentracije kome je dodato 4,4 cm<sup>3</sup> fosfatnog pufera – „kontrolni rastvor“), i za ekstrakt pre tretiranja H<sub>2</sub>O<sub>2</sub> (1 cm<sup>3</sup> ekstrakta kome je dodato 4,0 cm<sup>3</sup> fosfatnog pufera – „blank“ rastvor). Kapacitet neutralisanja H<sub>2</sub>O<sub>2</sub> se izračunava prema jednačini (1).

Antioksidativna aktivnost primenom ovog testa određena je i za standardni antioksidans L-askorbinsku kiselinu ( $0,781\text{--}25 \mu\text{g cm}^{-3}$ ).

#### TBA-MDA test

TBA-MDA test je jedan od najčešće korišćenih testova za kvantitativno praćenje procesa lipidne peroksidacije *in vitro*. Zasniva se na zagrevanju ispitivanog uzorka sa tiobarbiturnom kiselinom (TBA) u kiseloj sredini, kada dolazi do reakcije TBA i MDA – krajnjim produktom lipidne peroksidacije nastalim degradacijom hidroperoksida i građenja ružičastog hromogena ( $[\text{TBA}]_2$ -malondialdehid adukt) sa apsorpcionim maksimumom na 532 nm. Ovaj obojeni kompleks nastaje kondenzacijom 2 mol TBA i 1 mol MDA samo iz lanaca masnih kiselina koje sadrže bar tri dvostrukе veze [22,23]. Antioksidativna aktivnost etanolnog ekstrakta jagode primenom TBA-MDA testa određena je po metodi razvijenoj za rastvore karotenoida, flavonoida i potencijalnih antioksidanasa [24–26], sa određenim modifikacijama. „Uzorak“ čine  $0,3 \text{ cm}^3$  metanolnog rastvora PL90 ( $1\times 10^{-2} \text{ mol dm}^{-3}$ ) i etanolnog ekstrakta lista jagode ( $0,00195\text{--}0,03125 \text{ mg cm}^{-3}$ ) u odnosu 2:1 (V/V). Lipidna peroksidacija je inicirana dodavanjem  $0,2 \text{ cm}^3$  vodenog rastvora hidrofilnog termalnog azo-inicijatora AAPH ( $2,2\times 10^{-2} \text{ mol dm}^{-3}$ ) u toku 3 h na temperaturi od  $40^\circ\text{C}$ , zaštićeno od svetlosti. Nakon inkubacije u reakcionu smešu je dodato po  $1 \text{ cm}^3$  vodenog rastvora TCA (5,5%),  $0,5 \text{ cm}^3$  metanolnog rastvora BHT ( $1\times 10^{-3} \text{ mol dm}^{-3}$ ) i  $0,5 \text{ cm}^3$  TBA ( $4,2\times 10^{-2} \text{ mol dm}^{-3}$  u  $5\times 10^{-2} \text{ mol dm}^{-3}$  NaOH). Smeša je inkubirana 10 min na  $65^\circ\text{C}$  a zatim centrifugirana 5 min na 13800 obr./min. Porast apsorbancije supernatanta na 532 nm predstavlja apsorpcioni maksimum stvorenog TBA-MDA kompleksa.

Određena je i apsorbancija rastvora PL90, u kome je inicirana lipidna peroksidacija, tretiranim sa TBA („kontrola“) i rastvora PL90, bez inicijacije lipidne peroksidacije, takođe tretiranim sa TBA („blank“).

Procenat inhibicije lipidne peroksidacije se izračunava prema jednačini (2):

Inhibicija lipidne peroksidacije (%) =

$$= \frac{100(A_K - A_U)}{(A_K - A_B)} \quad (2)$$

TBA-MDA test je urađen i sa metanolnim rastvorom kvercetina kao predstavnikom prirodnih antioksidanasa ( $0,00625\text{--}0,025 \text{ mg cm}^{-3}$ ) i metanolnim rastvorom BHT ( $0,0125\text{--}0,05 \text{ mg cm}^{-3}$ ), predstavnikom sintetskih antioksidanasa.

#### Sposobnost heliranja jona gvožđa (FIC test)

FIC (*Ferrous ion-chelating*) aktivnost se može koristiti kao test za antioksidanse i to je mera smanjenja apsorbance gvožđa (II) i ferozin kompleksa na 562 nm.

Sposobnost heliranja je određena u skladu sa metodom koju su opisali Dinis i saradnici [27] sa određenim modifikacijama [28,29]. Rastvoru ekstrakta različitih koncentracija ( $1 \text{ cm}^3$ ,  $0,031\text{--}2,0 \text{ mg cm}^{-3}$ ) dodato je  $3,75 \text{ cm}^3$  etanola i  $0,05 \text{ cm}^3$   $2 \text{ mM FeCl}_2\cdot 4\text{H}_2\text{O}$ . Reakcija je inicirana dodavanjem  $0,2 \text{ cm}^3$   $5 \text{ mM}$  ferozina. Smeša je inkubirana na sobnoj temperaturi u toku 10 min, nakon čega je izmerena apsorbanca na  $562 \text{ nm}$ . Smeša etanola, ferozina i  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  bez test uzorka se koristi kao „kontrola“ a smeša etanola i uzorka bez ferozina i  $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$  se koristi kao „blank“. EDTA je referentni standard za test. Niža vrednost apsorbancije označava bolju FIC aktivnost, odnosno gvožđe-jon helirajuću sposobnost test uzorka. Sposobnost heliranja jona gvožđa, FIC aktivnost (%) je računata korišćenjem jednačine (1).

Svi eksperimenti rađeni su u tri ponavljanja. Statička obrada podataka rađena je u MS Office Excel programu.

## REZULTATI I DISKUSIJA

### Prinos ekstrahovanih supstanci, sadržaj ukupnih fenola i flavonoida u ekstraktu lista jagode

Ranijim istraživanjima pokazano je da prinos ekstrahovanih supstanci, sadržaj ukupnih fenola i antioksidativna aktivnost biljnih ekstrakata zavise od primenjene tehnike ekstrakcije. Dhanani i sar. [30] su u svojim istraživanjima pokazali da je u etanolnim i vodeno-etanolnim ekstraktima biljne vrste *Withania somnifera* dobijenim konvencionalnom ekstrakcijom uz refluks veći sadržaj ukupnih fenola u odnosu na iste ekstrakte dobijene ultrazvučnom ili mikrotalasnom ekstrakcijom. Prinos ekstrahovanih supstanci, kao i sadržaj ukupnih fenola i flavonoida u etanolnom ekstraktu lista jagode dobijenom ekstrakcijom uz refluks na temperaturi ključanja prikazani su u Tabeli 1.

*Tabela 1. Prinos ekstrahovanih supstanci, sadržaj ukupnih fenola i flavonoida u etanolnom ekstraktu lista jagode; b.m. – biljni materijal*

*Table 1. The yield of extracted substances, total phenols and flavonoids content in the ethanolic extract from strawberry leaves*

Ekstrahovane supstance g/100 g b.m.	Ukupni fenoli mg GKE/g b.m.	Ukupni flavonoidi mg RE/g b.m.
$35,82\pm 1,075$	$427,46\pm 12,824$	$17,39\pm 0,870$

Biosinteza flavonoida odvija se u biljci gde oni učestvuju u svetloj fazi fotosinteze u toku koje katalizuju prenos elektrona. Proces biosinteze flavonoida može se stimulisati UV zračenjem, interakcijama izazvanim mikroorganizmima, ali i hemijskim stresom. Na stvaranje fenolnih jedinjenja utiču pored svetlosti, temperatura, zemljишte i drugi faktori [31]. S obzirom na to da je ispitivana sorta jagode gajena u plasteniku gde je tem-

peratura u sunčanim periodima iznad 40 °C, verovatno je visoka temperatura jedan od uzroka jako visokog sadržaja fenolnih jedinjenja u biljnem materijalu.

### Antioksidativna aktivnost

#### DPPH test

DPPH test se zasniva na reakciji razmene atoma vodonika između antioksidansa i stabilnog DPPH radikala. U reakciji dolazi do redukcije intenzivno ljubičasto obojenog DPPH radikala do odgovarajućeg hidrazina, što se spektrofotometrijski prati preko pada apsorbancije na 517 nm [32]. Na slici 1 je prikazan stepen neutralisanja DPPH radikala etanolnim ekstraktom lista jagode.

Vreme inkubacije ekstrakata ima uticaja na stepen neutralisanja slobodnog DPPH radikala i to za sve ispitivane koncentracije. Stepen neutralisanja DPPH radikala ispitivanim ekstraktom veći je za inkubirani uzorak (sl. 1).  $EC_{50}$  vrednosti etanolnog ekstrakta lista jagode, standardnih komponenti i sintetskih antioksidanasa prikazane su u Tabeli 2.

**Tabela 2.**  $EC_{50}$  vrednosti ( $\mu\text{g cm}^{-3}$ ) etanolnog ekstrakta lista jagode, standardnih komponenti i sintetskih antioksidanasa;  $EC_{50}$  – koncentracija ekstrakta potrebna za neutralisanje 50% početne koncentracije DPPH radikala

**Table 2.**  $EC_{50}$  values ( $\mu\text{g cm}^{-3}$ ) of ethanolic extract from strawberry leaves, standard components and synthetic antioxidants

Uzorak	Bez inkubacije	20 min inkubacije
Ekstrakt	19,46±0,584	7,91±0,237
Standardna komponenta		
L-Aksorbinska kiselina	3,29±0,10	3,29±0,10
Rutin	14,14±0,424	4,39±0,132
Sintetički antioksidans		
BHT	–	21,0±0,63
BHA	–	7,11±0,213

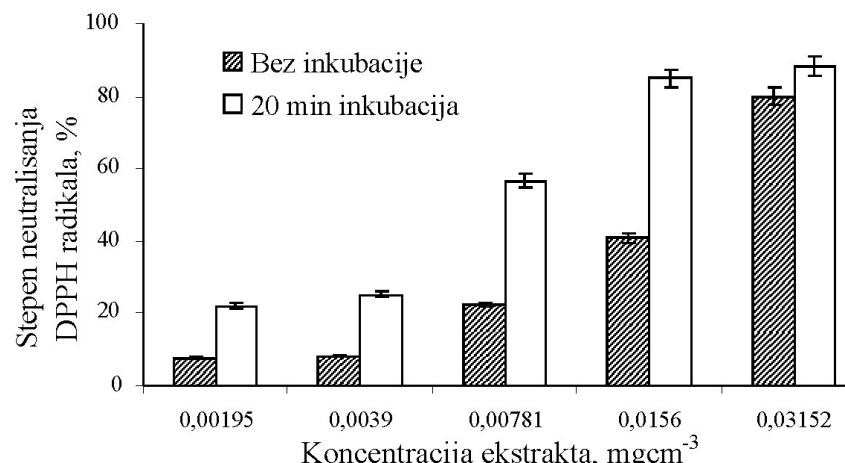
Ekstrakt pokazuje bolju antioksidativnu aktivnost od sintetskog antioksidansa BHT, približno istu aktivnost kao i BHA i nešto slabiju aktivnost od L-askorbinske kiseline i rutina. Obzirom da je BHT jedan od najčešće korišćenih antioksidanasa ali sa štetnim efektima po organizam [33], na osnovu dobijenih rezultata može se zaključiti da ispitivani ekstrakt predstavlja alternativu ovom antioksidansu sa potencijalnom primenom u farmaceutskim, kozmetičkim i prehrambenim proizvodima.

Izuzetno visoka antioksidativna aktivnost ekstrakta najverovatnije dobrim delom potiče od fenolnih jedinjenja za koje je utvrđen visok sadržaj (427,46 mg GKE/g b.m.). Antioksidativno delovanje fenolnih jedinjenja uglavnom se pripisuje prisustvu hidroksilne funkcionalne grupe u njihovoј strukturi [34,35]. Antioksidativno dejstvo ispitivanog ekstrakta ne potiče samo od fenolnih jedinjenja, već je posledica sinergističkog dejstva ovih jedinjenja sa nekim drugim biomolekulama izolovanim iz biljnog materijala. Podaci o ovakvim ispitivanjima antioksidativne aktivnosti nisu pronađeni u dostupnoj literaturi.

#### FRAP metoda

FRAP metoda [20,36–38] se zasniva na redukciji  $[\text{Fe}^{3+}\text{--TPTZ}]$  kompleksa do intenzivno plavog (apsorpcioni maksimum 593 nm)  $[\text{Fe}^{2+}\text{--TPTZ}]$  kompleksa u kise-loj sredini. Koncentracija  $\text{Fe}^{2+}$  ekvivalenta (FRAP vrednost) u ekstraktu je očitana direktno sa kalibracione krive [14] na osnovu koje se određuje koncentracija  $\text{Fe}^{2+}$  u uzorcima i preračunava na masu ekstrakta. U tabeli 3 su prikazane FRAP vrednosti (mg  $\text{Fe}^{2+}/\text{g s.e.}$ ) ekstrakta lista jagode, L-askorbinske kiseline i sintetskog antioksidansa BHT.

Rezultati prikazani u tabeli 3 pokazuju da najveću FRAP vrednost a time i najbolju redupcionu sposobnost, odnosno antioksidativnu aktivnost pokazuje sintetski antioksidans BHT. Ispitivani ekstrakt pokazuje skoro dva



**Slika 1.** Stepen neutralisanja DPPH radikala etanolnim ekstraktom lista jagode.

**Figure 1.** The capacity of neutralizing of DPPH radicals from ethanolic extract from strawberry leaves.

puta bolju redupcionu sposobnost od L-askorbinske kiseline. Na osnovu prikazanih rezultata, a saglasno antioksidativnoj aktivnosti ekstrakta određenoj primenom DPPH testa, može se reći da su i u ovom testu za antioksidativnu aktivnost najverovatnije dobrom delom odgovorni fenoli i flavonoidi.

*Tabela 3. FRAP vrednosti etanolnog ekstrakta lista jagode, L-askorbinske kiseline i sintetskog antioksidansa BHT; s.m. – suva materija*

*Table 3. FRAP values of ethanolic extract from strawberry leaves, L-ascorbic acid and synthetic antioxidant BHT*

Uzorak	FRAP vrednost, mg Fe <sup>2+</sup> /g s.m.
Ekstrakt	284,51±14,226
L-Askorbinska kiselina	145,10±10,157
BHT	810,92±40,546

#### H<sub>2</sub>O<sub>2</sub> test

Rezultati ispitivanja antioksidativne aktivnosti ekstrakta primenom H<sub>2</sub>O<sub>2</sub> testa (stepen neutralisanja H<sub>2</sub>O<sub>2</sub> – „H<sub>2</sub>O<sub>2</sub> scavenging“ u zavisnosti od koncentracije ekstrakata) su prikazani na slici 2a, a na slici 2b je prikazana antioksidativna aktivnost L-askorbinske kiseline primenom ovog testa.

Stepen neutralisanja H<sub>2</sub>O<sub>2</sub> etanolnim ekstraktom lista jagode zavisi od koncentracije ispitivanog ekstrakta. Sa ekstraktom koncentracije 0,5 mg cm<sup>-3</sup> postiže se stepen neutralisanja H<sub>2</sub>O<sub>2</sub> od 30,47%. Sa povećanjem koncentracije ekstrakta iznad 0,5 mg cm<sup>-3</sup> stepen neu-

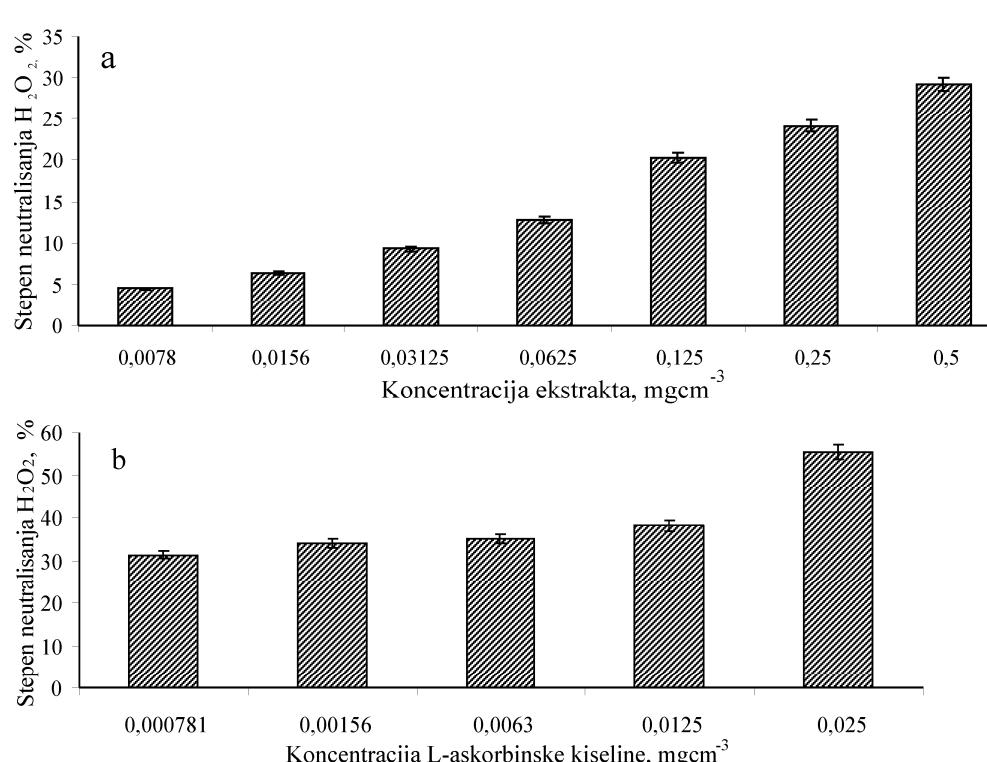
tralisanja H<sub>2</sub>O<sub>2</sub> se neznatno menja. Ispitivani ekstrakt pokazuje manju antioksidativnu aktivnost od L-askorbinske kiseline, koja u koncentraciji 0,025 mg cm<sup>-3</sup> neutrališe oko 55% H<sub>2</sub>O<sub>2</sub>.

H<sub>2</sub>O<sub>2</sub> je u odnosu na druge reaktivne kiseonične vrste (npr. hidroksil radikal, OH<sup>•</sup>; superoksid anjon radikal, O<sub>2</sub><sup>•-</sup>), manje reaktiv, ali može biti toksičan jer dovodi do stvaranja hidroksil radikala u ćelijama, zbog čega je uklanjanje H<sub>2</sub>O<sub>2</sub> kao i O<sub>2</sub><sup>•-</sup> veoma važno za zaštitu ćelija. Neutralisanje H<sub>2</sub>O<sub>2</sub> vodenim ekstraktima lista jagode može se pripisati prisutnošću fenolnih jedinjenja [39].

#### TBA-MDA test

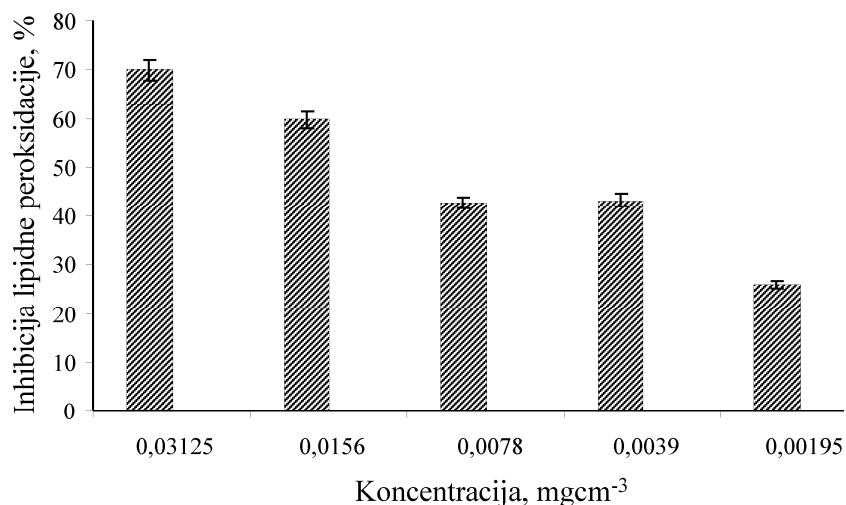
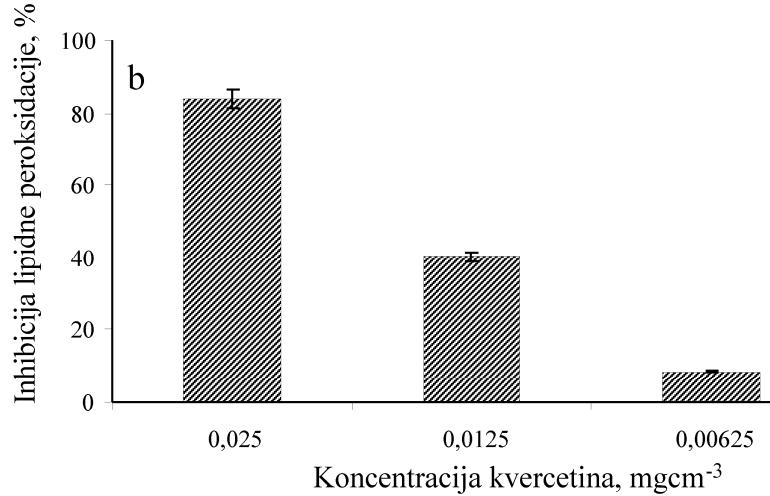
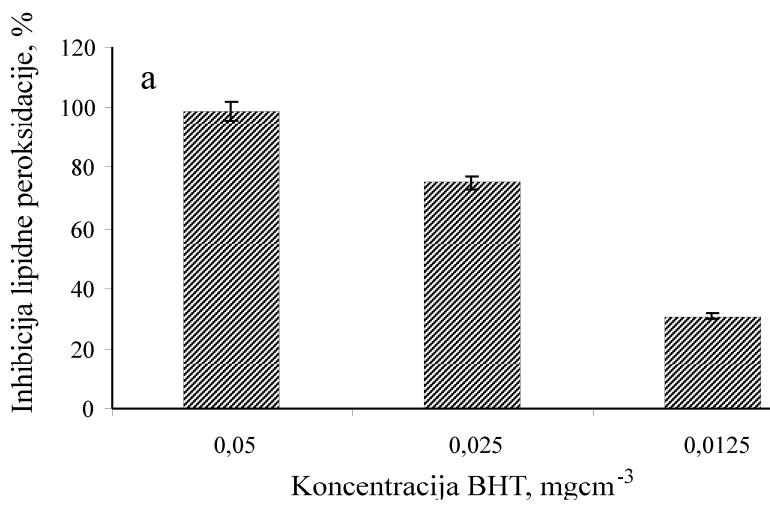
Na slici 3 je prikazan stepen inhibicije lipidne peroksidacije etanolnim ekstraktom lista jagode, a na slici 4 stepen inhibicije lipidne peroksidacije metanolnim ras-tvorima sintetskog antioksidansa BHT (slika 4a) i standarda kvercetina (slika 4b).

Najveći stepen inhibicije lipidne peroksidacije (99%) postiže se pri koncentraciji 0,05 mg cm<sup>-3</sup> BHT. Kod kvercetina se najveći stepen inhibicije lipidne peroksidacije (84%) postiže sa koncentracijom od 0,025 mg cm<sup>-3</sup>. Na osnovu prikazanih rezultata vidi se da je ispitivani ekstrakt pokazao dobru, koncentracijski zavisnu, antioksidativnu aktivnost. Sa koncentracijom ekstrakta od 0,03125 mg cm<sup>-3</sup> postignuta je inhibicija lipidne peroksidacije od 70%. Poređenjem antioksidativnih aktivnosti ekstrakta i poznatih antioksidanasa (kverce-



*Slika 2. Stepen neutralisanja vodonik peroksida etanolnim ekstraktom lista jagode (a) i L-askorbinskom kiselinom (b).*

*Figure 2. The capacity of neutralizing of hydrogen peroxide by ethanolic extract from strawberry leaves (a) and L-ascorbic acid (b).*

*Slika 3. Inhibicija lipidne peroksidacije etanolnim ekstraktom lista jagode.**Figure 3. The inhibition of lipid peroxidation by ethanolic extract of strawberry leaves.**Slika 4. Inhibicija lipidne peroksidacije metanolnim rastvorima BHT (a) i kvercetina (b).**Figure 4. The inhibition of lipid peroxidation by methanolic solutions of BHT (a) and quercetin (b).*

tina i BHT), može se zaključiti da dobijeni ekstrakt pokazuje visoku antioksidativnu aktivnost pod opisanim uslovima.

Fenolna jedinjenja direktno smanjuju količinu nastalih peroksi radikalata. Na osnovu brojnih *in vitro* ispitivanja se došlo do zaključka da je 1,2-dihidrosupstitucija (*ortho*-supstitucija) prstena B (tzv. kateholna struktura) ključna za antioksidativnu aktivnost fenolnih jedinjenja [40,41]. Ova jedinjenja su najverovatnije velikim delom odgovorna za visok stepen inhibicije lipidne peroksidacije etanolnim ekstraktom lista jagode.

#### *FIC test*

Sposobnost heliranja jona gvožđa etanolnog ekstrakta lista jagode i rastvora EDTA prikazana je na slici 5. Ekstrakt lista jagode sorte Zenga zengana dostigao je maksimum od 67,90% pri koncentraciji od  $2 \text{ mg cm}^{-3}$  (slika 5a). Helirajući efekat EDTA (opseg koncentracija  $0,004\text{--}0,25 \text{ mg cm}^{-3}$ ) je znatno bolji od ekstrakta (slika 5b). Maksimum aktivnosti je postignut pri koncentraciji  $0,25 \text{ mg cm}^{-3}$  i iznosi 96,34%.

Postoje naučni dokazi o pozitivnoj korelaciji između sposobnosti heliranja jona gvožđa i sadržaja ukupnih fenola [42]. Sposobnost heliranja jona metala koju pokazuju fenolna jedinjenja u funkciji je njihove karakteristične strukture, broja i položaja hidroksilnih grupa [43]. Antioksidativna aktivnost određena primenom

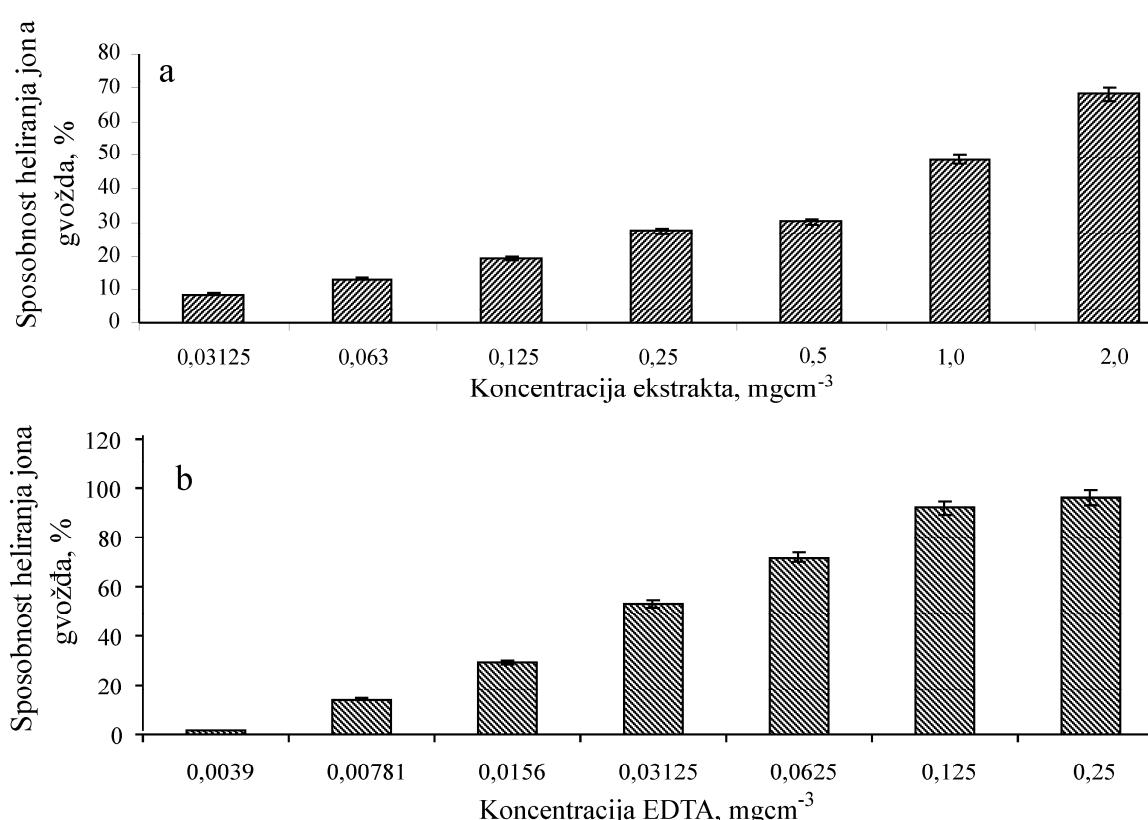
ovog, kao i u slučaju prethodnih testova verovatno je posledica visokog sadržaja fenolnih jedinjenja.

Prikazani podaci u ovom radu ukazuju da antioksidativna aktivnost etanolnog ekstrakta lista jagode najverovatnije potiče većim delom od fenola, ali je i rezultat njihovog sinergističkog delovanja sa ostalim bioaktivnim jedinjenjima prisutnim u ekstraktu.

#### ZAKLJUČAK

U etanolnom ekstraktu lista gajene jagode sorte Zenga zengana utvrđen je visok sadržaj ukupnih fenola dok je sadržaj ukupnih flavonoida znatno niži. Ispitivani ekstrakt pokazao je dobru, koncentracijski zavisnu antioksidativnu aktivnost, nezavisno od primenjene antioksidativne metode.

Poslednjih godina intenzivirana su ispitivanja biljnih ekstrakata koji predstavljaju alternativu sintetskim antioksidansima. Ispitivani ekstrakt lista gajene jagode pokazao je značajnu antioksidativnu aktivnost i predstavlja potencijalni izvor prirodnih antioksidanasa sa mogućom primenom u prehrabenoj, farmaceutskoj i kozmetičkoj industriji. Dalja istraživanja biće usmerena u pravcu ispitivanja antiinflamatornih, diuretskih i antikancerogenih aktivnosti, kao i detaljnijoj analizi hemijskog sastava dobijenog ekstrakta.



Slika 5. Sposobnost heliranja jona gvožđa etanolnog ekstrakta lista jagode (a) i EDTA (b).

Figure 5. The iron ions chelating ability of ethanolic extract from strawberry leaves (a) and of EDTA (b).

## Zahvalnica

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**SUMMARY****ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT FROM CULTIVATED STRAWBERRIES' LEAVES (*Fragariae folium*)**

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(Scientific paper)

Strawberry is a member of the rose family (*Rosaceae*, subfamily *Rosoideae*, tribe *Potentilleae*) in the genus *Fragaria*. The cultivated varieties of commercial strawberries usually were designated as *Fragaria ananassa*. Root, leaf, flower and fruit have the healing properties. The strawberry leaves extract is used for blood cleaning, for treatment of oral inflammation, diarrhea, various gastro-intestinal inflammation, and hemorrhoids, as well as a diuretic. So far, many positive biological effects of strawberries (anticancer, antioxidant and anticoagulant effect) have been proven. The purpose of this study was to evaluate the antioxidant potential of ethanolic extract from cultivated strawberries (*Fragariae folium*, varieties *Senga Sengana*) by using different antioxidant assays (DPPH, FRAP, FIC, H<sub>2</sub>O<sub>2</sub> and TBA-MDA). Ethanolic extract from strawberry leaves was obtained by reflux extraction at the boiling temperature. Total phenols and total flavonoids content was determined spectrophotometrically by the method of Folin-Ciocalteu and by method with AlCl<sub>3</sub>, respectively. In the extract was determined high content of total phenols, while the total flavonoid content is much lower. The concentrations of extract required to neutralize 50% of the initial concentration of DPPH radicals ( $EC_{50}$ ) after 20 min incubation and immediately after adding DPPH radical solution were 7.91 and 19.46 µg cm<sup>-3</sup>, respectively. Extract was achieved the maximum iron ions chelating ability (67.89%) at a concentration of 2 mg cm<sup>-3</sup>. Inhibition of lipid peroxidation of 70% was achieved by extract concentration of 0.03125 mg cm<sup>-3</sup>, while the maximum neutralization of H<sub>2</sub>O<sub>2</sub> (30.47%) was achieved by extract concentration of 0.5 mg cm<sup>-3</sup>. FRAP value of the investigated extract is 284.51 mg Fe<sup>2+</sup>/g of dry extract. Presented results of the antioxidant activity show that the obtained extract from the cultivated strawberry leaves is a potential source of natural antioxidants.

**Keywords:** Strawberry leaves (*Fragariae folium*) • Senga sengana • Total phenols • Total flavonoids • Antioxidant activity

# Start-up of a free water surface constructed wetland for treating olive mill wastewater

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## Abstract

An olive mill's existing evaporation pond was separated into five cells and transformed into a free water surface constructed wetland. The constructed wetland was used as a post-treatment stage for olive mill wastewater (OMW). Wastewater was previously treated by an aerobic trickling filter. The influent concentrations in the constructed wetland were 27400, 4800, 105 and 770 mg.L<sup>-1</sup> for COD, phenols, ortho-phosphate and TKN, respectively. Despite the rather high influent concentrations, the performance of the constructed wetland was very good since after the 60-day start-up operation period it achieved removal rates of about 94, 95, 95 and 98% for COD, phenols, ortho-phosphate and TKN, respectively. The major pollutant removal processes can be attributed to both biological processes occurring in the wetland and photo-oxidation. Laboratory-scale experiments with OMW from fifth cell of the wetland revealed that the net contribution of photo-oxidation after 112 h of simulated solar radiation at 765 W/m<sup>2</sup> (i.e., about 38 days of sunlight irradiation) was 18 and 31% removal for COD and phenols, respectively. In the constructed wetland, the total removal reached 81 and 86% for COD and phenols, respectively, for the same time period (38 days).

**Keywords:** Constructed wetlands, free water surface flow, olive mill wastewater.

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Although olive oil extraction is rather important for Mediterranean countries, as almost all worldwide production (95%) is produced there [1], the olive mills are also major environmental pollutants. Olive mill wastewater (OMW) is produced from three-phase olive mills and is characterized by its high organic (110–220 kg COD/m<sup>3</sup>) and phenolic load [1]. Therefore OMW treatment is imperative in order to protect the environment. As olive mills are usually small family enterprises they cannot handle the cost of a conventional treatment facility.

Although CWs have been used to treat a variety of wastewaters, only few attempts to treat OMW have been presented previously [2–6]. In these studies all three CW types were used to treat OMW, although the majority of these applications used VF systems [2,4,6], while HSF [7] and FWS [3] systems have only been used once. To minimize the pollutant load and reduce phytotoxicity [8], the majority of these applications used various pre-treatment stages including coagulation [2], electrochemical oxidation [4], and bio-

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logical trickling filters [6]. In the studies in [3] and [5] no pre-treatment stage was used, however the OMW was strongly diluted with tap water until it reached a dilution factor of 10 [3].

The present study presents the results of the start-up period of a full-scale FWS CW treating pre-treated OMW in a Mediterranean climate. OMW is first treated in a trickling filter and is then introduced into the FWS CW. Laboratory-scale photo-oxidation experiments were performed to estimate the quantitative contribution of photo-oxidation to the total pollutant removal. To the best of our knowledge, this is the first study on the effectiveness of a full-scale CW in treating OMW.

## MATERIALS AND METHODS

### Trickling filter

The olive mill plant is located near Amfilochia city in Aitoloakarnania Prefecture (Western Greece). OMW produced in the olive mill is initially treated by a trickling filter. The trickling filter is a metallic cylindrical tank 1.8 m in diameter and 3 m in height. It is filled with random-type high-density polyethylene (HDPE) media, with a specific area of 188 m<sup>2</sup> m<sup>-3</sup>, a specific weight of 47 kg m<sup>-3</sup>, and filter porosity 95%. To achieve natural aeration the filter has twelve ventilation valves

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at its base and its top is open to the air. OMW enters the filter through a rotating distributor, thus ensuring uniform distribution of the wastewater and then is drained to the filter's base. The treated OMW is led into the bottom of a plastic cylindrical recirculation tank of 30 m<sup>3</sup> total volume, where it is mixed with the raw wastewater from the olive mill plant. This diluted wastewater from the plastic tank is continuously recirculated to the top of the filter at a flow rate of 160 L min<sup>-1</sup>, using a pump. Influent concentrations ranged from 34000 to 46000 mg L<sup>-1</sup> for COD, from 4000 to 10000 mg L<sup>-1</sup> for phenol, from 700 to 1100 mg L<sup>-1</sup> for TKN, and from 100 to 180 mg L<sup>-1</sup> for ortho-phosphate. Effluent concentrations ranged from 11500 to 27500 mg L<sup>-1</sup> for COD and from 2600 to 4900 mg L<sup>-1</sup> for phenols, from 600 to 1000 mg L<sup>-1</sup> for TKN, and from 70 to 110 mg L<sup>-1</sup> for ortho-phosphate. The treatment system removes about half of the organic and phenol loads, while nutrients (nitrogen and phosphorus) are also removed at lower percentages [9]. Treated wastewater overflows from the recirculation tank into the FW CW.

#### Description of the full-scale constructed wetland

The full-scale CW is an FWS divided into five cells by soil barriers (Fig. 1). To avoid OMW leakage, the base of the CW was lined with clay. The CW has an incline of 2% and the five cells cover a surface area of 400, 350, 700, 350 and 250 m<sup>2</sup> for cells 1–5, respectively (total area 2050 m<sup>2</sup>). The first two cells, the closest to the influent entry point, were kept unplanted due to the high pollutant concentrations present in these cells that are toxic for plant growth, while the three last cells were planted with common reeds (*Phragmites australis*) in March 2011. Reed growth was rapid in the following two months. At the end of April, pre-treated OMW was introduced into the first cell for one day until all the cells were flooded. From this point in time, OMW did not flow in or out of the CW or between the cells until the end of June. The CW operated under batch conditions for 60 days (a hydraulic residence time (HRT) of 60 days). The CW had an initial mean depth of 0.5 m, thus initial OMW volumes in the FWS CW were 200, 175, 350, 175 and 125 m<sup>3</sup> for cells 1–5, respectively (total OMW volume 1025 m<sup>3</sup>). In real operation conditions the CW will receive a maximum flow rate of 30 m<sup>3</sup>/d, while the maximum hydraulic loading rate (HLR) and organic loading rate (OLR) will be 14.6 L m<sup>-2</sup> d<sup>-1</sup> and 285 g COD m<sup>-2</sup> d<sup>-1</sup>, respectively. The CW's minimum HRT under continuous flow will be 35 d. Due to high evapotranspiration (ET) rates, the depth of the CW reduced to 20 cm after 60 days. Liquid samples (38 samples per campaign) were collected in six monitoring campaigns from April to June from the influent, effluent, and various other points of each cell, in order to cover the whole CW area. The CW operated

for only 60 days, as during this period the OMW level in the CW was minimal due to high ET rates. In each monitoring campaign the CW depth was also measured in order to estimate ET values and correct measured pollutant concentrations. To calculate these removal efficiencies the pollutant concentration in cell 1 at day 0 was used as the influent value and the concentration in cell 5 at day 60 was used as the effluent value.

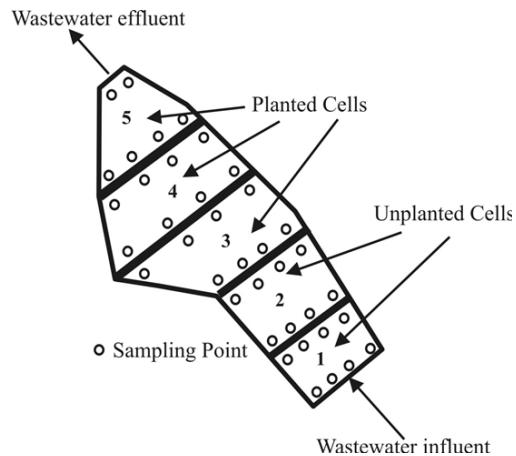


Figure 1. Full-scale free water surface flow constructed wetland layout (scale 1:600).

#### Photo-oxidation experiments

Photo-oxidation of centrifuged (4000 rpm for 20 min) wastewater samples taken from the fifth cell at day 0, was performed using a Suntest XLS+ solar light simulator from Atlas (Germany) equipped with a xenon arc lamp (2.2 kW) and special glass filters restricting the transmission of wavelengths below 290 nm. An average irradiation intensity of 765 W m<sup>-2</sup> (range between 300 and 800 nm) was maintained throughout the experiments and was measured by an internal radiometer. Chamber and black panel temperatures were regulated by the pressurized air cooling circuit and monitored using thermocouples supplied by the manufacturer. Irradiation experiments were performed using a cylindrical 250 mL Duran® glass UV-reactor with a flat flange lid with three necks (Lenz, Germany; light absorbance  $\lambda < 300$  nm) with a thermostatic jacket. A tap water cooling circuit was used to prevent any heating of the solution and keep the temperature at 24±1 °C. Aliquots (10 mL) were collected at different time intervals, and the concentration of total phenols and COD was analyzed according to the procedures described below. To assess biological activity, a control experiment was also carried out under dark conditions. In this experiment the same OMW was placed in a vessel and aliquots were collected at the same time intervals with the ones from the photo-oxidation experiment. During this experiment (data not shown) no alterations in COD or phenol concentrations were observed, leading to the con-

clusion that no significant microbial activity took place. Equivalent days of sunlight were determined according to the following equation based on the OECD guidelines [10,11] for the testing of chemicals:

$$\text{Days of sunlight} = \frac{h \times r}{0.75 \times 12}$$

where  $h$  represents hours of irradiation by the Xenon lamp of Suntest simulator,  $r$  is the ratio of intensity of the xenon radiation to that of the sunlight in the studied period, 0.75 is the correction factor for diurnal variation of natural sunlight, and 12 is the conversion of hours to days.

#### Water sample analysis

The water samples were analyzed for COD, TKN, orthophosphate (OP), and phenols. The open reflux method was employed to measure COD, the titrimetric

method was used to measure TKN, and the stannous chloride method was used to measure phosphorus [12]. Phenol (with respect to syringic acid) concentrations were determined spectrophotometrically according to the Folin–Ciocalteu method [13] using a JASCO V-530 UV/Vis spectrophotometer. Meteorological data (air temperature, atmospheric pressure, air humidity, wind velocity and direction, and total precipitation) were collected from the closest meteorological station in Kompoti ([www.meteo.gr](http://www.meteo.gr)). Figure 2 presents time series charts for mean air temperature and total rainfall. During the CW operation the mean air temperature was 24.7 °C and the total precipitation was 90 mm, while the OMW temperature in the CW varied from 14 to 23 °C.

Meteorological parameters significantly affect pollutant removal efficiency in CW systems [14]. For example, high amounts of precipitation tend to dilute wastewater, thus effluent concentrations appear to be

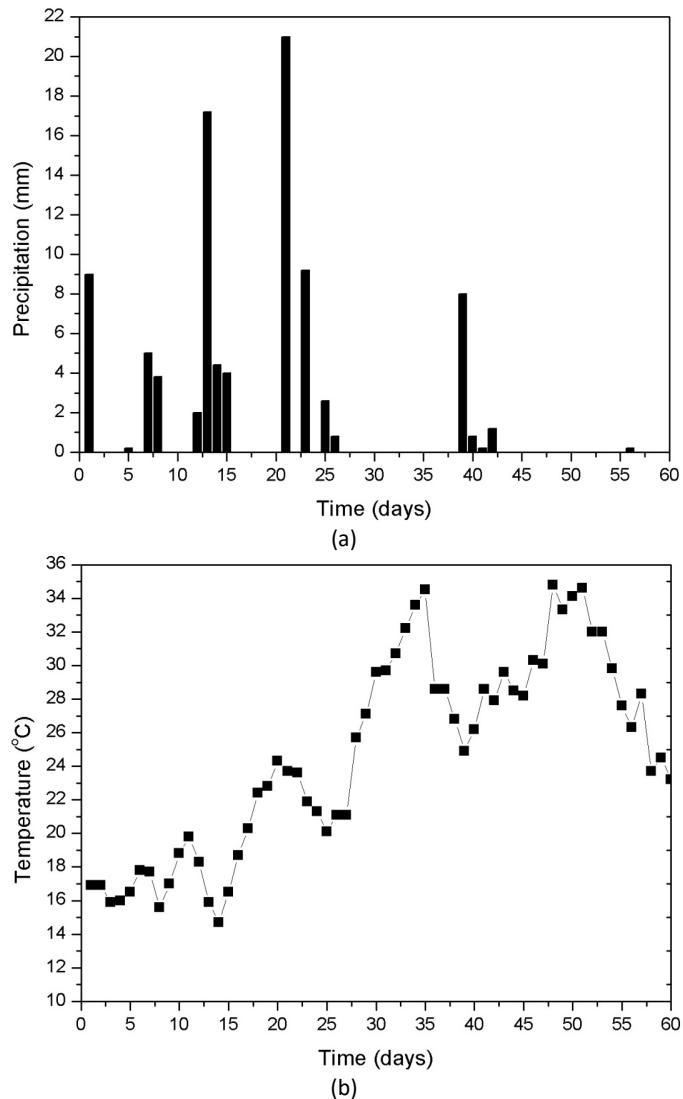


Figure 2. Time series charts for: a) air temperature and b) precipitation during the study period.

lower than expected and removal efficiencies higher. On the contrary, during periods of high temperature and solar radiation, water levels can decrease due to increased ET values, leading to underestimation of removal efficiency. In order to avoid over – or under – estimation of pollutant concentrations, the measured values were corrected by estimating OMW volumes in each cell in each sampling campaign. During the study period, the total precipitation was 90 mm, which added  $185 \text{ m}^3$  of water into the whole CW. At the end of the 60-day study period, the final depth of the CW was 0.2 m, resulting in an average ET value of  $6.5 \text{ mm d}^{-1}$  ( $\times 60 \text{ d} = 39 \text{ cm}$  over 50 cm initial depth).

## RESULTS AND DISCUSSION

The mean concentrations of COD and phenols in the raw OMW were 45000 and 9500 mg L<sup>-1</sup>, respectively. The filter effluent concentrations, when OMW was introduced into the CW, were 27400 and 4800 mg L<sup>-1</sup> for COD and phenols, respectively.

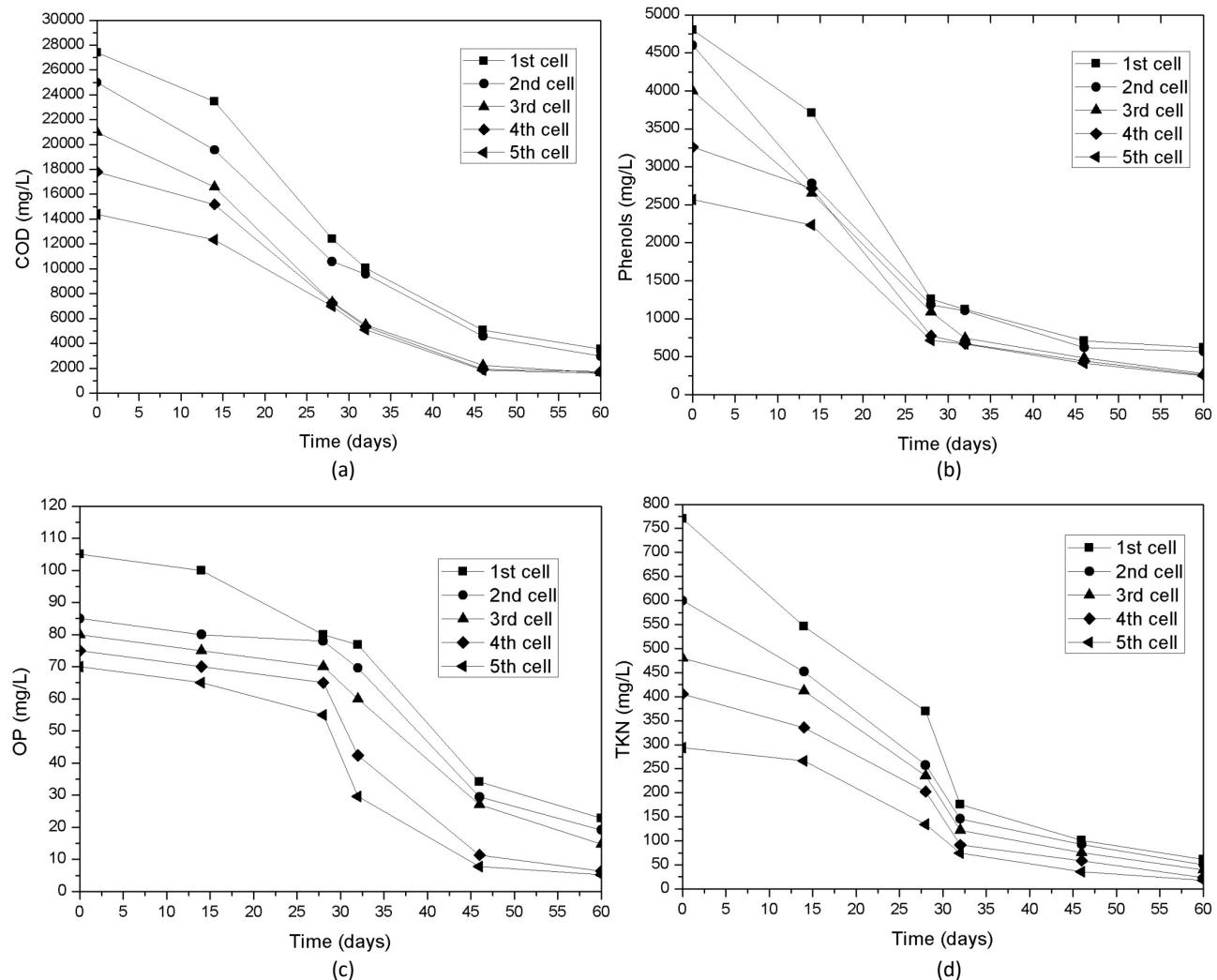


Figure 3. Time series charts of corrected pollutant concentration in the FWS CW for: (a) COD, (b) phenols, (c) OP and (d) TKN.

Figure 3 presents time series charts for COD, phenols, TKN and TP from samples of OMW taken from the FWS CW (mean values of concentrations are presented with a relative standard deviation not exceeding 5%). Mean pollutant concentrations of all the wastewater samples taken from each cell were used for the time series charts. Pollutant concentrations at the different sampling points in each cell did not show great variations (standard deviations were 801, 961, 1.3 and 2.5 for COD, phenols, TP and TKN, respectively), thus implying that OMW was homogenized in all cells. These homogenized pollutant concentrations in each cell occur due to the fact that CW was operated under batch conditions and OMW did not flow among the cells. At the beginning of the experiment (day 0) influent concentrations were 27400, 4800, 191 and 770 mg L<sup>-1</sup> for COD, phenols, TP and TKN, respectively. At day 0, when the entire CW was flooded, all pollutant concentrations in cells 2–5 were lower than in cell 1, indicating that the soil barriers acted to reduce the pollutant loads, possibly due to suspended solids reten-

tion. Pollutant concentrations decreased with time in all cells and followed similar trends. After 60 days of operation significant removal of all pollutants was achieved, as total removal efficiencies were recorded as 94, 95, 95 and 98% for COD, phenols, OP and TKN, respectively.

COD, phenols and TKN show similar trends of concentration decrease, as organic matter and nitrogen are mainly removed by microbial activity, which is enhanced in periods with high air temperatures [14]. Although OMW temperature values were higher (23 °C) at the end of the 60-day study period, the observed decrease in concentrations of COD, phenol and TKN was limited, possibly because the remaining pollutants are not easily bio-degradable. On the other hand, OP concentrations showed a more gradual decrease with time, as OP is mainly removed through precipitation in FWS CWs.

Although the monitoring program lasted only 60 days, the FWS CW achieved high pollutant removal rates in this short operation period. However, the measured final effluent concentrations were 3960 mg L<sup>-1</sup> for COD (corrected value 1584 mg L<sup>-1</sup>, Fig. 3a), 656 mg L<sup>-1</sup> for phenols (corrected value 248 mg L<sup>-1</sup>, Fig. 3b), 13 mg L<sup>-1</sup> for OP (corrected value 5.2 mg L<sup>-1</sup>, Fig. 3c), and 45 mg L<sup>-1</sup> for TKN (corrected value 18 mg L<sup>-1</sup>, Fig. 3d). These measured concentrations remain above legislation limits, as according to the EU Directive 1991/271/EEC, COD, total nitrogen and total phosphorus limits are 120, 10 and 2 mg L<sup>-1</sup>, respectively. Therefore, further treatment should be achieved by modifying the treatment facility in two ways: a) by introducing flocculation and sedimentation prior to the trickling filter treatment in order to reduce organic load and b) by recalculating the CW's effluent in order to increase HRT and lead to lower pollutant concentrations. Another issue for the operation of this treatment system is the sludge that accumulates mainly at the bottom of the first two unplanted compartments. This sludge can be easily removed periodically and treated. Co-composting of this sludge with other agro-industrial by-products is a viable and low cost solution, as many composting applications have used olive mill wastes as co-composting materials [15].

In previous studies the main CW types used for OMW treatment were HSF [7] and VF [2,4,6]. Results of only one FWS application have been published [3]. While for all CW types COD removal efficiencies present similar values (subsurface flow CWs ranged from 69 [7] to 73% [6] and FWS was 86% [3], subsurface CWs treated higher organic matter surface loads (ranging from 15 [2] to 6589 g m<sup>-2</sup> d<sup>-1</sup> [6], compared to the FWS (5-15 g m<sup>-2</sup> d<sup>-1</sup> [3]). The FWS CW of the present study appears to perform better than the other CW's as the organic matter surface load was significantly higher (57

g m<sup>-2</sup> d<sup>-1</sup>). VF CWs are more efficient in OMW treatment, as their organic matter and phenol removal efficiencies were higher compared with the other CW types. However, they do have one major drawback in OMW treatment and that is pore clogging due to the high concentration of suspended solids [5]. CWs appear to be extremely efficient in OMW treatment. Additionally, they have low construction and operational costs, and therefore could be an attractive treatment method for owners of small olive mills.

The photo-oxidation experiment lasted 112 h and samples were taken every hour for the first 6 hours and then twice a day. The 112-h duration of the experiment was chosen as it corresponds to approximately thirty-eight days of sun radiation in this part of Greece, according to data from the local meteorological station. Figure 4 presents the photo-oxidation kinetic profiles for COD and phenols, respectively. COD and phenol concentrations gradually decreased with time and at the end of the experiment their removal rates were 18 and 31%, respectively. In comparison, effluent concentrations in the CW at day 38 were 5200 mg L<sup>-1</sup> for COD and 672 mg L<sup>-1</sup> for phenols resulting in removal rates of 81 and 86%, respectively. These rates indicate that photo-oxidation is an important pollutant degradation process that is also costless as it only requires solar energy. The differences between the removal efficiencies of FWs CW and the photo-oxidation experiment were very high, and appear to prove that biological oxidation is the dominant mechanism of organic matter and phenol degradation.

In the photo-oxidation experiment phenol concentrations increased during the first hours of the experiment. This could be attributed to the split of multiphenolic substances to phenols. According to [1], in the first phase of phenol photo-oxidation, aromatic intermediates are formed which increase the total phenol concentration. Figure 4 also presents the exponential fit for COD and phenol degradation during the photo-oxidation experiment. It appears that for both phenols ( $R^2 = 0.945$ ) and COD ( $R^2 = 0.944$ ), second order decay was satisfactory. This second order decay is expressed by FOCUS [16] as a bi-phasic pattern of degradation, where a fast initial decrease in pollutant concentrations is often followed by a slower decline.

## CONCLUSIONS

This study examined: a) the pollutant removal efficiency of a FWS CW receiving pre-treated OMW and b) the contribution of photo-oxidation to the pollutant removal process. The photo-oxidation contribution appeared to be limited, since following 112 h of laboratory simulated solar radiation at 765 W m<sup>-2</sup> (corresponding approximately to thirty-eight days of sun radiation) removal rates of COD and phenols were 18 and

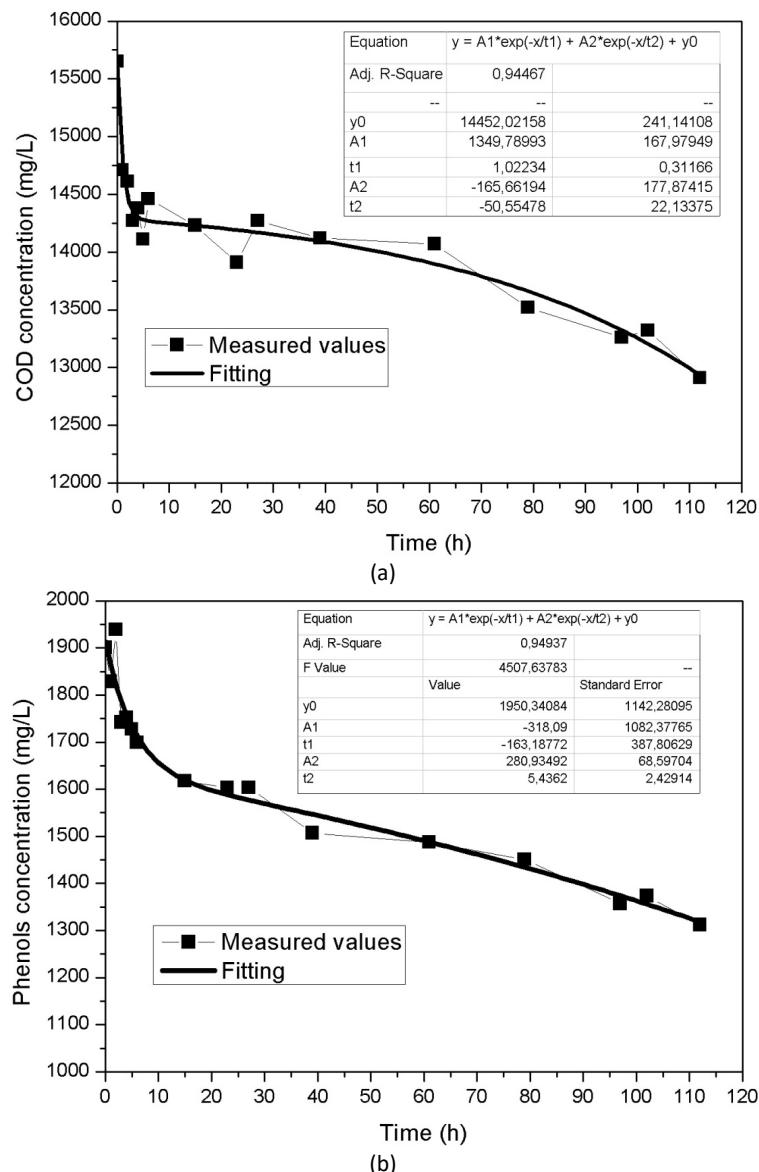


Figure 4. Time series charts and fitting of the photo-oxidation experiment for: a) COD and b) phenols.

31%, respectively, while for the same period, the total removal rates in the full-scale CW reached 81 and 86% for COD and phenols, respectively. The 60-day operating period of the full-scale FWS CW showed that:

- The full-scale FWS CW was quite efficient in treating pre-treated OMW, as it managed to reduce COD from 27400 to 3960 mg L<sup>-1</sup>, phenols from 4800 to 656 mg L<sup>-1</sup>, TKN from 770 to 45 mg L<sup>-1</sup>, and OP contents from 191 to 13 mg L<sup>-1</sup>.
- The pollutant surface loads applied in this study were significantly higher than those applied in other FWS CWs reported in the literature.
- Despite the high removal efficiencies, the final effluent concentrations still exceed legislation limits for irrigation use or environmental disposal.

Results from the present and previous studies indicated that CWs are extremely effective in treating

OMW, which, in combination with their extremely low construction and operation costs, make them a very attractive treatment method for owners of small olive mills.

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## IZVOD

### IZGRADNJA VESTACKE MOCVARE U CILJU TRETIRANJA OTPADNE VODE NASTALE USLED RADA MLINA ZA MASLINE

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(Naučni rad)

Postojeći evaporacioni bazen koji se nalazi u sklopu mlina za masline je podešten u pet čelija i transformisan u sistem mokrih polja. Ovako koncipiran sistem korišćen je kao faza tretmana otpadnog voda koje nastaju u procesu prerade masline. Otpadna voda je prethodno tretirana u aerisanom kapajućem filteru, a potom uvođena u mokra polja. U influentu mokrih polja, koncentracija hemijske potrošnje kiseonika, COD, je bila  $27400 \text{ mg L}^{-1}$ , fenola  $4800 \text{ mg L}^{-1}$ , ortofosfata  $105 \text{ mg L}^{-1}$  i ukupnog Kjeldal azota, TKN,  $770 \text{ mg L}^{-1}$ . Uprkos velikoj koncentraciji zagađujućih materija u influentu, učinak mokrih polja je bio veoma dobar s obzirom na to da je nakon 60 dana od početka rada sistema dostigao uklanjanje zagađenja od 94% za COD, 95% fenola, 95% orto-fosfata i 98% TKN. Najveći deo uklanjanja polutanata iz vode se može pripisati biološkim procesima u mokrim poljima i foto-oksidaciji. Eksperimenti na laboratorijskom nivou u pet čelija sa predmetnom otpadnom vodom su pokazali da je neto doprinos foto-oksidacije posle 112 sati simulirane solarne radijacije na  $765 \text{ W/m}^2$  (što odgovara ukupnom zračenju od oko 38 dana sunčevog zračenja) bio 18% za COD i 31% fenola. U konstruisanim mokrim poljima, za isti period od 38 dana, stepen uklanjanja zagađenja je bio 81% za COD i 86% za fenol.

*Ključne reči:* Mokra polja • Optadna voda  
• Prerada masline



# **RETRACTION NOTICE TO**

## **Environmental cadmium and zinc concentrations in liver and kidney of european hare from different serbian regions**

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This article has been retracted at the request of authors.

The retraction has been made because the authors admitted that they have done dual submission of the text and drawings from article named "Environmental Cd and Zn Concentrations in Liver and Kidney of European Hare from Different Serbian Regions: Age and Tissue Differences" published in Bull. Environ. Contam. Toxicol. **90** (2013):203–207, DOI 10.1007/s00128-012-0901-7.

One of the conditions of submission of a paper for publication are that authors confirm that their work is entirely originally written, someone else's data and/or text are appropriately cited or quoted and permission has been obtained for use of copyrighted material from other sources. Therefore, the retracted article represents a serious improper usage of the scientific publishing system.

Apologies are offered to readers of the journal Hem. Ind. since this abuse was not detected during the submission, i.e., peer review process.

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