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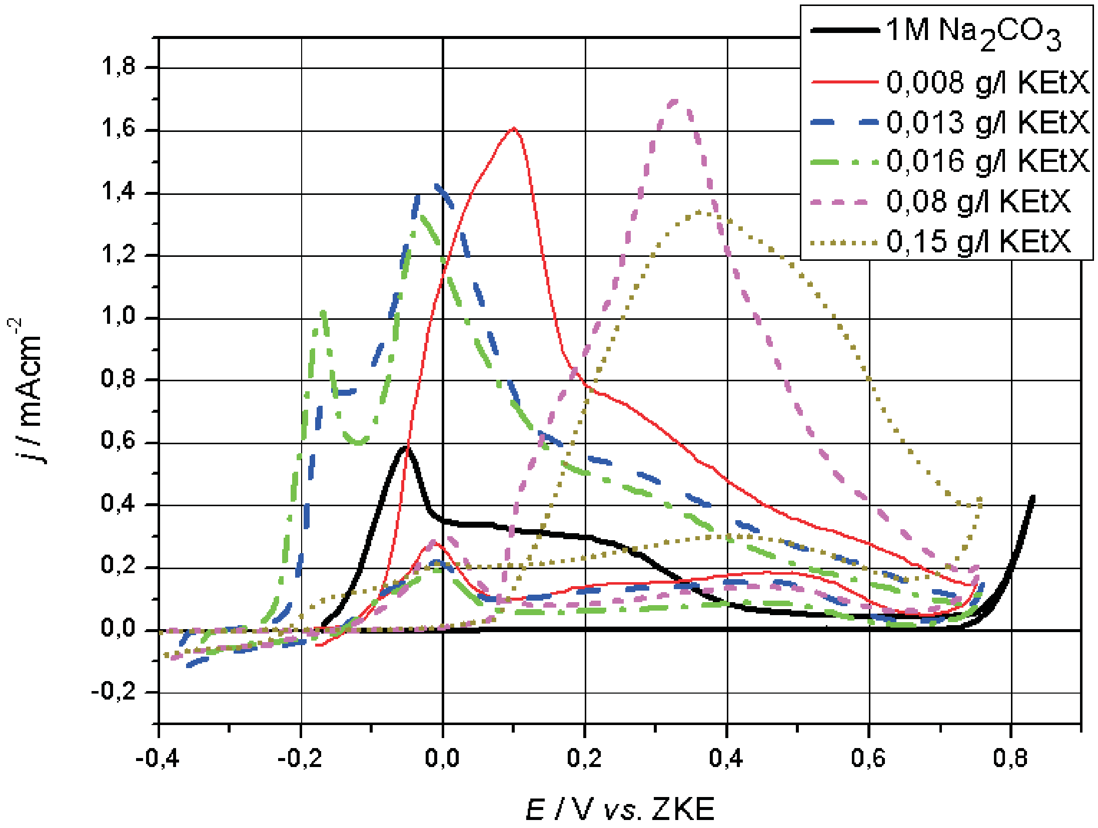
Hemijska industrija

Časopis Saveza hemijskih inženjera

Chemical Industry



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Mogućnosti oporavka i perspektive hemijske industrije Srbije u svetlu održivog razvoja*

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Izvod

Od zapadanja u lavirint globalne ekonomsko-finansijske krize krajem 2008, u Srbiji se sve više govori o "reindustrijalizaciji" kao temeljnom načelu zvanične razvojne strategije. Uprkos tome veoma su slabi izgledi da se ekonomski tokovi odmah i bitno unaprede obnovom zatečene industrijske strukture. Hemijska industrija Srbije nije u mnogo povoljnijoj poziciji u odnosu na ostalu. Danas je uostalom veoma teško uočiti oštru predmetnu razliku između različitih industrijskih grana u svetu modernih tehnologija, pa hemijska industrija u Srbiji ima izglede za oporavak, samo ako posluje u skladu sa zahtevima globalne tržišne konkurencije i modernih tehnoloških prožimanja. Iskustva u proizvodnji i razmeni današnjih sve složenijih hemijskih proizvoda u skladu sa svetskim industrijskim tokovima, govore da zemlje u reformi, kao i mnoge zemlje u razvoju, imaju potencijale da se prilagode novim tehnološkim i ekonomskim uslovima, odnosno da restrukturiraju i modernizuju svoje kapaciteta na bazi ekonomije znanja.

Ključne reči: hemijska industrija, produžen period krize, ekonomska struktura, strane direktne investicije, privatizacija, reindustrijalizacija, tehnološka rekonstrukcija, reorganizacija, ekonomska i ekološka efikasnost, održiva industrija.

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Trenutno ni jedna strategija ekonomskog oporavka u Srbiji ne daje vidljive i održive kvalitativne rezultate u industrijskoj proizvodnji pa i hemijskoj industriji. Kada je krajem 2010. godine Vlada Srbije donela (za sada jedinu zvanično usvojenu, ali nezvanično odmah napuštenu) „strategiju“ pod naslovom Postkrizni model ekonomskog rasta i razvoja Srbije 2011–2020 [1], računalo se iznad svega na daleko bolje buduće rezultate realnog sektora, pre svega industrijske i poljoprivredne proizvodnje, energetike, rudarstva,…, kao i na realizaciju povećanja proizvodnje u cilju izvoza.

Međutim, trenutno se više ne govori o numeričkim projekcijama tog dokumenta, u kome je pretpostavljen prosečni godišnji rast industrijske proizvodnje čak od 6,8%, za čitavu deceniju. Umesto toga ostaje opora istina da bruto domaći proizvod Srbije u 2012. nije dostigao pretkrizni nivo iz 2008. godine, kao i da je industrijska proizvodnja u Srbiji na samom početku pretrpela najteži „udar“ krize sa padom od 12,6% u 2009, godini u odnosu na prethodnu, onda minorni rast tokom naredne dve godine, i konačno ponovni pad u 2012. za 2,3%. Državnoj upravi zemlje, privredi i građanima očigledno tek predstoje prave strateške odluke i teške mere radi oporavka realnog sektora i reindustrijalizacije ekonomije, u meri u kojoj je to moguće.

Hemijska industrija u tehnološkom i ekonomskom okruženju – pojmovna razgraničenja

Hemijska industrija je tipičan primer pojma koji se brzi i kvalitativno menja, upravo onim tempom kojim se menja i sama tehnologija zasnovana na znanju, postupku, procesima, sirovinama, energiji, kao i samim proizvodima i načinu njihove upotrebe. To je u najopštijem određenju grana industrije koja se bavi hemijskom preradom prirodnih i veštačkih, biljnih, životinjskih i mineralnih sirovina, kao i otpadaka drugih industrija. Zato ona, na izvestan način, obuhvata i većinu industrijskih grana, s obzirom da se služi njihovim sirovinama, odnosno kombinovanim industrijskim procesima [2]. Temeljna odrednica današnje hemijske industrije je hemijsko inženjerstvo, struka, bez koje nema hemijske industrije. Hemijsko inženjerstvo je grana inženjerstva koja primenjuje prirodno–tehničke nauke kao što su fizika i hemija, prirodne nauke kao što su biologija, biohemija, mikrobiologija, kao i matematičke i ekonomske nauke, čime spisak nije zaključen. Moderno hemijsko inženjerstvo se sve više usmerava na relevantne materijale i tehnike kao što su nanotehnologije, goriva ćelija, biomedicinsko inženjerstvo [3]. Uporedo sa hemijskim inženjerstvom uopšte, razvijaju se uporedo i sledeće dve inženjerske podgrupe: 1) dizajniranje industrijske proizvodnje kao i različitih operacija na fabričkim industrijskim postrojenjima relevantnim procesima (hemijsko procesno inženjerstvo) i 2) razvoj novih i

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prilagođavanje starih supstanci za proizvodnju i pakovanje hrane, pića, kozmetike, sredstava za higijenu, farmaceutskih materijala, kao i mnogih drugih proizvoda. Međutim, razvoj savremenih i novih tehnologija hemijskom inženjerstvu i industriji daje nove sadržaje, koji zavise ne samo od nauke i tehničkih sposobnosti ljudi u proizvodnom procesu, već i od veoma brzih i dinamičnih promena koje se dešavaju u strukturi potreba i načinu potrošnje današnjeg čoveka [4]. Hemijska industrija obuhvata proizvodnju proizvoda od gume i plastičnih masa, a time se posredno širi i na automobilsku i elektronsku industriju, zatim na proizvodnju baterija i drugih elektrohemijskih izvora energije, kao i na energetiku uopšte. Zato bi, prema potpisniku ovog rada, najodrživije određenje hemijske industrije u ovom trenutku bilo bi ono koje podvlači da je hemijska industrija u većoj ili manjoj meri prisutna u svim inženjersko-industrijskim poslovima u kojima materijal, procesi i uslovi zahtevaju, između ostalog, korišćenje hemijske nauke i struke – hemijskog inženjerstva, i na njemu zasnovanih inovacija. Ali za promene u proizvodnoj i potrošačkoj strukturi najzaslužnije su tehnološke i organizacione inovacije koje, sve više zahtevaju sistematično, organizaciono i ekonomsko upravljanje tim procesima [5].

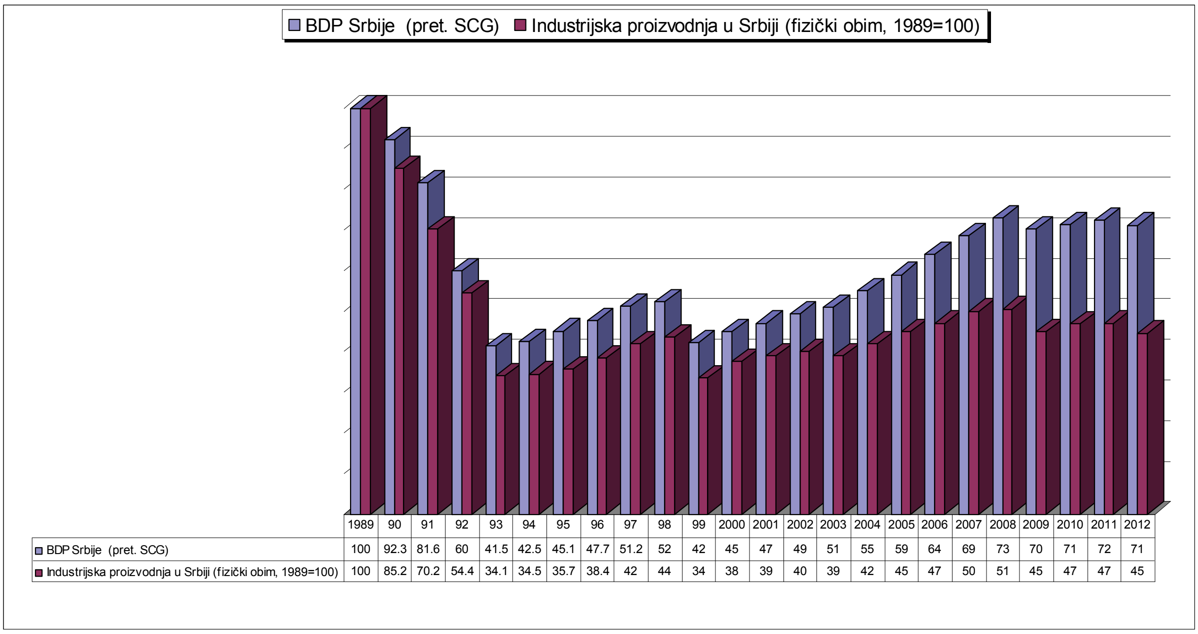
Brojne teškoće izazvane krupnim globalnim promenama, tranzicijom komandnih ekonomija ka tržišnom sistemu, kao i najnovijom globalnom ekonomsko–finansijskom krizom, dovele su u nezavidan položaj hemijsku industriju u mnogim zemljama u tranziciji (ZUT), a posebno u današnjoj Srbiji. Ne bi bilo dobro da se, samo na osnovu trenutnih podataka, tvrdi da je ona u kolapsu. Međutim, za vidljiv oporavak su neophodne brojne strateške (nacionalne), sistemske i operativne mere, kako države u okviru tzv. industrijske politike, tako i one operativne u okviru posebnih poslovnih strategija kompanija koje se bave hemijskom industrijom.

Industrija i ekonomska devastacija Srbije – polazna postavka problema

Radi bazne ilustracije ekonomskih činjenica i faktora urušavanja industrije u Srbiji, tokom poslednje dve decenije, najpre valja podsetiti da je bruto domaći proizvod (BDP) današnje Srbije u 2012. iznosio 71% referentne vrednosti iz 1989, dok je obim njene industrijske proizvodnje bio svega 45% u odnosu na 1989. (slika 1).

Udeo industrijskih sektora u okviru ekonomske aktivnosti bivše SFRJ, tokom osamdesetih godina XX veka kretao se do 40%, a u Srbiji već 2009. pao je na manje od 20% (slika 2) a 2011. na ispod 18%. Pri tome je bitno pomenuti i to da je industrija u Srbiji 1989. godine zapošljavala preko milion radnika, dok je ukupan broj zaposlenih u martu 2010. godine iznosio je 320 hiljada [1].

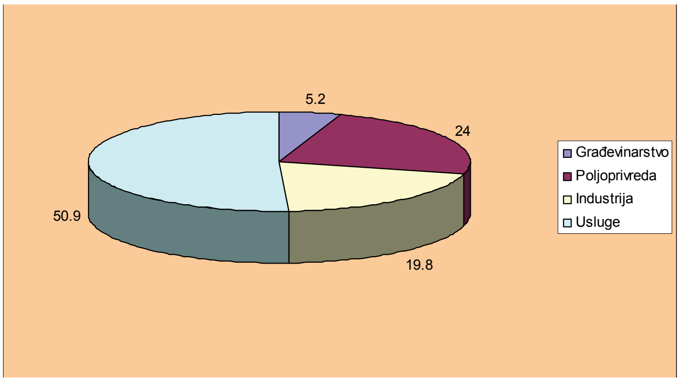
Drugi bitan ekonomski indikator odnosi se na udeo industrije u zaposlenosti. Na slici 3 prikazan je učinak predistorije i reformi u industriji Srbije od 2001. godine do 2008. godine, koja se uzima kao poslednja pretkrizna godina, bar u Srbiji. Podaci sa slike 3 jasno pokazuju da je među odabranim zemljama u prikazanom periodu neznatno smanjena zaposlenost samo u indus-



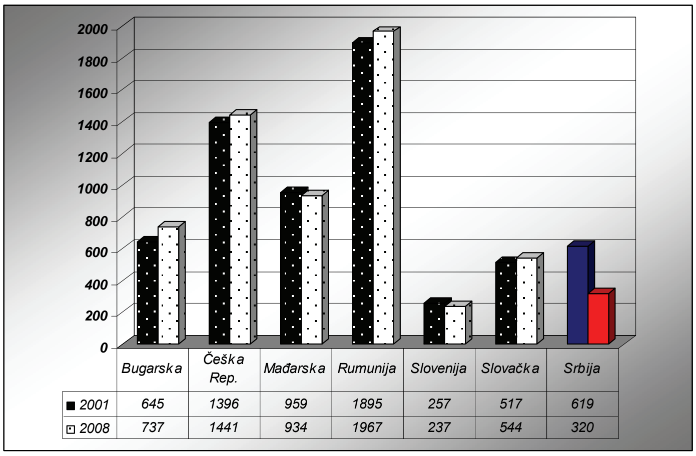
	1989	90	91	92	93	94	95	96	97	98	99	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
BDP Srbije (pret. SCG)	100	92.3	81.6	60	41.5	42.5	45.1	47.7	51.2	52	42	45	47	49	51	55	59	64	69	73	70	71	72	71
Industrijska proizvodnja u Srbiji (fizički obim, 1989=100)	100	85.2	70.2	54.4	34.1	34.5	35.7	38.4	42	44	34	38	39	40	39	42	45	47	50	51	45	47	47	45

Slika 1. Dugoročno dvodecenijsko propadanje industrije u Srbiji - pad izraženiji nego smanjivanje BDP. Izvor: Nacionalni zavod za statistiku i kalkulacija autora.

Figure 1. Long-term two-decade degradation of the Serbian industry – a decline more marked than the GDP reduction. Source: National Statistic Bureau and the author's calculation.



Slika 2. Struktura BDP Srbije 2009. Industrija u zaostajanju, rast usluga (RZS).
Figure 2. GDP structure of Serbia in 2009. Industry sector falling behind, growth of services sector (RSB).



	Bugarska	Češka Rep.	Mađarska	Rumunija	Slovenija	Slovačka	Srbija
■ 2001	645	1396	959	1895	257	517	619
⊡ 2008	737	1441	934	1967	237	544	320

Slika 3. Promene u industrijskoj zaposlenosti Srbije i relevantnih zemalja u tranziciji od 2001- 2008. godine (u hiljadama). Izvor: RZS, nacionalne statistike.
Figure 3. Industrial employment changes in Serbia and relevant transition countries 2000- 2008 (in thousands) Source: RSB, national statistic.

triji Mađarske i Slovenije, dok je u svim ostalim tranzicionim privredama povećana. Izuzetak je Srbija u čijoj je industriji u isto vreme zaposlenost bukvalno prepolovljena, tako da je sa 619 hiljada pala na svega 320 hiljada, pri čemu je ovaj trend nastavljen u toku recesije, sve do početka 2013. godine. Jedno od objašnjenja moglo bi da se odnosi na okasneli početak reformi i restrukturiranja srpske ekonomije u odnosu na ostale zemlje zbog nepovoljnih političkih okolnosti i promena koje su nastupile tek 2000. godine.

Istini za volju, zaposlenost u industriji Srbije bila je u permanentnom, blažem ili intenzivnijem, smanjivanju sve od 1989. godine. Za poređenje je verovatno najpogodnija Bugarska, u čijoj je industriji danas zaposleno više nego dvostruko radnika u odnosu na Srbiji, pri čemu je broj zaposlenih 2008. bio veći nego 2001. Pri tome treba naglasiti da je Bugarska prošla tranzicioni period i, kao članica EU u daleko većoj meri je izložena konkurenciji, sa manjim mogućnostima da štiti svoju industriju.

Jedan od pokušaja novog strateškog pozicioniranja privrede Srbije bio je pomenuti „postkrizni model“ prema kome je prosečni godišnji rast srpske industrije u drugoj deceniji XXI veka trebalo da iznosi 6,9% (građevinarstva 9,7%, usluge 5,5%) kako bi došlo do pozitivne promene ekonomske strukture, odnosno povećanja udela realnog sektora i proizvodnje razmenljivih dobara (prerađivačka industrija i poljoprivreda) i smanjenja udela usluga u okviru BDP. Tu opštu „strategiju“ trebalo je da konkretizuje Strategija i politika razvoja industrije Republike Srbije 2011–2020 [6]. Sudeći po izjavama zvaničnika iz ekonomskih resora Vlade Republike Srbije, početkom 2013. bez obzira što je opšta „strategija“ iz 2010. napuštena u smislu kvantifikacijskih projekcija, zadržana je vizija „novog modela rasta“ kao i osnovne strukturni ciljevi „industrijske strategije“ [6]. Pošto je učešće realnog sektora u BDP-u sa 32% 2001, još pre početka krize (2008) smanjeno na ispod 24%, po tim strateškim pretpostavkama, ukupna industrija, posebno prerađivačka (kojoj pripada i hemijska) sa navodnim natprosečnim rastom od 7,3% godišnje, imala bi priliku da se oporavi i rekonstruiše, tako da bi konačno učešće ove industrije u BDP poraslo sa 13% na 14,7% u BDP na kraju perioda [1].

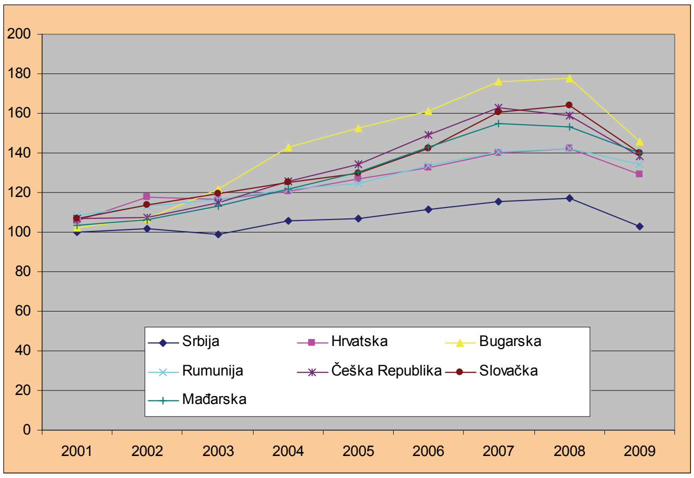
Razume se da bi takav scenario izuzetno pogodovao hemijskoj industriji. Međutim, pretpostavke „novog modela rasta“ imaju smisla samo kao ilustracija proporcija pretpostavljenog povećanja ekonomske aktivnosti, do koga će, na ovaj ili onaj način doći, nakon izlaska iz krize koja traje sve od 2009. godine. A faktički izlazak Srbije iz recesije moguć je tek kada se prevaziđe nivo BDP iz 2008, što bi uz prosečan rast od 2–3% (industrije od 3–4%) bilo moguće tek 2014 ili 2015.

Učešće pojedinih tehnoloških sektora u bruto dodatoj vrednosti (BDV – kao preciznijoj meri doprinosa pojedinih ekonomskih aktivnosti u odnosu na BDP) u 2008. godini govori da pozicija hemijske industrije nije zanemarljiva. Nažalost, nisko-tehnološki sektor u kome dominiraju prehrambeni i tekstilni proizvodi davao je čak 50,7% BDV, dok su srednje-niže tehnologije davale 25%, srednje-više 16,4%, a visoke (*high tech*) svega 7,5% BDV. Međutim, hemijska industrija kao podsektor u okviru srednje-višeg tehnološkog sektora već 2008. davala je 7,7% BDV, pri čemu su proizvodi od gume i plastike (srednji-niži tehnološki sektor) doprinosili BDV sa 6%, tako da se na ovaj način obuhvaćen doprinos najvećeg dela hemijske industrije (isključujući farmaceutsku) može proceniti na oko 13% BDV. Tako obračunat, ovaj doprinos je na trećoj poziciji po udelu u BDV, iza prehrambenog sektora (29,9%) i metalskog koji je tada iznosio 13,1%, ali je njegovo učešće faktički prepolovljeno nakon povlačenja stranog investitora iz vlasništva *US Steel Serbia* , marta 2012.

Industrija Srbije na udaru ekonomskih i političkih lomova

Tradicija današnje hemijske industrije Srbije proističe iz planske industrijalizacije tokom šezdesetih i sedamdesetih godina XX veka. Međutim, izgradnja brojnih fabrika bazne industrije druge Jugoslavije donela je relativno brz tehnološki progres, pa i dobru relativnu poziciju hemijske industrije tadašnje Srbije. Zajedno sa ostalim industrijama, opsluživala je tržište tadašnje Jugoslavije, koja je po veličini ekonomije delila 36. i 37. mesto u svetu sa Poljskom [7]. Međutim, slaba strana takve industrijalizacije je struktura, sistem funkcionisanja i način trajanja. Hemijska industrija u Srbiji se delila na laku i tešku, prema fizičkim karakteristikama procesa i sirovina, a ne prema tržišnim kriterijumima. To implicira netržišnu alokaciju resursa i shodno tome poslovanje koje je imalo smisla jedino u uslovima samoupravljanja, odnosno tzv. reprodukcionih celina, bez međusobne i prave međunarodne konkurencije. Sistem je funkcionisao u uslovima samoupravne međuzavisnosti čitave ekonomije i države, što je bilo moguće samo pre pada Berlinskog zida i ulaska Kine i drugih novih industrijskih džinova na svetsku trgovinsku scenu. Rušenje Berlinskog zida i raspad zajedničkog jugoslovenskog tržišta raspršili su iluzije o mogućem nastavku prethodne prakse. Ako se tome doda učinak međunarodnih ekonomskih sankcija prema Srbiji, uvedenih od strane Saveta bezbednosti OUN 31. maja 1992, a zatim hiperinflacije, sloma finansijskog pa i fiskalnog sistema [7–9] onda postaju jasniji prethodni uspon i pad socijalističke i postsocijalističke industrije Srbije.

Slika 4 pokazuje da je industrija Srbije pretrpela daleko veće štete od ostalih uporedivih industrija u okruženju, između ostalog i usled bombardovanja NATO, kao i aktuelne globalne i nacionalne ekonomske krize. Ne bi bilo korektno iz ovoga izvesti zaključak da bi srpska hemijska i druge industrije danas bile u mnogo povoljnijem položaju da nije bilo NATO agresije. Naime, svi faktori koji su doveli do međunarodne izolacije i ispadanja srpske države i privrede iz demokratskih pro-



Slika 4. Uporedni prikaz kumulativnih indeksa industrijske proizvodnje u Srbiji i relevantnim ZUT. Izvor: RZR na osnovu podataka UNECE Common Database, derived from national and Interstate Statistical Committee, Eurostata i Nacionalnih statistika tranzicionih zemalja.
Figure 4. Comparative review of cumulative indices of the industrial production in Serbia and relevant transition countries. Source: RSB, based on the dates from the UNECE Common Database, derived from the National and Interstate Statistical Committee, Eurostat and national statistics of the transition countries.

cesa i reformi uzrokovali su dugoročno loše stanje i gotovo doveli do kolapsa industrije [9,10].

Tranzicija privrede Srbije nakon 2000. usledila je nakon političkih promena, ali se odigravala po scenariju primenjenom u većini zemalja u reformi, u znaku brze deregulacije, liberalizacije i privatizacije, ne vodeći računa o specifičnim okolnostima. To je, između ostalog, vodilo ka intenzivnijem, ali privremenom rastu deviznog priliva (donacije, prodaja kapitala preduzeća, strane direktne investicije – SDR, inozaduživanje), a onda, u populističkom maniru, ka dinamičnom porastu svih vidova potrošnje, uz daleko brži rast usluga i smanjenje učešća realnog sektora u BDP, razume se i ekspanziju uvoza. Raspoloživi podaci govore da je BDP Srbije u poslednjoj godini pre krize porastao u odnosu na 2000. godinu za 63%, dok je industrijska proizvodnja u to vreme uvećana svega za 30%, da bi nakon četvorogodišnje krize u 2012. godini BDP bio veći 57% u odnosu na 2000, a industrijska proizvodnja jedva 18%.

Proces privatizacije sa programima restrukturiranja, modernizacije i reorganizacije u Srbiji nije dao rezultate koji su očekivani. Naravno da je privatizacija bila neophodna. Sve što je preostalo od kvalitetne industrije ZUT prošlo je kroz čistilište privatizacije i novog tržišnog prilagođavanja. Međutim, Srbija je u svemu kasnila, pa i u privatizaciji, u kojoj su najpre, većinski privatizovane uglavnom uspešne kompanije koje se bave proizvodnjom duvana, piva, cementa, ulja, mineralne vode,..., i samo mali deo tzv. teške industrije (Valjaonica aluminijuma i bakra, Sevojno, i nažalost samo privremeno, Sartid – *US Steel Serbia*). U zvanično uspešne privatizacije ulazi i deo hemijske industrije, posebno industrija guma (*Michelin* – Tigar, Pirot).

Hemijska industrija Srbije u tranziciji, krizi 2009. i dalje…

Pokazalo se da infrastrukturni, geopolitički pa i tradicionalni socio–kulturni uslovi u približno podjednakim ekonomskim pretpostavkama odnose prevagu u privlačenju SDI (posebno u industrijskom sektoru) bez kojih kao da nije bilo moguće sprovesti privatizaciju. Valjalo je početi oprezno i kvalitetno sa programima oporavka, restrukturiranja i privatizacije [11,12]. Osnovni rezultati ukupnog skora mogu se videti na osnovu poređenja makroekonomskih performansi prerađivačke industrije iz 2001. i onih iz prve krizne 2009. godine (tabela 1).

Promene performansi nakon 2001. pokazuju da čitava ovdašnja industrija prolazi kroz nova iskušenja reformi i ekonomske politike, a posebno krize od 2009. Promene su se ispoljavale najviše na pad zaposlenosti i proizvodnje, gašenje privatizovanih preduzeća, otpuštanju zaposlenih, neblagovremeno isplaćivanje preuzetih finansijskih obaveza, loše upravljanje, kao i otkazivanje mnoštva ugovora sa neuspelom realizacijom (tabela 2).

Tabela 1. Osnovne makroekonomske performanse prerađivačke industrije: promene od 2001–2009. Izvor: podaci RZS
Table 1. Basic macroeconomic performances of processing industry: changes 2001–2009. Source: NSB data

Karakteristika	2001	2009
Učešće u BDP, %	18	13
Zaposlenost	619000	331136
Izvoz, % robnog izvoza	93	95
Uvoz, % robnog uvoza	73	81
Zarade (u odnosu na prosek), %	87	80
Br. preduzeća	12538	18509
Br. velikih preduzeća	489	277

Iz podataka u tabelAMA 1 i 2 vidi se da je srpska prerađivačka industrija tokom reformi bitno smanjila učešće u BDP, ali je u daleko većoj meri pala zaposlenost u njoj. Hemijska industrija u tom pogledu ne stoji ništa bitno drugačije. Od 66 preduzeća obuhvaćenih privatizacijom, poništeno je 17 privatizacionih ugovora, 6 preduzeća je bilo u restrukturiranju, a preostalo za privatizaciju još 3. Procenat neuspešnih privatizacija, međutim, ne meri se samo brojem raskinutih ugovora, već i kvalitetom rada preduzeća u podsektoru. Treba

Tabela 2. Privatizacija prerađivačke industrije 2002–2010. Izvor: RZR
Table 2. Privatization of processing industry 2002–2010. Source: RSB

Sektor/podsektor	Privatizovano		Restrukturiranje		Poništeni ugovori		Preostala privatizacija	
	Broj	%	Broj	%	Broj	%	Broj	%
Prerađivačka industrija	878	100	58	100	258	100	40	100
Prehrambeni proizvodi, piće i duvan	202	23,0	3	5,0	48	18,6	12	30,8
Tekstil i tekstilni proizvodi, koža i predmeti od kože	91	10,4	7	11,7	44	17,1	2	5,1
Drvna industrija i papir	144	16,4	4	6,7	58	22,5	10	25,6
Hemijski proizvodi, guma i plastika	66	7,5	6	10,0	17	6,6	3	7,7
Proizvodi od ostalih minerala	77	8,8	4	8,3	23	8,9	1	2,6
Metali i metalni proizvodi	116	13,2	6	10,0	24	9,3	2	5,1
Ostale mašine i uređaji	40	4,6	4	6,7	10	3,9	3	7,7
Električni i optički uređaji	68	7,7	6	10,0	10	3,9	7	17,9
Saobraćajna sredstva	38	4,3	17	28,3	16	6,2	–	–

imati u vidu da je hemijska industrija veoma heterogena, kao i da je u nju (kao statistički podsektor 24) uključena i farmaceutska industrija. Prema relevantnim podacima u 2009. zabeleženo je veoma loše poslovanje u ovom podsektoru koji je zapošljavao 20.813 radnika, ali je bilo aktivno svega 627 preduzeća [12]. Izražena tržišna koncentracija govori da je svega 40 velikih preduzeća sa najvećim udelom u poslovnom prihodu (86%) u okviru podsektora, u ukupnom broju preduzeća učestvovalo sa 6,4%. Međutim, uglavnom zbog lošeg stanja pojedinih kompanija, podsektor je imao ukupne gubitke od čak 17,6 milijardi dinara, pri čemu su glavni gubitaši bili Petrohemija, Pančevo, sa 9,5 milijardi i Azotara, Pančevo, sa 1,7 milijardi dinara. Dodatni problemi bili su sve slabija ukupna likvidnost, produžetak rokova plaćanja na 239 dana u 2009, pad tržišta za 25,36%, dok su troškovi servisiranja bankarskih kredita bili čak 19% viši nego za prosek privrede Srbije. Drastično smanjivanje broja zaposlenih u industriji vodilo je ka povećanju produktivnosti rada. Međutim to nije moglo da nadomesti gubitak u prihodima u odnosu na ostale ekonomske sektore u zemlji (pre svega sektor usluga) tako da su zarade prerađivačke industrije s početka tranzicionog perioda od 87% proseka u zemlji za 2001, u 2009. godini iznosile samo 80% prosečnih u toj godini (tabela 1).

Tako se desilo da se, nakon jednog veoma problematičnog modela većinski insajderske privatizacije po zakonu od 1997, u toku novog talasa privatizacije i (na osnovu Zakona od 2001.) daleko odlučnije krene u privatizaciju većinskom prodajom kapitala. Međutim, model praktično nije bio dovoljno osmišljen, jer su, najpre uspešno većinski privatizovane kompanije čije tržište nije moglo do dođe u pitanje (duvan, cement itd) i samo mali deo tzv. teške industrije. U direktnim stranim investicijama dominirale su one koje se tiču usluga. Uporedo sa odmicanjem krize, danas sve više dominiraju one koje se realizuju ali uz ogromne finansijske podsticaje vlade, radi „očuvanja radnih mesta“. To može da bude razumljivo u „socijalnom“ pogledu, ali samo dotle dok ovakva praksa ne dođe u sukob sa zakonima koji štite slobodnu konkurenciju i bitno ograničavaju državnu pomoć.

Već je rečeno da je učešće usluga u privredi Srbije prešlo je preko polovine BDP, ali je učešće čitave industrije palo na manje od 20%. Kriza je obelodanila drastičnu ranjivost privrede i označila urušavanje industrijske strukture u kojoj je zapostavljen realni sektor. Bazna hemijska produkcija pretrpela je ogromne štete ne samo zbog liberalizacije, loše privatizacije ili nekvalitetnog restrukturiranja, već i zbog korupcije koja je pratila veliki deo postupaka, a u javnosti kulminirala, u aferi „Azotara Pančevo“, koja je u trenutku nastanka ovog rada u istražnom postupku. Ne bi bilo dobro da se taj postupak protegne u nedogled i tako dodatno umanje šanse za investiranje ili ukrupnjavanje, odnosno modernizaciju hemijske industrije koja je neophodna nakon krize.

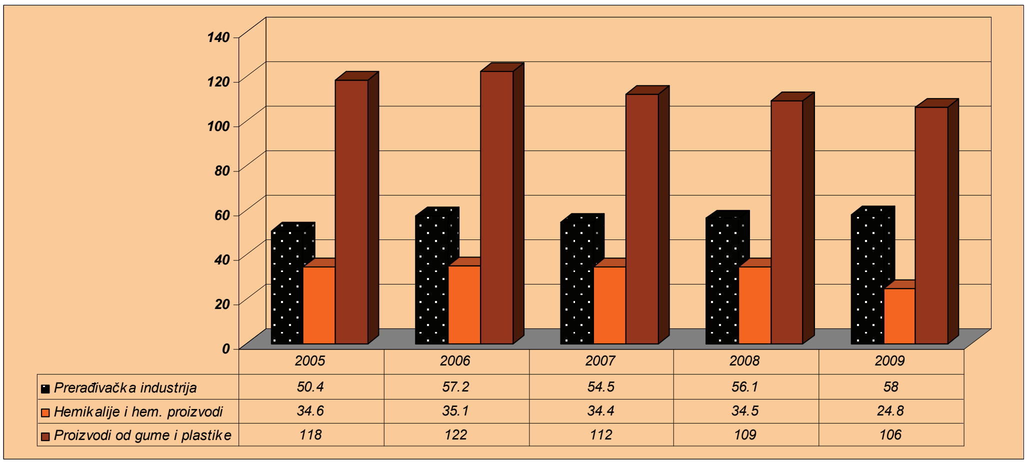
Međunarodna pozicija hemijske industrije Srbije

Kakva je relativna pozicija hemijske industrije Srbije u međunarodnom okruženju? Jadan od pokazatelja mogao bi da bude bilans uvoz–izvoz. U robnom izvozu iz Srbije učešće prerađivačke industrije koja predstavlja najveći deo ukupne industrijske proizvodnje, od 2001––2010. povećano sa 93 na 95%, pri čemu je i u robnom uvozu to učešće povećano sa 73 na 81% (tabela 1). Međutim, treba imati u vidu da je gotovo permanentno nakon 2000. godine pogoršavan ukupni bilans robne razmene sa inostranstvom. Udeo razmenljivih dobara u izvozu je smanjivan, a udeo usluga donekle porastao, što je dovelo u pitanje održivost međunarodne likvidnosti u krizi, naročito tokom drugog talasa krize 2012. Međunarodne trgovinske relacije najbolje se vide kroz pokrivenost vrednosti uvoza izvozom, odnosno procentualno učešće izvoza u uvozu. Taj parametar je u Srbiji tokom poslednje decenije za čitavu ekonomiju iznosio znatno ispod 60% u proseku, sa maksimumom od 59%, i tendencijom pogoršanja u uslovima recesije. I takav relativni uspeh ostvarivan je, razume se, zahvaljujući samo malom delu suficitarnih proizvodnji među kojima je i poljoprivreda. Ako je reč o prerađivačkoj industriji, njena pokrivenost uvoza izvozom kretala se oko 55% u proseku sa tendencijom blagog povećanja do 58% za 2009. (slika 5).

Detaljnija analiza stanja tokova uvoza-izvoza hemijske industrije Srbije nije potpuno moguća zbog neažurnih i nespecificiranih podataka koje nudi ovdašnja statistika. S obzirom na heterogenost hemijske industrije, moglo bi se reći da ona generalno u pogledu izvoza i uvoza stoji daleko lošije od ostatka prerađivačke industrije, s obzirom na to da tokom relativno povoljnih godina 2005–2008. nije uspela da pokrije vrednost uvezenih proizvoda izvozom sa više od 35%. Podaci bi verovatno bili lošiji da su posebno prikazan uvoz-izvoz hemijskih proizvoda bez farmaceutskih, koji su uračunati takođe u okviru podsektora 24. Međutim, u hemijsku industriju spada i podsektor 25, u koji spadaju proizvodi od gume i plastike. Ovaj podsektor ostvaruje suficit, tako da se pokrivenost uvoza izvozom u njemu pretkriznih godina kretala do 122% (za 2006), sa tendencijom pogoršanja, pri čemu je taj procenat u 2009. pao na 106%.

Protekle dve decenije učinile su dovoljno očiglednim posledice loše postsocijalističke evolucije i kasnijih reformi hemijske industrije Srbije, tako da se za njeno trenutno stanje i perspektive nameću sledeći zaključci:

– prvo, kompleks nasleđene hemijske industrije danas je u poziciji veoma različitih oštećenja, ne samo po podsektorima i oblastima, već i po karakteru proizvoda i tržišnih pozicija pojedinih preduzeća koja su nastala u prethodnom periodu;



Slika 5. Pokrivenost uvoza izvozom (% učešće) prerađivačke i hemijske industrije Srbije 2005–2009. Izvor: privredna komora Srbije i kalkulacija autora.
Figure 5. Coverage of imports by exports (% of participation) in the Serbian chemical and processing industry 2005–2009. Source: Serbian Chamber of Commerce and the author's calculation.

– drugo, te razlike se odnose, kako na tehnološko stanje, tako i ekonomsko-finansijsku situaciju pojedinih kompanija;

– treće, mnoge od tih kompanija neće moći da prežive u konkurenciji i pri susretu sa novim problemima prilagođavanja domaćem i međunarodnom tržištu;

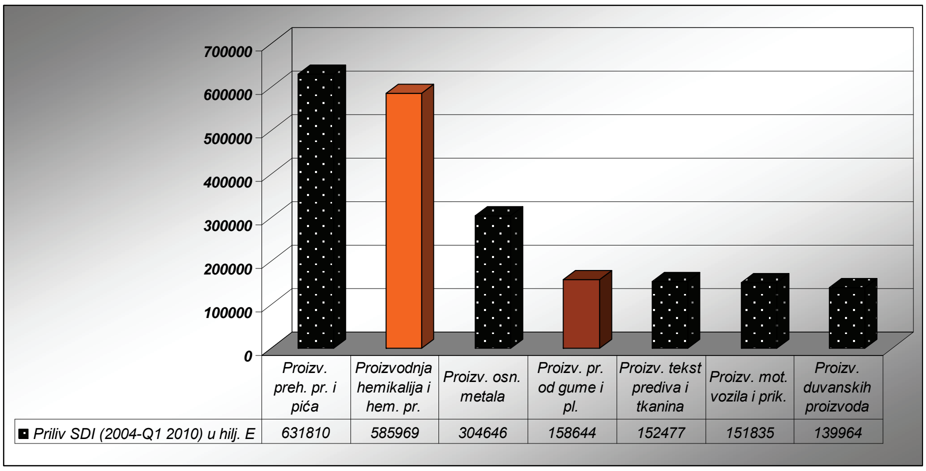
– četvrto, proces restrukturiranja koji u pojedinim kompanijama traje gotovo unedogled, imajući u vidu gubitke, lanac nelikvidnosti i dalje zaostajanje za tehnološkim promenama, ne omogućava delu hemijske industrije i privredi Srbije da zauzmu povoljniju poziciju za izlazak iz krize.

Industrijska politika, održivi razvoj i hemijska industrija

Tzv. „reindustrijalizacija“, shvaćena kao ekonomsko-politički zadatak došla je na dnevni red tek nakon propasti koncepta „novog modela rasta“ koji je implicirao posebne podsticaje za kvalitativni rast industrije. Među prvim i najvažnijim granama na koje se računalo, nalazi se hemijska industrija. Naime tzv. „targetirani sektori“ prema strategiji reindustrijalizacije bile su „srednje i visoke tehnološke oblasti“ kao što su hemijska industrija, proizvodnja mašina i uređaja, proizvodnja saobraćajnih sredstava i elektronska industrija, kojima je pridodata i prehrambena industrija. [1,2,13]. Ovakav izbor „prioriteta reindustrijalizacije“ možda potiče od prožimanja hemijske sa ostalim granama industrije, ili pak iz procene njenih resursa odnosno sposobnosti da privuče nove investicije. Ako se napravi pregled ukupnih stranih direktnih investicija, onda se vidi da su u periodu od 2004. do prvog kvartala 2010. godine ulaganja u industriju iznosila čak 24% ukupnih. Pri tome je učešće investicija u prerađivačku industriju iznosilo čak 20% ukupnih SDI, po godinama varirajući od 14% (2007.), čak do 30% (2009.). Ulaganja u proizvodnju hemikalija i hemijskih proizvoda u prikazanom periodu bila su na drugom mestu u okviru prerađivačke industrije sa ukupno 585 miliona evra, što je iznosilo 4,37% ukupnih SDI. Međutim, ako bi se tome dodalo i oko 158 miliona evra uloženih u proizvodnju proizvoda od gume i plastike, ova dva podsektora zajedno važili bi kao liderski u privlačenju stranih ulaganja (slika 6).

Privatizacija kao većinska prodaja (po Zakonu od 2001.) donela je tek retke primere uspešne tranzicije hemijske industrije iz tzv. socijalističkog nasleđa, koji pokazuju da su kompanije mogle da se prilagode novim tržišnim uslovima i na održiv način da unapređuju ekonomsko-tehnološku poziciju. Taj koncept bio je samo odgovor na neuspeli pokušaj tzv. svojinske transformacije u kojoj je na osnovu Zakona 2007. bilo moguće kupiti odnosno privatizovati manje od polovine vrednosti kapitala domaćih preduzeća, čime je u velikoj meri bila blokirana privatna poslovna inicijativa, na kojoj počiva konkurencija. Međutim, sudeći po današnjim rezultatima, oba koncepta bila su pogrešna. To pokazuje obim industrijske proizvodnje kao i podaci o rastu industrije, kako u devedesetim godinama XX veka, tako i veoma loši rezultati prosečnog rasta industrijske proizvodnje nakon 2000. godine. Uprkos zakonskim mogućnostima, privatizacija se odvijala veoma problematično i protivrečno, tako da je od 2002. do 2012. godine privatizovano ukupno 2350 preduzeća, pri čemu je svaki treći kupoprodajni ugovor raskinut, pri čemu broj raskida nije identičan sa brojem neuspešnih privatizacija. Kompanije gubitaši koje su 2002. godine bile u gubitku 100 miliona evra, u privatizovanoj formi kraj decenije završile su sa dobitkom od 200 miliona evra.

U tzv. društvenim preduzećima pre privatizacije bilo je zaposleno 680 hiljada radnika a 2012. je u njima bilo zaposleno 286 hiljada. Broj zaposlenih u privatizovanim preduzećima za jednu deceniju smanjen je za 58%, ali je



Slika 6. Priliv stranih direktnih investicija u hiljadama evra od 2004–2010. Izvor: NBS.
Figure 6. Foreign Direct Investments inflow in thousands of EUR 2004–2010. Source: National Bank of Serbia.

prihod uvećan za 69%, pri čemu treba imati u vidu da 537 firmi još nije našlo kupca. Zbirni prihodi države od privatizacije (iako predstavljaju samo deo cene kapitala) ukupno su iznosili preko 2 milijarde evra. Međutim, problem koji ostaje i multiplikuje se, ne samo zbog efekata privatizacije, već i zbog posledica krize, predstavljaju nenaplaćena potraživanja od strane čak oko 50.000 radnika prema preduzećima u kojima su radili. Uprkos formalnim nastojanjima mnogih vlada (i posebnog obećanja prethodnog Cvetkovićevog kabineta pri preuzimanju vlasti 2008.) da će problem biti rešen prodajom državne imovine, drugi talas krize produbio je problem nanaplativih potraživanja i računa uopšte, tako da će taj „doprinos“ privatizacije formiranju novog „ekonomskog sistema“ verovatno predstavljati neku vrstu negativne i veoma teško rešive „ostavštine za budućnost“. Sve u svemu, rezultati privatizacije u Srbiji su prilično višedimenzionalni i protivrečni. Ne može se reći da su u celini negativni, iako po posledicama, naročito na polju zaposlenosti, izgleda tako. Naime, u svetu je već davno utvrđeno da tehnološki progres donosi zemlji po definiciju višu produktivnost, ali smanjuje udeo zaposlenih, posebno u industriji, kao što se dešavalo u razvijenim zemljama od šezdesetih godina XX veka [14].

Onda su nastupile posledice zbivanja u vezi sa privatizacijom i restrukturiranjem realnog sektora, koji je naročito nakon 2001. pretrpeo ogromnu devastaciju. Industrija se, i pored dugotrajnih i neselektivnih subvencija iz državnog budžeta (tabela 3) nije snašla na otvorenoj tržišnoj sceni. Sve što je urađeno u privatizaciji zbilo se veoma brzo ali nedovoljno transparentno, a kriza je faktički dokrajčila one segmente industrije koji nisu bili preferirani od strane ekonomske politike. Industrija tekstila i obuće, ne samo zbog globalnog restrukturiranja i pomeranja ka istoku, u Srbiji su faktički nestale sa scene.

Državne subvencije iz budžeta ili posebnih kredita prestale su samo za prehrambenu industriju i to tek 2005. godine. Ostalo je da funkcioniše samo ono što je moglo u bilo kojoj od zemalja u okruženju. Uprkos tome, sadašnje stanje industrijske proizvodnje pokazuje da je ona više od ostalih sektora ranjiva na spoljne krizne udare, tako da je u prvoj godini krize (2009.) industrija Srbije „potonula“ čak oko 12%, a kasniji minimalni rast nije mogao da podigne njen nivo u 2012. čak ni do stanja pre bombardovanja (1998.).

Restrukturiranje i zaposlenost kao ključni izazovi hemijske industrije Srbije

Ključni empirijski nalazi govore da je hemijska industrija nešto bolje, ili možda „manje loše“ od ostalih sektora prošla u deindustrijalizaciji tokom prve decenije XXI veka. To pokazuju podaci sa slike 7 iz koje sledi da ukupna industrija Srbije u 2011. bila na nivou indeksa 106,8 u odnosu na inače veoma nisku bazu iz 2000. (=100) dok je hemijska industrija u referentnom odnosu dospela do indeksa 155,7.

Međutim, da bi u uslovima otvorene privrede dovoljno konkurentno proizvodila plastične mase, smole, veštačka vlakna, lakova i boje, kao i neorganske kiseline, sode, poljoprivredne hemikalije, ovdašnjoj hemijskoj industriji ovog podsektora neophodno je mnogo veće tržište, kao i obim proizvodnje od onoga koji ona trenutno ostvaruje, što u srednjem roku, bez masovnih investicija u ovaj sektor nije moguće ostvariti. Ali, kada su u pitanju finalni proizvodi kao što su autogume, proizvodi od plastike, farmaceutski proizvodi, baterije i akumulatori, stvari stoje nešto drugačije. Indirektan pregled onoga što je ostalo od hemijske industrije Srbije daje struktura izvoznih proizvoda Srbije u novije vreme. Ono što se tokom krize može proizvesti i izvesti ne mora da znači da je održivo na duži rok, ali zaslužuje posebnu pažnju i vizuelni prikaz koji sledi na slici 8.

Tabela 3. Budžetska sredstva po programima Vlade za subvencije, odnosno kreditnu podršku privredi, u mil. RSD. Izvor: Ministarstvo za ekonomiju i regionalni razvoj, 2011
Table 3. Budgetary funds by the Government programmes for subsidies, or credit support to economy, in millions of RSD. Source: Ministry of Economy and Regional Development, 2011

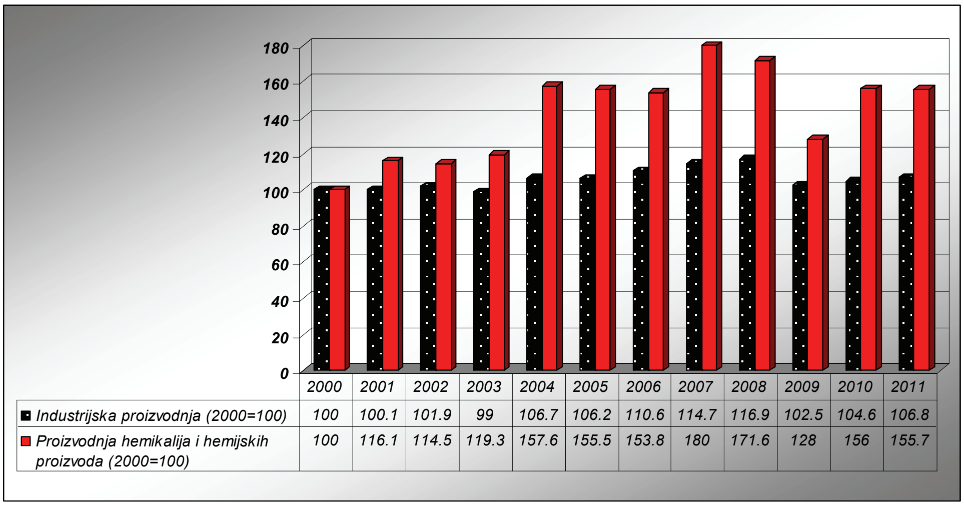
Oblast industrije	2002	2003	2004	2005	2006	2007	2008	2009	I–IX, 2010
Saobraćajna sredstava	1.658,8	1.848,0	1.640,0	1.347,5	1.435,0	1.365,2	1.839,7	1.653,7	1.229,4
Metalski kompleks	1.305,6	1.408,6	882,5	805,5	754,0	621,7	696,5	710,3	909,5
Metalurgija	1.210,0	843,0	743,5	496,5	211,5	297,0	96,1	95,0	11,0
Tekstil i koža	372,7	419,8	379,2	390,0	360,5	122,7	151,0	143,0	198,2
Hemijska industrija	397,7	430,5	420,5	406,0	330,5	295,5	215,2	343,4	13,9
Prerada nemetala	182,5	305,0	231,5	210,5	285,5	45,5	172,2	74,2	111,0
Električne mašine i aparati	237,7	376,5	244,0	156,0	120,5	130,5	247,6	230,2	205,0
Prehrambena industrija	137,5	211,0	65,0	–	–	–	–	16,4	–
Drvna industrija	132,0	121,0	149,5	174,0	161,0	12,5	16,5	3,0	78,0
Industrija – ukupno	5.634,5	5.963,4	4.755,7	3.986,0	3.658,5	2.890,6	3.434,8	3.269,2	1.526,6
Građevinarstvo	127,5	138,0	167,0	199,0	160,0	121,0	46,2	32,3	7,2
Ukupno (I+G)	5.762,0	6.101,4	4.922,7	4.185,0	3.818,5	3.011,6	3.481,0	3.301,5	1.533,8

Srbija je u toku prve decenije XX veka veoma zapustila ne samo realni sektor ekonomije već i finalnu industrijsku proizvodnju, tako da u strukturi izvoznih proizvoda dominiraju sirovine, poluproizvodi ili proizvodi veoma niskog nivoa obrade, kao što su kukuruz, smrznuto voće i povrće, metalične sirovine... Od finalnih proizvoda u nešto većoj meri u izvozu je prisutna jedino odeća, kao proizvod nisko-tehnološkog sektora. Tek u novije vreme (januar 2013.) prvo mesto i izvozu zauzela su putnička motorna vozila i prikolice, zahvaljujući početku masovnije serijske proizvodnje i izvoza vozila kompanije Fiat, Srbija. Međutim, dominantan motiv države u podsticanju te kompanije su postojeća i nova radna mesta koja se iz budžeta finansiraju sa po 10.000 evra, što je vrlo problematično sa stanovišta evropskih standarda konkurencije i kontrole državne pomoći [14]. Veće učešće hemijske industrije u izvozu, podrazumeva se uz odgovarajući napredak tehnologije, porast produktivnosti i unapređenje ekonomskih performansi, moglo bi da vodi dvostrukoj koristi:

– sa jedne strane, ka *povećanju tržišne realizacije*, obima proizvodnje i zaposlenosti, u uslovima oskudne domaće tražnje;

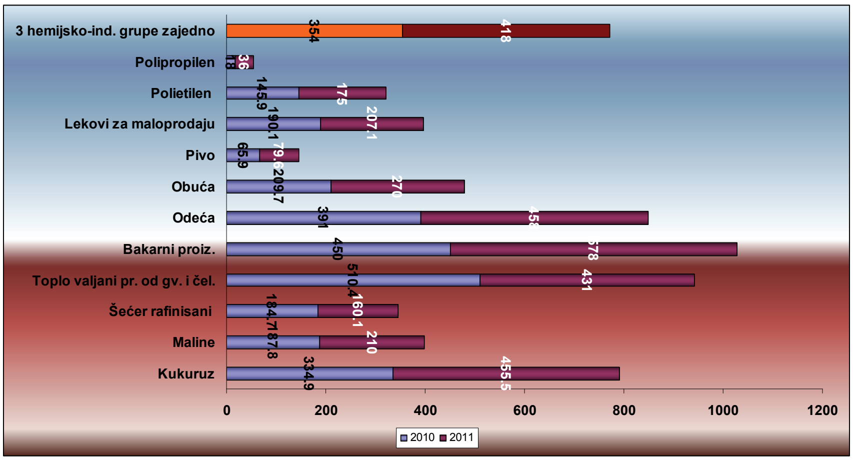
– sa druge strane, ka daleko višoj efikasnosti, jer bi u pitanju bio izvoz proizvoda viših-srednjih i nižih-srednjih tehnologija, odnosno produkata višeg nivoa prerade, sa daleko većim strukturno-tehnološkim, finansijskim i deviznim učinkom. To je strateška šansa za srpsku hemijsku industriju, naročito za industriju autoguma, akumulatora, lekova dakle i niza plastičnih i drugih poluproizvoda.

Najveći izvozni aduti Srbije trenutno su putnički automobili, kao i metalične sirovine, pojedina bazna hrana. Što se tiče hemijske industrije ona nije jedna od



	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
■ Industrijska proizvodnja (2000=100)	100	100.1	101.9	99	106.7	106.2	110.6	114.7	116.9	102.5	104.6	106.8
🟥 Proizvodnja hemikalija i hemijskih proizvoda (2000=100)	100	116.1	114.5	119.3	157.6	155.5	153.8	180	171.6	128	156	155.7

Slika 7. Hemijska i ostala industrijska proizvodnja – tokovi 2000–2011.
Figure 7. Chemical and other industrial manufacturing – trends 2000–2011.



Slika 8. Najznačajniji izvozni proizvodi Srbije prema vrednosti izvoza 2010. i 2011. (u mil. evra). Izvor: RZS.
Figure 8. The most important Serbian exportation products by exports value in 2010 and 2011 (in millions of EUR). Source: RSB.

vodećih u izvozu, ali ako se saberu rezultati izvoza polietilena, polipropilena kao i farmaceutskih proizvoda, ukupni skor u izvozu bio bi treći po veličini, naročito ako bi se tome dodali izvozni rezultati od autoguma. Međutim, laka hemijska industrija proizvodi gotove proizvode kao što su lekovi, hemijski proizvodi, guma, kozmetika. Nova tehnološka rešenja sve više potiru oštre granice između hemijske i ostalih industrija. Statistička i druga evidencija uglavnom se baziraju na nacionalnim računima koji evidentiraju ostvarenu novododatu vrednost ili udeo u ostvarenom bruto domaćem proizvodu, što nije najbolji parametar ekonomske, ekološke pa i socijalne efikasnosti.

Budućnost hemijske industrije na platformi održivog razvoja

Sve okolnosti iz prošlosti kao i faktori koji oblikuju potrebe za budućnost govore da hemijska industrija nije prevaziđena i da ona po prirodi stvari, sve više postaje umrežena u ukupni doprinos niza industrija i tehnologija modernom tehnološkom progresu. On dolazi pre svega iz tržišnih podsticaja, odnosno iz prožimanja tehnološke i ekonomske sfere, iz učinka tehnološkog razvoja, naročito novih tehnologija i njihove kreativne kombinacije, kao i usled uticaja globalizovane ekonomije i međunarodne konkurencije sa druge strane.

Nakon krize, prema sadašnjim globalnim prognozama od 2014. stopa održivog rasta BDP u Srbiji mogla bi da iznosi do 4% u proseku za narednih deset godina. To ostavlja mogućnost da industrijska proizvodnja raste po stopi do 6%, pri čemu bi hemijska industrija, odnosno njen perspektivni i relativno zdrav ostatak mogao da beleži rast proizvodnje od oko 5,5%, s obzirom na nešto povoljniju polaznu poziciju, kao i preostale probleme subjekata u restrukturiranju. Međutim, mnogo je važniji kvalitet takve obnovljene hemijske industrije, koja bi morala da ide u pravcu koncepta održive industrije (*sustained industry*) u okviru modela održivog razvoja.

Pojam *održivog razvoja* se veoma široko upotrebljava, ali je mali broj polazišta dovoljno kvalifikovan. Ova, relativno fluidna kategorija, koju je najpre odredio *Brundtland Report 1987* kao takav razvoj koji omogućava pravo današnje generacije da živi bolje, ne uskraćujući to pravo budućim generacijama [13], u sebi sadrži:

– ekonomsko–tehnološku dimenziju koja se tiče održive privrede, ekonomije zasnovane na znanju i sve većeg uticaja nematerijalnih faktora kao što su znanje, informacije, kultura;

– socijalnu dimenziju koja podrazumeva stalno smanjivanje siromaštva i društvenu inkluziju;

– ekološku dimenziju (bolju zaštitu životne sredine, održivo gazdovanje prirodnim resursima i bolje stanje prirode uopšte).

Da li hemijska industrija Srbije može da se razvija na održiv način, odnosno da se taj proces odvija u interesu budućih generacija, nudeći benefite sadašnjoj? Hemijska industrija Srbije po sebi verovatno trenutno ne predstavlja glavni razvojni faktor srpske ekonomije. Međutim, ni energetika ni poljoprivreda sa agroindustrijom (kao oficijelni strateški prioriteti) ne mogu bez hemijske industrije, novih kombinovanih tehnologija neophodnih u programima energetske efikasnosti ili konverzije energije, tehnika obnovljivih izvora energije, konzerviranja i pakovanja hrane, reciklaže… Ekološka sfera održivog razvoja ima mnogo primenljivih oblasti za sektor hemijske industrije, kao što su: zaštita i prečišćavanje voda i vazduha, kvalitet hrane, zdravstvena bezbednost.

Ključni adut hemijske industrije Srbije nisu nasleđena materijalna tehnologija, raspoloživi prirodni resursi, finansijski ili realni kapital, već ljudski kapital, koji je ključna uzdanica modernog „postindustrijskog“ privrednog razvoja. S obzirom na vladajući trend „dematerijalizacije“ proizvodnje, kao i na dominantnu ulogu znanja, obrazovanja, istraživanja i nauke u privrednom i ukupnom društvenom razvoju, ključna referentna prednost ovdašnje hemijske industrije mogao bi da bude potencijal domaćeg naučno-stručnog znanja koji, prema standardnim naučnim indikatorima vrednovanja, nije za potcenjivanje. Kooperacija industrije i naučno-istraživačkog potencijala univerziteta i instituta morala bi da postane daleko efikasnija, jednostavnija i jeftinija.

Međutim, izgledi farmaceutske industrije, industrije automobilskih guma, plastičnih masa i ploča, proizvoda ili tzv. kućne hemije, nešto su povoljniji u odnosu na industriju baznih hemijskih sirovina soli, kiselina, mineralnog đubriva. Pri tome je veoma bitan problem nedovoljno kvalitetnog ili neažurnog vođenja statistike proizvodnje, prometa, uvoza i izvoza, kao i ekonomskih performansi u okviru hemijske industrije i njenih podsektora. Nema pouzdanih podataka ni o toma kakav je uspeh imala privatizacija u ovom sektoru. Pretpostavka svakog budućeg napretka je bolja koordinacija između državnih statističkih službi ili odgovarajućih ministarstava sa Agencijom za privatizaciju. Brojne malverzacije, propale privatizacije, afere i istražni postupci oko privatizacije i restrukturiranja pojedinih prethodno velikih sistema u okviru hemijske industrije govore o smanjivanju korupcije i privrednog kriminala kao jednoj od prvih pretpostavki napretka.

Za povoljnije izglede i bolju budućnost hemijske industrije, kao i industrije uopšte neophodne su pojedine opšte strateške mere razvojne politike kao i mnoštvo posebnih mera koje se odnose na kratkoročno stanje. U tome bi trebalo poći od sledećih principa:

– država treba i može da pomogne u revitalizaciji hemijske industrije Srbije, što nikako ne znači da svi privredni subjekti mogu i treba da opstanu na tržištu koje se bitno promenilo u međuvremenu;

– ta pomoć ne može da bude isključivo ili dominantno finansijska, odnosno iz javnih fondova, već pre svega u podizanju kvaliteta obrazovanja stručnih kadrova za novu hemijsku industriju;

– Srbiji je neophodno ministarstvo za industriju, koje bi eventualno još obuhvatalo i energetiku, kao što ga ima i poljoprivreda kao strateška sektor;

– subjekti hemijske industrije, okupljeni u okviru poslovnih asocijacija kao što su ona za gumu i plastiku ili baznu hemiju, odnosno farmaceutsku industriju itd., uz pomoć privrednih komora, treba da pokrenu istraživački projekat u okviru koga bi se empirijski istražila trenutna ekonomsko–tehnološka slika u sektoru, registrovali najvažniji problemi na terenu koje statistika ne evidentira i zauzele zajedničke strateške pozicije sektora u pokušaju revitalizacije, kako prema državi, dobavljačima, kupcima, tako i strateškim partnerima izvan sektora;

– državne mere koje se odnose na podsticaj za oporavak i održivi razvoj hemijske industrije morale bi u kratkom roku da se odnose prvenstveno na logističku podršku, širenje mreže znanja i informacija, a u dugom roku na poreske i druge stimulacije čistije proizvodnje, energetske efikasnosti, reciklaže i razvoja ekološki efikasnijih „zelenih“ tehnologija.

Samo u tako izmenjenom ekonomsko-političkom okruženju i drugačijim mikro-inicijativama kompanija, može se govoriti o boljim perspektivama za održivu hemijsku industriju Srbije.

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SUMMARY

POSSIBILITIES FOR RECOVERY AND PROSPECTS OF THE SERBIAN CHEMICAL INDUSTRY IN THE LIGHT OF SUSTAINABLE DEVELOPMENT

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(Professional paper)

There are numerous dilemmas related to the meaning of common terms associated with modern economic sectors, and especially the ones concerning industry. Chemical industry is a typical example of a term which changes rapidly and qualitatively, exactly with the pace of changing of the very technology based on knowledge, procedure, processes, raw materials, energy, as well as on the products themselves and on the way of their use. Numerous difficulties caused by huge changes in global market, by transition of command economies towards market system, as well as by the latest global economic-financial crisis, have brought the chemical industry in modern Serbia to an unenviable position. We cannot generally claim that chemical industry is collapsing, but the recovery of the whole chemical industry, as well as of the industry in general, necessitates many favourable presumptions from the environment, as well as strategic, systemic and operative measures, of the state within the so-called industry policy, as well as of the very companies which deal with chemical industry. The re-industrialisation strategy, adopted officially during the first crisis blow, but to the full extent only during the prolonged crisis period in Serbia (2009-2013) should not be based on direct state incentives, but above all on the institutional infrastructure and business environment improvement which will lead to the investments in technological reconstruction and re-organisation of the entire sector. However, chemical industry cannot be observed as a chance for economic growth per se, nor it can lead to higher employment rate in such a short period of time, but above all to productive use of profession, or of growth potential based on knowledge factor. This is why a proper evolution and prosperity of the Serbian chemical industry can be comprehended, not only through contribution of one separate sector, but as complementary and useful technologies within many other industries such as food, construction, energy, and many others, from the medium technologies domain, as well as those from the high technologies domain, such as bio-medical, pharmaceutical or cosmetic technologies. In such manner, the participation of chemical industry in the re-industrialisation of Serbia should not be observed only as a contribution to the recovery of industry, but as a factor of sustainable development in all its components, economic, social and ecological.

Keywords: Chemical industry • Prolonged crisis period • Economic structure • Foreign direct investments • Privatization • Re-industrialisation • Technological reconstruction • Re-organisation • Economic and environmental efficiency • Sustained industry

Elektrohemijsko ispitivanje hladno deformisanog bakra u alkalnoj sredini u prisustvu kalijum-etilksantata

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Izvod

U radu su prikazani rezultati elektrohemijskog ispitivanja bakra u 1 M rastvoru Na_2CO_3 u prisustvu kalijum-etilksantata (KEtX) različitih koncentracija. KEtX je najčešće korišćen kolektor u procesima flotacione koncentracije sulfidnih minerala obojenih metala te je od značaja poznavanje uticaja njegovog prisustva u alkalnoj sredini na reakcije prisutnih metala, pre svega bakra. Ispitivanja su obavljena na uzorcima od bakra različitog nivoa deformacije 83, 91 i 99%. Uzorci su dobijeni postupkom hladnog izvlačenja žice koja je prethodno dobijena po *dip-forming* postupku. Za elektrohemijsko ispitivanje bakra koriščena je metoda ciklične voltametrije zato što su korozione karakteristike metala u određenoj sredini povezane sa potencijalom otvorenog kola elektroda od odgovarajućih metala i sa njihovim strujnim odzivom za vreme anodne polarizacije. Eksperimentima je utvrđeno da prisustvo KEtX u rastvoru 1 M Na_2CO_3 menja hemizam procesa kao i stepen oksidacije bakra pri anodnoj polarizaciji.

Ključne reči: bakar, stepen deformacije, natrijum-karbonat, anodna polarizacija, kalijum--etilksantat.

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Bakar je metal koji ima veliku primenu u tehnici zbog svojih osobina kao što su: koroziona otpornost, odlična toplotna i električna provodljivost [1].

Uprkos dobroj korozionoj postojanosti, gubici usled korozije bakra i njegovih legura su znatni. Čine se veliki napori da se korozija svede na minimum, jer je korozija proces razaranja metala, gde se stvaraju nekorisni, pa čak i štetni materijali [2].

Na dijagramu potencijal–pH za sistem bakar–voda (slika 1) uočavaju se oblasti u kojima je bakar termodinamički stabilan, u kojima korodira i u kojima je pasivan [2]. Može se zaključiti da je bakar pri negativnim potencijalima stabilan i ne korodira. Na pozitivnim potencijalima i pri pH 7–12 bakar postaje pasivan, to jest na površini se stvaraju jedinjenja (nerastvorne prevlake) koja su u ovoj oblasti potencijala i pH vrednosti, stabilna.

Ako ova prevlaka dobro prianja uz metal i nije porozna, čime se sprečava direktan kontakt bakra i rastvora, sprečava se i korozija. U slučaju oštećenja ovog filma korozija je moguća. Šrafirana oblast na dijagramu predstavlja uslove u kojima je korozija bakra intenzivna.

U toku ispitivanja, za vreme anodne polarizacije bakra u alkalnoj sredini, mogu se javiti sledeće reakcije [3–5]:

$$2Cu + H_2O = Cu_2O + 2H^+ + 2e^- \qquad (1)$$

$$Cu_2O + H_2O = 2CuO + 2H^+ + 2e^- \qquad (2)$$

Ove reakcije predstavljaju stupnjeve ukupne reakcije date jednačinom:

$$Cu + H_2O = CuO + 2H^+ + 2e^- \qquad (3)$$

Jednačine koje pokazuju elektrodne potencijale navedenih reakcija u funkciji od pH vrednosti, kao i izračunate vrednosti njihovih ravnotežnih potencijala za pH rastvora 10 prema standardnoj vodoničnoj elektrodi (SVE) i zasićenoj kalomelskoj elektrodi (ZKE), prikazane su u tabeli 1.

Najčešće korišćeni kolektori u procesu flotacije sulfidnih minerala su reagensi iz grupe ditiokarbonata (ksantata), kako zbog njihovih osobina, tako i zbog odnosa cena–efikasnost. Ksantat koji se najčešće koristi u flotaciji sulfidnih minerala jeste kalijumova so ksantatne kiseline – kalijum-etildikarbonat (kalijum-etilksantat – KEtX, KEX). Kalijum-etilksantat ($CH_3CH_2OCS_2K$) je organska supstanca koja se koristi u flotaciji i kao kolektor za flotiranje oksidnih minerala olova i srebra [6––9]. Iako je osnovni cilj dodatka KEtX-a da promeni ponašanje čestica od hidrofilnih u hidrofobne, hemijske i elektrohemijske reakcije koje se javljaju pri flotaciji, ne mogu se izbeći. Tokom niza godina aktuelna su istraživanja posvećena izučavanju hemijskih reakcija između sulfidnih minerala i KEtX koje dovode do formiranja hidrofobnih proizvoda [8]. U tim istraživanjima merenja su vršena na bakarnom sulfidu halkopiritu, $CuFeS_2$ i legurama na bazi bakra metodom ciklične voltametrije [9–14]. Tehničko–tehnološki razlozi uslovljavaju da se pretežno svi procesi flotacijske koncentracije izvode u

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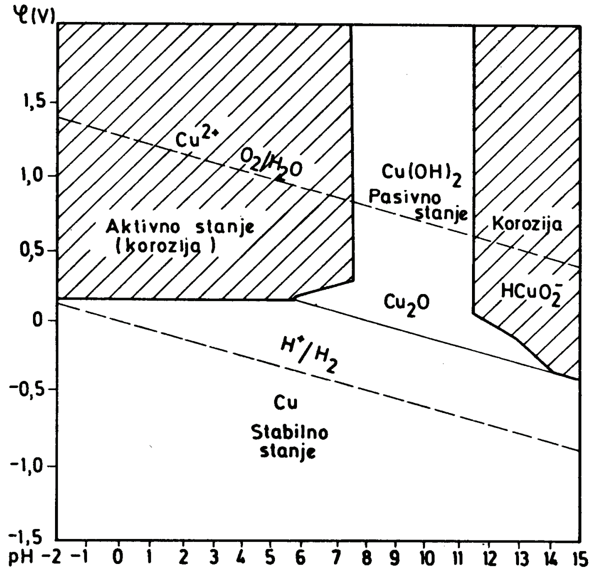


Slika 1. Zavisnost potencijal–pH za sistem Cu–H_2O na 25 °C [2].
Figure 1. Dependence of potential–pH for system Cu–H_2O at 25 °C [2]

baznoj sredini, pa je shodno tome i u ovim ispitivanjima uzeta bazna sredina.

Tabela 1. Ravnotežni potencijal elektrohemijskih reakcija na bakru u alkalnim rastvorima za pH 10
Table 1. The equilibrium potential of electrochemical reactions on copper in alkaline solutions at pH 10

Reakcija	E_0 (V) = f (pH)	E_0 prema SVE V	E_0 prema ZKE V
(1)	E_0 = 0,471–0,0591pH	–0,120	–0,362
(2)	E_0 = 0,669–0,0591pH	0,078	–0,164
(3)	E_0 = 0,570–0,0591pH	–0,021	–0,263

U literaturi mogu da se sretnu istraživanja rađena na platinskoj i bakarnoj elektrodi koje su uronjene u boratni rastvor sa ksantatom, kao i poređenje sa ponašanjem elektroda u čistom boratnom rastvoru [13,14]. Ta istraživanja imala su za cilj da objasne mehanizam površinskih reakcija u flotaciji sulfidnih minerala pri kontaktu delova od bakra sa tečnostima koje sadrže KEtX. Bakarna elektroda je najpre uronjena u boratni rastvor bez ksantata pri pH vrednosti 9,2 i podvrgnuta promeni potencijala od 0,01 V/s. Polarizacija je vršena od mirujućeg potencijala tj. od –0,318 V prema ZKE ka pozitivnijim vrednostima do 1,2 V prema ZKE. Rezultati su pokazali da pri anodnoj polarizaciji dolazi najpre do oksidacije bakra, a potom do pasivacije elektrode. U boratnom rastvoru bez ksantata mogu se uočiti dva pika od kojih je jedan širi od drugog, što ukazuje da postoje dva stupnja oksidacije: Cu do Cu_2O i Cu_2O do CuO. U literaturi prvi pik se objašnjava oksidacijom Cu do Cu_2O, a drugi pik je često dvostruk i pripisuje se oksidaciji Cu_2O do CuO [4]. Nagli porast gustine struje javlja se na 1,2 V prema ZKE, što odgovara izdvajanju gasovitog kiseonika. U boratnom rastvoru sa ksantatom na voltamogramu se javljaju četiri anodna pika. Ksantatni joni reaguju spontano sa površinom bakra i dovode do formiranja CuEtX i $Cu(EtX)_2$ [13,14]. Prvi pik je na potencijalu –0,650 V prema ZKE i predstavlja formiranje CuEtX, što je predstavljeno reakcijoma (4):

$$Cu + EtX^- = CuEtX + e^-,\ E_0 = -0{,}864\ V \qquad (4a)$$

ili

$$CuO + EtX^- + 2H^+ + e^- = CuEtX + H_2O,\ E_0 = 1{,}542\ V \qquad (4b)$$

Za koncentraciju 50 ppm KEtX, ravnotežni potencijal se uspostavlja na –0,657 V prema ZKE, a pri koncentraciji od 200 ppm KEtX, taj potencijal je –0,150 V prema ZKE. Drugi pik je na potencijalu –0,500 V prema ZKE i očekuje se da predstavlja formiranje $Cu(EtX)_2$ kao u reakciji (5):

$$Cu + 2EtX^- = Cu(EtX)_2 + 2e^-,\ E_0 = -0{,}588\ V \qquad (5)$$

Diksantogen se može formirati pri potencijalima od –0,2 do 0 V prema ZKE, oksidacijom jona ksantata po reakciji (6) [13]:

$$2EtX^- = (EtX)_2 + 2e^-,\ E_0 = -0{,}3306\ V \qquad (6)$$

Treći pik predstavlja oksidaciju bakra. Visina trećeg pika smanjuje se sa povećanjem koncentracije KEtX-a u rastvoru. To znači da opada vrednost gustine struje, što predstavlja pozitivan uticaj KEtX-a u zaštiti od korozije. Na četvrtom piku dešava se oksidacija CuEtX. Visina četvrtog pika je gotovo konstantna sa povećanjem koncentracije KEtX u boratnom rastvoru, samo se pomera ka pozitivnijim vrednostima potencijala [13,14]. To ukazuje na povoljnije uslove u zaštiti od korozije sa povećanjem koncentracije KEtX. Pokazalo se da dodatak KEtX u boratni rastvor menja oblik voltamograma [13,14].

U literaturi se nalaze i ispitivanja uticaja koncentracije KEtX kao flotacionog sredstva i pH rastvora na vreme uspostavljanja stabilne vrednosti elektrodnog potencijala halkopiritne elektrode [9,15]. Flotaciono iskorišćenje halkopirita od 100% može se postići u vrlo širokom opsegu pH od 2 do 10,5, kada se kao kolektor koristi dietil-diksantogen, dok se pH vrednost kreće od 4 do 12 kada je kolektor etilksantat. Eksperimentima je utvrđeno da je za uspešnu flotaciju halkopirita potrebno obezbediti uslove da pH ne prelazi 10 i da koncentracija KEtX bude 3×10^{-5} mol/l [15].

Takođe je poznato da se reagensi iz grupe ditiokarbonata (ksantata) razlažu u prisustvu jona obojenih metala na odgovarajući alkohol, ugljendisulfid i odgovarajuću bazu, pa razlozi istraživanja mogu imati ekonomski i ekološki aspekt. Ekonomski aspekt se ogleda u tome što, usled razlaganja ksantata, dolazi do gubitka kolektora. To predstavlja negativnu pojavu u tehnološkom procesu, jer dolazi do njegove povećane potrošnje. Ekološki aspekt može se sagledati kroz povećanu koncentraciju proizvoda razlaganja, naročito ugljendisulfida, koji je jako otrovan i ozbiljno narušava radnu sredinu. Sa druge strane, baza i alkohol odlaze na jalovište gde izazivaju dugotrajne ekološke posledice. Kinetika razlaganja ksantata igra važnu ulogu u flotaciji sulfidnih minerala i tretmanu flotacijske jalovine.

Cilj ovog rada je da utvrdi uticaj prisustva KEtX-a na korozione karakteristike bakra u jednomolarnom rastvoru natrijum-karbonata. Ispitivanja su obavljena na uzorcima od bakra različitog nivoa deformacije, kao jednog od činilaca koji bi mogao da utiče na stabilnost ovog metala pri njegovom korišćenju u sličnim uslovima [16–18]. U eksperimentima prikazanim u ovom radu korišćen je KEtX u različitim koncentracijama.

EKSPERIMENTALNI DEO

Elektrohemijski sistem koji je korišćen u svim ispitivanjima sastoji se od:

- elektrohemijske ćelije sa tri elektrode (radna, referentna i pomoćna),
- hardvera (PC, AD/DA konvertor PCI-20428W proizveden od strane Burr-Brown i analogni interfejs razvijen na Tehničkom fakultetu u Boru) [19,20] i
- softvera za merenje i upravljanje (LABVIEW platforma i specijalno razvijena aplikacija za elektrohemijska merenja).

U elektrohemijsku ćeliju, ispunjenu radnim rastvorom, uronjene su:

a) referentna elektroda (zasićena kalomelska elektroda, ZKE),

b) radna elektroda (bakarna žica dibijena različitim stepenima hladne deformacije),

c) pomoćna, odnosno platinska elektroda.

Za ispitivanje je korišćena bakarna žica prečnika 8 mm ($\varepsilon = 0\%$) proizvedena po dip-forming postupku [21––23]. Sa jednog trna uzet je uzorak žice prečnika 8mm koji je dalje podvrgnut neposrednom izvlačenju bez međufaznog žarenja po utvrđenom režimu kroz seriju matrica, do završnih dimenzija Ø 3,3; Ø 2,9; Ø 2,4; Ø 1,8 i Ø 0,8 mm za koje stepen hladne deformacije iznosi 83, 87, 91, 95 i 99 %, redom, uz zadržavanje određene količine žice nakon svakog provlaka. Za dalja elektrohemijska ispitivanja odabrani su uzorci žica prečnika 8 ($\varepsilon = 0\%$), 3,3 ($\varepsilon = 83\%$), 2,4 ($\varepsilon = 91\%$) i 0,8 mm ($\varepsilon = 99\%$).

Priprema za eksperiment je obuhvatala sledeće radnje:

- dvostruko ispiranje elektrohemijske ćelije, najpre običnom i destilovanom vodom, a zatim radnim rastvorom;
- mehaničko poliranje radne elektrode, najpre abrazivnim papirom, a zatim pomoću filca natopljenog suspenzijom destilovane vode i glinice;
- izolovanje središnjeg dela elektrode lakom, koji je premazan u više slojeva, dok su krajevi ostali neizolovani. Na jednom kraju je tako dobijen radni deo elektrode površine 1 cm^2, a drugi kraj je služio kao električni kontakt;
- radni deo elektrode je odmašćivan pomoću ugljentetrahlorida;
- ispiran je radni deo elektrode najpre vodom i vodenim rastvorom HNO_3 (1:1) radi uklanjanja eventualno formiranih oksida, zatim destilovanom vodom i radnim rastvorom i uranjan je u radni rastvor.

Osnovni rastvor korišćen u eksperimentu je 1 M Na_2CO_3 približne pH vrednosti 10. Njemu su dodavane različite količine kalijum-etilksantata između 0,008 i 0,15 g/l.

Eksperiment počinje merenjem potencijala pri otvorenom kolu, a dobijeni potencijal se naziva potencijalom otvorenog kola ili korozioni potencijal. Dobijeni rezultati prikazuju se na dijagramu $E = f(t)$. Pre početka merenja metodom ciklične voltametrije, podešeni su uslovi pod kojima je vršena polarizacija: vrednost potencijala otvorenog kola dobijena merenjem od koje se počinje sa polarizacijom, vrednost maksimalnog potencijala do kojeg se vrši merenje (0,750 V prema ZKE) i brzina polarizacije (0,01 V/s).

Praćenjem promene gustine struje pri promeni potencijala, dobijene su polarizacione krive (voltamogrami), koje su predstavljene na graficima kao funkcije $j = f(E)$. Ovde su jedinice gustine struje u mA·cm^{-2} i jačine struje u mA, iste, ako se gustina struje prikaže po 1 cm^2, zato što je radna površina elektrode 1 cm^2.

REZULTATI I DISKUSIJA

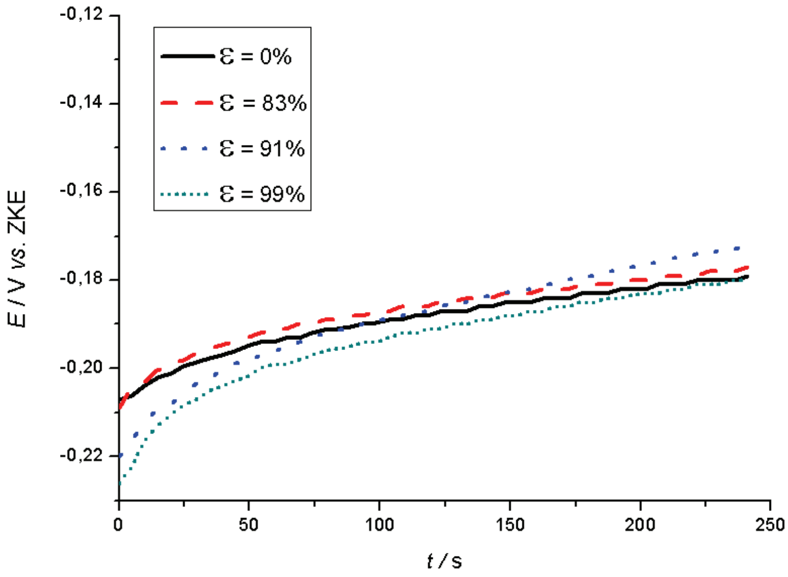
Merenja u 1 M natrijum-karbonatu

Radi poređenja karakteristika bakra u 1 M Na_2CO_3 bez dodatka i sa dodatkom KEtX, najpre su prikazani rezultati merenja potencijala otvorenog kola i voltamogrami dobijeni u rastvoru 1 M Na_2CO_3 bez prisustva KEtX.

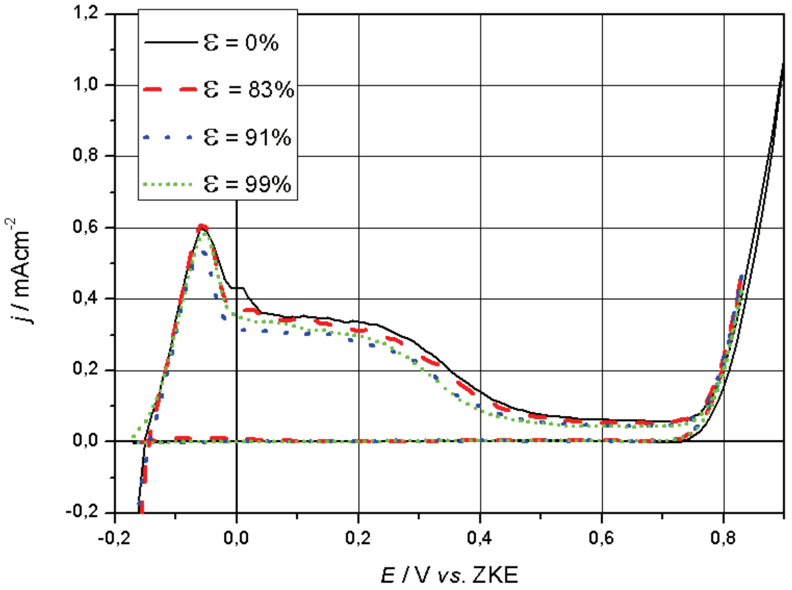
Merenje potencijala otvorenog kola vršeno je na uzorku bez deformacije i deformisanim uzorcima različitim stepenima defomracije: 83, 91 i 99 %. Merenje je trajalo 250 s u 1 M Na_2CO_3 (slika 2). Potencijal otvorenog kola raste sa vremenom veoma sporo i posle 250 s ni na jednom uzorku nije uspostavljena potpuno stabilna vrednost potencijala, mada je promena mnogo sporija nego u prvih 100 s. Vrednost potencijala posle 250 s se kreće u uskom opsegu od –0,187 do –0,193 V prema ZKE. Porast potencijala na samom početku merenja to jest njegovo pomeranje u pozitivnom pravcu, ukazuje da postoje promene na površini bakra, koje ga čine koroziono stabilnijim u posmatranom rastvoru.

U daljim eksperimentima, metodom ciklične voltametrije ispitivane su karakteristike hladno deformisanog bakra za vreme anodne polarizacije u rastvoru 1 M Na_2CO_3. Brzina promene potencijala bila je 0,01 V/s, a merenje je počelo posle 10 min čekanja na uspostavljanje relativno stabilnog potencijala.

Dobijeni voltamogrami u rastvoru 1 M Na_2CO_3 prikazani su na slici 3 i oni pokazuju da nema velikih razlika između visine pikova dobijenih za elektrode sa različitim stepenom deformacije, pa se može zaključiti



Slika 2. Potencijal otvorenog kola za bakarnu elektrodu u 1 M Na_2CO_3 rastvoru u funkciji vremena.
Figure 2. Open circuit potential of copper electrode in 1 M Na_2CO_3 solution as a function of time.



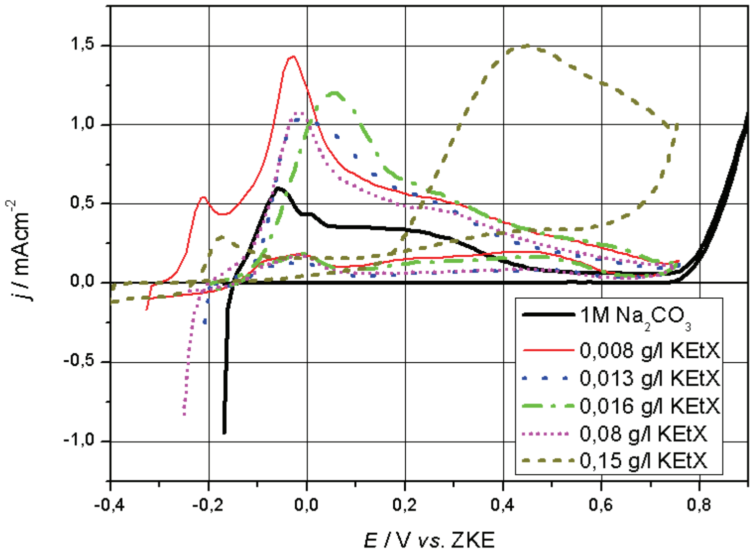
Slika 3. Voltamogrami snimljeni za bakarnu elektrodu u rastvoru 1 M Na_2CO_3 pri brzini promene potencijala od 0,01 V/s.
Figure 3. Cyclic voltammograms of copper electrode obtained in 1 M Na_2CO_3 solution at the sweep rate of 0.01 V/s.

da stepen deformacije između 83 i 99 % nema velikog uticaja na potencijal otvorenog kola, kao i na ponašanje bakra za vreme anodne polarizacije u 1 M rastvoru Na_2CO_3. To se objašnjava činjenicom da kod visokih stepena deformacije dolazi do formiranja slične, relativno homogene kristalne strukture, to jest obrazuje se tekstura deformacije. Prisutne male razlike u visini pikova mogu se objasniti verovatno prisutnim minimalnim razlikama u površini elektroda. Pri anodnoj polarizaciji dolazi do oksidacije bakra i na voltamogramima se pojavljuju dva pika, što ukazuje da postoje dva stupnja oksidacije. Prvi pik odgovara formiranju oksida bakra Cu_2O, a drugi pik odgovara formiranju CuO, po reakcijama (1) i (2), redom. Na ovim dijagramima pojavljuje se jedan dobro definisan strujni talas na potencijalu oko –0,06 V prema ZKE i drugi široki strujni talas na potencijalu oko 0,150 V prema ZKE. Nagli porast struje na oko 0,750 V prema ZKE odgovara početku izdvajanja gasovitog kiseonika.

Ako ove podatke uporedimo sa podacima koji se mogu naći u literaturi za bakarnu elektrodu uronjenu u boratni rastvor čija je pH vrednost 9,2, zapaža se da su podaci vrlo slični za eksperiment izveden u sličnim uslovima, pri promeni potencijala od 0,01 V/s [13,14].

Merenja u prisustvu KEtX

Rezultati elektrohemijskog ispitivanja hladno deformisanog bakra u prisustvu KEtX prikazani su na slikama 4–7. Jasno se uočava da dodatak KEtX menja mehanizam procesa pri anodnoj polarizaciji. Oblik voltamograma se značajno menja u odnosu na voltamograme snimljene bez prisustva KEtX, prikazane na slici 3.



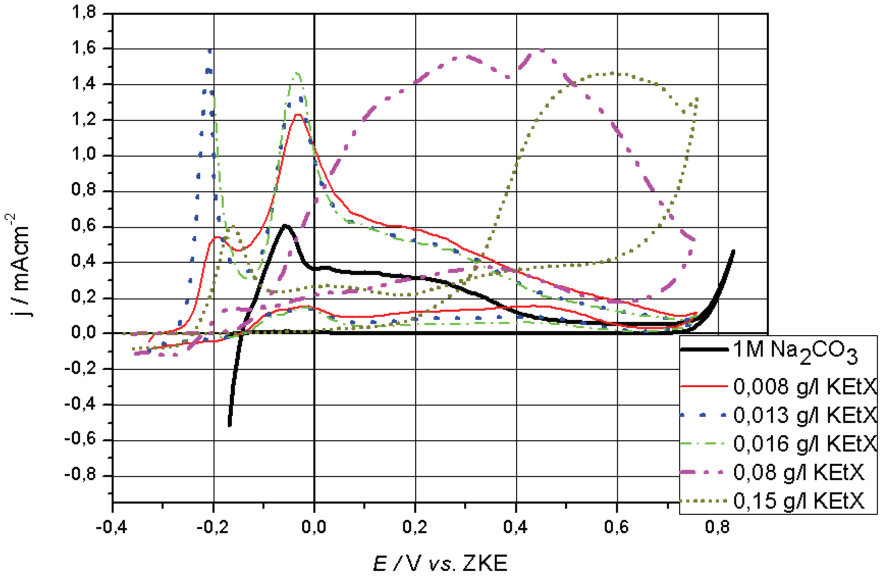
Slika 4. Voltamogrami snimljeni za bakarnu elektrodu sa ε = 0 % u rastvoru 1 M Na_2CO_3 bez i sa dodatkom (0,008– 0,15 g/l) KetX pri brzini promene potencijala od 0,01 V/s.
Figure 4. Cyclic voltammograms of copper electrode with ε =0 % obtained in 1 M Na_2CO_3 solution without and with different KEtX content (0.008–0.15 g/l) at the sweep rate of 0.01 V/s.

Voltamogrami su dobijeni polazeći od potencijala otvorenog kola, pri čemu su te vrednosti u opsegu od –0,323 do –0,387 V prema ZKE. Vrednosti relativno stabilnog potencijala otvorenog kola dobijene za različite koncentracije KEtX posle 100 s, date su u tabeli 2.

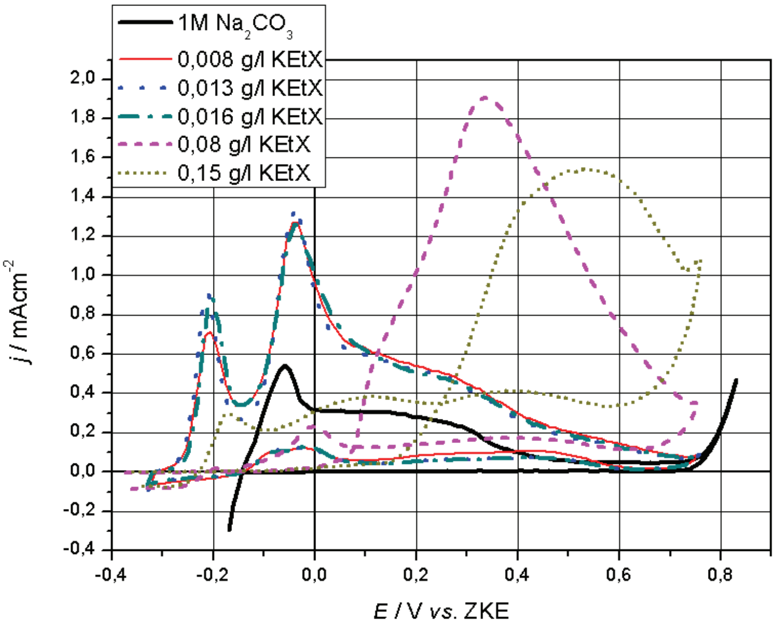
Promena potencijala vršena je ka pozitivnijim vrednostima brzinom od 0,01 V/s do početka izdvajanja kiseonika, to jest do 0,750 V prema ZKE. Ispitivanja su najpre rađena na uzorku bez deformacije, u rastvoru sa različitim koncentracijama KEtX (slika 4). Na voltamo-

Tabela 2. Vrednosti potencijala otvorenog kola nakon 100 s od uranjanja bakarne elektrode u rastvor za različite koncentracije kalijum-etilksantata, V prema ZKE
Table 2. Values of open circuit potential obtained after 100s of immersion of copper electrode in solution for various concentrations of potassium ethylxanthate, V vs. SCE

Stepen deformacije, ε / %	Koncentracija KetX, g/l				
	0,008	0,013	0,016	0,08	0,15
0	–0,238	–0,314	–0,255	–0,250	–0353
83	–0,317	–0,315	–0,364	–0,310	–0,387
91	–0,318	–0,318	–0,347	–0,320	–0,383
99	–0,318	–0,323	–0,379	–0,318	–0,380



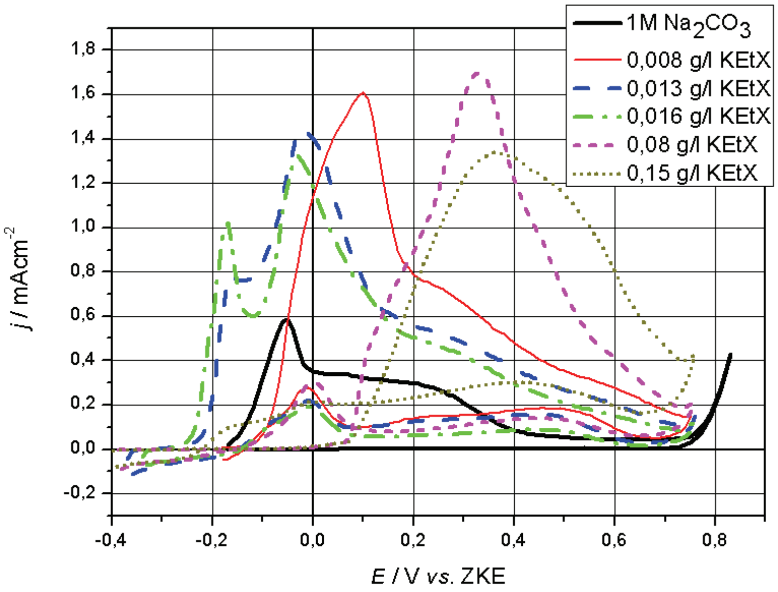
Slika 5. Voltamogrami snimljeni za bakarnu elektrodu sa ε = 83 % u rastvoru 1 M Na_2CO_3 bez i sa dodatkom (0,008–0,15 g/l) KetX pri brzini promene potencijala od 0,01 V/s.
Figure 5. Cyclic voltammograms of copper electrode with ε =83 % obtained in 1 M Na_2CO_3 solution without and with different KEtX content (0.008–0.15 g/l) at the sweep rate of 0.01 V/s.



Slika 6. Voltamogrami snimljeni za bakarnu elektrodu sa ε = 91 % u rastvoru 1 M Na_2CO_3 bez i sa dodatkom (0,008–0,15 g/l) KEtX pri brzini promene potencijala od 0,01 V/s.
Figure 6. Cyclic voltammograms of copper electrode with ε = 91 % obtained in 1 M Na_2CO_3 solution without and with different KEtX content (0.008–0.15 g/l) at the sweep rate of 0.01 V/s.

gramima koji su dobijeni u prisustvu ksantata uočava se novi oštar pik na potencijalu oko –0,03 V prema ZKE. Istovremeno, pik koji je pripisan formiranju Cu_2O znatno je viši od odgovarajućeg pika koji se pojavljuje pri ispitivanju u osnovnom rastvoru bez KEtX. Gustina struje na celom toku voltamograma, već u prisustvu najmanje količine ksantata, veća je nego bez prisustva ksantata. Ovo ukazuje na to da je formiranje oksida bakra u prisustvu ksantata znatno intenzivnije u odnosu na njihovo nastajanje u rastvoru koji ne sadrži ksantat. Znači da se procesi oksidacije bakra ubrzavaju ksantatom, mada u literaturi postoje primeri gde se on pojavljuje kao potencijalni inhibitor korozije bakra u nekim elektrolitima [13,14,24].

Pri manjim koncentracijama KEtX (<0,08 g/l) sve krive imaju sličan oblik i ne postoji bitna razlika u visini



Slika 7. Voltamogrami snimljeni za bakarnu elektrodu sa ε = 99 % u rastvoru 1 M Na_2CO_3 bez i sa dodatkom (0,008–0,15 g/l) KEtX pri brzini promene potencijala od 0,01 V/s.
Figure 7. Cyclic voltammograms of copper electrode with ε = 99 % obtained in 1 M Na_2CO_3 solution without and with different KEtX content (0.008–0.15 g/l) at the sweep rate of 0.01 V/s.

strujnih pikova. Pri većim koncentracijama KEtX u rastvoru (≥0,08 g/l) površina bakarne elektrode je prekrivena adsorbovanim molekulima KEtX i dobija se potpuno drugačiji oblik voltamograma. Početak izdvajanja gasovitog kiseonika je pri potencijalu od 0,750 V prema ZKE. Do promene oblika voltamograma dolazi usled promene mehanizma procesa i pojave piting korozije. Osim piting korozije dolazi i do formiranja jedinjenja bakra sa ksantatom po reakcijama (4) i (5) na površini elektrode, pa se na povratnom delu krivih javlja novi anodni pik koji odgovara oksidaciji tih jedinjenja CuEtX i $Cu(EtX)_2$ do CuO i ksantata ili CuO i diksantogena, po reakcijama (7) i (8):

$$CuEtX + H_2O = CuO + EtX^- + 2H^+ + e^-,\ E_0 = 1{,}542\ V \qquad (7)$$

$$Cu(EtX)_2 + H_2O = CuO + (EtX)_2 + 2H^+ + 2e^-,\ E_0 = 0{,}612\ V \qquad (8)$$

Naredni eksperimenti vršeni su sa deformisanim uzorcima, gde su stepeni deformacije 83, 91 i 99%, u rastvoru sa različitim koncentracijama KEtX. Dobijeni voltamogrami prikazani su na slikama 5–7, redom. Na ovim slikama prikazani su i voltamogrami za odgovarajuće deformacije dobijeni u čistom rastvoru 1 M Na_2CO_3, radi poređenja.

Kao i u eksperimentima sa nedeformisanim uzorkom, dodatak KEtX značajno utiče na oblik voltamograma, menjajući mehanizam reakcije u anodnom području. Pri povećanju potencijala polazeći od potencijala otvorenog kola, javlja se više anodnih pikova. Na najnegativnijem delu to jest na samom početku voltamograma, javlja se dobro definisan pik na oko –0,2 V prema ZKE. Termodinamički podaci koje daju Hepel i Pomianowski ukazuju na to da joni ksantata mogu reagovati spontano sa površinom bakra, što dovodi do obrazovanja ksantatnih jedinjenja CuEtX i $Cu(EtX)_2$ prema reakcijama (4a) i (5) [25–28]. Tako, pojava ovog strujnog pika na voltamogramima (slike 5–7), odgovara formiranju jedinjenja na površini bakarne elektrode, kako sa ksantatom tako i sa diksantogenom.

Diksantogen može biti formiran oksidacijom bakar ksantata, po reakciji:

$$2CuEtX + O_2 = 2CuO + (EtX)_2 \qquad (9)$$

Oksidacija bakar-ksantata do diksantogena po reakciji (9) je termodinamički moguća pri potencijalu od –0,2 do 0 V prema SVE [27]. Oksidacija bakar-ksantata do bakar-oksida CuO i diksantogena $(EtX)_2$ inhibira flotaciju minerala bakra etilksantatom na višim potencijalima [6,25,27].

Međutim, pri koncentraciji KEtX u rastvoru od 0,08 g/l i većoj ovaj pik izostaje. Drugi dobro definisani strujni talas na voltamogramu pri potencijalu oko –0,06 V prema ZKE, očekuje se da predstavlja oksidaciju Cu do Cu_2O i Cu_2O do CuO po reakcijama (1) i (2). Kod deformisanih uzoraka visina ovih strujnih pikova veća je u odnosu na odgovarajuće dobijene u rastvoru bez ksantata, što potvrđuje da je u prisustvu KEtX stepen oksidacije bakra povećan. Pri većim koncentracijama KEtX u rastvoru (≥0,08 g/l) stablina vrednost potencijala otvorenog kola (korozioni potencijal) uspostavlja se vrlo sporo i javlja se drugačiji oblik voltamograma, odnosno pojavljuje se široki pik u opsegu potencijala od 0,300 do 0,600 V prema ZKE u anodnom delu voltamograma, a oštar pik na početku voltamograma potpuno izostaje.

Ovakav oblik voltamograma dobijen u rastvoru sa većom koncentracijom KEtX, gde prvi pik izostaje, a drugi je širok, može se dovesti u vezu sa adsorpcijom KEtX na površini bakarne elektrode i sa formiranjem organometalnog kompleksa koji se specifično ponaša u toku anodne polarizacije, pa dolazi do piting korozije. U literaturi takođe, postoje podaci za bakarnu elektrodu uronjenu u boratni rastvor pri pH vrednosti 9,2 sa dodatkom ksantata u količini od 50 do 200 ppm, koji ukazuju na promenu oblika voltamograma u značajnoj meri u odnosu na voltamograme dobijene u čistom boratnom rastvoru [13,14], slično kao i u eksperimentima koji su predmet izučavanja u ovom radu. Elektrohemijska ispitivanja vršena u boratnom rastvoru sa ksantatom na bakarnoj elektrodi ukazuju na to da su ksantatna jedinjenja CuEtX i $Cu(EtX)_2$ odgovorna za hidrofobnost površine bakra [12,13].

Na povratnom delu voltamograma, javlja se novi anodni pik koji se verovatno može pripisati oksidaciji ksantata i formiranju diksantogena po reakciji (9) [25]. Formiranje diksantogena dešava se pri potencijalu od 0 do –0,1 V prema ZKE. Ovaj pik se javlja pri koncentraciji ksantata manjoj od 0,08 g/l kod svih deformacija, a pri većim koncentracijama ksantata (≥0,08 g/l) pik koji odgovara formiranju diksantogena izostaje, a javlja se piting korozija. Ovaj pik se ne uočava ni na voltamogramima koji se odnose na rastvor bez ksantata, slika 3.

ZAKLJUČAK

Rad predstavlja rezultat primene metode ciklične voltametrije za karakterizaciju hladno deformisanog bakra, pri anodnoj polarizaciji u alkalnom rastvoru, u prisustvu kalijum-etilksantata u različitim koncentracijama.

Potencijal otvorenog kola od trenutka uranjanja elektroda u elektrolit raste sa vremenom veoma sporo do dostizanja stabilne vrednosti, što ukazuje na formiranje zaštitnog sloja na površini bakra. Stepen deformacije u opsegu od 83 do 99 % nema značajnog uticaja na potencijal otvorenog kola; može se uočiti samo veoma mala promena u negativnom pravcu sa povećanjem stepena deformacije. Za čist rastvor 1 M Na_2CO_3 stabilna vrednost potencijala se kreće od –0,187 do –0,193 V prema ZKE, a u rastvorima sa dodatkom ksantata vrednosti su od –0,323 do –0,387 V prema ZKE.

Prisustvo kalijum-etilksantata u rastvoru 1 M Na_2CO_3 menja oblik voltamograma dobijenih pri anodnoj polarizaciji. To znači da KEtX kao kolektor utiče na promenu površinskih reakcija u flotaciji pri kontaktu bakra sa karbonatnim rastvorom. Rezultati su pokazali da pri anodnoj polarizaciji dolazi do oksidacije bakra i ksantata.

Pri malim koncentracijama KEtX (<0,08 g/l) u anodnom delu voltamograma pojavljuju se tri pika, pri čemu se prvi pripisuje reakciji između bakra i samog ksantata, odnosno formiranju organometalnog kompleksa na površini hladno deformisane bakarne žice. Drugi pik predstavlja oksidaciju Cu do Cu_2O i treći pik odgovara oksidaciji Cu_2O do CuO. Kod svih uzoraka visina strujnih pikova u prisustvu ksantata veća je u odnosu na pikove u rastvoru bez ksantata, što ukazuje na povećan stepen oksidacije.

Na povratnom delu voltamograma javlja se novi anodni pik koji se ne pojavljuje ni u odsustvu ksantata, ni pri većim koncentracijama KEtX od 0,08 g/l, a koji se može pripisati oksidaciji bakar ksantata do diksantogena.

Pri većim koncentracijama KEtX (≥0,08 g/l) uspostavljanje stabilne vrednosti potencijala na pojedinim uzorcima je vrlo sporo. Oblik voltamograma dobijen u ovim rastvorima je potpuno drugačiji, zbog promene mehanizma procesa. Prvi pik koji se dovodi u vezu sa reakcijom bakra sa ksantatom izostaje, a drugi strujni talas pri pozitivnom potencijalu između 0,3 i 0,6 V prema ZKE je širok i povezan je sa formiranjem ksantatnih jedinjenja koja se specifično ponašaju u toku anodne polarizacije i dovode do piting korozije. Formirana ksantatna jedinjenja odgovorna su za hidrofobnost površine bakra.

Zahvalnica

Ovaj rad je urađen u okviru projekta 34003, Osvajanje proizvodnje livenih legura sistema bakar–zlato, bakar–srebro, bakar–platina, bakar–paladijum i bakar–rodijum poboljšanih svojstava primenom mehanizma ojačavanja žarenjem, koji podržava Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

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SUMMARY

ELECTROCHEMICAL INVESTIGATION OF COLD WORKED COPPER IN ALKALINE SOLUTION WITH THE PRESENCE OF POTASSIUM ETHYLXANTHATE

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(Scientific paper)

This paper presents the investigation results of the electrochemical behavior of copper in 1 M Na_2CO_3 solution in the presence of potassium ethyl xanthate (KEtX) with different concentrations. Tests were conducted on copper samples obtained without deformation and with deformation of 83, 91 and 99%. Samples were obtained by cold drawing of wire, which was previously obtained by dip-forming procedure. Corrosion behavior of cold deformed copper wire is characterized by its open circuit potential and behavior during anodic polarization. Experiments were carried out in aqueous solution 1 M Na_2CO_3 with added various amounts of KEtX between 0.008 and 0.15 g/l. It was experimentally proved that the degree of deformation between 83 and 99% does not have a large effect on the open circuit potential, as well as on the behavior of copper during anodic polarization in 1 M Na_2CO_3. Voltammograms show no significant differences between peak heights obtained for different electrodes. The first peak which occurs at potential of around –0.06 V *vs*. SCE corresponds to the formation of copper oxide Cu_2O. The second wide peak is at potential of around 0.15 V *vs*. SCE and corresponds to the formation of CuO. Addition of potassium ethyl xanthate in alkaline 1 M Na_2CO_3 solution changes the mechanism of the process in anodic part, which is reflected in the change of shape of voltammograms. In presence of KEtX in concentration between 0.008 and 0.15 g/l on voltammograms a sharp peak appears at potential of about –0.2 V *vs*. SCE and corresponds to the oxidetion of xanthate. Current density, which determines the rate of the process which takes place at the electrode surface, yet in the presence of smallest amounts of KEtX (<0,08 g/l) is higher than in the absence of KEtX. It allows one to conclude that the processes of oxidation of copper accelerate in presence of potassium ethylxanthate.

Keywords: Copper • Degree of deformation • Sodium carbonate • Anodic polarization • Potassium ethylxanthate

The influence of packaging materials protective properties and applying modified atmosphere on packed dried apricot quality changes

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Abstract

The influence of protective properties of packaging materials and modified atmosphere on quality changes of dried apricot is shown in this paper. In our investigation, we used four different characteristic combinations of packaging materials with different barrier properties for packaging of dried apricot: polyester-polyethylene (PET/PE), paper/polyethylene (PAP/PE), paper/aluminum/polyethylene (PAP/Al/PE), polyester/aluminum/polyethylene (PET/Al/PE) and two different atmospheric conditions: normal and modified. Modified atmosphere was made under laboratory conditions: CO_2, about 30%, N_2, about 60%, and the rest is O_2. Over the 12-months storage period, the changes in the water content, water activity (a_w) and overall polyphenol content were monitored in the packed product.

Keywords: packaging, modified atmosphere, dried apricot.

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The packaging of dehydrated processed food products represents a very specific problem in terms of product protection. Due to its significant active surface, dried fruit and vegetables are a substrate sensitive to humidity and oxidation during the storage period. That is why it is necessary to choose the right packaging, capable to protect the product in the best way and to enable the longest possible quality of content after conservation.

When it comes to the packaging of dried products, there are special requirements which depend on the sensitivity of the packed dehydrated product. When their water content is reduced, fruit and vegetable products become very hydroscopic, sensitive to humidity, oxidation and light. Depending on the production, packaging and storage conditions, some physicochemical processes occur in this sensitive substrate, which further cause changes in the quality of the packaged product.

In order to prevent these changes, the packaging of this kind of products is carried out in an oxygen-free atmosphere. The best results are obtained by implementing inert gases in which case the air in the packaged product is being extracted or replaced. As a protective gas during packaging, usually nitrogen, carbon dioxide or their suitable combination is used. The dosing is performed after the air has been extracted from the packaging unit.

The packaging of food in modified atmosphere (MAP) is a special way of treating already prepared food which protects the food from oxidation, keeps it fresh and extends product shelf life without any change in its color [1–4].

The storing of food in a modified gas atmosphere can preserve quality and extend product shelf life, slowing down the chemical and bio-chemical spoilage processes and also slow down (and in some cases even prevent) the growth of microorganisms [5].

The properties of packaging material, such as mechanical and barrier, are very important when it comes to decision about which materials are to be used in packaging of different types of products. Exposure to various conditions during processing can lead to a number of physical/chemical changes.

Flexible and semi-hard/hard plastic materials and laminates are the materials most frequently used in modified atmosphere food packaging. Plastic materials constitute approximately one third of the material required for packaging in food industry and it is estimated that its use is going to increase even more in future [5].

Some of the properties that make plastic materials suitable for food packaging are: they are rather easy to mold, light, pretty translucent, solid and they are also heat insulators. The usual polyolefin films, such as PE and PP, are excellent moisture barriers [6].

If a polymer material does not satisfy all strict quality requirements for packaging and preservation of a product until it is used, then it is combined with other polymer materials in which case double-layer (duplex) or multi-layer polymer materials are obtained [7,8]. These materials possess better physical and mechanical properties, and especially better barrier properties

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than the mono-materials. This is due to the fact that by combining them, all the positive properties of mono-materials are gathered up. In this way, the area of the application of these materials is significantly extended [8,9].

Combined materials are consisted of a polymer and some other material (paper, cardboard, aluminum foil,…), for example, PE/PAP/PE, PET/A1/PE, metPET/ /PE, PE/PAP/PE/A1/PE,… Each material brings in its good properties and in this way it contributes to the optimal properties of the produced multi-layer or combined material [10].

In order to obtain optimal protective properties as a barrier against electromagnetic radiation, permeability of gas molecules and vapor, metal foil is also used, mostly aluminum foil cassated on polymer film. The thickness of the metal layer is 7 μm or more. The combined packaging material obtained in this way is light proof and very little gas molecule and vapor proof [11].

With the aim of improving barrier properties, a thin layer of aluminum (or other materials) can be applied on polymer films using metallization process. In this way, the metalized films barrier properties regarding electromagnetic radiation are improved (over 90%), as well as regarding gas molecules and vapor. Barrier properties improvement rate is conditioned by the mass (layer thickness) of the applied metal, as well as by the application on the surface of the polymer film [4,11,12].

The right choice of the packaging material for dried apricot is very important for keeping the quality of the dried fruit during storage due to its chemical structure and the changes that occur during the drying process.

From a nutritional point of view, apricot pericarp contains saccharides, organic acids and mineral elements (iron, boron and potassium), vitamins such as provitamin A, vitamins B and C and polyphenols. It was found that apricots are rich in phenolic substances, in addition to the aforementioned compounds. Phenolic compounds, such as catechin, epicatechin, *p*-coumaric acid, caffeic acid, ferulic acid and their esters have been identified in the fruits [13].

During the drying process, in the presence of carbon hydrates and proteins, that are the products of their decomposition, like peptides and amino acids, poly hydroxyl phenol transformation reactions in non-enzymatic oxidative browning reactions can be isolated by means of reducing water [14].

The moisture content is one of the most important factors in preserving the quality of dried products. During the storage period of dried fruit (with low moisture content and low water activity) moisture content and water activity increase, followed by the increase in activation energy of the reactions that are responsible for quality changes of packaged content. This leads to the production of HMF (hydroxymethylfurfural) and to the changes in colour compounds in the reactions of auto oxidative changes and nonenzymatic browning [14].

Water-soluble color compounds and brown pigments, which are the indicators of undesirable browning that occurs during the production and storage period, are polyphenols and HMF. During the storage period their content increases [15].

During the storage period of dried carrot and dried apple, the transformation of polyphenols occurs as the result of oxidative reactions of non-enzymatic browning [16,17]. During the first six months, the decreasing of the polyphenol content is almost linear. After six months storage period the polyphenol content rapidly increases in both cases.

After further water reduction, auto oxidation reactions become very pronounced. These reactions will reach their maximum peak at certain water content in the packed product. In addition, each food group has its own optimal moisture balance area in which the sum of all the changes is minimal, in which case, the food has extended shelf life period [12,18].

EXPERIMENTAL

Chemicals

Folin–Ciocalteu reagent, sodium carbonate and gallic acid were purchased from Merck® (KGaA, Darmstadt, Germany).

A sample

Dried apricot was packed under normal atmospheric pressure and in the modified atmosphere. The air was taken out from the sample and the gas mixture injected into the achieved vacuum. The sample was hermetically closed. A 100 g of dried apricot was packed into formed packaging material units. Dried apricot was packaged in four characteristic combinations of packaging materials with different barrier properties: polyester/polyethylene (PET/PE), paper/ /polyethylene (PAP/PE), paper/aluminum/polyethylene (PAP/Al/PE) and polyester/aluminum/polyethylene (PET/Al/PE). A 12 μm packaging material in the case of PET/PE i PAP/PE and 15 μm material for PAP/Al/PE and PET/Al/PE was used. Packed samples were kept at room temperatures (17 to 22 °C) and were exposed to the influence of light for 12 months.

Packaging materials barrier properties testing

Modified atmosphere sustainability was monitored by OXY-BABY device (Witt Gasetechik, Germany).

Dried packed apricot analyses

Moisture content was determined after drying of samples at 103±2 °C to constant mass (Laboratory dryer, Termodry, Raypa, Spain).

Water activity

Water activity is determined by avemeter (Termoconstater - Novasina , tip TN 2(RTD), Switzerland).

Total polyphenols are determined by the Folin–Ciocalteu method

The Folin-Ciocalteu method, based on the reduction of a phosphotungsten-phosphomolybdatecomplex by phenolics to blue reaction products, was used to determine the phenolic compounds. Sample (0.5 mL) was pipetted into cuvette and diluted with deionized water (1.5 mL). Subsequently, Folin–Ciocalteu reagent (0.05 mL) was added and the solution was incubated at 22 °C for 2 h. The absorbance readings were taken at 760 nm. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/100 g [19].

Instruments

UV/Vis spectrophotometer T80 (PG Instrument, England) was used for all measurements.

RESULTS AND DISCUSSION

Modified atmosphere was made in laboratory conditions. Initial values for all gas components of modified atmosphere in the moment of closing of packaging materials are shown in Table 1.

The measurement results are given in Figures 1–3. The most significant changes were observed after the first month in PAP/PE. In this package, the percentage of O_2 has been increased from the initial 1.43 to 17.53%, the percentage of CO_2 changed from the initial 31.53 to 1.53%, while the percentage of N_2 starting from 67.03 reached 80.93%. The PET/Al/PE showed the smallest change in CO_2 concentration, where the concentration of this gas was 28.84% at the beginning, 27.94% at the end of the first month, and 27.48% after 12 months. These further support good barrier characteristics of this packaging material.

Other combinations showed more significant changes in CO_2 concentration after the first month.

The smallest changes in nitrogen concentration were measured in PET/Al/PE, whereas other combinations showed insignificant increase.

Moisture content change

The dried product had 31.2% of moisture at the beginning. Following the humidity values during the storage of the samples packed under the atmospheric pressure in combination with PET/PE, PAP/PE, PAP/Al/

Table 1. Initial values of modified atmosphere

Packaging material	CO_2 Content, %	N_2 Content, %	O_2 Content, %
PAP/PE	31.53	67.03	1.43
PAP/AL/PE	30.17	68.76	1.07
PET/PE	32.60	66.43	0.97
PET/AL/PE	28.48	69.93	1.23

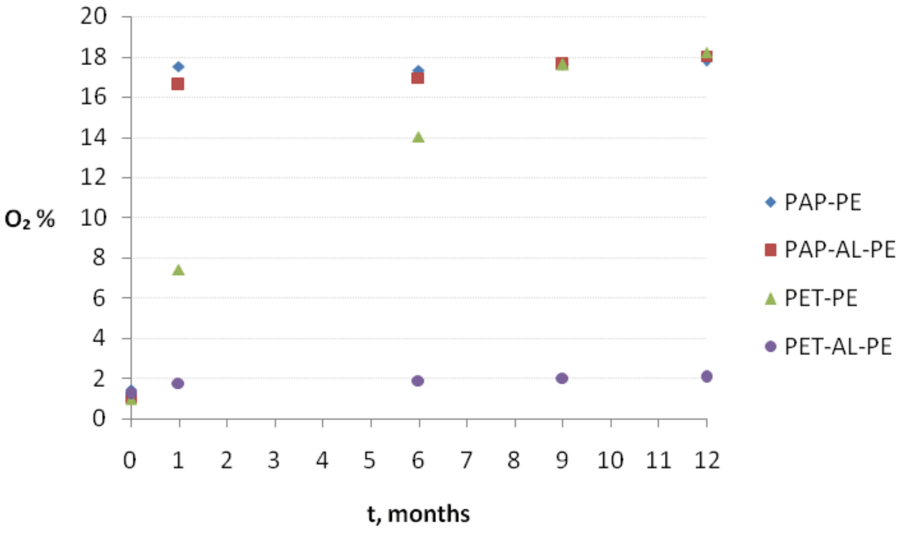

Figure 1. Changing the concentration of oxygen in modified atmospheric conditions.

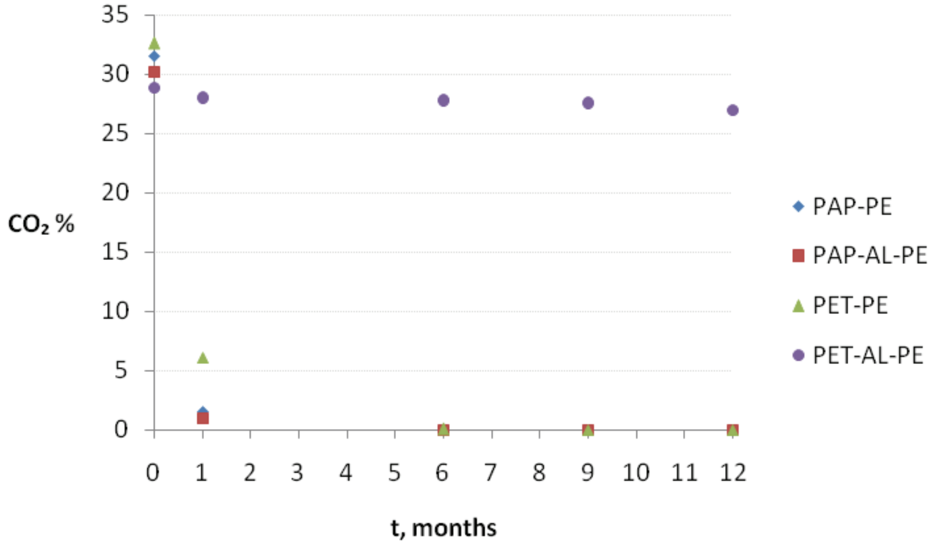

Figure 2. Changing the concentration of carbon dioxide in modified atmospheric conditions.

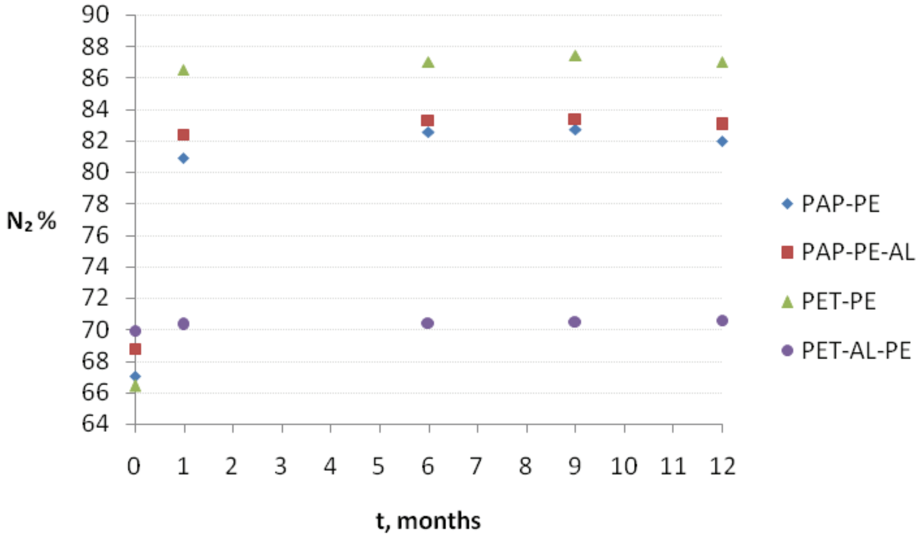

Figure 3. Changing the concentration of nitrogen in modified atmospheric conditions.

/PE, PET/Al/PE materials and according to Table 2, we can conclude that more changes occurred after 12 months, especially with samples packed in PAP/PE where the humidity concentration increased from 31.2 to 39.6%. PET/Al/PE showed the best moisture protection. In this package, the humidity concentration after 12 months reached 32.5%, which is 1.3% more than the initial value.

Modified atmosphere packaging showed the same tendencies as the corresponding packaging in atmospheric conditions (Table 3). The PET/Al/PE material combination sample showed the best protection from humidity in modified atmospheric conditions. In this package, the humidity concentration increased by 0.8%.

The water activity changes in the packed dried apricot during the storing are given in Tables 4 and 5.

Table 2. Changes of the moisture content (%) of packed dried apricots during storage under the normal atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	31.2	31.2	31.2	31.2
1	35.4	33.8	33.1	31.3
9	39.6	36.8	35.4	32.5
12	39.6	36.8	35.4	32.5

Table 3. Changes of the moisture content (%) of packed dried apricots during storage under the modified atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	31.2	31.2	31.2	31.2
1	34.5	33.0	32.0	31.3
9	38.0	35.0	34.0	32.0
12	38.0	35.0	34.0	32.0

Table 4. Changes of the water activity of packed dried apricots during storage under the normal atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	0.645	0.645	0.645	0.645
1	0.713	0.709	0.72	0.671
6	0.766	0.72	0.752	0.691
9	0.782	0.753	0.778	0.701
12	0.955	0.851	0.792	0.715

Table 5. Changes of the water activity of packed dried apricots during storage under the modified atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	0.645	0.645	0.645	0.645
1	0.704	0.711	0.718	0.66
6	0.75	0.732	0.737	0.689
9	0.772	0.761	0.763	0.693
12	0.912	0.831	0.81	0.698

Water activity changes start after the first month of the sample being stored. The biggest changes were noticed in samples packed under normal atmospheric pressure. According to Table 3 we can conclude that after 12 months the biggest changes were observed in PAP/PE samples, where the water activity value increased from 0.645 to 0.955. The smallest changes of water activity values were observed in samples packed into PET/Al/PE packaging material under the modified atmospheric conditions, where the value at the beginning was 0.645, after 9 months it was 0.693, while after 12 months it was 0.698 (Table 4). The increase of water activity is correlated with the increase of moisture content, and it depends on the packaging material and atmosphere conditions applied [14].

Observed polyphenol changes (Tables 6 and 7) point to the occurrence of change in cyclicities depending on packing material and storage time.

During the nine-month period, the total polyphenol content decrease occurs followed by an increase of water activity and moisture content. The biggest change occurs during the first month. The least amount of these changes was observed in the specimens packed into the PET/AL/PE combination. These results show that the reactions of nonenzymatic browning changed the polyphenol content, over the storage period.

After nine months, the total polyphenol content continues to increase, except in the PET/AL/PE combination, where the total polyphenol content decreased.

Table 6. Changes of the total polyphenol content (mg/100 g) of packed dried apricots during storage under the normal atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	0.137	0.137	0.137	0.137
1	0.063	0.038	0.105	0.053
6	0.063	0.016	0.038	0.053
9	0.018	0.015	0.038	0.040
12	0.078	0.070	0.050	0.037

Table 6. Changes of the total polyphenol content (mg/100 g) of packed dried apricots during storage under the normal atmospheric conditions

Time, months	Package			
	PAP/PE	PAP/Al/PE	PET/PE	PET/AL/PE
0	0.137	0.137	0.137	0.137
1	0.067	0.074	0.080	0.053
6	0.058	0.069	0.064	0.049
9	0.017	0.035	0.064	0.047
12	0.054	0.050	0.075	0.037

The smallest changes are ascertained in the specimens packed in modified atmospheric conditions, which points to the oxidation nature of the change and the influence of the applied packing materials barrier properties. The modified atmosphere conditions didn't affect the total polyphenol content in PET/AL/PE combination.

CONCLUSION

Moisture content, water activity and total polyphenol content changes are conditioned by the type, combination, barrier properties of the used materials and the packaging conditions applied.

According to the tests, it can be concluded that adequately applied modified atmosphere combination, choice of packaging materials, their combinations and barrier characteristics all bare great significance and influence the sustainability of packed dried apricot. PET/Al/PE combination provides the best protection to packed dried apricot.

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IZVOD

UTICAJ ZAŠTITNIH SVOJSTAVA AMBALAŽNOG MATERIJALA I PRIMENE MODIFIKOVANE ATMOSFERE NA PROMENE KVALITETA UPAKOVANE SUŠENE KAJSIJE

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(Naučni rad)

U ovom radu prikazan je uticaj zaštitnih svojstava ambalažnog materijala i primene modifikovane atmosfere na promene kvaliteta upakovane osušene kajsije. U istraživanju su za pakovanje osušene kajsije korišćene četri kombinacije ambalaže sa različitim barijernim svojstvima: poliestar/polietilen (PET/PE), papir/ /polietilen (PAP/PE), papir/aluminijum/polietilen (PAP/Al/PE) i poliestar/ aluminijum/polietilen (PET/Al/PE), kao i dva različita uslova atmosfere. Parametri koji su praćeni u ovom istraživanju su sadržaj vlage, aktivitet vode i sadržaj polifenolnih materija. Najveća promena u sadržaju vlage u upakovanoj osušenoj kajsiji je utvrđen kod PAP/PE u uslovima normalne atmosfere (od 31,2 na 39,6% nakon 12 meseci skladištenja). Porast aktiviteta vode bio je u korelaciji sa porastom sadržaja vlage i zavisi od primenjenog ambalažnog materijala i uslova pakovanja. Praćenjem vrednosti polifenola tokom skladištenja konstatovano je opadanje vrednosti kod uzoraka upakovanih u PET/Al/PE. Kod ostalih uzoraka utvrđena je cikličnost promena tokom skladištenja. Nakon jačeg pada vrednosti u periodu do 9 meseci, najviše izraženog kod uzoraka pakovanih u kombinaciju PAP/Al/PE u uslovima normalne atmosfere (0,015 mg/100 g) imamo nagli porast vrednosti tako da nakon 12 meseci najveće vrednosti polifenola imamo kod uzoraka pakovanih u PAP/PE u uslovima normalne atmosfere (0,078 mg/100 g). Rezultati istraživanja ukazuju na to da optimalna kombinacija primenjene modifikovane atmosfere i barijernih svojstava ambalažnog materijala pruža dobru zaštitu i dugotrajniju održivost upakovane osušene kajsije u periodu sprovedenog istrazivanja u trajanju od 12 meseci.

Ključne reči: Pakovanje • Modifikovana atmosfera • Osušena kajsija

Phenolic composition, antioxidant and antimicrobial activity of the extracts from *Prunus spinosa* L. fruit

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Abstract

Blackthorn (*Prunus spinosa* L.) is commonly used in food industry and phytotherapy. The contents of phenols, flavonoids, anthocyanins and antioxidative activity in extracts of blackthorn fruit were determined using spectrophotometric methods. The content of total phenol compounds varies from 15.33 to 20.94 mg GAE g^{-1} of fresh fruit. The content of total flavonoids is very low and ranges from 0.419 to 1.31 mg QE g^{-1} of fresh fruits. Anthocyanins content lies between 0.112 mg cyanidin 3-glucoside/g of fresh sample in ethanol extract and 0.265 mg of cyanidin 3-glucoside g^{-1} of fresh blackthorn fruit in methanol-water 50/50 (*V*/*V*) extract. The differences in total phenol compounds content depend on used extraction medium as a consequence of different polarity of used organic solvents and their mixtures, which selectively extract individual compounds. All explored extracts exhibited strong scavenging activity against DPPH radicals, which ranges from 32.05 to 89.10%. Phenolic acids (neochlorogenic and caffeic acids), flavonoids (quercetin and myricetin) and anthocyanins (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and peonidin-3-*O*-glucoside) were identified in investigated ethanol extracts by HPLC analysis. Ethanol extract shows significant antimicrobial activity against S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017 and antifungal activity against *Candida albicans* ATCC 10231. Blackthorn fruit extract exhibits a high phenolic content and a high antioxidant activity, and can be used as an antioxidant in food and pharmaceutical industries.

Keywords: phenolic compounds content, antioxidants, antimicrobial activity.

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Blackthorn (*Prunus spinosa* L.) is a perennial plant growing as a shrub on slopes of wide uncultivated areas, making a thick thorny mass, but it can also be found beside roads, along the channels and in shelterbelts against the wind. It grows in moderate continental climate in northern chemisphere. Blackthorn (*Prunus spinosa* L.) is used in phytotherapy for the treatment of many diseases related to various forms of cough; it is mild laxative, diuretic, spasmolytic and anti-inflammatory agent. It has anti-septic (due to the presence of tannins) effect and shows activity against inflammation of the mucosal layer of the digestive system [1,2].

Medicinal characteristics of blackthorn were shown by fruit, flowers, bark and root of the plant. Apart from phytotherapy, blackthorn is also used in food industry for the production of jams and various beverages: liqueur, wine, juice, compote and tea.

Antioxidative capacity and the content of biologically active compounds in wild fruit species have been previously examined. Due to the complexity of food composition, it is still unknown which dietary constituents are responsible for this behaviour, but it seems that antioxidants play a major role in the protection in plants. The main property of an antioxidant is its ability to trap free radicals which may oxidize nucleic acids, proteins, lipids [3,4]. Fresh fruit extracts are an excellent source of polyphenolic compounds, as free radicals scavengers, which can significantly alleviate the negative effect of free radicals in the organism. Therefore, they have an important role in the prevention of neurodegenerative diseases, cardiovascular diseases and cancer [5].

There is an increasing interest in the measurement and use of wild fruits as antioxidants for scientific research, as well as for industrial (dietary, pharmaceutics and cosmetics) purposes. Aqueous and aqueous-methanolic extracts obtained from six Bulgarian wild edible fruits have been studied for their antioxidant activity and polyphenol content [6]. *Sambucus ebulus* fruits exhibited the highest polyphenol content – 73.73±0.57 mg QE/g in aqueous extracts and 68.27±1.93 mg QE g^{-1} in aqueous-methanolic extracts, while *Prunus spinosa* showed the lowest polyphenol

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content of 9.44 and 9.15 mg QE g^{-1}, respectively. In both types of extracts, the antioxidant activity decreased as follows: *Sambucus ebulus* > *Crataegus monogyna* > *Rosa canina* > *Berberis vulgaris* > *Vaccinium myrtillus* > *Prunus spinosa.* High positive correlation was found between the polyphenol content and the antioxidant activity of these extracts.

In one of the previous studies, seven wild species from Poland were investigated: dog rose (*Rosa canina* L.), blackberry (*Rubus caesius* L.), elderberry (*Sambucus nigra* L.), blueberry (*Vaccinium myrtillus* L.), blackthorn (*Prunus spinosa* L.), rowan (*Sorbus aucuparia* L.) and wild strawberry (*Fragaria vesca* L.). The following order of polyphenol content was established: dog rose > elderberry > blueberry > blackthorn > blackberry > rowan > wild strawberry, while the order of antioxidant activity was: dog rose > blueberry > elderberry > blueberry > blackthorn > rowan > wild strawberry. Polyphenol content of fresh blackthorn fruit exhibited 402.67±12.44 mg GAE·100 g^{-1} f.w., and vitamin C content was 21.94±1.42 mg GAE·100 g^{-1} f.w. Fresh blackthorn fruit shows a significant antioxidant activity amounting to 14.17±3.06 mM Fe·100 g^{-1} f.w. measured by FRAP, and5.33±0.22 μM TE·g^{-1} f.w. by ABTS method. The achieved results indicated a high biological value of the analyzed wild fruit species. [7].

Fresh blackthorn flowers contain a cyanogenic glycoside, which makes investigated plants a mild laxative and diuretic agents. In addition, they contain flavonoids, rutin and hyperoside [3]. Among flavonoids, the following compounds have been isolated from the flowers of *Prunus spinosa* L.: kaempferol, quercetin, kaempferol 3-*O*-*α*-L-arabinofuranoside, quercetin 3-*O*-*α*-L-arabinofuranoside, quercetin3-*O*-*α*-D-xylopyranoside, kaempferol 3-*O*-α-L arabinofuranoside-7-*O*-*α*-L-ramnopyranoside [4,5], tannin, 2% of organic acids (malic, etc.), and water. Blackthorn stones are poisonous because they contain toxic glycoside amygdalin which produces hydrogen cyanide.

Quantitative studies revealed a high content of flavonoids in the flowers of the Polish population of blackthorn (about 2.7% as aglycones and 3.8% as glycosides) [8], while in the Romanian population of this plant it reached around 1.2% as aglycones [9]. Apart from flavonoids, the flowers of blackthorn contain A-type proanthocyanidins [10] and phenolic acids [11]. Studies conducted at the Department of Pharmacognosy, Medical University of Lodz, confirmed the presence of kaempferol, quercetin and their heterosides in the flowers and leaves of blackthorn. In flowers, flavonoids are present mostly in the form of monoglycosides, mainly kaempferol and quercetin 3-*O*-arabinosides. Leaves are abundant in diglycosides, mainly kaempferol 3,7-*O*-dirhamnoside [12]. The structure of these compounds was detected chemically and instrumentally (UV, IR, H-NMR, C-NMR and MS).

The content of flavonoids in the flowers of Romanian blackthorn population (1.16% of total flavone aglycones – quercetin) is the only well established value. It is known that flowers and leaves of blackthorn contain complex of flavonoids, derivatives of flavonol: kaempferol, quercetin and their glycosides with arabinose, rhamnose and xylose [9].

Fraternale *et al.* proved that the antioxidant activity of red berries has been correlated with their anthocyanin content. The results of this study indicate that the three most representative anthocyanins in *P. spinosa* fruit juice (cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside) are likely to play an important role in its antioxidant properties [14].

Despite the wide use of these wild fruits in Serbia, data regarding a complete phytochemical characterization are missing. Therefore, the aim of our work is further examination of phenolic composition (total phenols, flavonoids and anthocyanins), of fresh fruit extracts of *Prunus spinosa* L. from Southeast region of Serbia. The influence of solvent on the extraction of these compounds was investigated. Determination of compounds was performed using HPLC analysis. The antimicrobial activity of compounds from the ethanol extract was checked.

EXPERIMENTAL

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin and $AlCl_3$ were purchased from Sigma Chemical Co (St Louis, MO, USA). Folin–Ciocalteu's phenol reagent and sodium carbonate were purchased from Merck Chemical Suppliers (Darmstadt, Germany). Sodium chlorate buffer (pH 1.0) and acetate buffer (pH 4.5) were purchased from Merck Chemical Suppliers (Darmstadt, Germany).

All other chemicals used, including solvents, were of analytical grade. An Agilent 8453 UV/Vis spectrophotometer was used for absorbance measurements and spectra recording, using optical or quartz cuvettes with 1 cm of optical path. The pH measurements were made with Hanna Instruments pH-meter equipped with glass electrode.

Materials preparation

Blackthorn (*Prunus spinosa* L.) fruits (sloes) were collected in the phase of full ripeness in the region of Southeast Serbia, in October 2009. This region is classified as an ecological complex, with minor negative environmental influences.

Voucher specimens are deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade under the accession number: 16477, BEOU [15]. The plant species were identified by Mirjana Milenković, Faculty of Biology, University of Belgrade. Fresh blackthorn fruits were used for the extract preparation. The prepared extracts were kept in a refrigerator in dark bottles and were analyzed before use.

Preparation of herbal extracts

Fresh blackthorn fruits (*Prunus spinosa* L.) were ground up in a blender. Samples of 2 g of each fruit were weighed from the homogenized herb and extracted with solvents listed in Table 1.

The extraction was carried out in time intervals of 15 min three times with 30, 20 and 20 mL of solvent, respectively. The selected solvents were: water, ethanol (also ethanol–water: 50/50, *V*/*V*), and methanol (and methanol–water: 50/50, *V*/*V*). The extraction was performed in an ultrasonic bath. The extracts were filtered through a Buchner funnel and filter paper (blue collar), (CHMLAB, Spain), transferred into a standard 100 mL flask and the same solvent was added. The liquid extracts were stored in a refrigerator at 5 °C until analysis.

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin–Ciocalteu method [16]. Then, the absorbencies were measured at 765 nm. Gallic acid was used as standard. The calibration line was linear in the range of 0 to 2 mg L^{-1}. The total phenolic content was expressed as mg gallic acid equivalent (GAE) on 1 g of fresh fruit (f.f.). The result of each assay was obtained from three parallel determinations.

Determination of total monomeric anthocyanins

Total monomeric anthocyanin content of the plant extracts was determined using pH-differential method [17]. The colored oxonium form of antocyanins predominates at pH 1.0, and the colorless hemiketal form at pH 4.5. Finally, the absorbance of each sample was measured at $\lambda_{\max}$ 520 and 700 nm, respectively. $A_{\lambda\text{vis-max}}$ and A_{700} were absorbances of each solution at above mentioned wavelengths.

The total absorbance of each solution (A) was calculated from Eq. (1):

$$A = (A_{\lambda\text{vis-max}} - A_{700})_{\text{pH1.0}} - (A_{\lambda\text{vis-max}} - A_{700})_{\text{pH4.5}} \quad (1)$$

The content of the monomeric anthocyanin pigment (MAP) was calculated from Eq. (2):

$$MAP\ (\text{mg L}^{-1}) = (A \times MW \times DF \times 1000)/(\varepsilon l) \quad (2)$$

where ε is molar absorptivity (26.900), MW is the molecular weight of cyanidin-3-*O*-glucoside (449.2 g mol^{-1}), DF is the dilution factor for dilution of extracts by buffer, l is the length of cuvette. Monomeric anthocyanin pigment (MAP) was expressed as mg of cyanidin-3-*O*-glucoside L^{-1}.

Determination of total flavonoid content

The total flavonoid contents were determined using the spectrophotometric method based on the formation of yellow flavonoid complex with aluminum [18]. The absorbance was measured at 420 nm. Quercetin was used as a standard. The calibration line was linear from 0 to 30 mg L^{-1}. The total flavonoid content was calculated using the equation based on the calibration curve and expressed as mg of quercetin equivalent (*QE*) per g of fresh fruits.

Free radical scavenging activity

The free radical scavenging activity of the fruit extracts was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [19,20]. The antioxidant assay is based on the measurement of the loss of colour of DPPH solution by change of absorbance at 517 nm caused by the reaction of DPPH with the test sample. The reaction was monitored with a UV–Vis spectrophotometer.

The ability of extracts to inhibit DPPH (*RSC*, %) was calculated from the decrease of absorbance:

$$RSC\ (\%) = (1 - A_{\text{sample}}/A_{\text{blank}}) \times 100 \quad (3)$$

where A_{blank} is the absorbance of control (1×10^{-4} mol L^{-1} DPPH methanol solution) and A_{sample} is the absorbance of the test sample. The data were expressed as milligrams of quercetin equivalent (*QE*) per 100 g of fresh fruit (mg *QE* 100 g^{-1} f.f.).

Antimicrobial activity

The *in vitro* antimicrobial activity of ethanol extract of *Prunus spinosa* L. was tested against a range of laboratory control strains belonging to the American Type Culture Collection, Maryland, USA (except one, belonging to National Collection of Type Cultures, see below). The antibacterial activity was evaluated against two Gram-positive and three Gram-negative bacteria. Used Gram-positive bacteria were: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The Gram-negative bacteria in the assay were: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017. The antifungal activity was tested against two organisms *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231.

A disc-diffusion method was used for the determination of the antimicrobial activity of the extracts, according to NCCLS [20]. The inocula of the bacterial and fungal strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. 100 μL of suspension containing 1.0×10^{8} CFU mL $^{-1}$of bacteria and 1.0×10^{4} CFU mL^{-1} of fungal spores spread on Mueller-Hinton agar (MHA, Torlak) and sabouraud dextrose agar (SDA, Torlak) respectively, in sterilized Petri dishes (90 mm in diameter). The discs (9 mm in diameter, Macherey–Nagel, Düren, Germany) were impregnated with 20 and 50 μL of extracts (conc. 30 mg mL^{-1}) and placed on the inoculated agar. Negative controls were prepared using the same solvent (ethanol). Tetracycline (30 μg, Torlak) and Nystatin (30 μg, Torlak) were used as positive reference standards to determine the sensitivity of a strain of each tested microbial species. The inoculated plates were kept at 4 °C for 2 h and incubated at 37 °C (24 h) for bacterial strains and at 28 °C (48 h) for fungal strains. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Each assay in this experiment was repeated in triplicates.

High-performance liquid chromatography (HPLC) analysis

The fruit extracts were analyzed by the direct injection of the extracts, previously filtered through a 0.45 μm pore size membrane filter, into the Agilent Technologies 1200 chromatographic system equipped with the Agilent photodiode array detector (DAD) 1200 with RFID tracking technology for flow cells and a UV lamp, an automatic injector, and a Chemstation software. The column was termostated at 30 °C. After injecting 5 μL of the sample extract, the separation was performed in an Agilent-Eclipse XDB C-18 4.6 mm×150 mm column. Two solvents were used for the gradient elution: A – (H_2O + 5% HCOOH) and B – (80% ACN + 5% HCOOH + H_2O). The used elution programme was as follows: from 0 to 28 min, 0.0% B, from 28 to 35 min, 25% B, from 35 to 40 min, 50% B, from 40 to 45 min, 80% B, and finally for the last 10 min again 0% B. The detection wavelengths were 320 and 520 nm. The identification and quantification of phenolic compounds were performed using calibration curves obtained with standard solutions. The results are expressed as mg L^{-1} of fruit extracts.

Statistical analysis

The experimental results were expressed as mean value ± standard error of mean value of three replicates. In order to statistically estimate any significant differences among mean values wherever it was applicable, the data were subjected to a one-way analysis of variance (ANOVA test), and differences among samples were determined by Duncan's Multiple Range test using the Statistical Analysis System software (SAS) [22].

The data were reported as mean ± standard deviation (*SD*) for triplicates. The significance of inter-group differences was determined by the analysis of variance (ANOVA). The p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The total phenol content in the investigated samples of blackthorn (*Prunus spinosa* L.) fruits extracts was determined by Folin–Ciocalteu method and shown in Table 1.

The experimental results show that the content of total phenols in the investigated extracts was significant, ranging from 15.33 mg GAE g^{-1} f.f. (water) to 20.94 mg GAE g^{-1} f.f. (ethanol–water 50/50, *V*/*V*) extract.

Earlier, the total phenolic content in plum fruits which was reported ranged from 0.42–4.13 mg GAE g^{-1} f.f. [23]. Uzelac reported data regarding the phenol content in blackthorn (*Prunus spinosa* L.) fruits. It is considerably lower than our values and amounts from 0.546 to 0.86 mg GAE g^{-1} f.f. (obtained by extraction with 80% ethanol solution) [13]. The results of this study support the results of our work regarding the values of total phenols. Fraternale et al. [14] proved in their study that the polyphenolic content in *Prunus spinosa* L. was 83.5±2.5 mg/g DW, that is significantly higher than our values, which may be due to differences in the expression of results sincethey are expressed in relation to dry substance, and the results of the present study are expressed in the relation to the fresh fruit.

Egea *et al*. investigated the antioxidant activity and the phenolic composition of several wild fruits including *P. spinosa*. Based on the fact that the plants were collected from different areas of the Mediterranean region, the results obtained in our study showed differences in total phenolics content per gram of fresh fruit [24].

Barros *et al.* investigated the dried fruits of strawberry-tree, blackthorn and dog rose and found that the

Table 1. The total phenolic, anthocyanin and flavonoid content and antioxidant activity of fruit extracts (Prunus spinosa *L.)*

Sample	Total phenols content[a]	Anthocyanin content[b]	Flavonoid content[c]	*RSC* / %	RSC[d]
Ethanol extract	15.33±0.19	0.11±0.008	0.700±0.10	47.38±0.02	2.76±0.36
Ethanol–water extract (1:1)	20.94±0.74	0.238±0.03	1.242±0.09	72.12±0.05	4.25±0.06
Methanol extract	15.33±0.98	0.17±0.01	1.31±0.17	89.10±0,02	5.24±0.83
Methanol–water extract (1:1)	17.69±0.41	0.265±0.01	1.18±0.18	75.69±0.22	4.45±0.45
Water extract	12.17±0.19	0.12±0.005	0.42±0.013	32.05±0,85	0.86±0.08

[a]Expressed as mg GAE g^{-1} f.f.; [b]expressed as mg of cyanidin-3-*O*-glucoside g^{-1} f.f.; [c,d]expressed as mg QE g^{-1} f.f.

ethanolic extracts of the fruits contain considerable amounts of phenolic compounds, affecting the antioxidant capacity of the fruits. Due to the fact that in the Southeast region of Serbia, the local people consume the investigated fruits in their fresh form, we examined the free radical scavenging capacity, as well as the bioactive compounds content of the fresh fruits in our study [25].

The antioxidant activity of the plant extracts was determined by DPPH method. All tested extracts exhibited strong scavenging activity against DPPH radicals, which ranged from 32.05 to 89.10%. Correlation coefficient between the total phenols and antioxidant activity was very low (R^2= 0.325).

Nevertheless, there are reports with low correlation between total phenolic contents and radical scavenging capacity. The high free radical scavenging capacity of the wild plants might be attributed not only to phenolic composition, but also to the presence of other bioactive compounds, such as vitamins (ascorbic acid, tocopherols) and pigments (anthocyanins), as well as the structural interaction among these compounds [26].

The colour of blackthorn (*Prunus spinosa* L.) fruits extracts originates from the anthocyan content. Anthocyan content lies between 0.112 mg cyanidin 3-glucoside g^{-1} of fresh sample in ethanol extract, to 0.265 mg of cyanidin 3-glucoside g^{-1} of fresh blackthorn fruit sample in methanol-water 50/50, *V*/*V* extract (Table 1). The content of monomer anthocyans in all extracts is very similar. The correlation coefficient for monomer anthocyans and antioxidant activity is R^2 = 0.5072. This value is lower than the value reported by Uzelac (0.305 compared to 0.497 mg cyanidin-3-*O*-glucoside g^{-1} f.f., 80% ethanol solution). The highest amount of total anthocyanins was observed in dark purple fruits: 0.413 mg cyanidin-3-*O*-glucoside g^{-1} f.f. [13].

The total level of anthocyanins in *Prunus spinosa* L. juice was 55.1±5.6 mg g^{-1} DW, where the determined values were considerably higher than ours. In accordance with reported data for different fruits and vegetables, the total polyphenol and anthocyanin content appeared to contribute significantly to the antioxidant activity of sloe fruits (cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside) [14].

The content of total flavonoids in the investigated extracts of the plant fruit is low. The flavonid content ranges from 0.4 (water) to 1.3 mg (methanol) QE g^{-1} f.f. The correlation coefficient between the total flavonoids content and antioxidant activity is very high (R^2 = = 0.9618). On the basis of the obtained results we can conclude that the flavonoid compounds have significant contribution as antioxidants in the investigated plant fruits.

The data reported by Uzelac are lower than our values and amounts vary from 0.437 to 0.656 mg QE g^{-1} f.f. obtained by the extraction with 80% ethanolic solution [13].

The differences in phenolic compounds content depend on the used extraction medium as a consequence of different polarity of used organic solvents and their mixtures.

Using HPLC-DAD techniques, a comparative analysis of the quantities of phenolic compounds in investigated extracts was performed. The peaks were identified comparing their HPLC retention times and UV-Vis absorption spectra. Individual compounds and their content in tested extracts are shown in Table 2.

Phenolic acids (neochlorogenic and caffeic acids), flavonoids (quercetin and myricetin) and anthocyans (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, peonidin-3-*O*-glucoside) were identified in investigated extracts by HPLC analysis. Only anthocyanins were found in water extract, while in ethanol and ethanol-water extract, phenolic acids and flavonoids were determined.

Fraternale *et al.* [14] identified the following anthocyanins using HPLC analysis: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside chloride and peonidin-3-*O*-rutinoside chloride, which is in agreement with the results obtained in this study.

We chose the ethanol extract, which approximately has the same content of active substances as methanol extract, but it is not toxic, for the antimicrobial analysis.

The antimicrobial activity was analyzed by the measurement of the inhibition zone. Antimicrobial activity of the prepared ethanol fruit extracts (*Prunis spinosa* L.) is given in Table 3.

Table 2. HPLC Analysis of the fruit extracts (Prunus spinosa L.)

Time, min	Compound	Ethanol extract contents mg L^{-1}	Ethanol–water extract content mg L^{-1}	Water extract content mg L^{-1}
15.6	Neochlorogenic acids	12.26±0.2	16.95±0.3	–
19.8	Caffeic acids	2.12±0.1	9.73±0.2	–
20.8	Myricetin	–	8.86±0.2	–
23	Cyanidin-3-*O*-glucoside	1.1±0.1	0.9±0.1	1.1±0.1
24	Cyanidin-3-*O*-rutinoside	1.1±0.1	3.1±0.2	1.5±0.1
25	Peonidin-3-*O*-glucoside	–	1.2±0.1	2.2±0.1
26.5	Quercetin	4.02±0.2	3.83±0.2	–

Table 3. Antimicrobial activity of ethanol fruit extracts (Prunus spinosa L.); Zone of inhibition (mm); Data are expressed as the mean of three replicates ± standard deviation

Test microorganism	Extract (10 mg mL^{-1})		Reference antibiotic	Reference antimicotic	Negative control
	20 µL	50 µL	Tetracycline (30 µg)	Nistatin (30 µg)	Ethanol, 96%
Salmonella abony NCTC 6017	–	19.0±0.2	28.0±0.3	–	–
Escherichia coli ATCC 25922	11.0±0.1	24.0±0.3	30.0±0.3	–	–
Pseudomonas aeruginosa ATCC 9027	13.0±0.15	23.0±0.3	19.0±0.2	–	–
Bacillus subtilis ATCC 6633	–	–	36.0±0.4	–	–
Staphylococcus aureus ATCC 6538	13.0±0.2	18.0±0.2	34.0±0.3	–	–
Candida albicans ATCC 10231	14.0±0.2	23.0±0.3	–	20.0±0.3	–
Aspergillus niger ATCC 16404	–	–	–	19.0±0.3	–

Ethanol fruit extract (*Prunus spinosa* L.) showed antimicrobial activity against all tested microorganisms (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NCTC 6017) except *Bacillus subtilis* ATCC 6633. The antifungal activity was tested against two organisms *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. The investigated extract exhibits antifungal activity against *Candida albicans* ATCC 10231.

The antimicrobial activity of tested extracts was directly correlated to the quantity and structure of extracted compounds. Significant antimicrobial activity against S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017 was detected. The ethanol extracts exhibited antifungal activity against *Candida albicans* ATCC 10231.

CONCLUSION

From the medical point of view, the investigated extracts contain a high percentage of polyphenolic compounds and show a significant antioxidant and antimicrobial activity. Alcohol solutions have a higher content of phenols than water extracts. All extracts contain significant amount of anthocyanins. The content of flavonoids in alcohol solutions is uniformed and it is higher than the content of flavonoids in water extract. Therefore, alcohol solutions are more suitable for extraction processes. Ethanol extracts are more suitable than methanolic because they are not toxic. All tested extracts exhibited strong scavenging activity against DPPH radicals. Ethanol solution of the investigated plant showed antimicrobial activity against all tested microorganisms except *Bacillus subtilis*, and a species of mold, *Aspergillus niger*. On the basis of obtained results, we concluded that the investigated blackthorn (*Prunis spinosa* L.) fruit extracts have significant biological importance as antioxidant and microbiological agent.

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IZVOD

SADRŽAJ FENOLA, ANTIOKSIDATIVNA I ANTIMIKROBNA AKTIVNOST EKSTRAKATA PLODA TRNJINE (*Prunus spinosa* L.)

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(Naučni rad)

Trnjina (*Prunus spinosa L.*) se najčešće koristi u prehrambenoj industriji i fitoterapiji. Sadržaj fenola, flavonoida, antocijana i antioksidativna aktivnost određeni su primenom spektrofotometrijske metode. Sadržaj ukupnih fenolnih jedinjenja varira od 15,33 do 20,94 mg GAE g^{-1} svežeg voća. Sadržaj ukupnih flavonoida je veoma nizak, i kreće se od 0,419 do 1,31 mg QE g^{-1} svežeg voća. Sadržaj antocijana je od 0,112 mg cijanidin 3-glikozida g^{-1} svežeg uzorka u etanolnom ekstraktu do 0,265 mg cijanidin 3-glikozida g^{-1} svežeg voća u methanol–voda (50/50, *V*/*V*) ekstraktu. Svi testirani ekstrakti pokazuju visoku antioksidativnu aktivnost u odnosu na DPPH radikale, koja se kreće od 32,05 do 89,10%. Fenolne kiseline (neohlorogenska i kafeinske kiseline), flavonoidi (kvercetin i miricetin) i antocijani (cijanidin-3-*O*-glikozid, cijanidin-3-*O*-rutinozid i peonidin-3-*O*-glikozid) su identifikovani u ispitivanim ekstraktima HPLC analizom. Etanolni ekstrakti pokazuju značajnu antimikrobnu aktivnost prema bakterijama: S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 i *Salmonella abony* NCTC 6017 i antifungicidnu aktivnost prema *Candida albicans* ATCC 10231. Ekstrakti svežeg ploda trnjine pokazuju visok sadržaj fenolnih jedinjenja i visoku antioksidativnu aktivnost, pa se mogu koristiti kao antioksidansi u prehrambenoj i farmaceutskoj industriji.

Ključne reči: Sadržaj fenola • Antioksidanti • Antimikrobna aktivnost

Polysaccharides of higher fungi: biological role, structure and antioxidative activity

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Abstract

The fungal polysaccharides attract a lot of attention due to their multiple challenging biological properties, such as: anti-tumor, anti-viral, anticomplementary, anticoagulant, hypolipidemic, immunomodulatory and immune-stimulatory activities, which all together make them suitable for application in many quite distinctive areas, such as food industry, biomedicine, cosmetology, agriculture, environmental protection and waste water management. This article presents results with respect to biological properties, structure and procedures related to the isolation and activation of polysaccharides of higher fungi. It is considered and presented along with a review of the critical antioxidative activity and possible influence of the structural composition of polysaccharide extracts (isolated from these higher fungi) upon their antioxidative properties.

Keywords: polysaccharides, fungi, polysaccharide extracts, antioxidative properties.

REVIEW PAPER

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The mycotherapy became acknowledged in the past 50 years as a new approach to the treatment in contemporary medicine and immunology. A wide spectrum of strong medicinal effects of fungi comes from some of their components. Fungi contain numerous biologically active compounds, such as various polysaccharides (β-glucans, chitin and heteropolysaccharides), terpenes, phenols, essential amino acids, lectins, nucleotides, sterols and unsaturated fatty acids. Besides, they also contain many minerals and vitamins. The active components of fungi are today known for their anti-inflammatory and anti-tumor properties, prevention of cardiovascular diseases, antiparasitic, heaptoprotective and anti-diabetic, antimicrobial and antioxidative properties [1–4]. The active substances of fungi affect the maintenance of biological homeostasis and re-establishment of the balance and natural defence system of the organism. Nowadays, in the medicine and pharmacology of the Far East, numerous preparations made from medicinal fungi with antitumor and immunomodulating properties are produced in the form of capsules, ampoules, extracts and teas. The Japan lentinan, a polysaccharide obtained from the mushroom *Lentinula edodes* and krestin (PSK) obtained from the mushroom *Coriolus versicolor,* has been registered as anti–cancer drug since mid-1980s and it is among the first 10 anti-tumor preparations in that market [5]. Therefore, nowadays, fungi are considered to be functional food and a source of physiologically important components.

HIGHER FUNGI AS A SOURCE OF BIOLOGICALLY ACTIVE POLYSACCHARIDES

Polysaccharides of higher fungi are used as adaptogens and immunostimulators. The immunostimulatory effect of polysaccharides is prophylactic and it belongs to a non-invasive form of treatment, for instance the prevention of infectious disease and of tumor metastases. These molecules do not directly attack pathogens related to the cancer cells. They achieve their anti-tumor effect indirectly, through activation of various defensive immune responses [6–10].

The immunomodulating properties of polysaccharides of higher fungi include mitogenic activity, stimulation of the pluripotent stem cell in the process of hematopoiesis, activation of the alternative complement pathway and activation of the immune system cells such as macrophages, T helper cells (Th cells) and cytotoxic T cells (Tc cells), natural killer cells (NK cells) and B cells [15]. Recent investigations have shown that the receptors, such as the Complement Receptor Type 3 (CR 3, $\alpha_M\beta_2$ integrin or CD11b/CD 18) on phagocytes and NK cells and the Toll-like receptor group, i.e. TLR-2 and TLR-4, as well as Dectin-1 on the macrophage surface, are the main receptors that are specifically

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recognized and bound by polysaccharide molecules [11–14]. Interactions among them further stimulate the cascade of the immune response including the production of cytokines such as interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10) and interferon-γ (INF-γ) and the tumor necrosis factor-α (TNF-α) [15]. Polysaccharides of higher fungi have effects on the maturation, differentiation and proliferation of the immune system cells through the network of the spectrum of interleukines and interferons, Figure 1.

More than 650 types of medicinal fungi are known today. Besides the immunostimulatory and anti-tumor effects, their polysaccharides have additional beneficial effects such as the suppression of damages caused by cytostatics and radiation to the immune system cells [16–18].

Polysaccharides isolated from various types of fungi may significantly affect the support of the immune system of an organism in its fight against infectious disease such as parainfluenza viruses and rhinoviruses, herpes simplex and herpes zoster viruses, cytomegalovirus, Epstein–Barr virus, hepatitis viruses [19,20]. Lentinan obtained from the mushroom *Lentinula edodes* in combination with azidothymidine (AZT) reduces the expression of human immunodeficiency virus (HIV) on the surface of T cells to a significantly higher extent than only AZT application [4,21]. According to the medical protocols, all strategies applied in the treatment of acquired immunodeficiency syndrome (AIDS) are based on the activation of the immune system of the body, thus creating the most effective immunological response. Besides that, β-glucans of fungi are used today effectively in the treatment of dermatitis, such as bacterial foot infections in athletes [22].

The majority of active fungal polysaccharides, soluble or unsoluble in water, may be classified as dietetic fibres (β-glucans, chitin, various heteropolysaccharides). Fungal dietetic fibres protect the colon mucous membrane from detrimental effect of certain substances, and at the same time they serve as a good basis (prebiotics) for the stimulation of growth of beneficial bacteria (probiotics) that have already been present in the gastrointestinal tract and that prevent, through mutual interaction, the development and occurence of diabetes, atherosclerosis, hypertension and trombosis. Nowadays, preparations based on dietary fibres isolated from various types of fungi have a worldwide commercial value of USD 5-6 billion a year [4,22,23].

Globally, the number of newly registered patents based on polysaccharides of higher fungi has been increasing. The schizophyllan, curdlan, lentinan and scleroglucan have been used as nucleic acid-binding agents since 2003. The obtained complexes of polysaccharides (glucans) and nucleic acids are used as gene

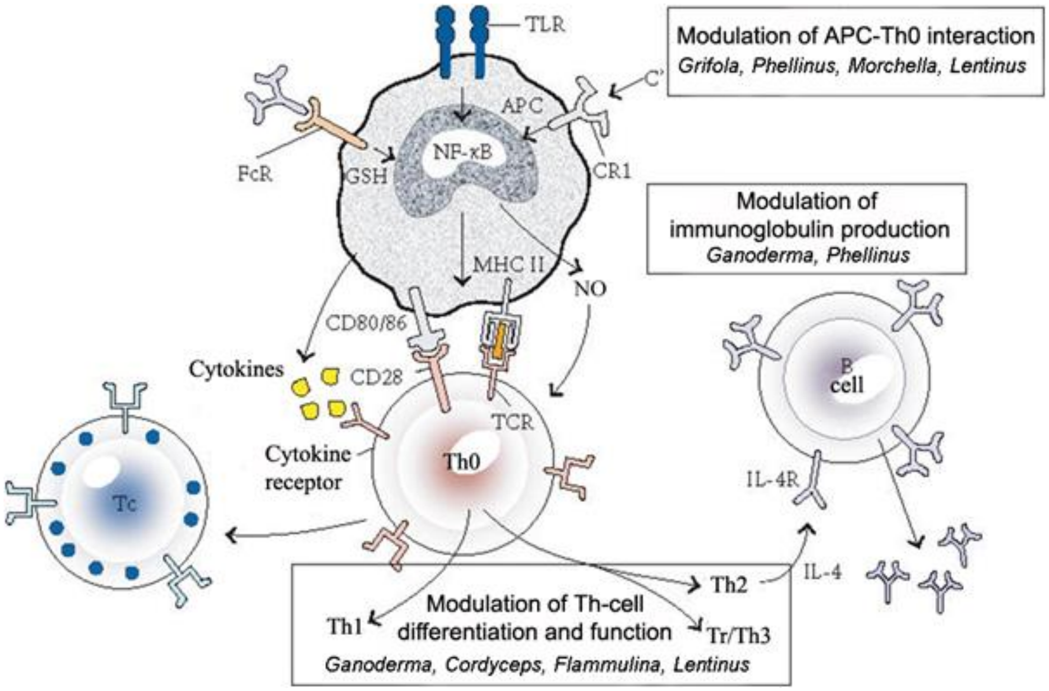

Figure 1. Schematic representation of the possible targets of the adaptive immune system for mushroom ingredients with immunomodulatory properties. APC: antigen-presenting cell; FcR: Fc receptor; TLR: Toll-like receptor; CR1: complement receptor type 1; C': activated complement; GSH: glutathione; MHC II: major histocompatibility complex class II; TCR: T-cell receptor; Th: helper T cells; Tc: cytotoxic T cells; Tr: regulatory T cells; NO: nitric oxide; IL-4: interleukin-4; IL-4R: interleukin-4 receptor; CD: cluster designation [15]. (Figure reprinted with permission from Hindawi Publishing Corporation).

therapy vectors, for the treatment of genetic diseases. Such complexes exhibit resilience to nucleases and serve as the protective structures of nucleic acids [22].

β-Glucans of higher fungi are used in cosmetology, in protective creams that prevent irritation and diseases of the skin. They are also used to protect the skin from ultraviolet radiation [22].

Structure of biologically active polysaccharides

About 650 types and 7 interspecific taxa from 182 genera of higher hetero- and homobasidiomycetes are known today, they contain pharmacologically active polysaccharides which may be isolated from the carpophore, mycelium or cultivation media [4,24].

β-D-glucans together with molecules of chitin, celluloses and glycoproteins form the fungal cell wall [23,24].

Lentinan from the mushroom *Lentinula edodes* has been the most studied fungal glucan, Figure 2. The basis of this molecule comprises the main chain made of glucose molecules connected by *β*-(1→3)-glycosidic linkage. One part of the glucose units from the main chain is substituted *via* the position of O-6, with side chains that are linked by *β*-(1→6)-glycosidic linkage. The molecular weight of lentinan approximately amounts to 500 kDa, the branching level is 0.40 (2/5)*, and solved in water it makes a triple helical conformation [25,26]. Among the immunomodulatory polysaccharides, which role and structure have been studied in detail, are grifolan, obtained from the fungus *Grifola frondosa*, schizophyllan, obtained from the fungi *Schizophyllum commune*, *Sclerotonia sclerotiorum* glucan (SSG), PSK and the polysaccharide-peptide complex (PSP), obtained from the fungus *Coriolus versicolor*.

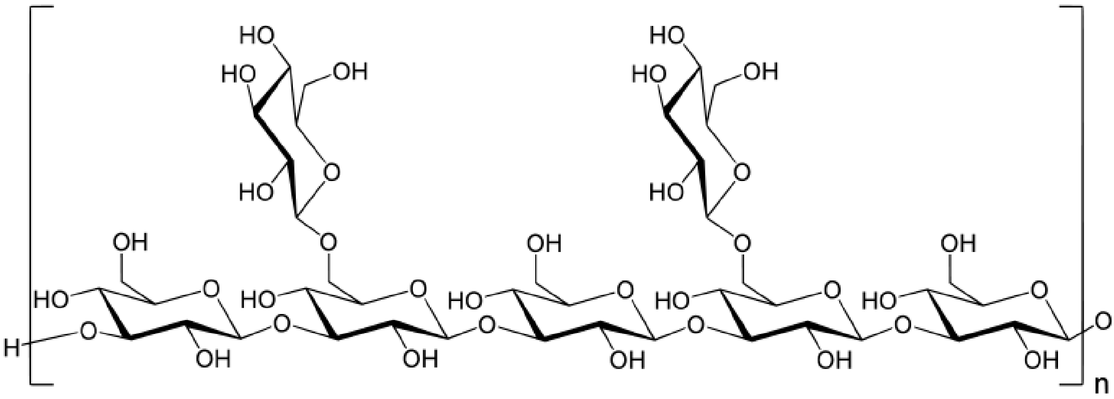

Figure 2. Chemical structure of lentinan [27]

Also, some other sugar molecules may be bound to molecules of *β*-D-glucan, such as mannose, xylose, galactose or uronic acids, and also peptides and protein molecules [24]. The investigation of their structure has confirmed that these macromolecules have a triple helical conformation, Figure 3 [28,29].

The biomodulating activity of various myco-D-glucans is depending on their chemical properties, such as molecular weight, branching level, solubility in water and tertiary structure. Research efforts focused on the structural identification of biologically active polysaccharides of higher fungi are hot topics today [31,32].

Isolation of polysaccharides

The extraction procedure and the method used for the purification of the obtained fractions of active polysaccharides depend in many aspects on the type of a fungus and physical properties of polysaccharides, such as solubility, molecule conformation, branching level, molecular weight. The selection of extraction methods depends on the biosynthesis site and the activity in the cell, transportation and their biological function. This is why non-polar organic solvents are used, for example, for substances that exhibit their activity in the inner part of the cell. However, the aqueous solutions are used for substances that exhibit their activity on the cell surface [33].

After two decades of intensive investigations related to the medicinal fungi, reliable procedures for effective extraction, fractioning and purification of polysaccharides from the carpophore or mycelium have been devised. In general, this scheme includes the extraction of polar compounds with the use of 80% ethanol, after which three successive extractions were applied, with water (100 °C, 3 h), 2% ammonium oxalate (100 °C, 6 h) and 5% sodium hydroxide (80 °C, 6 h) [5]. The first extraction results in a polysaccharide fraction that is dissolved in water, while the other two

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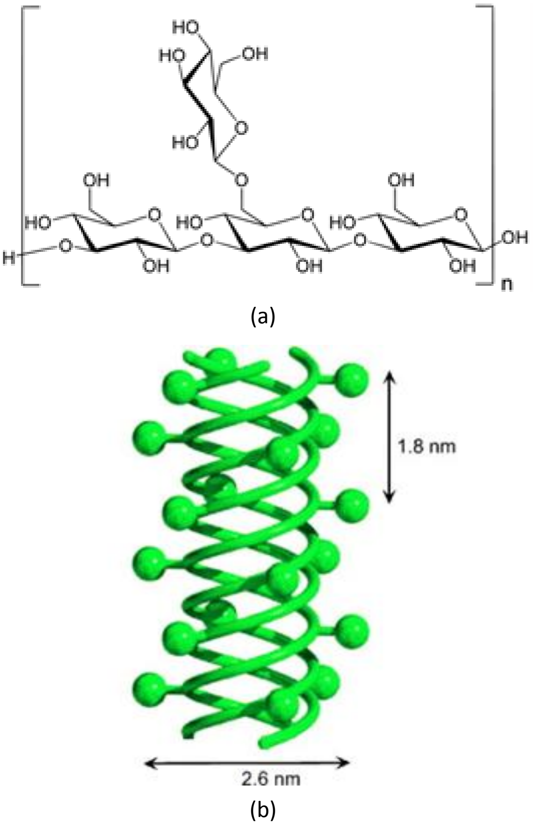
*The branching level is the number *β*-(1→6)-glycosidic linkages of the side chain divided by the number *β*-(1→3)-glycosidic linkages of the main chain.

fractions of polysaccharides are water-insoluble. Extracted polysaccharides may further be purified through the combination of various methods, such as fraction precipitation by ethanol in various concentrations, precipitation performed by acetic acid, ion exchange chromatography, affinity chromatography, gel filtration [24].



(a)

(b)

Figure 3. Repeating unit of schizophyllan (a); a representative model of the triple helix (b) [30]. (Figure reprinted with permission from Elsevier).

Through hot water extraction of the basidiomycete *Lentinula edodes* the polysaccharide lentinan was obtained, which was then followed by precipitation in 70% ethanol and lyophilization of the precipitate. Repeated precipitation in alcohol led to a polysaccharide extraction of 87.5% purity [34].

Activation of polysaccharides by chemical modification

Modified biologically active polysaccharides have significant application due to their improved properties, such as, for example, the increase in solubility in aqueous solvents [35]. There are various methods of chemical modification of fungal polysaccharides that yield the best defined products for further use. The two basic procedures by which modified polysaccharides of fungi can be obtained for clinical application are: a) the modification by Smith degradation and b) activation with the formolysis method. These procedures are the most successfully developed for the polysaccharides from the fungi *Ganoderma lucidum*, *Grifola frondosa* and *Leucopaxillus giganteus* (= *Triholoma gigantea*). Carboxymethylation is an additional chemical method that results in derivates of water soluble β-glucans, as opposed to the initial native polymers [24].

Enzymatic modification by cellulases, amylases and proteases is used to obtain modified biologically active glucans with a lower molecular mass [36,37]. Acetylated linear α-(1→4)-glucan which has a molecular mass of about 5 kDa, was obtained by enzymatic reduction of side chains from active hexose correlated compound (AHCC) and it is considered to be main active ingredient. AHCC is a product prepared by cultivation and enzymatic modification of several species of mushroom mycelia, including shiitake, grown in rice bran extract as the primary food source and contains a mixture of polysaccharides (~74% of AHCC from where ~20% being of the α-1,4-glucan type), proteins, lipids and minerals [38].

OXIDATIVE STRESS, FREE RADICALS, ANTIOXIDANTS

Free radicals (FRs) are involved in pathological processes (*diabetes mellitus,* HIV infection, autoimmune, neurodegenerative, coronary, malignant, pulmonary, inflammatory and many other diseases) and they are part of the complex pathophysiological mechanisms of cell damage [39–41]. When the chain of FRs reactions is started, they tend to spread in space and time, with an increasing effect. This propagation is reflected in the occurance of secondary FRs that continue to spread to a cascade of chain reactions [41].

The mechanisms by which these pathologies develop involve generally oxidative alteration of physiologically critical molecules, including proteins, lipids, carbohydrates and nucleic acids, along with modulation of gene expression and the inflammatory response.

The human organism has developed defense systems to deal with this oxidative stress. These include expression of enzymatic systems, *via* activation of Nuclear Factor Kappa B (NF-κB) transcription factors, especially superoxide dismutases, catalases, gluthation peroxidases and thioredoxin systems, which are recognized as being highly efficient in reactive oxygen species (ROS) detoxification. The main nonenzymatic antioxidants present in the human organism are gluthation, bilirubin, estrogenic sex hormones, uric acid, coenzyme Q, melanin, melatonin, α-tocopherol and lipoic acid [42,43,46].

NF-κB also plays a central role in the processes of inflammation and immunity. While certain NF-κB-regulated genes play a major role in regulating the amount of ROS in the cell, ROS has various inhibitory or stimulatory roles in NF-κB signaling. In such a way, ROS can directly interact with the components of the NF-κB pathway leading to its up- or down-regulation. In spite of this, the appearance of ROS in the nucleus might exclusively lead to the reduction of NF-κB binding to DNA, which may also cause inflammation processes and pathological conditions [43–45].

Oxidative degeneration of fats and oils in food has an significant impact on the quality and safety of food due to the occurrence of secondary, potentially toxic compounds. The addition of antioxidants is necessary to preserve the colour and taste of nutritious products, and to avoid vitamin degradation [46]. Antioxidants are not only used in the food industry, but also in cosmetology and in the pharmaceutical industry, as well as in the manufacturing of tyres and fuels (gas and biodiesel), in order to prevent oxidative degradation processes.

Natural antioxidants, such as vitamins (A, E and C), minerals (Se and Zn), proteins (transferrine, ceruloplasmin, albumin), glutathione and polyphenols and flavonoids possess the property of catching free radicals, thus inhibiting oxidative mechanisms that lead to degenerative diseases [41]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) are largely applied in food industry, where they are used as natural supplements to antioxidants for the purpose of improvement of their efficacy. However, despite their high efficacy in the process of catching free radicals, there are indications that some of synthetic antioxidants exhibit adverse, toxic effects [47, 48]. Therefore, increased attention is paid to investigations of new natural sources of antioxidants. These investigations are focused on the decrease of the production costs and on the improvement of solubility of such substances in emulsions used in the food industry. Some of well-known natural sources of antioxidants are isolated from spices such as oregano, thyme, dictamon, marjoram, lavander and rosemary, but have limited application due to their strong and specific taste. Additive industry had a turnover of USD 3.7 billion at the US market in the year 2007, based on antioxidants, with the tendency of further growth by about 3% *per annum* [49,50].

Today, the broader impact of proper nutrition on human health becomes increasingly important. Both investigations and clinical studies indicate that nutrition rich in foodstuffs of plant origin, such as fruits, vegetables, grains, sprouts, and the consumption of teas, red wine and juices ensures the reduction of risks of development of human disease [51], including cancer and cardiovascular diseases that are the leading cause of death in the modern world. Most antioxidative components in the typical nutrition comprising foodstuffs of plant origin belong to various classes of phenolic compounds. Phenolic acids, polyphenols and flavonoids in fruits and vegetables are classified into the group of non-essential dietary components. The bioactivity of phenols may be associated with their property to chelate metals, inhibit lipoxygenases and catch free radicals, because of which they are considered as basic components that have a favourable effect on health [52–55]. Some authors have pointed out that the antioxidating effect of foodstuffs of plant origin is in correlation with the content of complexes made by phenolic compounds with lignins and arabinoxylans. Such complexes isolated from barley and malt have shown twice higher antioxidative activity than the extract of free phenolic compounds from the same material [56,57]. Other authors believe that the antioxidative activity is not directly connected with free or bound phenolic compounds [58].

DETERMINATION OF ANTIOXIDANT ACTIVITY

The various methods are used in order to measure antioxidative properties that are appropriate for various levels of antioxidative activity, such as methods based on the transfer of electrons and hydrogen atom [59], the ability to chelate ferrous (Fe^{2+}) ions, the method of electron spin resonance (ESR), erythrocyte hemolysis, and the monitoring of the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), Table 1.

During the determination of antioxidative properties of the tested samples, which are primarily foodstuffs, one should take into consideration the fact that the total antioxidative capacity results from a combined activity of various antioxidants that are present in the tested material, their respective concentrations and possibilities for synergic or antagonistic reactions [46].

In order to determine antioxidative properties of the tested samples it is necessary to apply a number of different methods based on various reaction mechanisms. Some components may give completely different responses, depending on the applied method [52,54,59,60].

Antioxidants can exercise their protective properties at different stages of the oxidation process and by different mechanisms. There are two main types of antioxidants, namely, primary (chain breaking, free radical scavengers) and secondary or preventive. Secondary antioxidant mechanisms may include deactivation of metals, inhibition of breakdown of lipid hydroperoxides to unwanted volatile products, regeneration of primary antioxidants, singlet oxygen quenching, etc. [61]. But also it should be kept in mind that antioxidants often act *via* mixed mechanisms that combine different types of antioxidation.

Table 1. List of antioxidant methods [59,60]

Name of the method
Hydrogen Atom Transfer methods (HAT)
Oxygen radical absorbance capacity (ORAC)
Lipid peroxidation inhibition capacity (LPIC)
Total radical trapping antioxidant parameter (TRAP)
Inhibited oxygen uptake (IOC)
Crocin bleaching nitric oxide radical inhibition activity
Hydroxyl ($HO^{\bullet}$) radical scavenging activity by *p*-butrisidunethyl aniline (PNDA)
Scavenging of peroxide ($ROO^{\bullet}$) radicals
ABTS radical scavenging method
Scavenging of super oxide ($O2^{\bullet-}$) radical formation by alkaline (SASA)
Electron Transfer methods (ET)
Trolox equivalent antioxidant capacity (TEAC) decolourization
Ferric reducing antioxidant power (FRAP)
1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay
Cupric ion reducing antioxidant capacity (CUPRAC)
Total phenols by Folin-Ciocalteu
N,N-dimethyl-p-phenylenediamine (DMPD) assay
Other Assays
Total oxidant scavenging capacity (TOSC)
Inhibition of Briggs–Rauscher oscillation reaction
Chemiluminescence
Electrochemiluminescence
Fluorometric Analysis
Enhanced chemiluminescence (ECL)
TLC bioautography
Cellular antioxidant activity (CAA) assay
Dye-substrate oxidation method

Besides the mechanism of antioxidants effects, it is extremely important to understand the antioxidant and pro-oxidant behavior of bioactive substances according to their structure, chemical environment and the experimental conditions involved. For example, gallic acid and epigallocatechin gallate, antioxidative tea components, exhibit high antioxidative activity, but they may also act as pro-oxidants [62,63]. Moreover it is now well established that flavonoids can have a pro -oxidant effect [64] in the presence of Cu^{2+}. Also it is established that β-carotene behaves only as a radical-trapping antioxidant, when the partial oxygen pressure is below 20 kPa (normal ambient oxygen pressure). However, when the pressure increases, β-carotene loses its antioxidant activity and has an autocatalytic pro-oxidant effect which increases with its concentration [46,65].

An important factor, when antioxidant efficacy of compounds is evaluated, is also medium polarity. Antioxidants behave differently in media with different polarities and phase states, *e.g.*, antioxidants have been difficult to evaluate in oils and food emulsions due to the complex interfacial phenomena involved [66]. Many studies, concerning methods for measuring antioxidant activity in organic media, have shown that hydrogen bonding of solvents can induce a sharp change in the antioxidant capacity of phenol compounds and especially in their ability to shed an H atom [46]. Hydrogen bonding in the solvent also affects the antioxidant mechanism. Hence, quercetin does not function like chain-breaking antioxidant in tert-butyl alcohol, which is a protic medium, but rather as retarder, while it acts like chain breakers in chlorobenzene, which is an aprotic solvent [67].

Moreover, the natural antioxidants have been difficult to evaluate in oils and food emulsions in view of the complex interfacial affinities between involved air-oil and oil-water interfaces and the questionable conditions and methodology used to follow oxidation [66]. This is clearly illustrated by Porter's polar paradox [68] in which polar antioxidants, *e.g.*, Trolox, are more often active in lipid solutions than apolar antioxidants, *e.g.*, α-tocopherol, whereas apolar antioxidants are more efficient in emulsion media than their polar homologues. This paradox is based on the interface properties of antioxidants, on their partitioning in multiphase media [66] and on the fact that lipid oxidation is initiated at the system interfaces.

Apart from the complexity of the entire process of determining the antioxidant activity of the various components, it must be kept in mind that *in vitro* assays can only rank antioxidant activity for their particular reaction system and that their relevance to *in vivo* health protective activities is uncertain. Therefore, it is prudent to use more than one type of antioxidant assay to measure antioxidant activities, and to include at least one assay that has biological relevance.

ANTIOXIDATIVE PROPERTIES OF FUNGI

Besides many plants, fungi are significant sources of antioxidative components. Some edible fungi, such as *Agaricus bisporus*, *Grifola frondosa*, *Hericium erinaceus*, *Flammulina velutipes*, *Lentinula edodes*, *Pleurotus eryngii* and *Pleurotus ostreatus* have the significant antioxidative activity and the ability to catch free radicals [69––72]. Such properties have also been proven in case of fungi that are important in medicine, such as *Agaricus brasiliensis*, *Sparassis crispa*, *Phellinus linteus*, *Ganoderma lucidum* and *Inonotus obliquus* [73–76]. It is generally accepted that extracts of fungi comprise many components and each of them has its own biological effect. The method of extraction, *i.e.*, the type of the applied solvents for the extraction directly affects antioxidative properties of fungi [73,77,78]. It has been established that extracts from fungi that have been obtained by water extraction comprise various polysaccharides, phenolic compounds and proteins, including lectins [74–77,79]. Fungi also accumulate a range of various secondary metabolites, which include, besides phenols, polyketides, terpenes, flavonoids and steroids [3,4].

Antioxidative properties of polysaccharide extracts of higher fungi

The antioxidative activity of polysaccharides is attributed to their ability to scavenge free radicals, their reduction property and ability to chelate Fe^{2+}, lipid peroxidation inhibition, erythrocyte hemolysis and the increase of activities in eukaryotic as well as in prokaryotic cells of enzymes that take part in antioxidative processes, such as SOD, CAT and GPx [80–84]. In some researches, besides the increase in the enzyme activity, antioxidative properties are also attributed to the property of polysaccharides to induce an increased expression of antioxidative enzymes, such as SOD and GPx [85].

Polysaccharide extracts of higher fungi with antioxidative properties are most frequently obtained through the extraction procedures that involve hot water and alcohol precipitation, and alkaline extraction [75––77,80,86]. The concentration of polysaccharides, as bioactive compounds, depends a lot on the stage of development of the fruiting bodies, time period that has elapsed from the moment of picking until the commencement of the investigation, storage conditions and the additional purification of extracts [77,87–90]. The total content of glucans in relation to the total content of polysaccharides varies, depending on the type of the fungus and the applied extraction procedure. It has been established that glucans in extracts primarily possess β-glycosidic linkages. Polysaccharide extracts contain glucose, as the prevailing monosaccharide component, and to a smaller or greater extent some other sugars [75–77,79,86,90].

The ability to scavenge free radicals depends on the size of the carbohydrate molecule; polysaccharides have a greater ability of neutralizing free radicals than monosaccharides, but that antioxidative activity is still rather low. Polyelectrolytes, such as sulfated or phosphorylated glucans, lipopolysaccharides and surprisingly-mannans, exhibit significantly stronger free radicals scavenging ability [84,91,92].

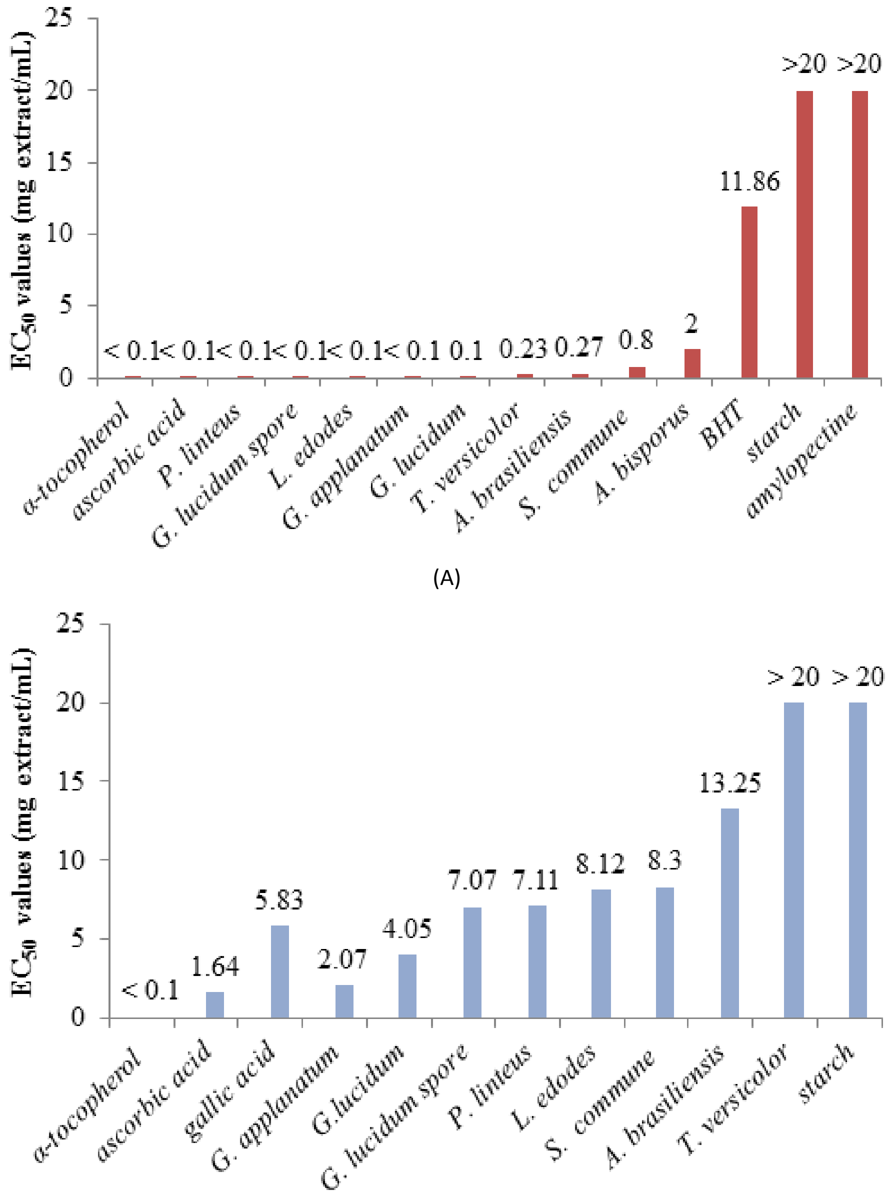
The ability to scavenge free radicals of polysaccharides molecules may be conditioned by the presence of hydrogen from specific, certain monosaccharide units, and the type of their binding in side branches of the main chain. This is how the direct connection between the monosaccharide composition, side chain conformation and ability to catch DPPH radicals of polysaccharides isolated from the filtrate that has been obtained by submerse cultivation of the fungus *L. edodes* has been determined. It has been detected that the presence of glucopyranose units that are linked by β-(1,6)-glycosidic linkages and arabinose bound by (1,4)-linkages in the side chains has a significant impact on the capacity of catching free radicals as compared to ascorbic acid, BHA and citric acid [84].

By measuring of EC_{50} values when testing the antioxidative activity by the DPPH method of polysaccharide extracts, obtained by hot water extraction from the fruiting bodies of the fungus *P. linteus*, the commercial extract of spores of *G. lucidum*, and polysaccharide extracts of the fungi *G. applanatum* and *L. edodes*, additionally purified by dialysis (MWCO 8000––10000 Da), it has been observed that they are comparable with EC_{50} values of the standard commercial antioxidants α-tocopherol and ascorbic acid, Figure 4. The values of EC_{50} for polysaccharide extracts of fungi *S. commune*, *A. brasiliensis, T. versicolor* and *G. lucidum* are, according to this method, 15–120 times lower than the values of EC_{50} for BHT. Starch and amylopectine have exhibited a very low ability of protoning DPPH radicals [75,76,86,90]. Being rapid, simple and independent of sample polarity, the DPPH method is very convenient for the quick screening of many samples for radical scavenging activity [93].

The polysaccharide extract isolated by water extraction from the fungus *G. atrum* has revealed a significantly higher efficacy in protoning free DPPH radicals than the chromatographically purified polysaccharide fractions of the same extract. In accordance with the obtained results, it has been concluded that other components that are present in the extract, such as proteins, peptides, amino acids, phytosterols, nucleotides, organic acids and microelements, as well as their potential interactions and synergic effects, can have a significant impact on the increase of antioxidant properties [79].

Polysaccharide extracts of higher fungi have been proven effective in the prevention of lipid peroxidation in the linoleic acid model system, Figure 4. Extracts with a higher share of non-polysaccharide, primarily phenolic components, are more effective in the protection of lipids from oxidative damages *in vitro* [75,76,86].

By testing the level of inhibition of lipid peroxidation in the linoleic acid model system of polysaccharides isolated from the fungus *L. edodes*, a high correlation between the content of some monosaccharide



(A)

(B)

Figure 4. The antioxidant activity of the hot water polysaccharide extracts from P. linteus, A. brasiliensis, A. bisporus, S. commune fruiting bodies, G. lucidum spore and polysaccharide extracts from L. edodes, G. applanatum, G. lucidum and T. versicolor fruiting bodies purified by dialysis (MWCO: 8.000–10.000) in scavenging ability on DPPH (A) and inhibition of lipid peroxidation evaluated in the linoleic acid model system (B) expressed as EC_{50} (mg/mL) values which are the effective concentrations of each mushroom extract that are required to show 50% antioxidant properties. A lower EC_{50} value corresponds to higher antioxidant activity of the mushroom's extract. Standards: ascorbic acid, α-tocopherol, gallic acid, BHT, starch and amylopectine.

units in polysaccharide extracts and inhibitory activity has been determined. The inhibitory activity increases with the increase of concentrations of mannose and rhamnose, and decreases with the decrease in the content of arabinose and glucose [84].

Influence of phenolic compounds in polysaccharide extracts on antioxidative properties

Polysaccharides in the fungal cell wall may be bound by covalent (ester) linkeages with proteins via remains of tyrosine and with ferulic acid that has resulted from the lignin degradation process [94,95]. Also, it has been detected that with aging, the structure and chemical composition of the fungal cell wall has been changed. Older cells are featured by a layered wall that has thickened in the interior, and it comprises pigments and other materials that may be found in the wall of young cells [96].

By measuring the ability to scavenge hydroxyl radicals by electron paramagnetic resonance (EPR) of polysaccharide extracts obtained by hot water extraction and dialysis (MWCO: 6000–8000) from the fruiting bodies of the fungus *G. tsugae* at various stages of maturity, as well as from the mycelium and fermentation filtrate, a lower efficacy was found as compared to non dialyzed extracts of this fungus, which indicates that the components of smaller molecular masses that are present in extracts and that have not been exposed to dialysis contribute significantly to antioxidant activity [80,97].

In recent investigations of polysaccharide extracts of higher fungi, obtained in various ways (hot water or alkaline extraction, additional dialysis), the presence of phenolic compounds has been detected to various extents [76,86,98]. The intensity of the colour of an extract is in positive correlation with the increased content of the total phenols, meaning the larger content of phenols – the darker colour of the extract [85]. A darker colour of the extracted polysaccharide was associated with a higher antioxidant activity.

According to the structural analogy with oxidated derivatives of monophenols that occur as intermediary products in the polyphenol oxidase driven browning from damages inflicted to the tissue of plants, it seems that during extraction of polysaccharides, when fungal tissue is damaged, the phenol oxidase has access to the phenolic compounds of the extracts [99], the oxidations leading to browning then occur [100,101]. The polyphenol oxidases, such as tyrosinase, that were first discovered in mushrooms, are widely spread in the phylogenetic scale and catalyze the hydroxylation of monophenols and/or *o*-diphenols, that further oxidate into derivatives of *o*-quinones, which polymerize to form brown-coloured pigments generally known as melanins [102,103].

By testing the reductive ability of water and alkaline polysaccharide extracts, very high correlation between the total content of phenols and reductive ability has been observed [76,77,86,90]. In water polysaccharide extracts, isolated from the fungal fruiting bodies that are additionally purified by dialysis and alcohol precipitation, a very high correlation with the total content of α-glucans has been detected, besides very high correlation with the total content of phenol [76]. With the increase of the total content of phenol and α-glucans the reductive ability also increases, which indicates a possible creation of phenolic and α-glucan complexes in the investigated polysaccharides of fungal extracts.

In the analysis of the FT-IR spectra of these polysaccharide extracts, the presence of typical absorption bands was detected, that corresponds to the structures that are typical for polysaccharides, as well as absorption peaks typical for protein structures, and additionally, absorption peaks that correspond to frequences typical for aromatic structures, aliphatic groups and phenols have been detected, which all indicate the presence of aromatic compounds originating from pigments [76]. The FT-IR spectrum of the polysaccharide extract of the fungus *G. applanatum* contains absorption band in the area typical for uronic acids, which reveals the presence of free or esterified C=O groups, as structural components of one (or more) polysaccharides which may be found in the tested extract, Figure 5. *G. applanatum* is a lignicolus fungus, which indicates the presence of enzyme systems that participate in lignin degradation processes. *cis*-ferulic acid is a yellow-coloured compound that shows a FT-IR band in the 1600 cm^{-1} region [104] which is indicative for aromatic C=C conjugated with C=O and/or COO^{-} groups [105]. During the extraction of polysaccharide extracts by hot water, the native protein structure is disturbed, but the procedure is insufficiently effective for the breaking of covalent interactions between the remains of tyrosine and polysaccharides, as well as ferulic acid and polysaccharides [94,106].

By testing the antioxidative potential of the complex of oligosaccharides and ferulic acid, the greater antioxidative ability has been confirmed, as compared to free ferulic acid [107]. Even more, these complexes have exhibited a higher antioxidative potential than ascorbic acid [108].

Influence of proteins in polysaccharide extracts on antioxidative properties

The analysis of the protein content in polysaccharide fungal extracts obtained by hot water extraction, and of extracts that have been additionally exposed to dialysis and alkaline extracts, has confirmed the presence of these compounds in various quantities [75,76,79,98]. Even with thermal treatment full depro-

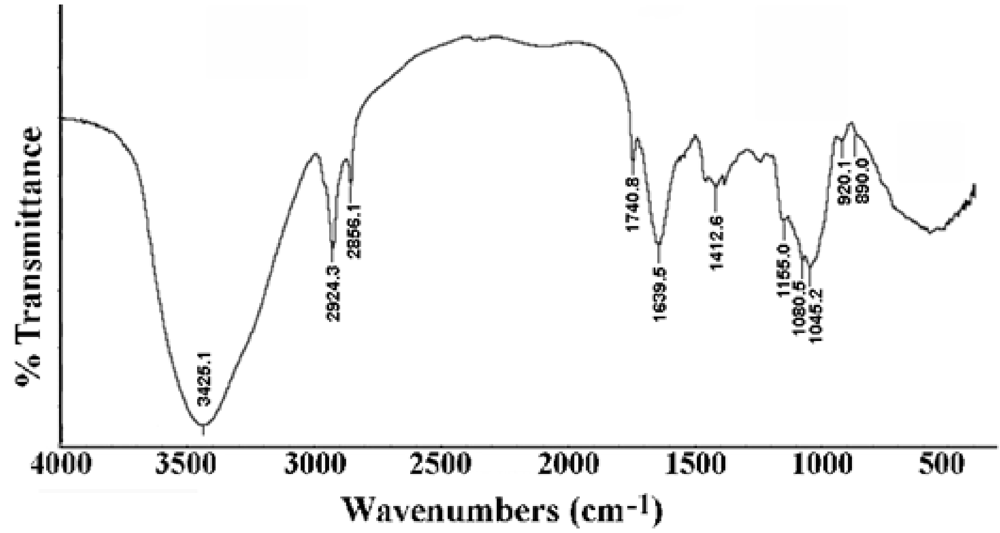


Figure 5. FT-IR Spectrum of polysaccharide extract obtained from fruiting bodies of G. applanatum [76]. (Figure reprinted with permission from Elsevier). FT-IR assignments, wave number (cm^{-1}): 3000–3500 stretching vibration (str) O–H and N–H; 2920–2930 str of asymmetric (CH_2); 2950–2850 str of aliphatic (C–H); 1690–1750 str (C=O) of free acid group and of (C=O) of ester group; 1600–1650 str of amide I, C=O and C=C aromatic; 1520–1410 str of amide II (CN stretching, NH bending); 1410–1400 str of aliphatic C–C chain; 1410–1310 str of OH of phenolics; 1200–900 finger prints of carbohydrates, namely 1155–1150 str of C–O–C; 1080 str of β-glucans 1020 str of C–O; 920 str of α-glycosidic linkage; 890 str of β-glycosidic linkage.

teinisation of polysaccharide extracts cannot be achieved.

Investigations relating to the ability to scavenge superoxide and hydroxyl radicals have revealed that the quantity of proteins (peptides) that are present in polysaccharide – protein complexes has an impact on the properties [109]. Extracts of lentinan and schizophyllan, polysaccharides of *Lentinula edodes* and *Schizophyllum commune*, in which the presence of proteins has been detected in traces, have not exhibited a significant antioxidative activity. On the contrary, the polysaccharide-protein complex (PSK) that has been obtained from the mycelium of *Coriolus versicolor* and polysaccharide extracts of the fruiting bodies of *Ganoderma lucidum* and *Grifola umbellata* with a higher share of proteins have proven to be more effective in antioxidative activity [109].

In recent investigations of the DPPH-TEAC antioxidative activity of polysaccharide extracts of higher fungi, no correlation with protein content was detected, and only a small correlation was observed, with the occurrence of reactive oxygen species (ROS) in an *in vitro* culture of leukemia cells K625, indicating a limited or no role for protein in scavenging activity and in the generation of ROS [74].

By testing the ability to chelate Fe^{2+} of polysaccharide extracts of higher fungi obtained by hot water extraction, moderate correlation with the total protein content was detected, which is explained by the catalyzation of the oxyidation of Fe^{2+} by tyrosinases that had remained present in the protein mixture after extraction, and contributed to the chelating ability [75]. The correlation between the total content of phenols and chelating ability in all extracts was high. All polysaccharides extracts were more effective chelators of ferrous ions than citric acid whose chelating effect does not exceed 10% [75,90].

Moderate correlation was determined between the reducing ability on Fe^{3+} and the total protein content, which may be a consequence of the presence of reductive amino acids of cystein, methionine and tyrosine in proteins of the extract [75,90].

Toxic effect

Individual substances that have proven to be good antioxidants exhibit some adverse, cytotoxic effects, depending on the tested physiological conditions and the applied dosage.

For example, epigallocatechin-3-gallate (EGCG) which is isolated from green tea leaves induces the activation of protein caspases-3 and c-Jun N-terminal kinases (JNKs) sometimes known as stress-activated kinases (SAPKs), which belongs to the group of mitogen-activated protein kinases (MAPKs) that have a role in the process of programmed cell death, i.e. apoptosis. This effect depends on the dosage and the time of exposure, and it particularly occurs at higher concentrations. It is possible that low concentrations of EGCG activate MAPK, leading to antioxidant responsive element (ARE)-mediated gene expression, whereas higher concentrations and sustained activation of MAPKs lead to apoptosis [50,110].

The expression of genes encoding antioxidative and Phase II detoxification enzymes is induced in cells exposed to electrophilic compounds and phenolic antioxidants. Induction of these enzymes is regulated at the transcriptional level and is mediated by a specific enhancer, ARE, found in the promoter of the enzyme's gene [111]. Involvement of the ARE in controlling constitutive gene expression implies a critical role of the enhancer in the maintenance of cellular redox homeostasis under both stressed and non-stressed conditions [111].

Some synthetic antioxidants that are largely applied as supplements to natural antioxidants may cause adverse toxic effects under certain conditions [47,48]. BHA, which is very often used as additive in food industry, may have negative effects on the regulation of the activity of MAPK depending on the dosage [112]. The induction of apoptosis in hepatocytes of rats *in vitro*, with the addition of a larger quantity of BHA is achieved through direct release of cytochrome C [113].

Cytotoxic effects of polysaccharide extracts of higher fungi on primary cells have not been reported up-to-date [114,115].

CONCLUSION

Investigations of antioxidative properties of polysaccharide extracts obtained from various species of higher fungi reveal their obvious antioxidative potential and the consequtive possibility for their application as the new potential antioxidants.

The antioxidative activity of extracts is exhibited even after a thermal treatment is applied in order to isolate extracts the most effectively from the cell wall of higher fungi. The antioxidative activities depend on the content of polysaccharides, phenols and proteins in polysaccharide extracts.

Immunomodulatory, anti-tumor and antioxidative properties of polysaccharide extracts of higher fungi have been shown and supported by our data indicating that they might be effectively applied as dietary supplements in everyday nutrition. Good results in the prevention of lipid peroxidation indicate the application of polysaccharide extracts of higher fungi as functional additives in the preservation of the stability of foodstuffs [116,117].

β-Glucans are applied in cosmetology as protective creams that prevent irritation and diseases of the skin, also stimulating the skin cell proliferation and collagen biosynthesis [22,118,119]. Polysaccharide extracts of fungi with antioxidative properties have commercial application in creams that provide protection from ultraviolet rays and from other detrimental effects caused by activities of free radicals that lead to damages and aging of the skin.

Investigations of factors that have influence upon the antioxidative properties of polysaccharide extracts are in the progress. The investigations are focused on the antioxidative mechanisms of action and further studies on the complexity of structural interactions between polysaccharide, phenols and protein components of the extracts.

Very high correlations between the total content of phenols in polysaccharide extracts of higher fungi have been detected in the investigations related to the reducing ability and chelating ability of Fe^{2+} [90]. The quantification of total phenols using Folin-Ciocalteu reagent is based on electron transfer reactions [120]. Therefore, when one determines phenolic compounds, one has to take into account the presence of other compounds in the extracts, such as organic acids, sugars and amino acids that may also take part in the electron transfer processes. Even if one puts aside the presence of these compounds, the various phenolic compounds give various results with the Folin-Ciocalteu reagent. While catechin, rutin, caffeic acid and gallic acid behave in a similar way, some flavonoids exhibit less absorption than expected, which leads to a wrongful interpretation of results. Therefore, it is necessary to identify precisely the nature, as well as the structure of phenolic compounds responsible for the antioxidant activity of polysaccharide extracts of higher fungi.

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IZVOD

POLISAHARIDI VIŠIH GLJIVA: BIOLOŠKA ULOGA, STRUKTURA I ANTIOKSIDATIVNA AKTIVNOST

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(Pregledni rad)

Polisaharidi gljiva privlače veliku pažnju zbog svojih interesantnih bioloških svojstava kao što su: antitumorno, antivirusno, antikomplementarno, antikoagulaciono, hipolipidemijsko dejstvo, kao i imunomodulatorska i imunostimulativna aktivnost, što ih sve čini pogodnim za primenu u mnogim oblastima, među kojima su i prehrambena industrija, kozmetika, biomedicina, poljoprivreda, zaštita životne sredine i otpadnih voda. Oksidativna oštećenja prirodnih i industrijski pripremljenih namirnica predstavljaju veliki ekonomski problem na globalnom nivou, s obzirom da direktno utiču na promenu organoleptičkih osobina proizvoda, pri čemu dolazi i do stvaranja potencijalno toksičnih jedinjenja. Zbog toga se danas sve veća pažnja posvećuje istraživanjima novih prirodnih izvora antioksidanasa, među kojima su veoma značajni i polisaharidni ekstrakti različitih bazidiomiceta. U ovom radu su prikazana biološka svojstva, struktura, kao i postupci izolovanja i aktivacije polisaharida viših gljiva. Dat je i pregled antioksidativne aktivnosti polisaharidnih ekstrakata viših gljiva i razmatran je uticaj strukturnih karakteristika ovih ekstrakata na antioksidativna svojstva.

Ključne reči: Polisaharidi • Gljive • Polisaharidni ekstrakti • Antioksidativna svojstva

Analysis of interrelations between wheat protein fractions composition and its technological quality by combined multivariate and univariate statistics

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Abstract

Data on protein fractions' proportion, obtained with RP-HPLC and technological quality parameters for 29 wheat cultivars grown in Serbia and Croatia, were used for studying of interrelations among wheat protein fractions with different solubility and molecular weight properties by multivariate (PCA) analysis. Obtained trends were used as the base for investigations related to differentiation of technological quality among wheat cultivars with different combination of protein fractions' compositions using univariate statistics (ANOVA followed by Duncan's test) in order to draw out information about interrelations between protein fractions proportion in wheat cultivars and their technological quality. Analysis based on the first four PCA factors (89.04% of variability) pointed out interdependencies between: 1) high content of albumins and globulins, low gliadins content and gliadins/glutenins ratio, high ω-gliadins, LMW glutenins and low α-gliadins share in total protein with low water absorption, high energy and high resistance to extensibility ratio, 2) high albumin and globulin content and high proportion of ω-gliadins in total protein and low extensigraph extensibility, 3) high share of high molecular weight glutenins (HMW-GS) in total proteins, high extensigraph resistance/extensibility ratio and 4) high γ-gliadins share in total protein and low extensigraph resistance/extensibility ratio.

Keywords: wheat, genotype, quality properties, albumins and globulins, gluten proteins, RP-HPLC.

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The content and composition of wheat protein are considered to be among the major factors that influence on the technological properties and quality of final products of wheat processing [1,2].

The influence of wheat protein fractions on dough rheological properties and end use quality of wheat has been the subject of numerous studies that included the use of sodium dodecil sulphate polyacrilamid gel electrophoresis (SDS-PAGE) [3–5], capillary electrophoresis [6], electrophoresis on chip [7] or high performance liquid chromatography (HPLC) [4,8–11] for fractioning of wheat proteins by their molecular weights.

The gluten content has proven to be positively correlated with some of the properties of wheat technological quality, such as farinographs dough development and stability [12], and water absorptions and dough development time, whereas the negative correlation is confirmed with extensigraphs resistance and resistance, and extensibility ratio [1]. The properties of gluten are also influencing the parameters used for evaluation of dough rheological properties. The partial explanation and prediction of some dough rheological properties, like extensigraph energy, can be obtained from results of rapid methods like gluten index [1], which are depending on gluten molecular structure or even better by modification of gluten index procedure which includes also the enzymatic activity that has influence on gluten composition [13].

The quantity and ratio of two main gluten fractions, glutenin and gliadin, have also notable influence on dough properties and bread making quality [14]. It is well known that glutenins are responsible for dough elasticity, whereas gliadins are more viscous and influence mainly on dough extensibility [15]. According to Torbica *et al.* [13] increase of gliadin and glutenin ratio explained the drop of extensigraph energy.

The glutenins are, based on electrophoretic mobility, recognized as high molecular weight glutenins (HMW-GS) with molecular weight from 80000 to

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130000 [16] and low molecular weight glutenins (LMW-GS) with molecular weight from 30 000 to 50 000 [17].

HMW-GS composition depends on origin from specific genome and it is accepted to be one of the most important genetic factors determining empirical properties of dough [18]. Therefore, numerous research results were reported which connect influence of composition of HMW-GS with technological quality of wheat [4,5,9,19], but almost all reported studies HMW-GS are interpreted based on genetic alleles structure and are not divided by different molecular weights. The approach of analysis of influence of presence and quantity of glutenin fractions with different molecular weights on wheat technological quality was recently reported by Živančev *et al*. [7].

Regarding the influence of LMW-GS on dough rheological properties it was reported by Luo *et al*. [3] that they play important role in determining dough extensibility, while role in determining dough strength is less important and is related to HMW-GS.

In academic literature, the gliadins are divided according electrophoretic mobility in acid conditions into *α*-, *β*-, *γ*- and *ω*-classes [20]. Thomson *et al*. [21] found that molecular weight of *α*, *γ* and *ω* gliadins are approximately 35000, 45000 and 58000, respectively, using the small angle X-ray scattering. In the past, the relative contribution of specific composition of gliadin subunits in determining of wheat dough properties was not quite clear [22], except in the case of some subunits of *ω*-gliadins which were reported to affect the dough extensibility [23]. The recent study by Wang *et al*. [24] confirmed that others subunits of *ω*-gliadins could influence on dough strength, whereas Gobaa *et al*. [25] showed that high amount of *α*-gliadins has improved dough extensibility. However, Gil-Humanes *et al*. [26] in their research did not confirm the important role of *γ*-gliadins on dough rheology.

The albumins and globulins, fractions of wheat proteins soluble in water and salt solution, represent only several wheat endogenous enzymes [27] and enzyme inhibitors [28]. Their effect on dough properties and their role on dough strength and baking properties are not thoroughly investigated [29,30].

The basic statistic including analysis of correlations between pairs of variables was mainly used in the above-mentioned research about dependence between protein fractions and dough rheological properties. The utilization of multivariate statistics for explanation of interdependencies should be considered, having in mind that wheat dough is complex matrix in which all components interact resulting in final rheological properties, which was not found in reported research in this field [31].

In this study the interrelations among wheat protein fractions with different solubility and molecular weight properties are going to be studied for cultivars grown in south of Pannonian Plain by multivariate analysis. The obtained trends will further be used as the base for investigations related to differentiation of individual technological quality parameters among wheat cultivars with different combination of protein fractions proportion in order to draw out information about interrelations between protein fractions proportion in wheat cultivars and their technological quality.

EXPERIMENTAL

The examinations have been carried out on 16 bread wheat cultivars (Srpanjka, Žitarka, Divana, Aida, Felix, Zlata, Ilirija, Ružica, Sana, Seka, Renata, Golubica, Soissons, Olimpija, Vulkan and Tihana) grown in Croatia at the Agricultural Institute Osijek and 16 bread cultivars grown in Serbia (Vojvodina, Angelina, Dragana, Ljiljana, NS rana 5, Pobeda, Bastijana, Evropa 90, NS 40S, Simonida, Kantata, Etida, Renesansa, Zvezdana, Rapsodija and NS3-5299/2) at the Institute of Field and Vegetable Crops, Novi Sad, in 2009.

The protein content of wheat kernels was measured by Infratec 1241 (Foss Tecator, Höganäs, Sweden), the Falling Number was determined by ICC method No 107/1 [32], whereas wet gluten and gluten index of flour samples were determined according to ICC method No. 155 [33]. The dough rheology was measured by the Brabender (C.W. Brabender, Duisburg, Germany) equipments farinograph and extensigraph (ICC methods No. 115/1 [34] and 114/1 [35], respectively).

The procedure for extraction of wheat proteins and RP-HPLC method were performed according to Wieser *et al*. [36]. Perkin Elmer LC 200 chromatograph (Perkin Elmer Instruments, Waltham, USA) was utilized with a Supelco Discovery Bio Wide Pore C18 column (300 Å pore size, 5 μm particle size, 4.6 mm×250 mm i.d.) purchased from Sigma-Aldrich, Steinheim, Germany. Solvents were consisted of water and acetonitrile (ACN), comprised of 0.1% (*V*/*V*) trifluoroacetic acid (TFA). 20 μL samples were injected for analyses. Wheat protein fractions were eluted with a linear gradient from 24 to 58% ACN over 30 min at 1 mL/min, using a column temperature of 50 °C. All samples were scanned by UV absorbance at 210 nm in duplicate. The gained chromatograms were examined by Total-Chrom software package (Perkin Elmer Instruments, Waltham, USA). The peak areas (expressed in arbitrary units, AU) under albumins-globulins, gliadins and glutenins chromatograms were totalized and used as a direct extent of total wheat proteins content. Furthermore, the share of all protein fractions in total extracted wheat proteins was calculated.

The data were statistically analyzed by Statistica 10.0 (StatSoft Inc., USA) software [37]. Principal component analysis (PCA) was used to investigate associations among wheat protein fractions. Further on, ANOVA was used for testing of differentiation of quality parameters among cultivars for which PCA indicated differentiation related to main factors. Also, correlations between factors and protein fraction associated with them were calculated. Mean values of three measurements of considered parameters were used for statistical data analysis.

RESULTS AND DISCUSSION

The descriptive statistics of technological quality parameters and quantities of protein fractions with different solubility properties (albumins+globulins, gliadins and glutenins), their ratios and shares of glutenin and gliadin fractions from different molecular weight ranges in total protein in examined cultivars determined by RP-HPLC method are provided in Table 1.

Except for gluten index values, which were high for all examined cultivars indicating absence of gluten degradation under influence of unfavorable environmental factors, the ranges of others parameters were broad, indicating wide differentiation of protein composition and quality and thus representing reliable base for intended analysis of interrelations (Table 1).

Principal component analysis was conducted for parameters on composition of protein fractions from Table 1. The contribution of identified factors to total variability of sample set indicated that first four PCA factors explain 89.04% variability of protein composition in investigated wheat cultivars (Fig. 1), with individual contribution of each of first four factors over 10%. The contribution of other factors was lower and based on this remark the further analysis was performed based on analysis of first four PCA factors.

Further step in data analysis was to identify which protein fractions are mainly explaining identified principal components. For this purpose the correlations of protein fractions with identified principal components were calculated (Table 2). The highest correlation coefficient were obtained between total protein content and total content of gluten proteins and both gluten fractions and the first PCA factor, share of α-gliadins and LMW glutenins in total protein and GLI/GLU ratio and second PCA factor, content of albumin and globulin and share of *ω*-gliadins in total protein and third PCA factor and share of γ-gliadins and HMW glutenins in total protein and the fourth PCA factor. For the protein fractions associated with the same PCA factors, meaning that they demonstrate similar trends, it can be sup-

Table 1. Minimal, maximal and average values and standard deviation of quality parameters of wheat cultivars and content (AU s/mg flour) of protein fractions of wheat cultivars used for examination; GI = gluten index; FN = falling number (s); WA = water absorption (%); DDT= dough development time (min); DS = degree of softening (BU); E = energy (cm^2); R = resistance (BU); EXT = extensibility (mm); R/EXT = ratio resistance/extensibility. PT = total extractible proteins content as summ of AG, GLI and GLU content; GT = total gluten content (sum of GLI and GLU content); AG = content of albumins and globulins; GLI = giladins content; GLU = glutenins content; GLI/GLU = ratio gliadins/glutenins; ω/P = share of omega gliadins in total protein; α/P = share of alpha gliadins in total protein; γ/P = share of gamma gliadins in total protein; HMW/P = share of high molecular weight glutenins in total protein; LMW/P = share of low molecular weight glutenins in total protein

Parameter	minimal value	maximal value	average value	Standard deviation
GI	81	100	95	5.15
FN	111	436	299	78.87
WA	56.9	68.3	62.1	2.84
DDT	1.5	10.3	3.0	1.69
DS	10	125	62	27.08
E	42	128	83	27.64
R	140	360	235	58.05
EXT	132	209	169	20.17
R/EXT	0.67	2.52	1.42	0.41
PT	69.4	116.0	85.8	9.68
GT	60.5	103.1	73.5	9.51
AG	8.8	20.6	12.3	2.34
GLI	21.5	42.4	28.2	4.71
GLU	36.2	60.7	45.3	5.77
GLI/GLU	1.23	1.95	1.63	0.21
ω/P	2.29	7.63	3.99	1.14
α/P	19.80	32.55	28.05	2.95
γ/P	16.69	27.17	20.82	2.35
HMW/P	5.49	12.89	8.87	1.25
LMW/P	18.5	28.86	23.86	2.41

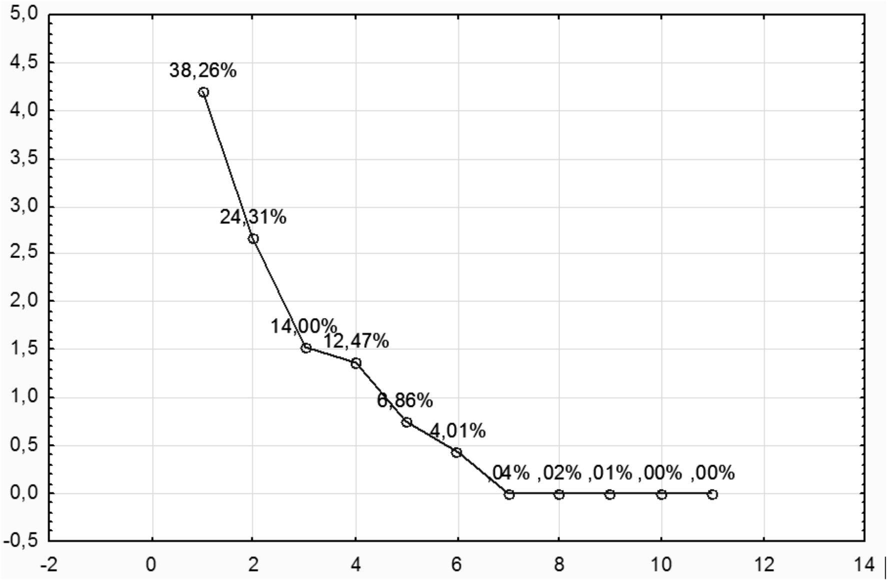

Figure 1. Contribution of PCA factors to the variability among protein fractions of examined cultivars.

posed that joint influence of associated fractions on wheat technological quality will be expressed.

The positions of examined cultivars in factorial plane presenting first *vs*. second and third *vs*. fourth PCA factor are presented in Figure 2. The groups of cultivars with extreme positions in factorial plane in respect to observed PCA factors are circled in the graphs, and the ranges of their coordinates in factorial planes with identification of groups of cultivars differentiating based on each of identified PCA factors are provided in Table 3.

Based on visual presentation from Figure 2 examined cultivars were grouped in respect to their differentiation by PCA factors with grouping of four cultivars with extreme negative coordinates in group A (A1, A2, A3, A4 for first four PCA factors, respectively), with extreme positive coordinates in group C (C1, C2, C3, C4 for first four PCA factors, respectively) and remaining cultivars in group B (B1, B2, B3, B4 for first four PCA factors, respectively). The lists of cultivars grouped as extremely differentiating for each PCA factor are provided in Table 3.

The significance of differences for all technological quality parameters (Table 1) and for all protein fractions (Table 1) were tested for groups of cultivars (Table 3) obtained in above explained manner. The differences were tested with ANOVA followed by Duncan’s test for each observed parameter individually. The parameters for which significant differences were obtained are presented in Table 4.

Differentiation of formed groups based on the first

Table 2. Interrelations of PCA factors with protein fractions; PT = total extractible proteins content as sum of AG, GLI and GLU content; GT = total gluten content (sum of GLI and GLU content); AG = content of albumins and globulins; GLI = giladins content; GLU = glutenins content; GLI/GLU = ratio gliadins/glutenins; ω/P = share of omega gliadins in total protein; α/P = share of alpha gliadins in total protein; γ/P = share of gamma gliadins in total protein; HMW/P = share of high molecular weight glutenins in total protein; LMW/P = share of low molecular weight glutenins in total protein.

Parameter	PCA factor 1	PCA factor 2	PCA factor 3	PCA factor 4
PT	–0.921	0.228	0.125	–0.280
GT	–0.920	0.340	–0.043	–0.183
AG	–0.070	–0.436	0.694	–0.414
GLI	–0.713	0.654	–0.045	–0.245
GLU	–0.987	–0.113	–0.032	–0.068
GLI/GLU	0.555	0.804	0.008	–0.182
ω/P	0.111	0.473	0.726	0.039
α/P	–0.134	0.720	–0.320	0.461
γ/P	0.436	0.210	–0.398	–0.606
HMW/P	–0.449	0.012	0.270	0.636
LMW/P	–0.541	–0.663	–0.418	–0.020

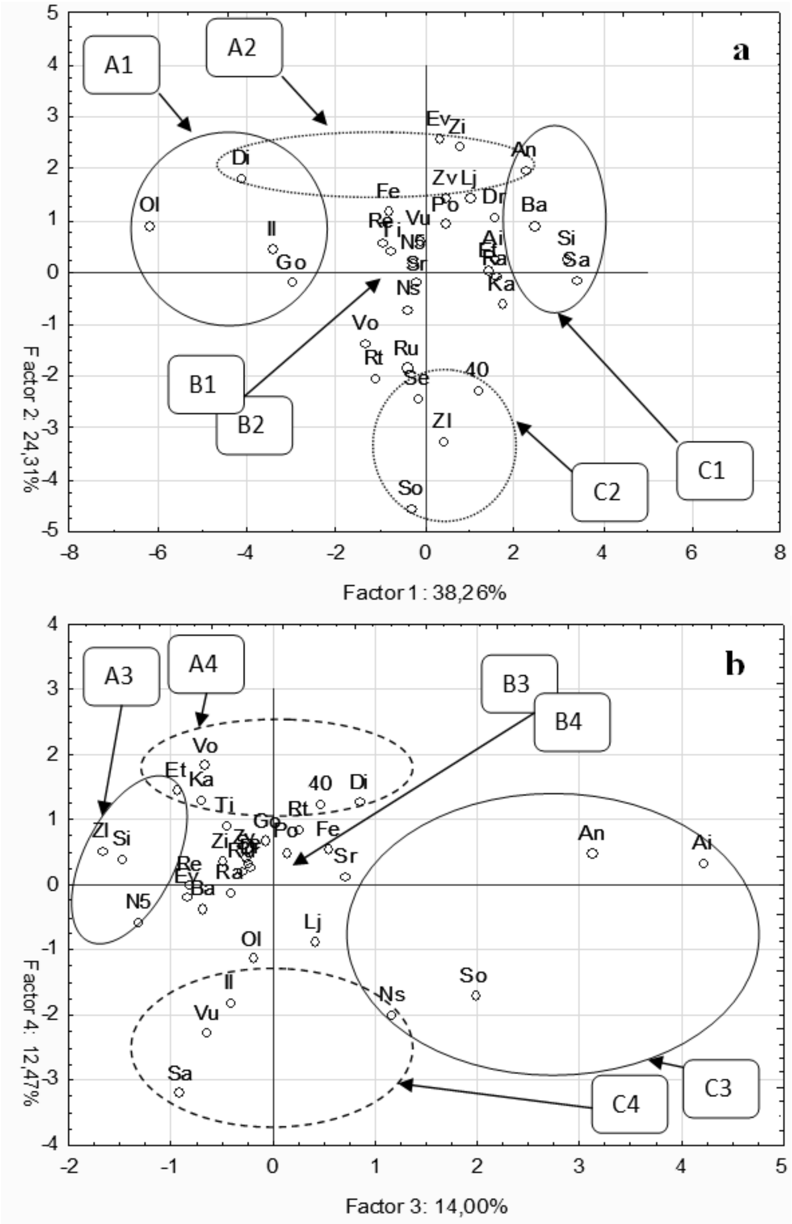

Figure 2. Position of examined cultivars in factorial plane. I – factorial plane in coordinates of PCA factors 1 and 2; II – factorial plane in coordinates of PCA factors 3 and 4 Groups A1,A2, A3, A4, B1, B2, B3, B4, C1, C2, C3, C4 defined in table 4 and explained in text; Sr = Srpanjka; Zi = Žitarka; Di = Divana; Ai = Aida; Fe = Felix; Zl = Zlata; Il = Ilirija; Ru = Ružica; Sa = Sana; Se = Seka; Rt = Renata; Go = Golubica; So = Soissons; Ol = Olimpija; Vu = Vulkan; Ti = Tihana; Vo = Vojvodina; An = Angelina; Dr = Dragana; Lj = Ljiljana; N5 = NS rana 5; Po = Pobeda; Ba = Bastijana; Ev = Evropa 90; 40 = NS 40s; Ka = Kantata; Ra = Rapsodija; Si = Simonida; Et = Etida; Re = Renesansa; Zv = Zvezdana; Ns = NS3-5299/2.

PCA factor, which is mostly influenced by total protein and gluten content, has confirmed existence of statistically significant differences in total extractable proteins and gluten content, but the difference in total glutenins content was also registered among groups (Table 4, PCA Factor 1). Group A1, characterized with statistically higher protein, gluten and glutenins content was significantly different from other two groups also in respect of higher gliadins content. The cultivars from group A1 are characterized with longer dough development time than cultivars from other two groups and higher energy than cultivars from group C1 with the lowest proteins and gluten content. This observation confirms multiply expressed fact [1,2,38,39] that higher protein content results in stronger dough with longer mixing times and higher energy.

On the other hand cultivars from group C1 (Table 4, PCA Factor 1) with statistically lowest proteins and gluten content were also characterized with significantly lower gluten index, falling number and higher degree of softening than other two groups indicating that the cultivars in this group are probably character-

Table 3. Groups of cultivars differentiating in respect to observed principal components with ranges of their coordinates in factorial planes

PCA factor	Group	Cultivar	Range of coordinates in factorial plane	
			From	To
1	A1	Divana, Olimpija, Golubica, Ilirija	–6.16	–3.00
	B1	Others	–1.34	1.73
	C1	Sana, Angelina, Bastijana, Simonida	2.25	3.41
2	A2	Soissons, Zlata, Seka, NS-40s	–4.54	–2.26
	B2	Others	1.46	–2.04
	C2	Divana, Angelina, Žitarka, Evropa90	1.83	2.59
3	A3	Zlata, Simonida, NS rana 5, Etida	–1.68	–0.95
	B3	Others	0.85	–0.92
	C3	NS3-5299/2, Soissons, Angelina, Aida	1.16	4.23
4	A4	Sana, Vulkan, NS3-5299/2, Ilirija	–3.20	–1.81
	B4	Others	–1.68	1.25
	C4	Divana, Kantata, Etida, Vojvodina	1.28	1.84

ized with more expressed activity of grain enzyme complex. This observation points out at the conclusion that the cultivars with lower protein content are more susceptible to initiation of enzymatic processes which was not reported in academic literature till now.

Differentiation of formed groups, based on the second PCA factor (Table 4, PCA Factor 2), which is mostly influenced by gliadin/glutenin ratio and the share of low molecular weight glutenins and gliadins (α-gliadins) in total protein, leads to several conclusions. Namely, the C2 group of varieities with statistically higher content of albumins and globulins,

Table 4. Differentiation of protein fractions' content and technological quality parameters among groups of cultivars differentiated by PCA factors; PT = total extractible proteins content as summ of AG, GLI and GLU content; GT = total gluten content (sum of GLI and GLU content); GLU = glutenins content; GLI = giladins content; GI = gluten index; FN = faling number value (sec); DDT = dough development time (min); DS = degree of softening (BU); E = energy (cm^{2}); AG = content of albumins and globulins; GLI/GLU = ratio gliadins/glutenins; α/P = share of alpha gliadins in total protein; ω/P = share of omega gliadins in total protein; LMW/P = share of low molecular weight glutenins in total protein; WA = water absorption (%); R = resistance (BU); R/EXT = ratio resistance/extensibility; EXT = extensibility (mm); γ/P = share of gamma gliadins in total protein; HMW/P = share of high molecular weight glutenins in total protein; means with the same letter do not differ significantly

Group	Parameter									
	TP	*GT*	*GLU*	*GLI*	*GI*	*FN*	*DDT*	*DS*	*E*	
	PCA factor 1									
A1	108.3^{a}	96.1^{a}	38.6^{a}	575^{a}	96.5^{a}	358^{a}	5.7^{a}	42^{a}	104^{a}	
B1	84.6^{b}	72.1^{b}	27.6^{b}	445^{b}	96.0^{a}	303^{a}	2.7^{b}	59^{a}	84^{ab}	
C1	73.4^{c}	62.4^{c}	22.4^{c}	399^{b}	88.9^{b}	194^{b}	2.4^{b}	103^{b}	56^{b}	
	PCA factor 2									
	AG	*GLI*	*GLI/GLU*	*α/P*	*ω/P*	*LMW /P*	*WA*	*E*	*R*	*R/EXT*
A2	10.6^{c}	48.0^{a}	1.94^{a}	30.11^{a}	2.55^{c}	20,98^{c}	64.5^{a}	62^{b}	205^{b}	1.34^{b}
B2	12.1^{b}	45.9^{a}	1.64^{b}	28.29^{a}	3.96^{b}	23,84^{b}	62.3^{a}	83^{ab}	229^{b}	1.37^{b}
C2	15.3^{a}	37.9^{b}	1.30^{c}	23.91^{b}	5.68^{a}	26,95^{a}	58.1^{b}	107^{a}	319^{a}	1.97^{a}
	PCA factor 3									
		AG			*ω/P*				*EXT*	
A3		10.0^{b}			3.02^{b}				186^{a}	
B3		11.9^{b}			3.84^{b}				170^{ab}	
C3		16.9^{a}			5.96^{a}				143^{b}	
	PCA factor 4									
		γ/P			*HMW/P*				*R/EXT*	
A4		24.6^{a}			7.7^{b}				1.20^{b}	
B4		20.5^{b}			8.8^{b}				1.40^{ab}	
C4		18.5^{b}			10.8^{a}				1.87^{a}	

ω-gliadin and low molecular weight glutenin share in total protein accompanied with statistically lower gliadin content, gliadin/glutenin ratio and *α*-gliadin share in total protein, was characterized with statistically lower water absorption and statistically higher energy resulting from higher resistance and higher resistance to extensibility ratio. This remark provides understanding of protein composition that causes low water absorption and high extensigraph resistance as undesirable characteristics of wheat cultivars. On the other hand, the cultivars from group A2 with lower content of albumins and globulins and lower shares of *ω*-gliadins and low molecular weight glutenins in total protein, did not reveal differentiation in technological quality parameters from group B2.

Analysis of differentiation of protein fractions composition and technological quality parameters among groups of differentiated cultivars, based on the third PCA factor (Table 4, PCA Factor 3), which is mostly influenced by albumins and globulins content and share of *ω*-gliadins in total proteins, reveals that the cultivars from group C3 with significantly higher albumins and globulins content and significantly higher share of *ω*-gliadins in total proteins express significantly lower extensigraph extensibility in respect to cultivars from group A3, regarding mentioned properties that have opposite performance. This observation points out at possible role of increased shares of mentioned proteins' fractions in shortening of dough extensibility.

The analysis of significance of differences among the mean values of protein fractions and technological parameters values for cultivars from groups, formed on the basis of fourth PCA factor (Table 4, PCA Factor 4), which is mostly influenced by the share of *γ*-gliadins and high molecular weight glutenins in total proteins, indicates that higher share of *γ*-gliadins in total proteins leads to lower ratio of extensigraph resistance and extensibility, while higher molecular weight of glutenins' share in total proteins causes the opposite dough behavior.

Explained observation obtained by multivariate approach to the consideration of influence of wheat protein fractions and their proportion on dough rheological properties partly confirms already determined influence of individual wheat protein fractions on rheological properties of dough [40], pointing out also the dependencies of rheological properties on interrelations of protein fractions and their overall proportion have to be reconsidered and more thoroughly analyzed in future investigations.

CONCLUSION

Applied combination of multivariate and univariate statistical techniques points out at the following interrelations among protein fractions and technological quality parameters of investigated wheat cultivars:

- Cultivars with low total extractible proteins and total gluten content tend to be more susceptible to enzymatic processes in dough.
- Cultivars characterized with higher content of albumins and globulins and low molecular weight glutenin share in total protein accompanied with lower gliadin content, gliadin/glutenin ratio and α- gliadin share, are characterized with low water absorption, high energy and high resistance to extensibility ratio.
- Combination of high albumins and globulins content and high share of *ω*-gliadins in total proteins results in lower extensigraph extensibility.
- High HMW-GS share in total proteins results in higher extensigraph resistance/extensibility ratio, while high *γ*-gliadins share in total proteins has the opposite effect.

Obtained results point out at the possibility of successful application of combination of multivariate and differential statistics for investigation of interrelations of wheat composition components and its technological quality, providing useful approach for innovative analysis in corresponding research areas.

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IZVOD

ANALIZA MEĐUZAVISNOSTI SASTVA FRAKCIJA PROTEINA I TEHNOLOŠKOG KVALITETA PŠENICE PRIMENOM KOMBINACIJE MULTIVARIJANTNE I UNIVARIJANTNE STATISTIKE

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(Naučni rad)

Podaci o odnosu frakcija proteina pšenice dobijeni primenom RP-HPLC i parametri tehnološkog kvaliteta 32 sorte pšenice uzgajane u Srbiji i Hrvatskoj su korišćeni za izučavanje međuzavisnosti frakcija proteina pšenice različite rastvorljivosti i molekulskih masa primenom multivarijantne (PCA) analize. Utvrđeni trendovi su upotrebljeni kao osnova za ispitivanje diferenciranja tehnološkog kvaliteta među sortama okarakterisanim različitim kombinacijma proteinskih frakcija uz primenu univarijantne statistike (analiza varijanse praćena Dankanovim testom značajnosti razlika) sa ciljem da se dobije informacija o međuzavisnosti između sastava frakcija proteina sorti pšenice i njhovog tehnološkog kvaliteta. Analiza zasnovana na prva četiri PCA faktora (89.04% varijabilnosti) ukazuje na zavisnosti između: 1) visokog sadržaja albumina i globulina, niskog sadržaja glijadina i odnosa glijadina i glutnina, visokog sadržaja ω-glijadina, niskomolekularnih glutenina i niskog udela α-glijadina u ukupnim proteinima sa niskom moći upijanja vode, visokom energijom i visokim odnosnim brojem, 2) visokog sadržaja albumina i globulina i visokog udela ω-glijadina u ukupnim proteinima i niske rastegljivosti na ekstenzogramu, 3) visokog udela visokomolekularnih glutenina u ukupnim proteinima i visokog odnosnog broja i 4) visokog udela γ-glijadina u ukupnim proteinima i niskog odnosnog broja.

Ključne reči: Pšenica • Genotip • Tehnološke osobine • Albumini i globulini • Gluten • RP-HPLC

Novi postupak prerade industrijskog otpada koji sadrži flotoreagense na bazi ksantata

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Izvod

U ovom radu je opisan novi laboratorijski postupak prerade industrijskog otpada na bazi ksantata i dat poluindustrijski postupak prerade sa definisanim tehnološkim parametrima. U prvoj fazi prerade se izdvajaju komponente koje ulaze u sastav otpadnog ksantata. Druga faza prerade predstavlja tretman izdvojenih komponenata u prvoj fazi, pri čemu se izdvojeni ksantati i diksantogenati upotrebljavaju za sintezu alkiltionkarbamata, koji nalaze primenu u flotaciji kao selektivni flotoreagensi. Laboratorijskim postupkom prerade dobijeni su tionkarbamati u prinosu od 69,7–87,7%, dok se poluindustrijskim postupkom, za odabrane šarže odgovarajućeg sastava, tionkarbamati dobijaju u prinosu od 74,2–80,5%.

Ključne reči: otpadni ksantat, diksantogenat, tionkarbamat, natrijum-ksantogenacetat.

NAUČNI RAD

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Flotacioni reagensi na bazi ksantata mogu se lagerovanjem u dužem vremenskom periodu transformisati u industrijski otpad. Osim toga, ako se reakcija sinteze ili proces sušenja ne vode po definisanim parametrima u industrijskim pogonima u kojima se proizvode flotoreagensi, mogu nastati otpadne šarže koje se lageruju kao otpad. Ovaj otpad predstavlja opasnost po čovekovu sredinu pa se mora zbrinuti na propisani način, ali se i može iskoristiti za dobijanje korisnih industrijskih proizvoda. Naime, tretmanom se industrijski otpadni ksantat može prevesti u komercijalni proizvod iz grupe tionkarbamatnih flotoreagenasa koji imaju izraženu selektivnost u primeni. Alkiltionkarbamati, koji se dobijaju tretmanom otpadnog ksantata, nastaju u drugoj fazi prerade tretmanom sa odgovarajućim aminom i natrijum-hipohloritom, ukoliko je polazni reaktant izolovani diksantogenat. Ukoliko je u prvoj fazi prerade izdvojen ksantat, deluje se sa natrijum-monohloracetatom (NaMXAc) i alkilaminom, pri čemu se, takođe, dobijaju alkiltionkarbamati.

Tionkarbamati su jedinjenja koja u osnovi predstavljaju derivate tiokarbaminske kiseline [1] i to tiol- i tion-estre. Njihove strukturne karakteristike, kao što je direktna veza tioacil-grupe i azota, doprinose njihovoj izraženoj biološkoj aktivnosti [2]. Ova jedinjenja poseduju veoma širok spektar delovanja, pa se industrijski proizvode i koriste kao fungicidi [3–5], baktericidi [4,6], herbicidi [7,8], germicidi [9], pesticidi [10–12], insekticidi [13,14] itd. Osim toga, alkiltionkarbamati nalaze primenu kao ubrzivači polimerizacije i selektivni flotoreagensi [15]. Tiokarbamati se mogu dobiti reakcijom *O*,*S*-diestra ditiokarboksilne kiseline u vodenom ili alkoholnom rastvoru sa primarnim ili sekundarnim aminima, kao i reakcijom hlorida *O*-estra monotiokarbonske kiseline sa aminima [16]. Tiokarbamati se, takođe, dobijaju reakcijom, u jednom stupnju, alkalnih ksantata, amina i oksidacionog sredstva [17]. Sinteza tiokarbamata iz tiola i izocijanata se može izvoditi u prisustvu katalizatora sa i bez prisustva rastvarača [18]. Osim poznatih postupaka sinteze [19–23], tiokarbamati se mogu dobiti i reakcijom natrijum- ili kalijum-ksantata u vodenom rastvoru sa primarnim ili sekundarnim aminima i u prisustvu elementarng sumpora [24]. Tiokarbamati se mogu dobiti i reakcijom ksantata i amina u prisustvu nikal(II)-sulfata heptahidrata kao katalizatora [25], a reakcijom oksidacije aminskih soli ksantogene kiseline pomoću vodonik-peroksida ili natrijum-hipohlorita ostvaruje se visok prinos izopropiltionkarbamata [26]. Oksidacijom aminskih soli ditiokarbaminskih kiselina pomoću amonijum-peroksodisulfata kao oksidacionog sredstva dobijaju se tiuramdisulfidi [27], pa su analogno dobijeni tionkarbamati oksidacijom aminskih soli ksantogene kiseline upotrebom kalijum-peroksodisulfata кao oksidacionog sredstva [28].

Sinteza *N*-alkil-, *N*,*N*-dialkil- i *N*-cikloalkil-*O*-izobutiltionkarbamata se odigrava reakcijom natrijumove soli izobutilksantogen sirćetne kiseline (NaiBXAc) i odgovarajućih amina. Takođe, opisani su katalizovani postupci sinteze: reakcijom izobutil-alkohola, amina i bis-(benzotriazolil)metantiona u prisustvu katalizatora 1,5--diazabiciklo[5.4.0]undek-5-ena, reakcijom KiBX i amina u prisustvu katalizatora Pd na aktivnom uglju [29] i

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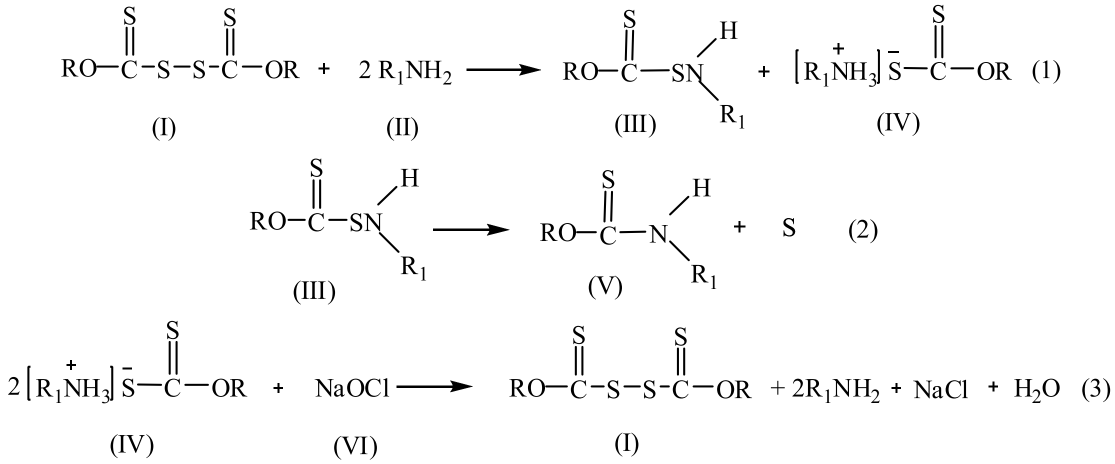
reakcijom natrijumove soli izobutilksantogen sirćetne kiseline i odgovarajućih amina u prisustvu nano katalizatora [30].

Cilj istraživanja u ovom radu je optimizacija uslova laboratorijskog tretmana otpadnog industrijskog ksantata i definisanje tehnoloških parametara odgovarajućeg poluindustrijskog postupka. U tom smislu je najpre utvrđen hemijski sastav otpadnog ksantata, zatim su razdvojene njegove komponente i na kraju je izvršena reakcija diksantogenata sa primarnim i sekundarnim aminima uz upotrebu natrijum-hipohlorita kao oksidacionog sredstava [26], pri čemu se dobijaju tionkarbamati kao komercijalni proizvodi:



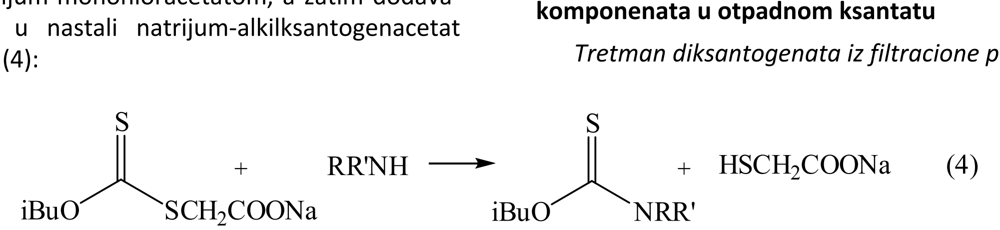
$$\underset{(I)}{RO-\overset{\overset{S}{\|}}{C}-S-S-\overset{\overset{S}{\|}}{C}-OR} + \underset{(II)}{2\,R_1NH_2} \longrightarrow \underset{(III)}{RO-\overset{\overset{S}{\|}}{C}-SN(H)R_1} + \underset{(IV)}{[R_1\overset{+}{N}H_3]\overset{-}{S}-\overset{\overset{S}{\|}}{C}-OR} \quad (1)$$

$$\underset{(III)}{RO-\overset{\overset{S}{\|}}{C}-SN(H)R_1} \longrightarrow \underset{(V)}{RO-\overset{\overset{S}{\|}}{C}-N(H)R_1} + S \quad (2)$$

$$2\underset{(IV)}{[R_1\overset{+}{N}H_3]\overset{-}{S}-\overset{\overset{S}{\|}}{C}-OR} + \underset{(VI)}{NaOCl} \longrightarrow \underset{(I)}{RO-\overset{\overset{S}{\|}}{C}-S-S-\overset{\overset{S}{\|}}{C}-OR} + 2R_1NH_2 + NaCl + H_2O \quad (3)$$

U ovim reakcijama, diksantogenat (I) reaguje sa alkilaminom (II), pri čemu nastaju međuprodukt sulfenamid (III) i aminska so alkilksantogene kiseline (IV) (reakcija (1)). U sledećem stupnju (reakcija (2)), sulfenamid se razlaže na sumpor i alkiltionkarbamat (V). Dalje, oksidacijom aminske soli ksantogene kiseline (IV) pomoću natrijum-hipohlorita (VI) nastaje diksantogenat (I) (reakcija (3)), a zatim se reakcija nastavlja sukcesivno.

Ksantat, koji se izdvaja iz industrijskog otpada, prerađuje se do odgovarajućeg alkiltionkarbamata reakcijom sa natrijum-monohloracetatom, a zatim dodavanjem amina u nastali natrijum-alkilksantogenacetat [29], reakcija (4):



$$iBuO-\overset{\overset{S}{\|}}{C}-SCH_2COONa + RR'NH \longrightarrow iBuO-\overset{\overset{S}{\|}}{C}-NRR' + HSCH_2COONa \quad (4)$$

Nakon izdvajanja proizvoda tionkarbamata, iz dobijenog natrijum-tioglikolata se, dodavanjem hlorovodonične kiseline i ekstrakcijom sa diizopropil-etrom, dobija tioglikolna kiselina.

EKSPERIMENTALNI DEO

Laboratorijski postupak tretmana otpadnog ksantata

Laboratorijski postupak razdvajanja komponenata u otpadnom ksantatu. U čašu zapremine 1 dm^3 ubaci se 100 g otpadnog ksantata, doda 400 cm^3 vode i uključi magnetna mešalica, tako da se mešanje vrši u toku 30 min. Nakon toga smeša se filtrira na Bihnerovom levku, filtraciona pogača ispira sa 200 cm^3 vode, a potom suši u vakuum sušnici na 30 °C (10 mm Hg). Pri tome se dobija 80 g etildiksantogenata, čija je struktura potvrđena FTIR i ^1H-NMR podacima [26]. Filtrat se prebaci u levak za odvajanje, i gornji deo, koji predstavlja organsku fazu – izobutil-alkohol, se odvaja od vodenog dela (izdvojeno je 1,7 cm^3 izobutil-alkohola). Vodena faza se analizira volumetrijski u cilju određivanja količine prisutnog ksantata, alkalija, tritiokarbonata i sulfida. Na osnovu standardne volumetrijske metode analize [31,32] utvrđeno je da se u vodenom delu nalazi 0,52 g natrijum-hidroksida, 5,5 g kalijum-izobutilksantata i 1,1 g kalijum-tritiokarbonata. Svi ispitivani uzorci otpadnog ksantata su tretirani po ovom postupku.

Laboratorijski postupak tretmana izdvojenih komponenata u otpadnom ksantatu

Tretman diksantogenata iz filtracione pogače. U trogrli balon od 250 cm^3, opremljen povratnim kondezatorom, levkom za kapanje, termometrom i magnetnom mešalicom, doda se 100 cm^3 vode i 20,65 g (0,075 mol) 98% etildiksantogenata i uključi mešalica. U reakcionu smešu se lagano dodaje 12,25 cm^3 (0, 15 mol) of 68% etilamina tokom jednog časa, tako da temperatura polagano raste od 19 do 30 °C. Nakon toga, dodaje se kap po kap 20,50 g (0,075 mol) natrijum-hipohlorita (130 g aktivnog Cl_2/1000 cm^3 rastvora). Temperatura reakcione smeše raste do 45 °C u toku dodavanja od 1,0 h, kada je reakcija završena. Tokom reakcije izdvajaju se koloidne čestice sumpora, čija količina odgovara stehiometriji reakcije. Reakciona smeša se profiltrira na Bihnerovom levku, pri čemu se izdvaja sumpor kao filtrataciona pogača, a filtrat je *N*-etil-*O*-etiltionkarbamat, koji je dispergovan u vodenoj fazi. Filtrat se prebaci u levak za odvajanje, odvoji gornja organska faza koja predstavlja proizvod reakcije. Proizvod se prečišćava vakuum destilacijom i hvata frakcija na 105–106 °C (660 Pa), pri čemu se dobija 18,80 g bezbojnog *N*-etil-*O*-etiltionkarbamata (81,8%). Čistoća dobijenog proizvoda određena gasnohromatografskom metodom (GC) iznosi 97,1%.

Na analogan način sintetisana su i drugi tionkarbamati.

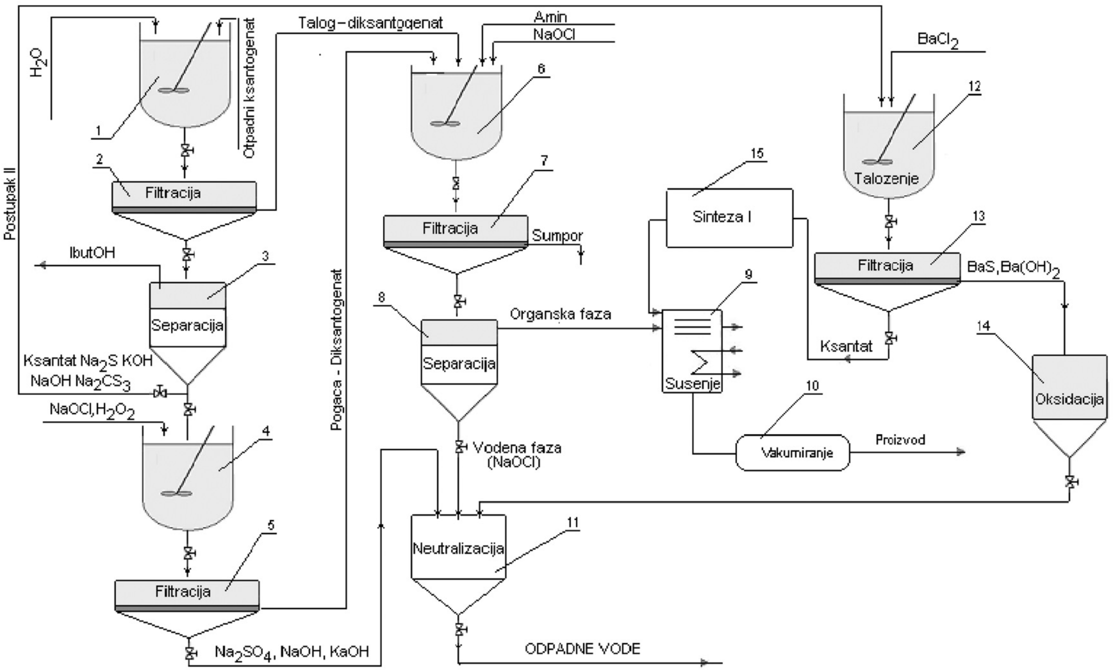
Tretman filtrata. Filtrat dobijen treiranjem otpadnog ksantata se prebaci u reakcioni balon od 2 dm^3 snabdeven mešalicom, levkom za kapanje, termometrom i povratnim hladnjakom. Zatim se doda 300 cm^3 vode i pomoću levka za kapanje dodaje 10 % rastvor $BaCl_2$ dok ne prestane izdvajanje čestica suspenzije. Nakon toga nastala suspenzija se filtrira na Bihnerovom levku, talog odvaja i tretira sa 30 cm^3 30% H_2O_2 do potpunog rastvaranja, a zatim neutrališe i ispušta u vodu. Izdvojeni filtrat (rastvor ksantata) se koristi kao polazni reaktant za reakcije sinteze *N*-alkil- i *N*,*N*-dialkil--*O*-izobutiltionkarbamata.

Sinteza N-alkil- i N,N-dialkil-O-izobutiltiokarbamata iz ksantata i alkilamina. Izdvojeni filtrat, koji predstavlja rastvor izobutil-ksantata (iButX) (0,6 mol), se prebaci u reakcioni balon od 2 dm^3 koji je snabdeven mešalicom, levkom za kapanje, termometrom i povratnim hladnjakom. Uključi se mešalica i pomoću levka za kapanje dodaje pripremljen rastvor natrijum-monohloracetata (NaMXAc) [28] 0,5 mola, uz održavanje temperature reakcione smeše od 35 do 40 °C u toku 2 časa, pri čemu nastaje natrijumova so izobutilksantogensirćetne kiseline (NaiButXAc). Zatim se, takođe, pomoću levka za kapanje, dodaje smeša 46 g (0,6 mol) 60,0% etilamina i 80 g (0,6 mol) 30% rastvora natrijum-hidroksida uz održavanje temperature reakcione smeše od 40 do 45 °C u toku 2 časa. Nakon završetka reakcije, reakciona smeša se razdvaja u levku za odvajanje, organska faza se ispira razblaženom hlorovodoničnom kiselinom (1:1) i vodom do pH 6, a iz vodenog dela se izdvaja tioglikolna kiselina. Organska faza se suši anhidrovanim natrijum-sulfatom, filtrira i destiliše frakcionom vakuum destilacijom, pri čemu se dobija 77,0 g (78,0 %) *N*-etil-*O*-izobutiltionkarbamata. Čistoća dobijenog proizvoda određena gasnohromatografskom metodom (GC) iznosi 98,0%.

Na analogan način sintetisana su i drugi tionkarbamati.

Poluindustrijski postupak tretmana izdvojenih komponenata u otpadnom ksantatu

Šema tehnološkog postupka tretmana industrijskog otpada koji potiče od otpadnog ksantata je prikazana na šemi 1.

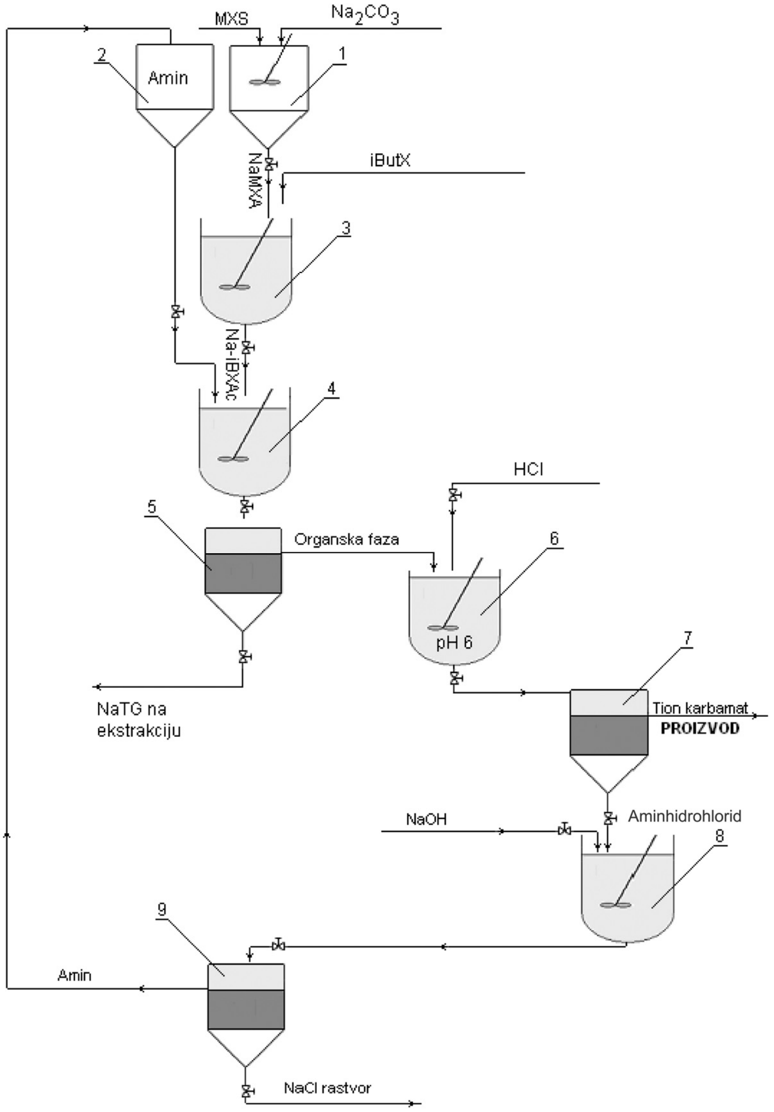


Šema 1. Šema tehnološkog postupka tretmana otpadnog ksantata.
Scheme 1. Scheme of waste xanthate treatment.

Novi postupak za preradu otpadnog ksantata, prikazan na šemi 1, počinje tako što se u mešaču – pozicija 1, otpadni ksantat tretira industrijskom vodom, filtrira – pozicija 2, separiše – pozicija 3, pri čemu se izdvaja izobutil-alkohol kao gornja organska faza, a vodeni deo se oksidiše pomoću natrijum-hipohlorita ili vodonik-peroksida – pozicija 4, filtrira– pozicija 5, filtrat prebaci u neutralizator – pozicija 11, a nakon toga ispušta u otpadne vode, a proizvod (filtraciona pogača) koristi kao reaktant – pozicija 6, za sintezu alkiltionkarbamata pomoću alkilamina i natrijum-hipohlorita. Nakon filtracije – pozicija 7, izdvaja se kao pogača sumpor, a filtrat se prebaci na separaciju – pozicija 8. Organska faza se suši – pozicija 9, vakuumira – pozicija 10 i izdvojeni proizvod (*N*-alkil- ili *N,N*-dialkil-*O*-izobutiltionkarbamat) pakuje. Vodena faza iz separatora – pozicija 8, se prebaci u neutralizator – pozicija 11, i ispušta u otpadne vode.

Druga ispitana mogućnost definisanog poluindustrijskog postupka se odnosi na tretman vodenog dela iz separatora – pozicija 3, šema 1. Vodeni deo iz separatora – pozicija 3, se prebaci u taložnik – pozicija 12, u koji se dodaje 10% rastvor $BaCl_2$ radi razdvajanja prisutnog ksantata od ostalih primesa koje se talože (sulfidi, tritiokarbonati, alkalije). Reakciona smeša se zatim prebaci u filter uređaj Nuč – pozicija 13, filtraciona pogača se oksidiše – pozicija 14, prebaci u neutralizator – pozicija 11 i ispušta u otpadne vode. Filtrat iz Nuča – pozicija 13, koji je rastvor ksantata u vodi, se prebaci u tehnološki deo za sintezu – pozicija 15 (predstavljena kompletno na šemi 2), pri čemu se dobija, takođe proizvod *N*-alkil- ili *N,N*-dialkil-*O*-izobutiltionkarbamat.

Iz šeme 2 se vidi da nakon sinteze natrijumove soli monohlorsirćetne kiseline (MXS) u reaktoru – pozicija 1, nastala so reaguje sa kalijum-izobutilksantatom (izdvojenim filtracijom, šema 2 – pozicija 13) u reaktoru 3.



Šema 2. Šema tehnološkog postupka tretmana izobutilksantata izdvojenog iz otpadnog ksantata (Sinteza I – pozicija 15, Šema 1).
Scheme 2. Scheme of isobutylxanthate (obtained by waste xanthate treatment (Synthesis I – position 15, Scheme 1).

Aminoliza nastale natrijumove soli etilksantogen sirćetne kiseline se vrši u reaktoru – pozicija 4, pomoću amina, koji se dodaje iz dozera za amin – pozicija 2. Nakon toga, organska faza se izdvaja u separatoru – pozicija 5 od vodenog rastvora natrijum-tioglikolata (NaTGK) koji se, dalje, neutrališe i ekstrahuje tioglikolna kiselina. Izdvojena organska faza se neutrališe pomoću hlorovodonične kiseline (1:1) u neutralizatoru – pozicija 6, separiše u separatoru – pozicija 7, a proizvod *N*-alkil-ili *N*,*N*-dialkil-*O*-izobutiltionkarbamat suši, vakumira i pakuje. Vodeni deo iz separatora – pozicija 7, koji predstavlja aminsku so hlorovodonične kiseline, se neutrališe pomoću natrijum-hidroksida – pozicija 9, u neutralizeru – pozicija 8, nakon čega se izdvaja amin u separatoru – pozicija 10, koji se ponovo vraća u proces – pozicija 2, a vodeni rastvor natrijum-hlorida ispušta u otpadne vode.

Instrumentalne metode

Gasnohromatografska analiza je rađena na aparatu Perkin-Elmer 8700, koji je opremljen plameno-jonizujućim detektorom i punjenom kolonom sa 5% OV-210 na Gas-Chrom Q: dužina 2 m, prečnik 0.3175 cm (1/8''). Uslovi izvođenja gasno hromatografske analize: temperatura injektora: 250 °C; temperatura detektora: 270 °C; temperatura kolone programski mod: 50 °C (5 min) → 10 °C/min → 130 °C (15 min); noseći gas: azot (čistoća 99,99%) – protok 1 cm^3/min; protok vazduha: 250 cm^3/min (čistoća 99,99%); protok vodonika: 25 cm^3/min (čistoća 99,99%).

^{1}H-NMR spektri su snimani na aparatu Bruker AC 250 na 250 MHz. Spektri su snimani na sobnoj temperaturi u deuterisanom hloroformu ($CDCl_3$) u 5 mm kivetama. Infracrveni spektri (FTIR) su dobijeni na aparatu FTIR BOMEM (Hartmann&Braun).

REZULTATI I DISKUSIJA

Laboratorijski postupak tretmana industrijskog otpadnog ksantata

U eksperimentalnom delu ovog rada opisan je laboratorijski postupak tretmana otpadnog ksantata, pri čemu se dobijaju polazni reaktanti za sintezu alkiltionkarbamata. Prva faza u ovom postupku predstavlja razdvajanje i identifikaciju prisutnih komponenata. U tabeli 1 su predstavljeni rezultati dobijeni u prvoj fazi laboratorijskog tretmana otpadnog ksantata. Uočava se da sastav otpadnog ksantata u tretiranom uzorku varira, s tim da je diksantogenat uglavnom prisutan u većoj količini (78 do 84%), osim u uzorku 2 (11%). Uzorak 2 sadrži 44,3% natrijum-etilksantata, što ukazuje da nije došlo do oksidacije i značajne degradacije komercijalnog pakovanja. Kod ostalih uzoraka vidi se da je došlo do oksidacije (prisustvo diksantogenata) i delimične degradacije proizvoda (prisustvo alkohola i alkalija). Prisustvo tritiokarbonata varira od 0,8 do 1,5%. U uzorku 2 određeno je, takođe, prisustvo 1,5% Na_2CS_3, što ukazuje na prisustvo veće količine alkalije koja je reagovala sa ugljen-disulfidom. Pored toga, postoji mogućnost da je dodata veća količina od stehiometrijske ili da nije završena reakcija pripreme alkoholata u toku sinteze ksantata.

Diksantogenat izdvojen iz otpadnog ksantata se tretira po postupku opisanom u eksperimentalnom delu, pri čemu se dobijaju odgovarajući alkiltionkarbamati. Rezultati reakcije sinteze etiltionkarbamata, polazeći od etildiksantogenata izdvojenog iz otpadnog ksantata i različitih alkilamina u prisustvu oksidacionog sredstva natrijum-hipohlorita, prikazani su u tabeli 2.

Na osnovu rezultata predstavljenih u tabeli 2 i poređenja sa literaturnim podacima [25,26,28], može se zaključiti da se pri optimalnim uslovima sinteze u ovom radu, dobijaju dobri prinosi i čistoća *N*-alkil- i *N,N*-dialkil-*O*-etiltionkarbamata. Niži prinosi reakcije su postignuti u sintezi *N,N*-di-*n*-propil-*O*-etiltionkarbamata (78,5%) i, *N*-izopropil-*O*-etiltionkarbamata (76,3%), a najniži prinos je ostvaren u sintezi *N,N*-diizopropil-*O*--etiltionkarbamatima (69,7%).

Struktura svih sintetisanih *N*-alkil-. i *N,N*-dialkil-*O*--etiltionkarbamata prikazanih u tabeli 2, potvrđena je na osnovu dobijenih FTIR i ^{1}H-NMR spektara i u saglasnosti je sa literaturnim podacima [20].

Ksantat izdvojen iz otpadnog ksantata se tretira po postupku opisanom u eksperimentalnom delu za reakciju sinteze tionkarbamata. U reakciji izdvojenog ksantata sa natrijum-monohloracetatom nastaje natrijum-izobutilksantogenacetat (NaiButXAc), koji reakcijom sa alkilaminima daje odgovarajuće tionkarbamate. Rezultati dobijeni u reakcijama sinteze tionkarbamata, polazeći od kalijum-izobutilksantata, izdvojenog tretmanom otpadnog ksantata, predstavljeni su u tabeli 3.

Tabela 1. Sastav otpadnog ksantata lagerovanog u H. I. Župa Kruševac. Sulfidi nisu identifikovani (određeni)
Table 1. Composition of waste xanthate from Chemical Industry Župa, Kruševac

Uzorak	Diksantogenat		Alkalije		Ksantat		Alkohol		Tritiokarbonati	
	Naziv	Količina, %	Naziv	Količina, %	Naziv	Količina, %	Naziv	Količina, %	Naziv	Količina, %
1	Izobutil	82,0	NaOH	0,52	K-*i*-butil	5,5	izobutil	1,7	K_2CS_3	1,1
2	Etil	11,0	NaOH	1,43	Na-etil	44,3	–	–	Na_2CS_3	1,5
3	Etil	84,0	NaOH	0,56	Na-etil	–	–	–	Na_2CS_3	1,2
4	Izobutil	78,0	NaOH	0,90	K-*i*-butil	13,0	izobutil	2,7	K_2CS_3	1,0
5	Izobutil	83,0	NaOH	0,95	K-*i*-butil	12,0	izobutil	2,3	K_2CS_3	0,8

Tabela 2. Prinosi i čistoće tionkarbamata dobijenih postupkom tretmana etildiksantogenata izdvojenog iz otpadnog ksantata
Table 2. Yields and purity of thioncarbamates obtained by the treatment of ethyldixanthogenate obtained from waste xanthate

R.b.	Jedinjenje	Amin	Reakciono vreme h	Reakciona temperatura °C	Temperatura ključanja, °C		Prinos %	GC čistoća %
					Eksp.[a]	Lit. [15][b]		
1.	EtOC(S)NHEt	Et NH_2	2,0	33–40	108–109	108–110	81,8	97,1
2.	$EtOC(S)NEt_2$	Et_2 NH	2,7	35–42	115–120	109–120	82,5	97,2
3.	EtOC(S)NHPr	Pr NH_2	2,5	35–43	124–128	126–128	82,0	97,9
4.	$EtOC(S)NPr_2$	Pr_2 NH	3,2	37–45	128–130	130	78,5	98,0
5.	EtOC(S)NHi-Pr	i-Pr NH_2	3,1	38 -46	126–129	127–129	76,3	99,1
6.	EtOC(S)Ni-Pr_2	i-Pr_2NH	4,3	42–48	130–135	132–134	69,7	98,2

[a]Vakuum 25 mbar; [b]vakuum 26 mbar

Tabela 3. Prinosi i čistoća dobijenih tionkarbamata postupkom tretmana kalijum-izobutilksantata izdvojenog iz otpadnog ksantata
Table 3. Yields and purity of thioncarbamates obtained by the treatment of potassium isobutylxanthate isolated from waste xanthate

R.b.	Jedinjenje	Amin	Reakciono vreme h	Reakciona temperatura °C	Temperatura ključanja, °C		Prinos %	GC čistoća %
					Eksp.[a]	Lit. [15][b]		
1.	i-ButOC(S)NHEt	$EtNH_2$	35–45	2,2	121–123	120–122	78,0	98,0
2.	i-ButOC(S)NHnPr	$nPrNH_2$	35–50	2,1	131–133	130–132	82,4	98,1
3.	i-ButOC(S)NHnBu	$nBuNH_2$	30–45	2,0	141–144	140–142	77,6	97,3
4.	i-ButOC(S)NEt_2	Et_2NH	35–50	2,8	145–148	144–146	75,01	98,0
5.	i-ButOC(S)NPr_2	Pr_2NH	35–45	2,0	178–180	178–179	85,4	97,6
6.	i-ButOC(S)NBu_2	Bu_2NH	35–45	2,1	194–195	192–194	87,7	98,3

[a]Vakuum 25 mbar; [b]vakuum 20 mbar

Reakcije sinteze *N*-alkil i *N*,*N*-dialkil-*O*-izobutiltionkarbamata predstavljaju reakcije nukleofilne supstitucije u kojoj nukleofil (amin) napada tiokarbonilni ugljenik NaiButXAc. Struktura nukleofilne čestice utiče, kako na brzinu, tako i na prinos proizvoda reakcija [29]. Na osnovu rezultata prikazanih u tabeli 3, vidi se da su postignuti značajni prinosi proizvoda kao i stepeni čistoće, što ukazuje na visok stepen konverzije amina. Upoređujući ostvarene prinose sa prinosima do sada objavljenih komparativnih postupaka, može se zaključiti da su ovi niži, ali kako su polazni reaktanti izdvojeni iz otpadnog ksantata, može se smatrati da su zadovoljavajući [28].

Poluindustrijski postupak tretmana industrijskog otpadnog ksantata

Na osnovu laboratorijskih istraživanja tretmana industrijskog otpadnog ksantata i dobijenih rezultata, razvijen je poluindustrijski postupak tretmana, kojim se praktično dobijaju kao proizvodi tionkarbamati, a ostali sastojci, sadržani u otpadu, uspešno razdvajaju i lageruju. Opisani tehnološki postupak prerade otpadnog ksantata, u stvari predstavlja novi tehnološki postupak sinteze alkiltionkarbamata iz industrijskog otpada koji sadrži ksantat.

Rezultati poluindustrijske prerade otpadnog ksantogenata su prikazani u tabelama 4 i 5. U tabeli 4 su prikazani rezultati prve faze tretmana industrijskog otpada koji sadrži ksantat (sastav dat u tabeli 1).

Tabela 4 pokazuje utrošak sirovina i količina izdvojenih komponenti. Izdvojeni izobutil-alkohol se može upotrebiti za sintezu izobutilksantata, a diksantogenat i vodeni rastvor ksantata za drugu fazu prerade prikazane na šemama 1 i 2. U drugoj fazi prerade, tj.

Tabela 4. Tretman industrijskog otpadnog ksantata sastava prikazanog u tabeli 1
Table 4. Treatment of industrial waste xanthate presented in Table 1

Šarža r.b.	Sirovine[a]				Reakcioni uslovi			Izdvojene komponente		
	Voda m^3	$BaCl_2$ (10%) kg	H_2O_2 (30%) m^3	HCl (15 %) m^3	Temp. °C	Vreme, h		Alkohol dm^3	Diksantogenat kg	Ksantat kg
						Rastvaranja	Taloženja			
1	900	23,0	0,015	0,030	30,0	1,0	1,0	2,0	79,0	5,0
2	1000	27,0	0,016	0,330	32,0	1,5	1,2	–	9,5	40,0
3	1300	18,0	0,011	0,036	31,0	0,6	1,1	–	87,0	–
4	1000	30,0	0,017	0,037	32,0	1,3	1,2	5,1	82,0	8,0
5	1100	24,0	0,015	0,034	30,0	1,2	1,2	1,8	80,2	4,3

[a]Količina otpadnog ksantata je 100 kg

Tabela 5. Rezultati druge faze prerade izdvojenih komponenata (diksantogenata i ksantogenata – tabela 4)
Table 5. Results of the second step of separated components treatment (dixanthogenates and xanthogenates – Table 4)

Šarža r.b.	Proizvod	Reaktanti[a]						Reakcioni uslovi		Sporedni proizvodi		Temp. ključanja, °C		Proizvodi		
		Diksantogenat kg	Ksantat kg	$ClCH_2COOH$ kg	Na_2CO_3 kg	Etilamin m^3	NaOCl m^3	Vreme h	*t* °C	Tioglikolat kg	Sumpor kg	Eksp.[b]	Lit.	Prinos kg	Prinos %	GC[c] %
1.	iBuOC(S)NHEt	79,0[d]	5,0[d]	2,6	1,5	0,40	0,66	3,0	45,0	3,9	16,1	119–121	120–122 [28][e]	73,3	76,5	96,8
2.	EtOC(S)NHEt	9,5[f]	40,0[f]	30,5	17,3	0,03	0,04	4,2	47,1	43,2	3,2	106–109	108–110 [15][g]	37,2	74,2	95,1
3.	EtOC(S)NHEt	87,0[h]	-	-	-	0,22	0,37	4,0	45,0	-	29,4	107–110	108–110 [15][g]	104,6	80,5	96,2

[a] $ClCH_2COOH$ (96%), $EtNH_2$ (70%, $\rho = 0,88$ g/cm^3), NaOCl (161,1 g akt. Cl_2/1000 cm^3), Na_2CO_3 (95%); [b] vakuum 25 mbar; [c] GC čistoća; [d] reaktant dobijen u šarži 1 – tabela 4; [e] vakuum 20 mbar; [f] reaktant dobijen u šarži 2 – tabela 4; [g] vakuum 26 mbar; [h] reaktant dobijen u šarži 3 – tabela 4

tretmana izdvojenog diksantogenata i ksantata dobijaju se komercijalni proizvodi – odgovarajući tionkarbamati (tabela 5).

U tabeli 5 su prikazani rezultati poluindustrijske proizvodnje tionkarbamata polazeći od izdvojenih reaktanata u postupku tretmana tri šarže otpadnog ksantata različitog sastava. U prvoj šarži se koriste reaktanti izobutildiksantogenat i izobutilksantat dobijeni u prvoj šarži prerade, tabela 4, pri čemu nastaje proizvod *N*-etil-*O*-izobutiltionkarbamat. U ovom tretmanu su izdvojeni reaktanti tretirani oksidacionim sredstvom natrijum-hipohloritom i etilaminom. Dakle, budući da je izdvojena mala količina izobutil-ksantata, primenjena je oksidacija prikazana na šemi 1 – pozicija 4 i filtracija – pozicija 5, a nakon toga sinteza – pozicija 6. Na ovaj način se ksantat oksidiše u diksantogenat i zajedno sa već izdvojenim ksantogenatom u prvoj fazi tretmana upotrebljava kao reaktant u reaktoru 6, šema 1.

U drugoj šarži koriste se reaktanti etildiksantogenat i kalijum-etilksantat dobijeni u drugoj šarži prerade, tabela 4, pri čemu nastaje proizvod *N*-etil-*O*-etiltionkarbamat. Kako je prisutna značajna količina ksantata, u ovom postupku tretmana rađene su obe metode sinteze prikazane na šemama 1 i 2. Diksantogenat je tretiran analogno prethodnoj šarži, dok je ksantat tretiran natrijum-monohloracetatom – pozicija 3, šema 2 i vršena aminoliza etilaminom – pozicija 4, šema 2. U ovom postupku tretmana nastaju kao nusproizvodi tioglikolat i analogno prethodnom, sumpor.

U trećoj šarži koristi se etildiksantogenat dobijen u trećoj šarži prerade, tabela 4, pri čemu nastaje proizvod *N*-etil-*O*-etiltionkarbamat. Zbog prisustva samo diksantogenata, u ovom postupku tretmana rađena je samo oksidacija pomoću natrijum-hipohlorita u prisustvu etilamina, šema 1 – pozicija 6. Pri tome se dobija sumpor kao nus-proizvod, koji se izdvaja kao filtraciona pogača na filter uređaju – pozicija 7, šema 1.

Na osnovu rezultata dobijenih poluindustrijskim postupkom proizvodnje, može se zaključiti da se lagerovani otpadni ksantat može tretirati na dva načina. Nakon prve faze tretmana (tabela 4), definiše se procentualni sastav, odnosno učešće izdvojenih ksantata i diksantogenata u otpadnom ksantatu. Dalji tretman na poluindustrijskom nivou zavisi od definisanog sastava u prvoj fazi prerade. Ukoliko je značajno učešće diksantogenata u odnosu na ksantat, primenjuje se oksidaciona metoda u prisustvu odgovarajućeg amina. Ovim postupkom se minimalne količine prisutnog ksantata oksidišu do diksantogenata i dalje pripajaju izdvojenom diksantogenatu na tretman. Ukoliko je značajno prisustvo ksantata, tretman se vrši reakcijom aminolize prethodno sintetisanog ksantogenacetata sa odgovarajućim aminima. Na ovaj način se prikazanim poluindustrijskim postupkom izdvojeni ksantat i diksantogenat iz otpadnog ksantata, kompletno prevode u odgovarajući tionkarbamat.

U sve tri šarže tretmana prikazane u tabeli 5, ostvarena je značajna konverzija (preko 70%), a dobijeni komercijalni proizvodi tionkarbamati imaju visok stepen čistoće određen GC metodom.

Struktura izdvojenih komponenata i svih sintetisanih proizvoda je potvrđena savremenim instrumentalnim metodama [28,30].

ZAKLJUČAK

U radu je opisan novi laboratorijski postupak tretmana industrijskog otpadnog ksantata. Opisanim postupkom je izvršena identifikacija i razdvajanje upotrebljenog otpadnog ksantata na sastavne komponente. Iz izdvojenih sastavnih komponenata diksantogenata i ksantata izvršena je sinteza tionkarbamata u laboratorijskim uslovima. Takođe, opisan je poluindustrijski postupak sinteze *N*-alkil- i *N*,*N*-dialkil-*O*-izobutiltion karbamata, polazeći od industrijskog otpadnog ksantata, koji se izdvaja kao filtrat nakon prve faze tretmana, u vodi kao rastvaraču. Ovakav put sinteze daje dobre prinose, a kao nusproizvod nastaje natrijum-tioglikolat koji se zakišeljavanjem prevodi u tioglikolnu kiselinu, što sa aspekta čistih tehnologija predstavlja metodu koja se može koristiti u industriji. Takođe, u ovom radu je dat postupak sinteze tionkarbamata reakcijom diksantogenata, koji je izolovan iz industrijskog otpada kao filtraciona pogača. Ovakav put prerade je interesantan sa aspekta jednostepenog postupka u jednoj šarži i pored dobrih prinosa i izdvajanja sumpora kao nusproizvoda, koristi kao rastvarač vodu koja se nakon izdvajanja finalnog proizvoda može ispuštati u vodotokove.

Zahvalnica

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SUMMARY

A NEW PROCEDURE FOR THE TREATMENT OF AN INDUSTRIAL WASTE CONTAINING FLOTATION REAGENTS

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(Scientific paper)

Flotation reagents can be transformed to industrial waste if they are stored for a long period of time. Also, if synthesis or drying process is not performed under defined conditions in industrial plants, which produce flotation reagents, batch of waste may arise and be stored as a waste. The chemical composition of this waste depends on the phase in which it was created, but typically includes: unreacted alkali hydroxide, solvent - alcohol and trithiocarbonate and oxidation product – dixanthogenate. In this paper a new laboratory procedure for the treatment of such wastes is described. the identification and separation of industrial waste components is also included. From the separated dixantogenate and xanthate a laboratory synthesis of thioncarbamates is given. In addition, a semi-industrial treatment of waste xanthate is presented. Synthesis of *N*-alkyl and *N,N*-dialkyl-*O*-isobutylthioncarbamates were obtained from the filtrate obtained in the first step. As a by-product, sodium thioglycolate was produced. This by-product is transformed to a thioglycolic acid by the addition of an acid. Also, the synthesis of thioncarbamates from dixanthogenates, isolated from industrial waste as a cake, is desribed. Described waste treatment is additionally interesting due to the production of sulphur as another by-product. laboratory synthesis gave thioncarbamates in yields from 69.7 to 87.7%, while the semi-industrial process for the selected batches produced thioncarbamates in yields from 74.2 to 80.5%. Taking into account the importance of the synthesized compounds as selective flotation reagents, a new procedure of their synthesis from industrial waste is characterized by good yields and purity of the obtained compounds, the simplicity of process, low environmental impact and short reaction times of synthesis.

Keywords: Waste xanthate • Dixanthogenate • Thioncarbamate • Sodium xanthogenacetate

Study on Mg^{2+} removal from ammonium dihydrogen phosphate solution by an emulsion liquid membrane

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Abstract

Mg^{2+} is extracted from ammonium dihydrogen phosphate ($NH_4H_2PO_4$) solution by an emulsion liquid membrane (ELM) using mono-(2-ethylhexyl) 2-ethylhexyl phosphonate (HEHPEHE) as a carrier, sulfonated liquid polybutadiene (LYF) as a surfactant and kerosene as a solvent. To study the extraction efficiency and advantages of the ELM process in the separation of Mg^{2+}, the effects of various operating conditions on the extraction – HEHPEHE volume fraction, reaction temperature, treat ratio (emulsion phase/external phase), phase ratio (membrane phase/internal phase), agitation speed, extraction time, internal phase concentration**,** surfactant LYF concentration and initial pH of $NH_4H_2PO_4$ solution are experimentally investigated and discussed. The results show that Mg^{2+} in $NH_4H_2PO_4$ solution can be effectively removed by the ELM process. An extraction efficiency of more than 83.1 % is attained at the optimized parameters and superior-grade $NH_4H_2PO_4$ can be obtained by two levels of extraction.

Keywords: wet process phosphoric acid, $NH_4H_2PO_4$ solution, Mg^{2+}, ELM, HEHPEHE.

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$NH_4H_2PO_4$ is used for flame retardant and drip-irrigation fertilization, which needs high pure $NH_4H_2PO_4$. In the past, the high purity of $NH_4H_2PO_4$ is manufactured with thermal-process phosphoric acid. The cost of thermal-process phosphoric acid is very high. The yellow-phosphorus manufacturers are closed one after the other because of the pressure from the energy consumption and environment protection. The cost of yellow phosphorus, as a basic raw material of thermal--process phosphoric acid is becoming higher and higher. Therefore, the low cost of wet process phosphoric acid (WPA) are gradually paid attention to in recent years. However, there are some undesirable impurities (Fe^{3+}, Al^{3+} and Mg^{2+}) in WPA. They will lower the quality of $NH_4H_2PO_4$ products. To get the superior grade $NH_4H_2PO_4$, WPA should be purified.

Improving the pH of the solution, usually between 4–4.5, the most of the metal ions can be removed, but there are still some Mg^{2+} which can cause formation of troublesome water-insoluble substance in the following concentration and crystallization process. The main ingredient of water-insoluble substance is magnesium-containing phosphate. Therefore, Mg^{2+} must be removed before concentrating the neutralized $NH_4H_2PO_4$ solution. Several methods based on solvent extraction [1,2] are used to remove Mg^{2+} industrially, however, in comparison with the solvent extraction process, an ELM process has main advantages such as very high mass transfer rates due to very thin liquid membrane and large interfacial areas between aqueous and organic phases, simultaneous extraction/stripping in one stage and small quantities of expensive extractant.

Since Li first applied an ELM to the separation of hydrocarbons [3], ELMs came to be an effective tool for the purification or separation of various materials, involving the transport of precious metal ions [4–6], rare earth elements [7], drugs [8–12], phenols [13–15], fructose [16–17], and the treatment of seawater [18] and waste water [19–22].

Consequently, in this paper, the ELM process is introduced to extract Mg^{2+} from the $NH_4H_2PO_4$ solution. The aim is to experimentally study the effects of various factors on the extraction efficiency using ELM.

EXPERIMENTAL

Materials

The solvent used in this work is kerosene. HEHPEHE is employed as an extractant produced by Luo Yang Zhong Da Chemical Company (China) (AR grade). The surfactant LYF is synthesized in our laboratory. Pure water is produced by Aquapro making-water machine (ABZ1-1001-P) in our laboratory.

Procedure

External aqueous solution (continuous phase) is prepared by dissolving magnesium sulfate heptahydrate in $NH_4H_2PO_4$ solution. Organic solution is prepared by dissolving HEHPEHE, as a carrier, and LYF as a surfactant in kerosene. Internal aqueous solution is prepared by dissolving hydrochloric acid in deionized

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water. A water-in-oil (W/O) emulsion (dispersed phase) is made by slow addition of the internal aqueous solution to the organic solution at optimal stirring speed (3000 rpm) by means of a motor-driven emulsifier (Shanghai Specimen Model Factory, China). The solution is stirred continuously for 5 min to obtain a white ELM as shown in Figure 1. This high shear-agitator produced high speeds, generating emulsions with small size droplets around 0.5–100 μm. The prepared ELM is added to specific volume of external aqueous solution. The contents are stirred by means of motor-driven at 300 rpm speed for a different transfer time.

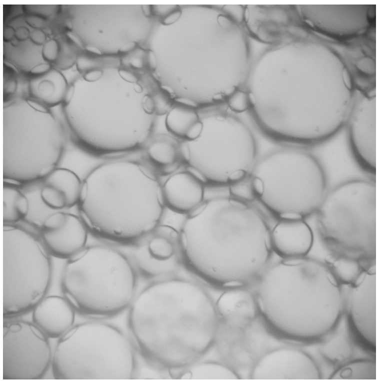

Figure 1. Samples of ELMs examined by a microscopic camera.

Parameters that could affect the extraction process

To study Mg^{2+} removal efficiency and advantages of the ELM process, it is necessary to optimize various parameters that could affect the process. The parameters to be optimized are the HEHPEHE volume fraction, reaction temperature, treat ratio (emulsion phase/external phase), phase ratio (membrane phase/ /internal phase), agitation speed, extraction time, internal phase concentration, surfactant LYF concentration and initial pH of $\mathrm{NH_4H_2PO_4}$ solution.

Analysis

The concentration of Mg^{2+} is determined by atomic absorption spectrophotometry (GF3000).

RESULT AND DISCUSSION

In the extraction process, the carrier (HA) reacts with the solute (Mg^{2+}) at the interface between organic and aqueous phases and forms an oil soluble complex according to the following equation:

$$nq\mathrm{Mg}^{2+}_{(a)}+\frac{q(s+2n)}{m}(\mathrm{HA})_{m(o)}\rightarrow \\ \rightarrow(\mathrm{Mg}_n\mathrm{A}_{2n}\times s\mathrm{HA})_{q(o)}+2nq\mathrm{H}^{+}_{(a)} \qquad (1)$$

where m is the aggregation number of HEHPEHE.

At the membrane solution phase - stripping solution interfaces according to the following equation:

$$(\mathrm{Mg}_n\mathrm{A}_{2n}\times s\mathrm{HA})_{q(o)}+2nq\mathrm{H}^{+}_{(a)}\rightarrow \\ \rightarrow nq\mathrm{Mg}^{2+}_{(a)}+q(s+2n)(\mathrm{HA})_{m(o)} \qquad (2)$$

The extraction ratio (*E*) is defined as followed:

$$E=\frac{M(\mathrm{Mg}^{2+}_{(i)})-M(\mathrm{Mg}^{2+}_{(r)})}{M(\mathrm{Mg}^{2+}_{(i)})}\times 100 \qquad (3)$$

E represents the efficiency of ELM; $M(\mathrm{Mg}^{2+}_{(i)})$: mole of Mg^{2+} in initial solution, mol; $M(\mathrm{Mg}^{2+}_{(r)})$: mole of Mg^{2+} in the raffinate.

Effect of carrier concentration

It is well known that the efficiency of ELM is directly affected by the concentration of the carrier. In a lower carrier concentration, the interface between the feed solution and membrane is not saturated by the carrier. The increasing of the carrier concentration in ELM will lead to two effects: the viscosity of membrane phase decreases and hence increases the extraction rate. At the same time, the stability of the emulsion will decrease when the carrier concentration is increased to a certain limit [23–26]. On the other hand, an increase in concentration of the carrier in the membrane phase will increase the extraction ability of the membrane phase. The effects of carrier concentration are shown in Figure 2.

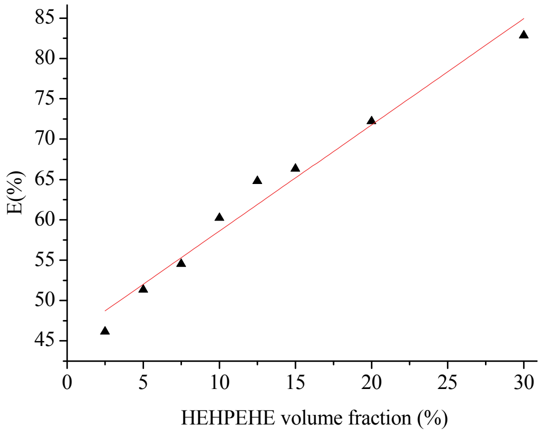

Figure 2. The effects of the HEHPEHE volume fraction (%) on the extraction ratio (E).

Effect of treat ratio (emulsion phase / external phase)

It seems that a large amount of Mg^{2+} and water could be transported into the internal phase at a high treat ratio. In fact, however, the net transport amount of Mg^{2+} is limited by the capacity of the internal phase. As seen from Figure 3, with the decreasing of treat ratio, the extraction ratio (E) decreases gradually. It is well known that HEHPEHE plays an important role in facilitating the Mg^{2+} transport into the internal phase. As the absolute amount of HEHPEHE in the extraction process is decreased, undoubtedly, the transport efficiency of Mg^{2+} also decreases.

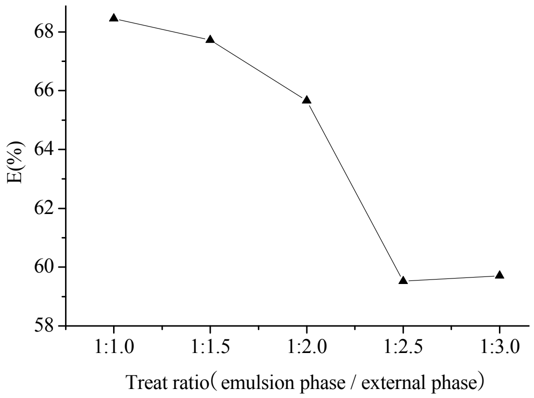

Figure 3. The effects of the treat ratio on the extraction ratio (E).

Effect of phase ratio (membrane phase / internal phase)

In order to form a stable and effective W/O emulsion, the phase ratio must exceed 1:1. With the increase of the phase ratio, the extraction and swelling rates are both strongly improved. The results are shown in Figure 4. This is due to the absolute amount of each component in the membrane phase is raised. It is well known that the HEHPEHE plays an important role in facilitating the Mg^{2+} transport into the internal phase. As the absolute amount of HEHPEHE in the membrane phase is increased, undoubtedly, the transport rate of Mg^{2+} also increases. Meanwhile, it is also demonstrated by Jing Qing Shen [27] that increasing the absolute amount of HEHPEHE and LYF enhances the swelling rate of the membrane because of their hydration properties.

Effect of extraction time

For obtaining the extraction equilibrium time experiments of effect of extraction time on the extraction ratio (E) are carried out. From Figure 5, it can be seen that the extraction equilibrium is achieved in 25 min.

So an extraction time of 25 min is used for all subsequent extraction and stripping experiments.

Effect of agitation speed

As the mixing speed increases, two phenomena occur: 1) the globule size decreases and 2) the breaking rate of the globules increases. A smaller globule size will lead to a larger transfer interfacial area between the feed and the membrane. This increased transfer area allows the extraction to occur at a higher rate, and explains the observations noted above. On the other hand, the higher rate of shear and subsequent higher rate of breakage of globules at the higher agitation speed values, the higher rate of breakage allows more leakage of the solute into the feed phase from the internal phase [28]. Therefore, from the Figure 6, the optimum value for agitation speed is found to be 350 rpm.

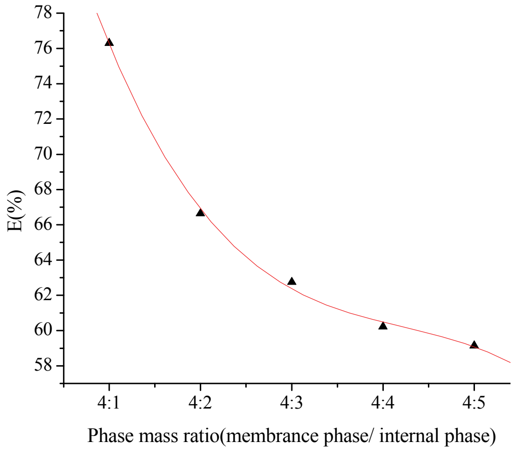

Figure 4. The effects of the phase mass ratio on the extraction ratio (E).

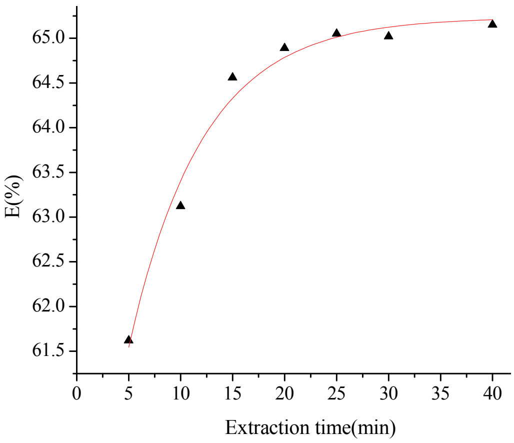

Figure 5. The effects of the extraction time on the extraction ratio (E).

Effect of initial pH of $NH_4H_2PO_4$ solution

The extraction efficiency of Mg^{2+} is obviously affected by the acidity of $NH_4H_2PO_4$ solution. As shown in Figure 7, the extraction efficiency increases with the increase of pH of $NH_4H_2PO_4$ solution. This may be explained as follows.

The strong acidity is favorable to form Mg^{2+}. However, the strong acidity is unfavorable to the ion-exchange reaction between Mg^{2+} and the carrier with an increase of H^+ concentration according to Eq. (1), because the equilibrium is shifted to the left side of the above extraction reaction with an increase in H^+ concentration. The final results are controlled by the competition of these two factors.

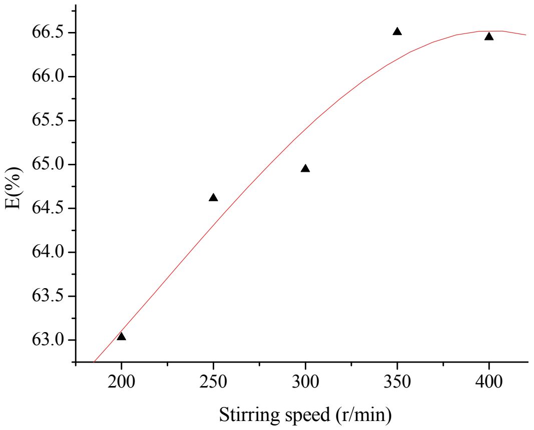

Figure 6. The effects of the agitation speed on the extraction ratio (E).

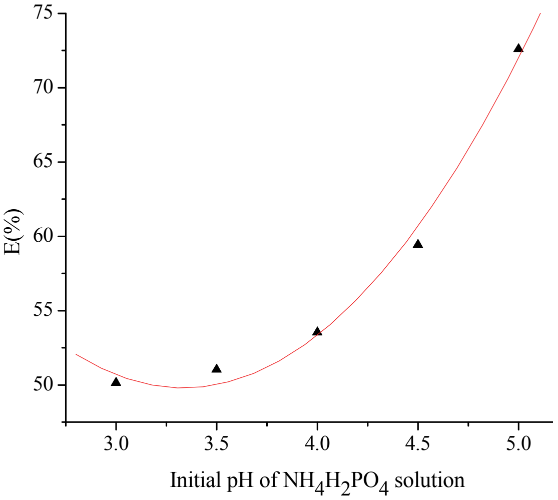

Figure 7. The effects of the initial pH of $NH_4H_2PO_4$ solution on the extraction ratio (E).

Effect of HCl concentration in internal phase

It is quite clear from equation (2) that the transport of Mg^{2+} through the liquid membrane is dependent on the H^+ concentration in the internal phase.

The results shown in Figure 8 indicate that the extraction efficiency decreases with an increase of the concentration of the stripping agents. If the amount of protons increases up to 1.0 and 2.0 M, the Eq. (2) will shift backward and there may be difficulties in dissociating the $MgCl_2$ from MgA_2. This causes the decrease in the efficiency.

Effect of surfactant LYF concentration in membrane

The surfactant concentration in the membrane phase is of crucial importance to any ELM system. While it ensures the stability of the membrane during the extraction, it also changes the viscosity of the membrane phase, so as to change the mass transfer resistance. Figure 8 shows the effects of various LYF concentrations on the extraction ratio (*E*). The figure indicates that the extraction ratio of Mg^{2+} is improved with the increase of LYF concentrations. However, when the LYF concentration increases to certain value about 2.0%, the extraction ratio (*E*) remains almost unchanged. On the other hand, however, an extremely low surfactant concentration cannot stabilize the membrane. As seen from Figure 9, a LYF concentration of 2.0% seems optimal.

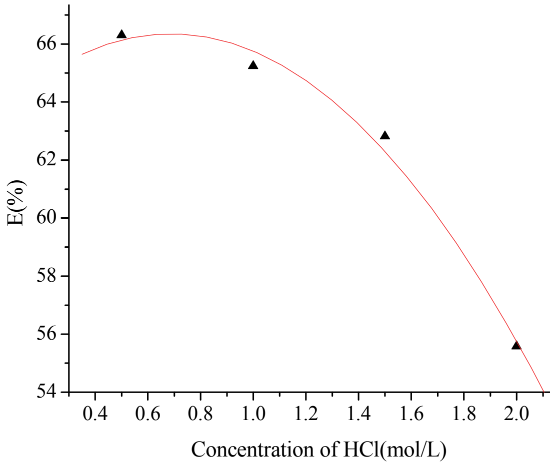

Figure 8. The effects of HCl concentration in feed solution on the extraction ratio (E).

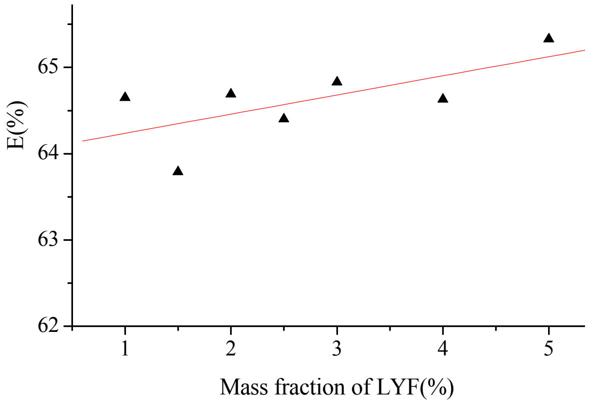

Figure. 9. The effects of the LYF concentration on the extraction ratio (E).

Effect of reaction temperature

The influence of temperature on the transport of Mg^{2+} through the liquid membrane containing HEHPEHE in kerosene is examined at 293, 297, 301, 305, 309 and 313 K.

As can be seen in Figure 10, the extraction efficiency increases as the temperature rises up. From the famous Van't Hoff equation: $\mathrm{dlog}\,D/\mathrm{d}(1/T) = -\Delta H/(2.303R) + \mathrm{const}$, ΔH value is 3.67 $\mathrm{kJ{\cdot}mol^{-1}}$, which shows that the extraction of Mg^{2+} with HEHPEHE is endothermic.

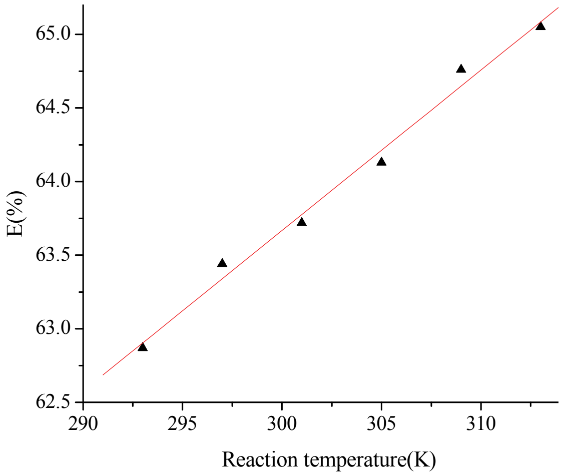

Figure 10. The effects of the reaction temperature on the extraction ratio (E).

Examination

A kind of practical wet-process phosphoric acid is neutralized to pH 4.5 with ammonia and filtered. The neutralized solution is then extracted under the above-mentioned optimal technological conditions. The superior grade $NH_4H_2PO_4$ is produced by the following concentration and crystallization process using the extracted solution. As Table 1 shows, the food grade $NH_4H_2PO_4$ can be obtained.

Table 1. Composition of $NH_4H_2PO_4$ product

Item	N, %	P_2O_5, %	Fe^{3+}, %	Mg^{2+}, %	Al^{3+}, %	Heavy metal Pb, %
Mass fraction	≥12	≥60.5	≤0.0003	≤0.0008	≤0.0002	≤0.0005
Item	As, %	F, %	SO_4^{2-}, %	pH	H_2O, %	Water-insoluble substance, %
Mass fraction	≤0.0050	≤0.0085	≤0.0020	4.5-4.8	≤0.2	≤0.05

CONCLUSION

Based on the results of this research on the removal of Mg^{2+} from $NH_4H_2PO_4$ solution with ELM, the following specific conclusions can be drawn:

1. ELM extraction is an effective method for the removal of Mg^{2+} from $NH_4H_2PO_4$ solution.

2. The optimized parameters are as follows: the HEHPEHE volume fraction: 30%; the reaction temperature: 35 °C; the treat ratio: 1:2; the phase ratio: 4:1; the LYF volume fraction: 1–1.5%, the agitation speed: 300 r/min, the extraction time: 25 min, the internal phase concentration: 0.5 mol/L and the initial pH of $NH_4H_2PO_4$ solution: 4.5.

3. The enthalpy change ΔH of the extraction process is 3.67 $kJ \cdot mol^{-1}$.

Acknowledgement

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IZVOD

PRIMENA TEČNIH MEMBRANA ZA EKSTRAKCIJU Mg^{2+} IZ RASTVORA AMONIJUM-DIHIDROGENFOSFATA

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(Naučni rad)

Joni Mg^{2+} su izdvojeni iz rastvora amonijum-dihidrogenfosfata ($NH_4H_2PO_4$) primenom tečnih membrana u obliku W/O emulzije (ELM); nosač je mono-(2-etilheksil)-2-etilheksilfosfonat (HEHPEHE), površinski aktivna materija je sulfonovani tecni polibutadien (LYF), a rastvarač je kerozin. Za definisanje efikasnosti ekstrakcije i prednosti ELM procesa pri separaciji Mg^{2+} praćeni su i diskutovani uticaji različitih operacionih uslova, na ekstrakciju HEHPEHE, na primer: reakciona temperatura, odnos teške i lake faze, odnos međupovršine i teške faze, brzina mešanja, vreme ekstrakcije, koncentracija teške faze, koncentracija površinski aktivne materije, i početna pH vrednost rastvora $NH_4H_2PO_4$. Dobijeni rezultati pokazuju da joni Mg^{2+} iz rastvora $NH_4H_2PO_4$ mogu biti efikasno uklonjeni primenom ELM procesa. Pri optimalnim parametrima procesa ostvarena je efikasnost ekstrakcije od 83.1%, a $NH_4H_2PO_4$ visoke čistoće (*superior-grade*) može se dobiti dvostepenom ekstrakcijom.

Ključne reči: Fosforna kiselina-mokri postupak • Rastvor $NH_4H_2PO_4$ • ELM proces • Ekstrakcija Mg^{2+}

An alkali catalyzed transesterification of rice bran, cottonseed and waste cooking oil

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Abstract

The biodiesel production by trans-esterification of three raw materials including virgin and used edible-oil and non-edible oil has been presented in this paper. A two step method following acidic and alkali catalyst was used for non-edible oil due to the unsuitability of using the straight alkaline-catalyzed trans-esterification of high FFA present in rice bran oil. The acid value after processing of rice bran, cottonseed and waste cooking oil was found to be 0.95, 0.12 and 0.87, respectively. The influence of three variables on percentage yield was studied at this stage, *i.e.*, methanol to oil molar ratio, reaction temperature and reaction time. The cottonseed oil, waste cooking oil and rice bran oil showed a maximum yield of 91.7, 84.1 and 87.1% under optimum conditions. The fuel properties of those three biodiesels have met standard biodiesel fuel results.

Keywords: biodiesel, rice bran oil, cottonseed oil, waste cooking oil.

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Besides coal and natural gas, with the exception of hydroelectricity and nuclear energy, the petroleum resources have been supplying the world energy needs. However, the rising concerns on depleting reserves of these natural resources, which are predicted to be consumed by the end of the next century and environmental problems that arise from the use of fossil fuel, have led the worldwide economy to endorse both the academic and industrial researchers to explore new sources of fuel [1]. One of the feasible sources, which is able to perform as well as the fossil fuel and also considered as environment-friendly is biodiesel, as a result of trans-esterification reaction. The straight vegetable oils have been used in the past; however, due to the issues of high viscosity, deposit formation in the injection system and poor cold start, it is extremely important to reduce the viscosity of oil [2]. There are various techniques that could be used to reduce the viscosity of vegetable oils, especially pyrolysis, micro-emulsion and trans-esterification [3].

Trans-esterification is a chemical reaction that occurs between triglycerides and alcohol in the presence of a catalyst with glycerol as a major by-product [4] as shown in Figure 1.

The advantage of transesterification is that it decreases the viscosity of the vegetable oils and improves the fuel properties of fatty acid esters. This is due to the fact that viscosity of vegetable oils is much higher than in diesel; there would have been several problems if these vegetable oils were directly used in diesel engine as a fuel [5].

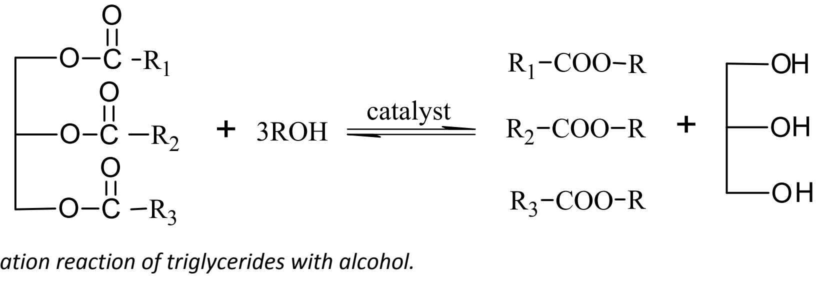

Figure 1. Trans-esterification reaction of triglycerides with alcohol.

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Global energy scenario

Today, in the modern age, with the increase in demand of fossil fuels, the reservoirs of fossil fuels are decreasing day by day due to its increasing demand in this era. According to an estimate, the reserves will last for 218 years for coal, 41 years for oil and 63 years for natural gas [6–8]. The economic growth during industrial revolution in the late 18th and 19th century has created serious problems for energy shortage. The world primary energy production is almost the same since those years, but consumption is rapidly increasing and creating an unbalance between demand and supply. It is believed that 50% more energy will be required round the world till 2030 [9,10] as shown in Figure 2.

The major part in this energy consumption is industry and transportation sector. Transportation is the second largest energy consuming sector. The most of the fossil fuel consumption for transportation sector is oil consumption, which is around 97.6%. The major oil demand is expected to be raised for transportation sector [11]. Although the major source of energy is oil and it is expected to be the largest source of energy, but dependence on oil share for energy is expected to decrease by 5% from current till 2035 [11].

Global environment issues

The climate change is believed to be the current most important global issue. The temperature of the Earth is rapidly increasing with the passage of time. If this may be allowed to rise at a rate of 2 degrees per year, there would be a drastic effect for human beings and as a result thousands of people may lose their lives just because of this issue [12]. The CO_2 levels in the atmosphere have increased from 280 to 350 ppm from the pre-industrial era till now. This increase in the CO_2 levels is mainly due to the aggressive use of fossil fuels [13]. Approximately, 8.6 billion metric tons (BMT) of carbon dioxide is expected to be released into the atmosphere from 2020 to 2035 [11,14]. Figure 3 shows the expected increase in CO_2 emissions till 2035 [11].

Most of the countries have started working towrds the renewable energy sources and almost all countries are seriously working on the reduction of the emissions. For this purpose, they have set standards and targets to their greenhouse gas (GHG) emissions to certain levels. Europe aims to reduce GHG emissions by one fifth till 2020. US president has announced to reduce GHG emissions by 28% till 2020. China has planned to control GHG emissions till 45% as from 2005. Japan has planned to cut GHG emissions to a target of 60–80% by 2050 from their emissions in current level [15]. Similarly India, Germany, Brazil, Pakistan and some other countries have made their targets to reduce GHG emissions as shown in Table 1.

The alternative renewable fuels therefore have received increasing attention for future utilization. In this respect the fermentation, transesterification, pyrolysis of biomass, industrial and domestic wastes have been proposed as alternative solutions. Among these different approaches, the trans-esterification seems to be a simple and an efficient method for fuel production [16].

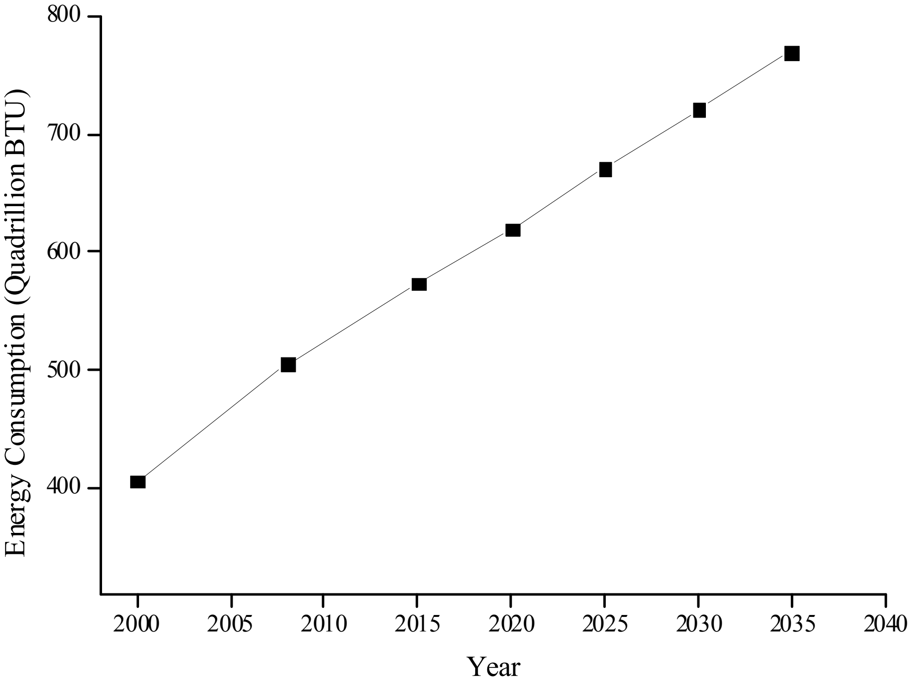

Figure 2. World energy consumption from 2000–2035.

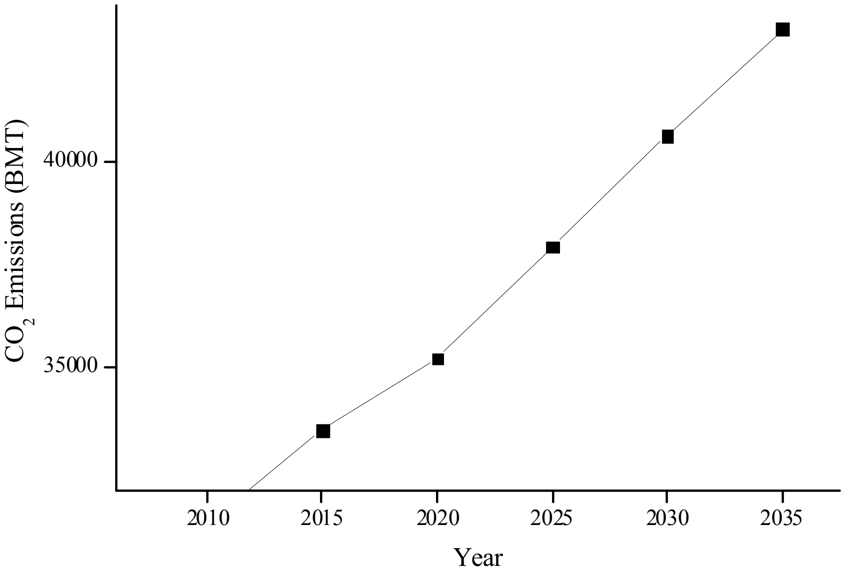

Figure 3. World CO_2 emissions.

The cost of biodiesel is higher than the conventional fossil fuel, but it can be controlled by using waste vegetable oil instead of fresh oil that has major contribution towards the increase in the price of biodiesel. For the maximum projection of biodiesel, the cost of biodiesel should be less as compared to fossil fuels, so that it can attract people towards this new change [17].

Technologies for biodiesel production

There are different technologies that can be used for the trans-esterification processes. This depends upon the amount of free fatty acids and the water content present in the feedstock. The alkali catalytic process is more effective when the FFA level is less than 1%. When the FFA contents of the raw material are greater than 1% then acid catalyzed process is preferred. Anyhow, this process requires higher catalyst concentration and high molar ratio that results in corrosion issues [18]. A brief description of the process used for trans-esterification reaction is presented in Figure 4.

The current research work investigates the optimal reaction conditions of biodiesel production from cottonseed oil, waste cooking oil and rice bran oil. It has been explored that the three oils used in this study could be viable for biodiesel production. Hence, it can contributed to the development of renewable energy resource.

MATERIALS AND METHODS

Crude rice bran oil (RBO) and cottonseed oil (CSO) were obtained from the local market while the waste cooking oil (WCO) was taken from the local fast food corner. Methanol (99.8%) and potassium hydroxide were purchased from Merck Chemicals.

Oil characterization

All three oils were analyzed for different properties which are listed in Table 2.

Experimental set-up

A lab scale experimental setup was developed for the transesterification reaction of biodiesel production. This was comprised of reaction flask, water bath, condenser and magnetic stirrer. The glass reactor was 1000 ml capacity having 3 necks, one for condenser and others for temperature detection and inlet of reactants. The temperature indicator was used to measure the reaction temperature.

Table 1. World energy targets

Country	Target
World	5.2% GHG emissions reduction till 2012 on 1990 levels
Germany	10% target of biodiesel
China	15% biodiesel share till 2020
UK	Renewable transport fuels accounting 10% till 2015
USA	4% soya biodiesel in 2016
India	10% renewable energy share till 2012
Pakistan	10% biodiesel share by volume till 2025

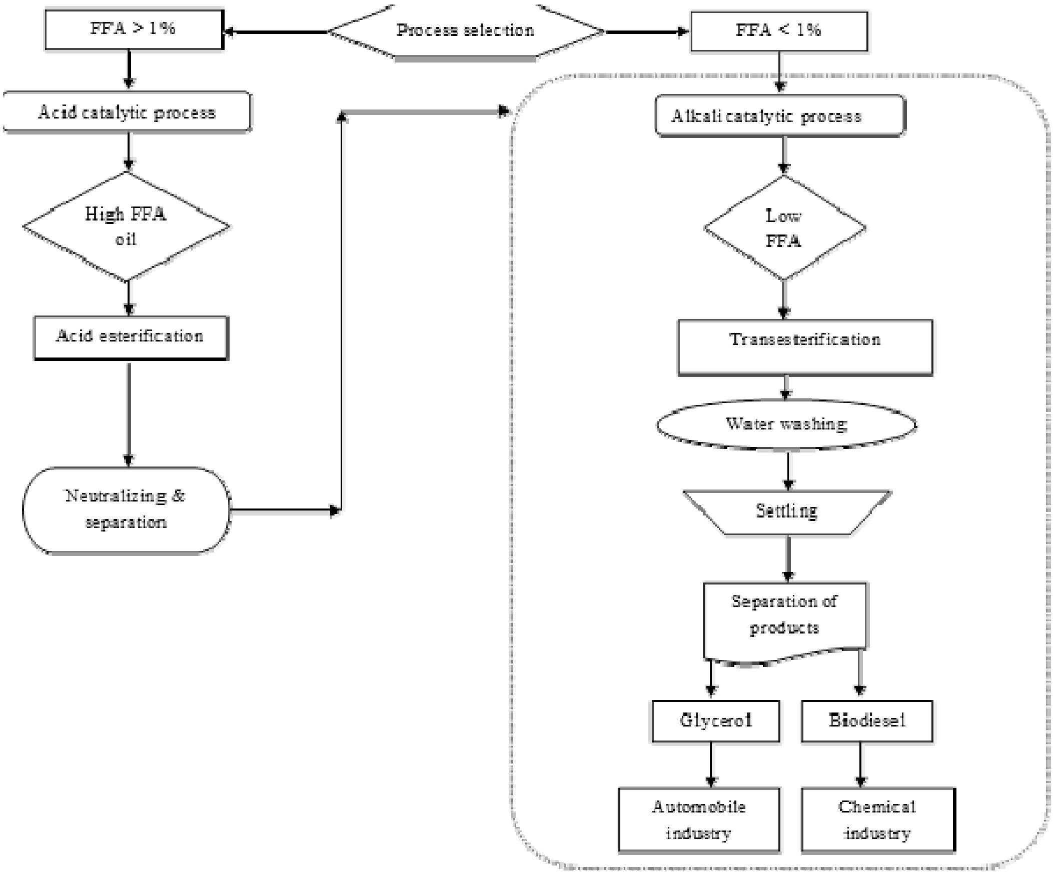

Figure 4. Steps for biodiesel production.

RESULTS AND DISCUSSION

Pretreatment and acid value determination

The three oil samples of cottonseed, rice bran and waste cooking oil were heated up to 40 °C to reduce the viscosity and then they were filtered to make them free of any unwanted suspended particles. Water is not required in the oil samples since it inhibits the process due to the saponification of the samples instead of trans-esterification. Therefore filtered oil samples were heated up to 105 °C to remove traces of water.

The acid value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 g of sample. The acid value of the three samples was measured using the Eq. (1):

$$\text{Acid value} = \left[\left(A-B\right) M\times 56.1\right]/W \qquad (1)$$

where A = KOH required for titration of sample, mL, B = KOH required for blank titration, mL, M = molarity of the KOH solution and W = sample used, g.

The acidity can either be measured as acid value or as free fatty acids (FFA) present [19]. Free fatty acids are the saturated or unsaturated monocarboxylic acids which are present in nature in fats, oils and greases but are not attached to glycerol backbones [20]. Higher amount of free fatty acids results in higher acid value.

Table 2. Physical and chemical properties of rice bran, cottonseed and waste cooking oil

Property	RBO	WCO	CSO
Palmitic acid C16:0	18.8	16.3	20.6±1.2
Stearic acid C18:0	2.4	5.63	4.6±0.31
Oleic acid C18:1	43.1	32.23	19.54±0.43
Linoleic acid C18:2	33.2	39.47	52.5±1.44
Linolenic acid C18:3	0.6	0	0.23±0.05
Arachidic acid C20:0	0.7	0.12	0.31±0.11
Kinematic viscosity at 40 °C, cSt	42.15	54.3	35.7
Acid value, mg KOH/g	38	0.87	0.12

For alkaline trans-esterification the free fatty acids value should be in the desired limits otherwise either the yield will be very low or reaction will not take place. Most researchers have different opinion about the level of FFA, but usually it is ranging from 0.5 to 3% for alkaline trans-esterification [21–24].

The acid value for cottonseed oil and waste cooking oil was found to be 0.12 and 0.87, respectively, but for the rice bran oil it was 38 and a two step acid catalyzed reaction was carried out to reduce it to the desired limit. In each step effect of molar ratio (methanol/oil) and the reaction time (45, 60, 75 and 90min) on acid value of the crude rice bran oil was studied. A 500 ml sample of rice bran oil was heated by placing it in the water bath. The sulfuric acid methanol solution was added in the reaction flask along with preheated oil sample. This was stirred for some time to make the solution homogeneous. The reaction mixture was poured into a separating funnel after the completion of this reaction. The impurities present were removed along with excess methanol and sulfuric acid. The acid value of the product was measured at the predefined intervals [4]. For the first step, the Acid Value of 3.6 mg KOH/g was selected, which consumed 75 min and molar ratio of 1:7 given in Figure 5.

This was used as a raw material for second step and the same experiment was repeated. The acid value 0.95 KOH/g was selected using minimum amount of alcohol and minimum time required, as shown in Figure 6. The final reaction mixture was separated using a separating funnel, washed, centrifuged and was used for further analysis.

Transesterification reaction

The experimental setup was established for trans-esterification process. All three oils were preheated before starting the reaction to avert moisture absorbance. A fresh prepared KOH-methanol solution was used in order to uphold the catalytic activity. The methanolic solution was added to all three reaction flasks. At proper intervals of time, the samples were withdrawn from reaction mixture and analyzed. The constant speed of 400 rpm was used throughout the reaction.

The product mixture from three reaction flasks was poured into separating funnel. The esters were separated from the lower aqueous glycerol layer under gravity and then were washed with distilled water. The washed esters were dried under vacuum and used for subsequent analysis.

Effect of methanol to oil molar ratio

The molar ratio of methanol to oil is very important factor as it affects the yield and also the cost of overall reaction. Higher molar ratio generates more esters than lower ratios in lesser time. The vegetable oils can be converted to biodiesel using 6:1 to 40:1 oil/alcohol molar ratio [25]. According to stoichiometric ratio, 3 mol of alcohol and 1 mol of triglyceride produce 3 mol of ester and 1 mol of glycerol for the trans-esterification reaction to occur. However, as trans-esterification is an equilibrium reaction, in practice, excess alcohol is required to move the reaction to forward direction. In this study, effect of methanol in the range

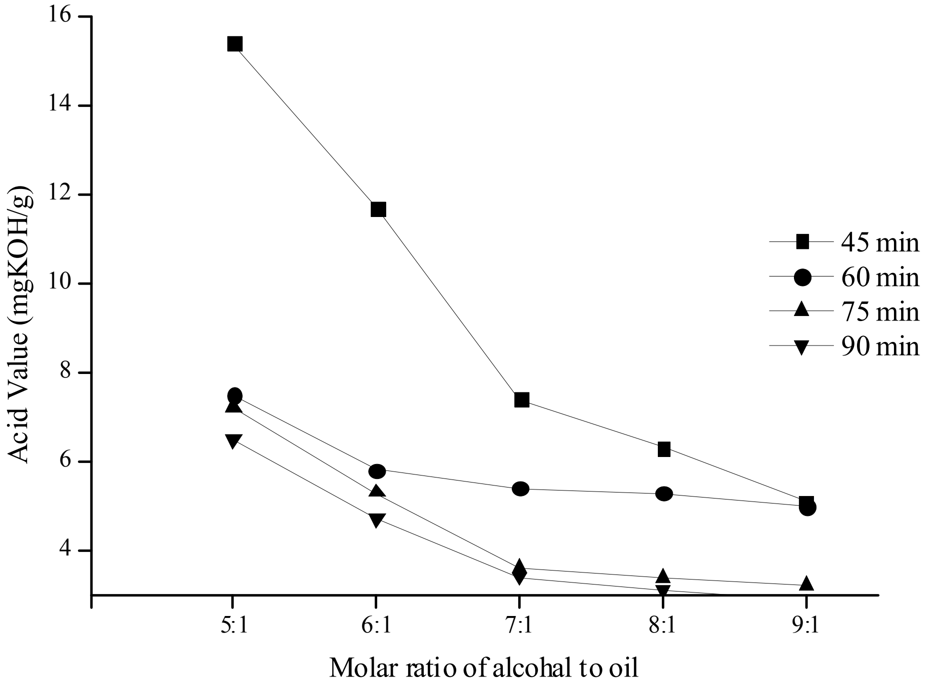

Figure 5. First step: acid value reduction.

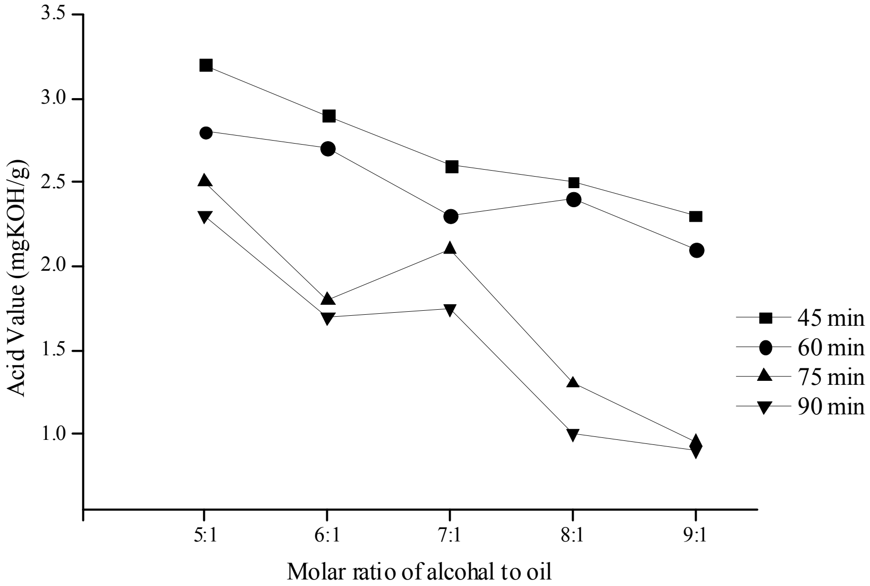

Figure 6. Second step: acid value reduction.

of 3:1 to 12:1 was investigated, keeping other process parameters fixed. The reaction temperature was kept constant at 55±1 °C, and reaction was carried out for 1 h at 400 rpm. The results are shown in Figure 7. Rice bran oil yield was found to be maximal at 6:1 molar ratio. However, after the molar ratio of 6:1, the conversion remained less. Waste cooking oil showed two different layers at a molar ratio of 3:1 due to the presence of un-reacted triglycerides. This showed that methanol delivered was not enough to perform the complete reaction. At a molar ratio of 6:1, a maximum yield of 86% was achieved. At molar ratio of 9:1; the yield was 87.3%. For cottonseed oil, the reaction was incomplete for a molar ratio less than 6:1 and no separation was observed during the separation process. It is observed that beyond molar ratio of 6:1 further addition of methanol had very little effect on % yield of biodiesel. With further increase it remained nearly constant. In addition, the higher molar ratio of alcohol to oil makes the solubility higher, which as a result inhibits the separation of glycerin. This presence of glycerin drives the equilibrium to backward direction and results in lower yield of esters [27].

Effect of reaction temperature

Reaction rate is strongly influenced by the reaction temperature. The trans-esterification reaction can even occur at room temperature provided sufficient time is

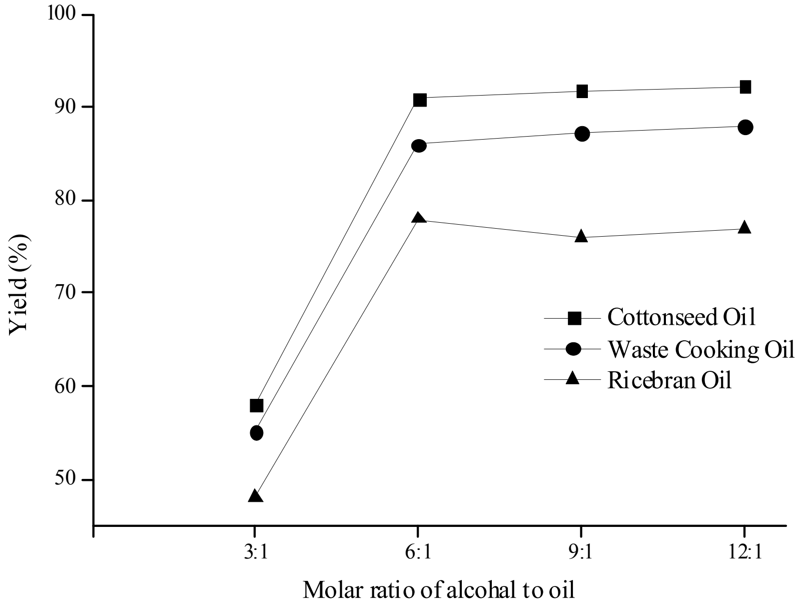

Figure 7. Effect of methanol to oil ratio on methyl ester yield.

given for the reaction to occur. The effect of reaction temperature in this study was investigated using the optimal parameters achieved in previous sections. Several investigators found that although the reaction is usually carried out at nearly boiling point of alcohol, the maximum yield has been reported at a temperature range of 60–80 °C at a molar ratio of 6:1 [23,27,28].

The effect of reaction temperature on the production of biodiesel is shown in the Figure 8. Temperature was changed in the intervals of 5 °C. It was found that biodiesel produced from cottonseed oil, waste cooking oil showed maximum yields at 60 °C and from rice bran oil at 55 °C. Since boiling point of methanol is 64.7 °C, at near temperatures, the methanol starts boiling and this affects the yield. The ester conversion is decreased when the temperature is higher than 60 °C although a reflux condenser was used to avoid methanol losses in this experiment.

Effect of reaction time

The influence of esterification reaction time on percent yield is shown in Figure 9. The percent yield increases with the reaction time. In reality most of the reaction is completed in the first few minutes. Most of the investigators found that 80% of reaction takes

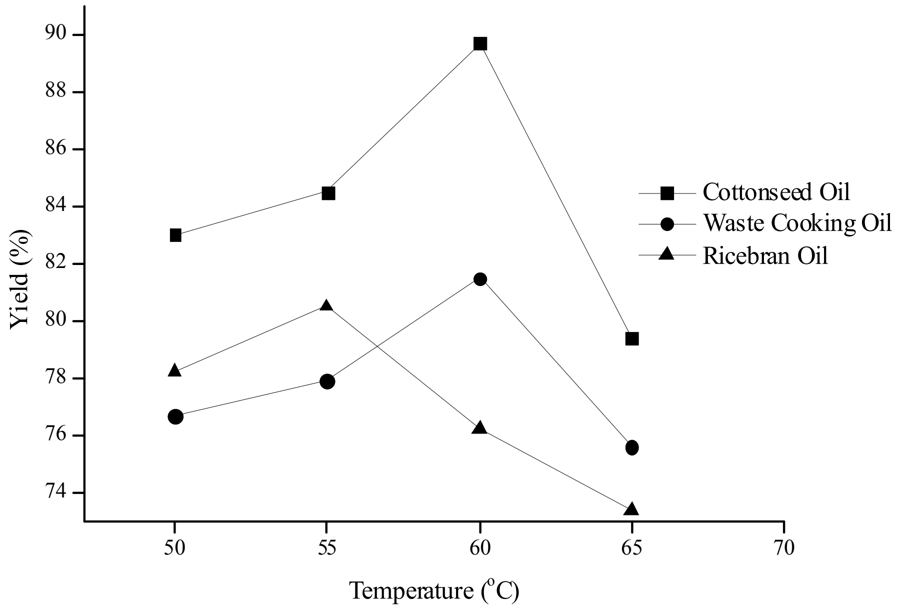

Figure 8. Effect of reaction temperature on % yield.

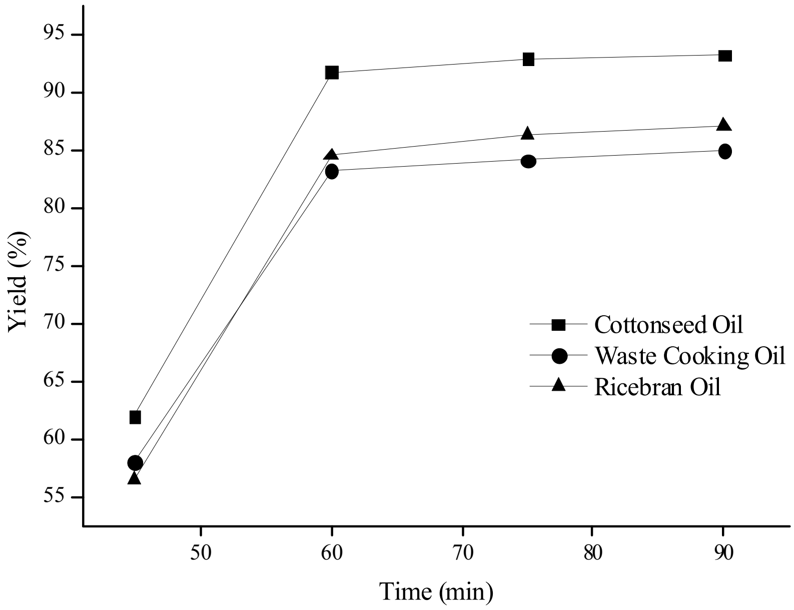

Figure 9. Effect of reaction time of % yield.

place in the first 5 min and the rest of conversion may take almost 1 h, in order to have 93–98% conversion of triglycerides [20, 27]. In this study time was changed from 45 to 90 min with interval of 15 min and effect on percent yield was studied. It was found that conversion increased very quickly with the reaction time between 30 and 60 min. It was observed that there was no noticeable change in the yield by increasing the reaction time for more than 60 min.

Effect of parameters

After the production of biodiesel, it was tested for different properties of the diesel such as specific gravity, kinematic viscosity, cetane no, cloud point and pour point. These were determined by using the ASTM D6751. The results are given in Table 3.

Table 3. Fuel parameters of methyl esters as compared to ASTM biodiesel

Property	Biodiesel D 6751-02	CSO Ester	RBO Ester	WCO Ester
Specific gravity	0.86–0.90	0.84	0.86	0.87
Kinematic viscosity, cSt	1.9–6.0	4.81	4.38	4.58
Cetane No, min	47–70	58	59.5	55.8
Cloud point, °C	No est. limit	6	8	4
Pour point, °C	–	3	-3	1
Flash point, °C	122–185	172	185	188

CONCLUSION

Three different vegetable oils, cottonseed, waste cooking and rice bran, were explored for their potential use as an alternative source for biodiesel production. It was observed that cottonseed oil showed a maximum yield of 91.7% at 60 °C, 6:1 molar ratio and consuming 60 min. However, rice bran oil gave a yield of 87% at 55 °C, 6:1 molar ratio, and 90 min, while waste cooking oil showed a maximum yield of 84% under optimum conditions of 60 °C temperature, molar ratio of 9:1 and consuming 75 min. This catalysis provides a simple and economic alternative method for biodiesel production. In addition, the separation of glycerol and soap (created by KOH) in this process could be easily isolated from the rich ester layer.

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IZVOD

BAZNO-KATALIZOVANA REAKCIJA TRANSESTERIFIKACIJE PIRINČANIH MEKINJA, SEMENA PAMUKA I OTPADNOG JESTIVOG ULJA

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(Naučni rad)

U ovom istraživačkom radu opisana je proizvodnja biodizela reakcijom transesterifikacije iz tri vrste sirovina, uključujući devičansko, korišćeno jestivo i nejestivo ulje. Zbog visokog sadržaja slobodnih masnih kiselina u pirinčanim mekinjama, proporcionalna bazno-katalizovana transesterifikacija je nepodobna, te je na nejestivo ulje primenjena dvostepena, kiselinsko i bazno katalizovana reakcija transesterifikacije. Vrednosti kiselinskog broja nakon tretmana pirinčanih mekinja, semena pamuka i otpadnog jestivog ulja bile su: 0,95, 0,12 i 0,87, redom. Uticaji tri promenljive na procenat prinosa (na primer molarni odnos metanola i ulja), temperaturu i vreme reakcije su ispitivani na ovom nivou istraživanja. Pri optimalnim uslovima, ulje iz semena pamuka, jestivog otpadnog ulja i ulja iz pirinčanih mekinja dalo je maksimalni prinos od: 91,7, 84,1 i 87,1%. Karakteristike dobijenog biodizela iz tri različite sirovine zadovoljavaju standarde propisane za biodizel kao gorivo.

Ključne reči: Biodizel • Ulje semena pamuka • Ulje pirinčanih mekinja • Otpadno jestivo ulje

Uticaj procesnih parametara na morfologiju i adsorpcione osobine nanokristalnog bemita

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NAUČNI RAD

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Izvod

Dobijanje različitih prelaznih faza Al_2O_3 dobrih adsorpcionih osobina poslednjih decenija privlači veliku pažnju istraživača kako sa aspekta istraživanja tako i sa aspekta primene ovih materijala. Prelazne Al_2O_3 faze predstavljaju metastabilne polimorfne oblike alumine koje nastaju pri dehidrataciji aluminijum-hidroksidnih (gibsita i bajerita) ili aluminijum-oksihidroksidnih prahova (bemita). U ovom radu sintetisan je nanokristalni bemit polazeći od rastvora natrijum-aluminata, dobijenog u Bajerovom postupku. U procesu neutralizacije sumpornom kiselinom, podešavanjem procesnih parametara i dodatkom glukoze, dobijen je nanokristalni bemit, prosečne veličine kristalita ispod 5 nm i specifične površine iznad 360 m^2/g. Za analizu faznog sastava, strukture i morfologije dobijenih prahova primenjene su sledeće metode: rentgenska difrakcija, XRD, Furije transformisana infracrvena spektroskopija, FT-IR, skenirajuća elektronska mikroskopija, SEM i niskotemperaturna adsorpcija azota. Rezultati su pokazali da je, bez obzira na uslove sinteze, a uz korišćenje glukoze, moguće dobiti nanokristalni bemitni prah, na relativno niskoj temperaturi. Pri tome je pokazano da karakteristike praha (struktura i morfologija) zavise od pH vrednosti polaznog rastvora kao i od vremena trajanja reakcije neutralizacije.

Ključne reči: natrijum-aluminat, nanokristalni bemit, neutralizacija, morfologija čestica, specifična površina, zapremina pora, raspodela pora po veličini.

Dostupno na Internetu sa adrese časopisa: http://www.ache.org.rs/HI/

Prelazne Al_2O_3 faze zbog svojih strukturnih karakteristika, a pre svega velike specifične površine, velike ukupne zapremine pora i uniformne raspodele pora, imaju izuzetno velike mogućnosti industrijske primene kao adsorbenti, katalizatori, membrane [1,2]. Polimorfni oblici Al_2O_3 dobijaju se u procesu kalcinacije aluminijum-hidroksidnih (AH) i aluminijum-oksihidroksidnih (AOH) prahova u temperaturnom intervalu od 220 do 1000 °C [2]. Bemit, kao aluminijum-oksihidrat, ima poseban značaj ne samo kao prekursor za dobijanje različitih faza alumine nego i kao materijal koji se koristi za dobijanje membrana, katalizatora, prevlaka, adsorbensa ili biokompatibilnog materijala [3–6].

Uobičajeni postupci za dobijanje bemita mogu se podeliti u tri grupe: *i*) precipitacija iz rastvora različitih soli aluminijuma, *ii*) hidroliza Al-alkoksida (ili nekih njegovih soli) pri različitim pH vrednostima i *iii*) termička dehidratacija gibsita. Karakteristično za sve ove metode je da se vrlo često koriste skupe i toksične sirovine, različiti aditivi za kontrolu brzina reakcija u tečnoj fazi i čestice bemita kao centri kristalizacije. Pored toga, pri primeni hidrotermalne metode koriste se povišeni pritisci i visoke temperature i do 200 °C, a sam proces može da traje od nekoliko časova do nekoliko dana [7–10].

Pri sintezi bemita veoma je važna kontrola procesnih parametara, kao što su pH vrednost, temperatura, pritisak, karakteristike polaznih sirovina, koji određuju morfološke karakteristike bemita poput stepena kristaličnosti, veličine kristalita, poroznosti, stepena aglomeracije. Poslednjih decenija, koje su obeležene intezivnim istraživanjima u oblasti dobijanja nanokristalnih materijala, velika pažnja je posvećena pronalaženju najpovoljnije metode za dobijanje nanokristalnog bemita unapred zadatih karakteristika [11]. Strukturne i morfološke karakteristike nanokristalnog bemita su značajne iz dva razloga. Kao prvo, omogućavaju njegovu mnogo veću primenu, a kao drugo, zbog izomorfne i topotaktičke fazne transformacije bemita u prelazne faze alumine određuju strukturne karakteristike ovih faza, a time i mogućnosti njihove primene.

Za dobijanje nanokristalnog bemita u ovom radu odabrana je metoda neutralizacije rastvora natrijum-aluminata sumpornom kiselinom. Rastvor natrijum-aluminata dobijen je u industrijskom Bajerovom procesu. Na taj način je izbegnuto korišćenje skupih i po okolinu štetnih alkoksida aluminijuma, čime se definiše i osnovna prednost predloženog postupka sinteze, a to je korišćenje jeftinih sirovina i sirovina koje su pogodne sa stanovišta ekologije [12]. Pored toga, za kontrolu procesa nukleacije bemita korišćena je glukoza kao

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netoksičan organski aditiv, pri čemu je ispitivan i njen uticaj na mogućnost dobijanja nanokristalnog bemita na niskoj temperaturi i atmosferskom pritisku. Glukoza, kao organski aditiv ima raznovrsnu ulogu pri sintezi raznih tipova materijala, međutim, podaci o njenoj primeni pri sintezi različitih Al_2O_3 faza prilično su ograničeni [13]. Prema našim saznanjima, glukoza je korišćena za dizajniranje poroznosti kao i za sprečavanje rasta čestica alumine pri hidrotermalnim uslovima, odnosno na visokim temperaturama (170 °C) [13,14]. Međutim, u ovom radu glukoza je korišćena na temperaturi od 70 °C, što je mnogo niža temperatura od onih koje se primenjuju pri hidrotermalnim uslovima dobijanja bemita i Al_2O_3 faza. Pokazalo se da je primena glukoze, pri uslovima sinteze koji su karakteristični za dobijanje gibsita i bajerita [12], dovela do neočekivanog efekta, odnosno do dobijanja nanokristalnog bemita. Moguće je da se glukoza preko svojih –OH grupa vezuje za aluminatne oblike koji nastaju u baznim rastvorima, obrazujući time slabe Al-komplekse koji blokiraju aktivne položaje za nukleaciju $Al(OH)_3$ faza, favorizujući time nukleaciju bemita.

Proces neutralizacije rastvora aluminata sastoji se iz dve faze: prve u kojoj dolazi do neutralizacije slobodnog NaOH i druge u kojoj dolazi do hidrolize natrijum-aluminata pri čemu se izdvajaju čvrste faze AH i AOH. Neutralizacijom razblaženih rastvora natrijum–aluminata (< 1 M NaOH) nastaje prah u kome dominira bajeritna faza [15], dok pri neutralizaciji koncentrovanih rastvora aluminata (> 2,5 M NaOH) nastaju prahovi u kojima domira faza gibsita [16,17]. Neutralizacijom rastvora aluminata srednjih koncentracija (1,0––2,5 M NaOH) dobija se prah koji predstavlja smešu bajerita i gibsita [18]. Dodatkom glukoze u rastvor aluminata niskih koncentracija u određenom masenom odnosu prema Al^{+3}, dobijen je prah koji je po sastavu nanokristalni bemit [19], prosečne veličine kristalita od 3 do 5 nm. Termičkom obradom ovako dobijenog bemitnog praha mogu se dobiti prahovi prelaznih faza alumine velike specifične površine i velike ukupne zapremine pora, što otvara široke mogućnosti za njihovu dalju primenu.

Cilj ovog rada je bio da se ispita uticaj procesnih parametara, pre svega pH područja neutralizacije rastvora natrijum-aluminata i vremena sinteze na fazni sastav i morfološke karakteristike sintetisanih prahova nanokristalnog bemita.

EKSPERIMENTALNI DEO

Kao polazni materijal za sintezu alumina prahova u ovom radu odabran je industrijski rastvor natrijum-aluminata dobijen Bajerovim procesom. Aluminijum-oksihidroksidni bemitni prahovi (AOH) sintetisani su u procesu neutralizacije rastvora natrijum-aluminata 1 M rastvorom sumporne kiseline, pri čemu je proces neutralizacije trajao 30, odnosno 120 min. Polazna koncentracija rastvora natrijum-aluminata je bila [NaOH], 0,82 M, [Al(III)], 0,52 M. Pre samog procesa sinteze u rastvor je dodata glukoza u određenom masenom odnosu prema Al^{+3} prisutnim u rastvoru aluminata. Nakon dodatka glukoze i postizanja temperature od 70 °C u rastvor je ukapavana sumporna kiselina uz intenzivno mešanje u zadatom vremenskom intervalu, pri čemu se pH vrednost rastvora menjala od 12 do 10,8 uz obrazovanje belog taloga. Dobijena čvrsta faza je odvojena iz suspenzije vakuum filtracijom, a preostaloj tečnoj fazi je ponovo dodavan rastvor sumporne kiseline do postizanja pH vrednosti od 9,5, nakon čega je vakuum filtracijom izdvojena čvrsta faza. Na ovaj način su dobijena po dva uzorka sintetisanih prahova u dva područja neutralizacije: pH 12–10,8 i pH 10,8–9,5. Oznake uzoraka i uslovi njihove sinteze dati su u tabeli 1.

Tabela 1. Sintetisani uzorci praha i uslovi njihove sinteze
Table 1. As-synthesized samples and conditions of their preparation

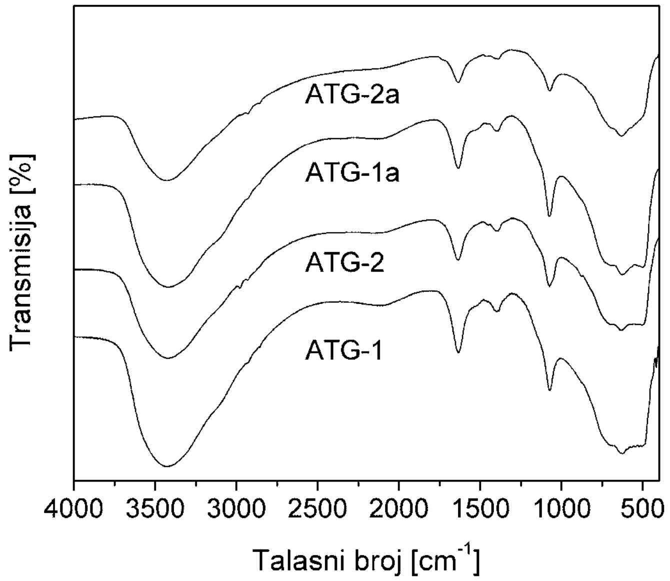
Oznaka uzorka	pH područje sinteze	Vreme sinteze, min
ATG–1	12–10,8	30
ATG–2	10,8–9,5	30
ATG–1a	12–10,8	120
ATG–2a	10,8–9,5	120

Strukturne promene uzoraka nastale tokom sinteze ispitivane su primenom Furije transformisane infracrvene spektroskopije (FT-IR, Nicolet–Nexsus 670 FT-IR spektrofotometar) i rentgenske difrakcije (XRD, PANalytical X'Pert PRO instrument). Za XRD ispitivanja korišćeno je CuKα zračenje talasne dužine od 0,15406 nm sa korakom od 0,03 °/s. Morfologija prahova je ispitivana primenom skenirajuće elektronske mikroskopije (SEM, JEOL JSM 6460 LV). Specifična površina uzoraka je određena niskotemperaturnom adsorpcijom azota (Quantachrom Autosorb–3B instrument) i izračunata primenom BET metode.

REZULTATI I DISKUSIJA

FT–IR spektroskopija sintetisanih prahova

FT–IR spektri sintetisanih prahova ATG–1, ATG–2, ATG–1a i ATG–2a prikazani su na slici 1 i pokazuju veoma široku traku na oko 3440 cm^{-1} koja potiče od vibracija valentnih –OH grupa. Traka koja dominira u ovoj oblasti, superponira se sa svim vibracijama karakterističnim za vibracije strukturne –OH grupe. Pored ove dominantne trake u FT–IR spektrima uzoraka, izražena je i traka koja potiče od deformacionih vibracija adsorbovane vode, a koja se javlja na oko 1640 cm^{-1}. U spektrima se javljaju i trake na oko: 1380 cm^{-1} koja potiče od vibracija površinskih –OH grupa, 1070 cm^{-1} koja je posledica deformacionih vibracija –OH grupe,



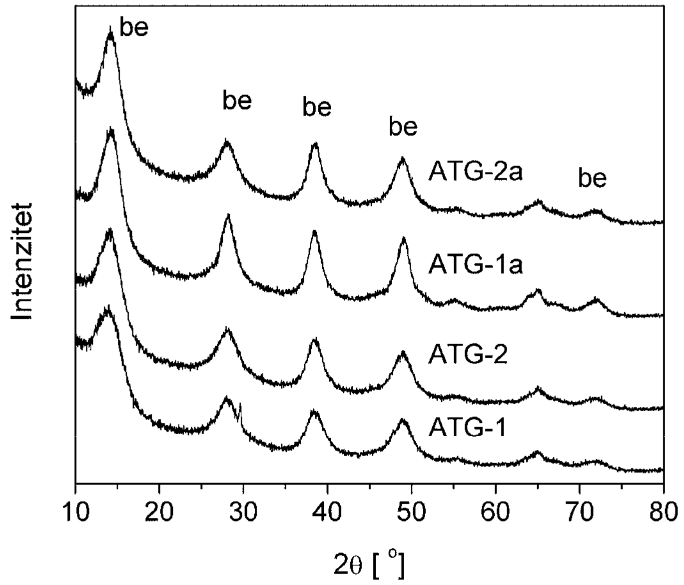
Slika 1. FT–IR spektri sintetisanih ATG prahova.
Figure 1. FT-IR Spectra of the as-synthesized ATG powders.

635 cm^{-1} koja potiče od Al–O valentnih vibracija [20––23]. Ove trake su karakteristične za bemit. Izražene trake koje potiču od različitih tipova vibracija –OH i H–O–H veza, ukazuju na dobro razvijenu specifičnu površinu sintetisanih prahova.

Sličnost FT-IR spektara sintetisanih prahova ukazuje na sličnost njihove strukture. Pri tome se može uočiti da je međusobna sličnost više izražena kod prahova koji su dobijeni pri netralizaciji u istom pH području (ATG–1 i ATG–1a u odnosu na prahove ATG–2 i ATG–2a). Pa su tako trake koje potiču od vibracija –OH grupa, izraženije kod uzoraka koji su sintetisani pri višem pH području, uzorci ATG–1 i ATG–1a.

Rentgenska difrakcija sintetisanih prahova

Difraktogrami sintetisanih prahova ATG–1, ATG–2, ATG–1a i ATG–2a pokazuju da su svi prahovi čista bemitna faza (JCPD No. 21–1307), sa prosečnom veličinom kristalita od 3 do 5 nm, slika 2. Ranije je pokazano da dodatak određene količine glukoze u razblažene rastvore aluminata inhibira kristalizaciju bajerita i favorizuje kristalizaciju nanokristalnog bemita [12]. Široki difrakcioni pikovi, kao i širina karakterističnog pika bemita na 2θ oko 14,4°, koja je znatno iznad 1,5° (iznosi oko 3°) ukazuju da je dobijen nanokristalni bemit. Položaj i širina ovog pika, koji odgovara difrakciji

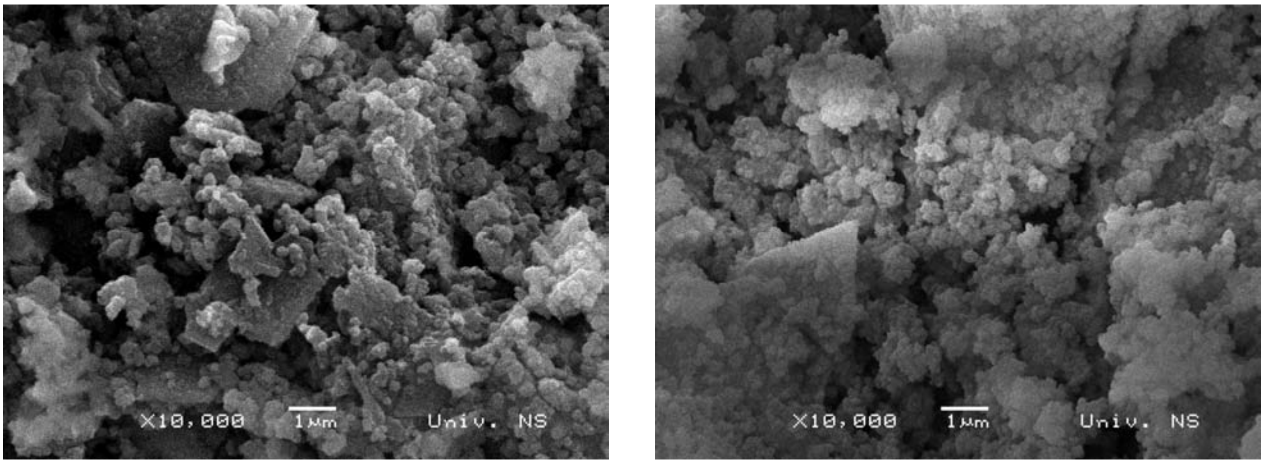


Slika 2. Difraktogrami sintetisanih ATG prahova (be – bemit).
Figure 2. XRD Patterns of the as-synthesized ATG powders (be – boehmite).

na (020) ravni, vrlo je osetljiva na efekat hidratacije, posebno u strukturi nanokristalnog bemita [9]. Odnosno, u zavisnosti od količine –OH grupa u strukturi nanokristalnog bemita dolazi do njegovog pomeranja (zbog toga se ne uzima u obzir pri određivanju parametra rešetke). Položaj ovog pika je povezan sa parametrom rešetke *b* i veličinom kristalita. Na osnovu difraktograma na slici 2 mogu se uočiti mala pomeranja ovog pika ka većim vrednostima 2θ koja se javljaju sa povećanjem pH vrednosti i sa produžavanjem vremena reakcije, međutim efekat trajanja reakcije neutralizacije je nešto izraženiji (uzorci ATG–1a i ATG–2a). Prema tome, sa smanjenjem pH vrednosti rastvora natrijum-aluminata i produžavanjem vremena neutralizacije dolazi do smanjenja prosečne veličine kristalita, uzorci ATG–1a i ATG–2a, slika 2. Razlike u veličini kristalita su manje izražene kod uzoraka ATG–1 i ATG–2, koji su dobijeni pri različitim pH vrednostima, ali kraćim vremenima neutralizacije. Najverovatnije, manja količina raspoloživih –OH grupa, pri nižim pH vrednostima sporije ulazi u strukturu bemita, posebno pri kraćim vremenima reakcije neutralizacije. Pored toga, prisustvo –OH grupa stabiliše strukturu nanokristalnog bemita [9].

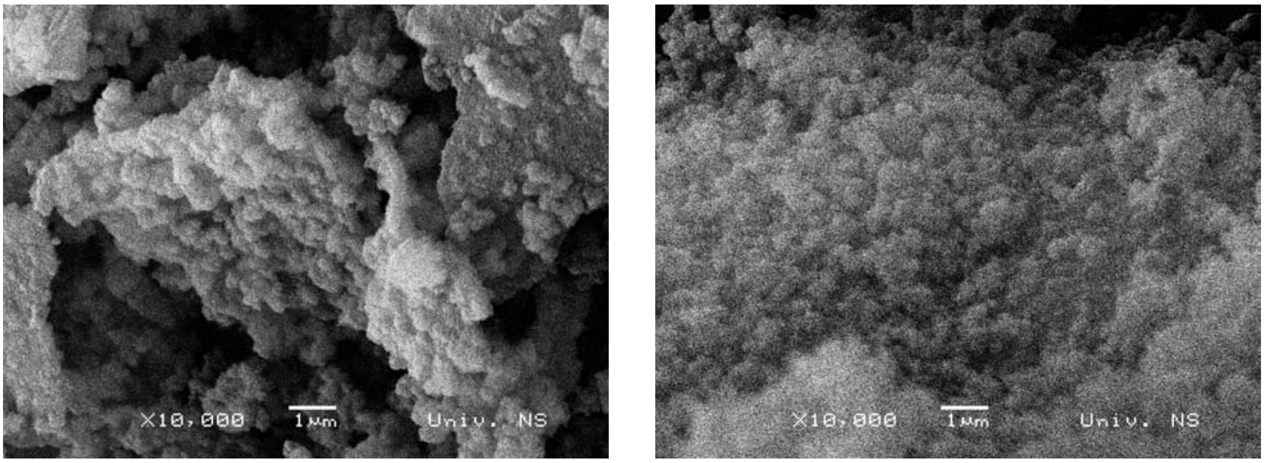
SEM analiza sintetisanih prahova

SEM snimci prikazani na slikama 3 i 4 ukazuju na to da se sintetisani prahovi sastoje od finih aglomerisanih nanokristalnih bemitnih čestica. Može se uočiti da stepen aglomeracije kao i veličina algomerata zavisi od procesnih parametara kao što su pH vrednost rastvora i vreme neutralizacije. Pri tome, vreme sinteze ima veći uticaj na morfologiju sintetisanih prahova od samog pH područja sinteze, s obzirom da se produžavanjem vremena neutralizacije na 120 min, slika 4, postiže homogenija struktura sa sfernim aglomeratima manjih veličina u odnosu na prahove dobijene pri vremenu sinteze od 30 min, slika 3. Prahovi koji su dobijeni pri kraćem



(a) (b)

Slika 3. SEM snimci sintetisanih prahova: a) ATG–1 i b) ATG–2.
Figure 3. SEM Images of the as-synthesized powders: a) ATG–1 and b) ATG–2.



(a) (b)

Slika 4. SEM snimci sintetisanih prahova: a) ATG–1a i b) ATG–2a.
Figure 4. SEM Images of the as-synthesized powders: a) ATG–1a and b) ATG–2a.

vremenu neutralizacije, ATG–1 i ATG–2, sadrže manje kristalite, koji su skloniji grupisanju i obrazovanju aglomerata različitih oblika i dimenzija, slika 3. Kod prahova sintetisanih pri dužem vremenu reakcije neutralizacije, ATG–1a i ATG–2a, prisutne su sferne čestice uniformnije veličine, slika 4, u odnosu na prahove ATG–1 i ATG–2.

Morfološke karakteristike sintetisanih prahova

Kao potvrda prethodno analiziranih rezultata, u tabeli 2 prikazane su vrednosti BET specifične površine, ukupne zapremine pora i srednjeg prečnika pora sintetisanih prahova. Vrednosti ukupne zapremine pora kao i srednjeg prečnika pora izračunate su iz BJH desorpcionih krivih. Specifične površine sintetisanih prahova su veoma visoke, čak iznad 390 m^2/g za prahove neutralisane pri kraćim vremenima. Na osnovu prikazanih rezultata može se uočiti tendencija smanjenja specifične površine sa produžavanjem vremena neutralizacije. Ovaj efekat je posledica promene morfologije praha koja se ogleda u smanjenju srednjeg prečnika pora, kao i u blagom povećanju ukupne zapremine pora, tabela 2.

Tabela 2. Specifična površina, ukupna zapremina i srednji prečnik pora sintetisanih prahova
Table 2. BET surface area, pore volume and the average pore diameter of the as–synthesized powders

Oznaka uzorka	Specifična površina, m^2/g	Ukupna zapremina pora, cm^3/g	Srednji prečnik pora, nm
ATG–1	390,7	0,381	4,52
ATG–2	393,6	0,398	4,64
ATG–1a	303,0	–	–
ATG–2a	361,1	0,402	3,73

Sve ovo je u saglasnosti i sa pokazanim rezultatima SEM analize koji su dati na slikama 3 i 4. Prema tome povećanje vremena sinteze utiče na morfologiju sintetisanih prahova dovodeći do formiranja manjih sferičnih aglomerata, ali utiče i na povećanje ukupne zapremine pora. Treba istaći da je uticaj vremena trajanja reakcije neutralizacije na morfološke karakteristike izraženiji u odnosu na pH područje u kojem se odvija reakcija.

ZAKLJUČAK

Na osnovu prikazanih rezultata može se zaključiti da je dodatak glukoze rezultovao dobijanjem jednofaznog nanokristalnog bemita. Pokazano je da je pri datim uslovima sinteze dobijen nanokristalni bemit velike specifične površine, iznad 360 m^2/g, i prosečne veličine kristalita ispod 5 nm. Rezultati su potvrdili da se upotrebom jeftinih i netoksičnih sirovina, kao što su aluminatni rastvor i glukoza mogu kontrolisati fazni sastav i morfologija sintetisanih prahova. Takođe je pokazano da procesni parametri, vreme trajanja reakcije neutralizacije i pH aluminatnog rastvora, utiču na strukturne i morfološke karakteristike dobijenog bemitnog praha. Uticaj pH vrednosti polaznog rastvora je izraženiji na strukturne karakteristike dobijenog praha. Nasuprot tome, vreme trajanja reakcije neutralizacije (izraženo preko vremena ukapavanja sumporne kiseline) ima znatno izraženiji efekat na morfološke karakteristike bemita. Pri dužem vremenu reakcije, dolazi do povećanja stepena aglomeracije sintetisanog praha, što rezultuje smanjenjem njegove specifične površine, povećanjem ukupne zapremine pora i smanjenjem srednjeg prečnika pora.

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SUMMARY

EFFECT OF PROCESS PARAMETERS ON THE MORPHOLOGY AND ADSORPTION PROPERTIES OF NANOCRYSTALLINE BOEHMITE

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(Scientific paper)

In the last decade, exploration of transition alumina phases with good adsorption properties has attracted a great research interest from both a fundamental and a practical point of view. The transition phases of alumina are metastable polymorphs of aluminum oxide formed through the thermal dehydration of aluminum trihydroxide and aluminum oxyhydroxide. Powder X-ray diffraction (XRD), Fourier-transformed infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and low-temperature nitrogen absorption studies were employed to trace the formation of the transition phases of alumina. In this work transition alumina powders were synthesized starting from sodium aluminate solution prepared from Bayer liquor. The neutralization of sodium aluminate solution was performed with the use of sulphuric acid, while glucose was added in the starting solution. In this way, the single phase nanocrystalline boehmite was obtained. As-synthesized boehmite powders have high surface area (above 360 m^2/g) and the average crystallite size less than 5 nm. The results showed that the properties of the powders (structure, morphology) are strongly influenced by the initial pH value of sodium aluminate solution, as well as by the duration of neutralization step.

Keywords: Sodium aluminate • Nanocrystalline boehmite • Neutralization • Morphology of particles • Specific surface area • Pore volume • Pore size distribution

Hemijski reaktori sa oscilirajućim tokom fluida

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Izvod

Ekonomski i ekološki pritisci, kao što su globalna konkurencija, povećanje energetskih i drugih troškova proizvodnje, zahtevi za visokim kvalitetom proizvoda i smanjenje otpada, primoravaju farmaceutsku, finu hemijsku i biohemijsku industriju na radikalne promene u proizvodnji. Jedan od efikasnih načina za sveobuhvatno poboljšanje proizvodnje (smanjenje troškova i bolju kontrolu reakcije) je prelazak sa tradicionalno šaržnih procesa na kontinualne. Međutim, reakcije od interesa za pomenute industrijske sektore su često spore, pa bi kontinualni cevni reaktori trebalo da budu nepraktično velikih dužina za režime strujanja koji obezbeđuju zadovoljavajuće prenose toplote i mase i uske raspodele vremena zadržavanja. Reaktori sa oscilirajućim tokom fluida (ROT) nude rešenje jer obezbeđuju kontinualni rad, strujanje blisko klipnom, visoke prenose mase i toplote, a pri tom se mogu koristiti male brzine strujanja, te su reaktori prihvatljivih dužina za spore reakcije. Ove pozitivne karakteristike se ostvaruju zbog jako dobrog mešanja u reaktoru, usled stvaranja vrtloga pri sudaru generisanog talasa fluida sa internim pregradama. Osim navedenih prednosti u ROT se obezbeđuju i niži smicajni naponi (za isti unos energije) u odnosu na reaktore sa mehaničkim mešalicama, što je značajno za aplikacije u biohemijskom i biomedicinskom inženjerstvu. U ovom preglednom radu je predstavljen princip rada ovih reaktora, različite konstrukcije i osnovni kriterijumi za projektovanje. Prikazani su reprezentativni rezultati istraživanja, koji jasno potvrđuju prednosti ovih uređaja u odnosu na konvencionalne i navedeni su primeri intenzifikacije procesa primenom ROT u različitim industrijskim sektorima, kao što su farmaceutski, polimerni, biogoriva, prerada otpadnih voda i dr.

Ključne reči: intenzifikacija procesa, reaktori sa oscilirajućim tokom, šaržni ka kontinualnim procesima, mešanje pomoću oscilacija fluida.

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OSNOVNE KARAKTERISTIKE REAKTORA SA OSCILIRAJUĆIM TOKOM FLUIDA

Procesna industrija se suočava sa različitim izazovima. Pre svega, društvo zahteva održivi razvoj industrije koja ne zagađuje životnu sredinu, više koristi obnovljive izvore energije i odlikuje se visokom energetskom efikasnošću. Ovi zahtevi ogledaju se u strožijim propisima o proizvodnji i upravljanju otpadom, emisiji CO_2, zagađenju vode i vazduha, itd. Sa druge strane, ekonomski zahtevi kao što su globalna konkurencija, brzi izlazak proizvoda na tržište, česte fluktuacije cena i dugotrajna ekonomska kriza, povećavaju pritiske na procesnu industriju. Društvo i ekonomija zahtevaju radikalno nova, ekološki efikasna i brza tehnička rešenja za trenutne izazove [1].

Razmatrajući različite pristupe u okviru hemijskog inženjerstva može se zaključiti da koncepti intenzifikacije procesa najviše obećavaju, pogotovo kada su povezani sa zelenom hemijom i procesnim sistemskim inženjerstvom [2]. Jedan od uspešnih primera intenzifikacije procesa su reaktori sa oscilirajućim tokom fluida koji se mogu primeniti za različite aplikacije u hemijskoj, biohemijskoj i farmaceutskoj industiji i zaštiti životne sredine. Ovaj tip reaktora omogućava da se spore reakcije, koje se uobičajno odigravaju u šaržnim sistemima, odvijaju kontinualno, što vodi do značajnih investicionih i operativnih ušteda i bolje kontrole toka reakcije. U ovom radu su predstavljeni osnovni principi funkcionisanja reaktora sa osilirajućim tokom i prikazani reprezentativni rezultati istraživanja koji potvrđuju prednosti ovog tipa reaktora u odnosu na klasične. Na kraju rada su navedeni primeri upotrebe, kako oni realizovani u industriji, tako i potencijalni koji su u fazi istraživanja i razvoja.

Jedan od najbitnijih fenomena u hemijskom inženjerstvu je mešanje fluida koje ključno utiče na prenos mase i toplote, performanse reakcije i uniformnost proizvoda. Kontinualni procesi često zahtevaju uređaje u kojima se ostvaruje dobro mešanje fluida, kod kojih je vreme zadržavanja precizno definisano, odnosno u kojima se ostvaruje strujanje fluida približno idealno klipnom strujanju [3]. Efikasan prenos mase i/ili toplote u toku kontinualnih procesa može se ostvariti pri turbulentnom strujanju fluida. Poboljšanje mešanja u nekim procesima je ostvareno na taj način što se u uređaj postavlja pakovani ili struktuirani sloj ili su u njega ugrađene pregrade. U šaržnim reaktorima se i

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danas mešanje najčešće obavlja preko dodatnih mehaničkih mešalica, a u pojedinim slučajevima se intenzivira ugradnjom pregrada. Mešanje pomoću oscilirajućeg toka nudi visoko efikasnu alternativu za klasično mešanje kako za šaržne, tako i za kontinualne procese [4].

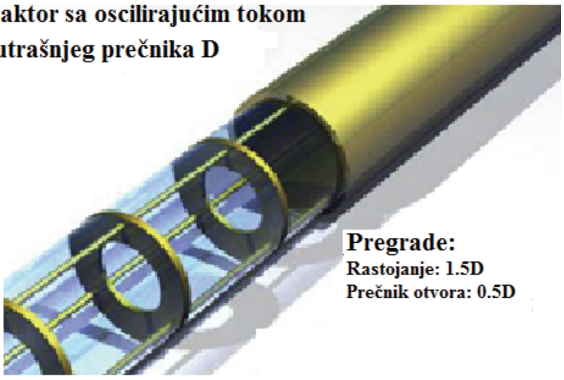
Mešanje pomoću oscilacija se istražuje poslednjih trideset godina, a najčešća primena je u cevnim reaktorima [5]. Ideja o načinu poboljšanja mešanja oscilacijama zasniva se na stvaranju kontrolisanih cikličnih vrtloga u zapremini fluida [6,7]. Takvo mešanje se najčešće ostvaruje u dugim cevima u kojima su ugrađene pregrade (raspoređene duž cevi), a tečnost ili višefazni fluid aksijalno osciluje delovanjem dijafragme, mehova ili klipova, koji se nalaze na jednom ili na oba kraja cevi. U drugom tipu uređaja iz ove klase, oscilacije se generišu mehaničkim pomeranjem pregrada u reaktoru, što je ređi slučaj primene [8]. Oscilatorno proticanje fluida kroz set pregrada podstiče formiranje vrtloga, samim tim i efikasno radijalno mešanje. Za male vrednosti amplitude i niske vrednosti frekvencije oscilacija, strujanje fluida je dobro definisano, a za velike vrednosti amplitude i visoke vrednosti frekvencije strujanje fluida postaje turbulentno [8]. Treba napomenuti da se pri oscilovanju fluida u cevi bez pregrada, ne javljaju vrtlozi, te se ne obezbeđuje željeni intenzitet mešanja. Nasuprot ovde obrađenih reaktora sa oscilirajućim tokom, treba razlikovati i reaktore sa periodičnim radom koji su takođe dosta iztraživani i primenjeni. Ovo su klasični cevni reaktori ili reaktori sa pakovanim slojem, koji rade periodično, tj. uvode se oscilacije u protoku, koncentraciji ili temperaturi u cilju poboljšanja same hemijske reakcije, prenosa mase ili iskorišćenja toplote i slično.

Reaktori sa oscilirajućim tokom (ROT) mogu raditi i kao šaržni i kao kontinualni uređaji, postavljeni bilo u horizontalni ili vertikalni položaj. Ispitivane su brojne različite konfiguracije pregrada, ali najčešće su korišćene one koje imaju jedan otvor na sredini (izgledaju kao prsten) postavljene na međusobnom rastojanju od oko 1,5 prečnika cevi. Upotreba ovakvih pregrada omogućava da strujanje bude aksisimetrično za relativno male vrednosti amplitude i niske vrednosti frekvencije oscilacija [8]. Na slici 1 je prikazana uobičajna konstrukcija reaktora sa oscilirajućim tokom.

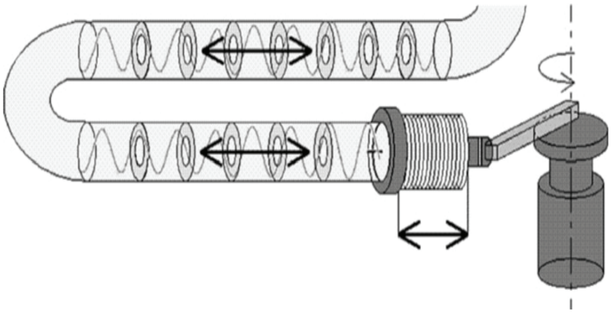
Na slici 2 je prikazana skica reaktora kod kojeg se oscilatorno kretanje fluida postiže delovanjem meha na jedan kraj reaktora, koji je povezan sa obrtnim cilindrom.

Postoje tri slična tipa reaktora sa pregradama u kojima se ostvaruje odlično mešanje [10]:

1. *Oscilirajući reaktor sa pregradama* (*eng. Oscillatory Baffled Reactor*). Reaktor u kojem se najčešće odvijaju šaržni i polušaržni procesi i uglavnom je postavljen vertikalno. U ovom uređaju oscilovanje fluida se postiže delovanjem klipa ili mehova koji su postavljeni sa donje strane reaktora ili pomeranjem seta pregrada delovanjem mehaničke sile čiji se izvor nalazi sa gornje strane.
2. *Kontinualni reaktor sa oscilirajućim tokom – ROT (eng. Continuous Oscillatory Baffled Reactor)*. Cevni reaktor koji se koristi za kontinualne procese i može biti postavljen horizontalno, vertikalno ili pod uglom. Ovaj tip reaktora sa pregradama je najčešće korišćen, te će njegove osnovne karakteristike i prednosti biti opisane u daljem tekstu.
3. *Cevni reaktor sa pregradama (eng. Tube Baffled Reactor)*. Po efektima, ovaj tip reaktora je sličan kontinualnom reaktoru sa oscilirajućim tokom, osim što se u ovom slučaju ne koriste oscilacije. Uniformno mešanje se ostvaruje strujanjem fluida kroz set perforiranih pregrada postavljenih u unutrašnjost reaktora, što dovodi do stvaranja vrtloga u prostorima između pregrada, slično kao kod tzv. statičkog miksera. Ovaj reaktor takođe omogućava dobar prenos mase i toplote kao i ROT.

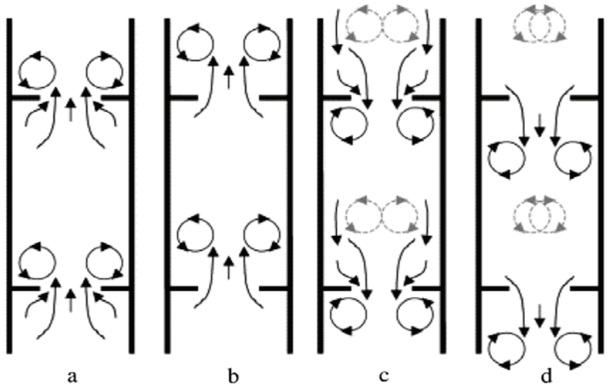


Slika 1. Reaktor sa oscilirajućim tokom. Unutrašnji izgled sa konstrukcijom pregrada. Adaptirano iz ref. [5] uz odobrenje izdavača Elsevier.
Figure 1. Oscillatory flow reactor. The reactor interior with baffles construction. Adopted from ref. [5] with the approval of publisher Elsevier.



Slika 2. Primer generisanja oscilacija u reaktoru sa oscilirajućim tokom. Pruzeto iz ref. [9] uz odobrenje izdavača Elsevier.
Figure 2. Illustration of oscillations generation in oscillatory flow reactor. Adopted from ref. [9] with the approval of publisher Elsevier.

U ROT celokupna zapremina fluida osciluje, a povratni tok fluida se periodično sudara sa pregradama formirajući vrtloge sa obe strane pregrada. Ovi vrtlozi obezbeđuju aksijalno i radijalno mešanje u prostoru između dve uzastopne pregrade duž celog reaktora, pri čemu intenzitet mešanja zavisi samo od uslova oscilovanja (amplituda i frekvencija oscilacija) [4,11,12]. Prednost oscilirajućeg toka je u tome što se može kontrolisati s dobrom preciznošću dajući pritom širok opseg režima strujanja fluida – od laminarnog do potpuno turbulentnog strujanja [4,6,13]. Na slici 3 prikazan je mehanizam mešanja pomoću oscilirajućeg toka.



Slika 3. Mehanizam mešanja pomoću oscilirajućeg toka. Shematski prikaz vrtloga u trenutku: a) početka udarnog talasa, b) maksimalne brzine udarnog talasa, c) početka povratnog talasa i d) maksimalne brzine povratnog talasa. Preuzeto iz ref. [7] uz odobrenje izdavača Elsevier.

Figure 3. Mixing mechanism in an oscillatory flow reactor. Schematic view of vortices in moment of: a) start of up stroke, b) maximum velocity in up stroke, c) start of down stroke and d) maximum velocity in down stroke. Adopted from ref. [7] with the approval of publisher Elsevier.

Zbog pojave vrtloženja, ukupno strujanje u ROT je blisko klipnom, što je poželjna slika proticanja za kontinualne reaktore. Kontrolisanje oscilatornih uslova omogućava da aksijalna disperzija bude minimizirana, a da raspodela vremena zadržavanja ne zavisi od brzine fluida na ulazu [6,11,14]. To znači da se i pri malim brzinama fluida, koje bi nominalno dale laminarni tok fluida, postiže veoma efikasno radijalno mešanje, kao pri turbulentnom toku u cevi (za šta su potrebne mnogo veće brzine strujanja). Zbog ovoga reaktor sa oscilirajućim tokom omogućava da se šaržni procesi sa dugim reakcionim vremenom (veće od 10 min) odvijaju kontinualno (*eng. Batch-to-continuous*) [4,15]. Nasuprot tome, standardni cevni reaktori su nepraktični za spore reakcije jer je potrebna veoma velika dužina reaktora pri radu sa brzinama koje daju približno klipno strujanje i dobar prenos mase i toplote (turbulentni režim).

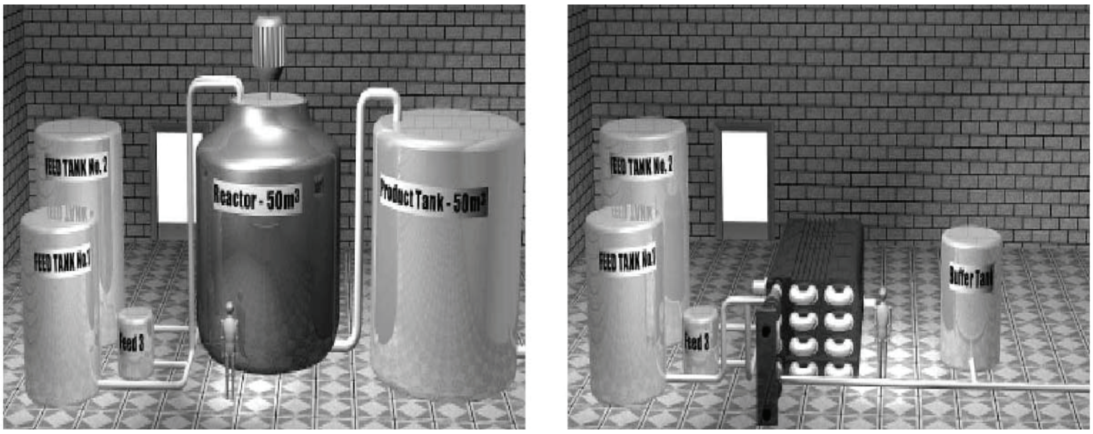
U reaktoru sa oscilirajućim tokom postiže se intenzivnije i uniformnije mešanje pri manjim smicajnim naponima u odnosu na standardne reaktore sa mehaničkim mešanjem (mešalicama), za iste vrednosti uložene energije [16–18]. Takođe, zbog dobrog mešanja koje se ostvaruje u ovom tipu reaktora, odnosno dobrog kontakta između faza, prenos mase i toplote je intenzivniji, tj. koeficijenti prenosa su znatno veći [6,15,19–21]. Mali smicajni naponi u ROT daju veliku prednost ovim reaktorima u odnosu na klasične mešače za reakcije sa osetljivim i nestabilnim komponentama, kao što su mikroorganizmi, ćelije i drugi biohemijski elementi. Karakteristike mešanja fliuda u ROT ostaju nepromenjene prilikom uvećanja razmera uređaja, odnosno nema gubitka efikasnosti mešanja [22]. Sa druge strane, karakteristike mešanja u reaktorima sa mešalicama veoma zavise od dimenzija sistema, te prilikom uvećanja razmera reaktora dolazi do stvaranja zona sa smanjenom efikasnošću mešanja [22].

ROT se može efikasno primeniti za intenzifikaciju različitih procesa u hemijskoj, biohemijskoj i farmaceutskoj industriji, što će biti prikazano u poglavlju 8. Tehničke i ekonomske prednosti su mnogobrojne i značajne, a dobar primer su dali Harvey i saradnici za reakciju saponifikacije sterilestra [4,23]. Oni su pokazali da je neophodna zapremina kontinualnog reaktora sa oscilirajućim tokom deset puta manja u poređenju sa zapreminom konvencionalnog šaržnog reaktora, za istu količinu proizvedenog sterola (slika 4). Takođe su pokazali da bi se ova reakcija mogla odvijati i na nižoj temperaturi i za znatno kraće vreme (12 min) nego u šaržnom reaktoru gde ova reakcija traje 2 h, a proizvodnja jedne šarže 24 h [4,23]. Potencijalne ekonomske pogodnosti koje nudi primena kontinualnog reaktora sa oscilirajućim tokom date su u tabeli 1 [10].

ISTORIJSKI RAZVOJ REAKTORA SA OSCILIRAJUĆIM TOKOM

Oscilatorno kretanje fluida kroz niz periodično postavljenih suženja pokazalo se kao dobar način ostvarivanja efikasanog kontakta između faza i povećanja brzine prenosa mase u višefaznim sistemima još kada je Van Dijick (1935) predložio kolonu sa vibracionim pločama (*eng. Reciproting plate column* – RPC) kao uređaj za ekstrakciju tečno–tečno [24,19,25]. Ploče u ovom tipu uređaja su pričvršćene za centralni nosač koji se pomera gore–dole, periodično, delovanjem zglobnog uređaja koji je postavljen na vrh kolone [25].

Četrdesetih i pedesetih godina dvadesetog veka za ekstrakciju urana iz fosforne kiseline primenjivale su se kolone sa pločama i kolone sa pakovanim slojem (*eng. Pulsed packed colum* – PPC) radi poboljšanja selektivne ekstrakcije. Sadržaj kolone je pulsirao ili direktnim delovanjem klipa ili pneumatski, naizmeničnim uduvavanjem i izduvavanjem vazduha [4]. U operacijama ekstrakcije urana, tečno–tečno, i danas se koriste pulsne kolone sa velikim protocima (60 m^3/h); paralelno sa



Slika 4. Intenzifikacija procesa proizvodnje sterola u reaktoru sa oscilirajućim tokom. a) Konvencionalni šaržni proces; b) intenzifikovani proces u ROT. Preuzeto iz ref. [4] uz odobrenje izdavača elsevier.
Figure 4. Intensifacion of sterol production utilizing oscillatory flow reactor. a) Conventional batch process; b) Intensified process in osscilatory flow reactor. Adopted from ref. [4] with the approval of publisher Elsevier.

Tabela 1. Prednosti reaktora sa oscilirajućim tokom u odnosu na konvencionalne šaržne i kontinualne reaktore [10]
Table 1. The advantages of oscillatory baffled reactors over conventional batch and continuous reactors [10]

Pogodnost	Obim	Napomena
Reakciono vreme	do 90%	Uniformno mešanje, poboljšani prenos mase i toplote omogućavaju značajno smanjenje reakcionog vremena u ROT
Manje sporednih proizvoda	do 90%	Zbog bolje kontrole hemijske reakcije i rada reaktora, u ROT nastaje manja količina otpada
Veći prinos	do 20%	Veći prinos u ROT se ostvaruje usled manje količine nepoželjnih proizvoda, i smanjenja drugih gubitaka
Energetska ušteda	do 75%	Značajno manja uložena energija za mešanje u poređenju sa klasičnim mešalicama; bolje kontrolisana razmena toplote
Kapitalni troškovi	do 50%	Klipno strujanje u ROT se postiže pri malim brzinama (mali nominalni Re) što vodi ka značajno kraćim reaktorima, time i manjim neophodnim prostorom -niži kapitalni troškovi
Ušteda katalizatora	do 100%	U nekim reakcijama kao što je oksidacija aromatičnih jedinjenja, katalizator se može potpuno eliminisati iz procesa

pulsnim kolonama za ekstrkciju urana koriste se i ekstraktori različitih konstrukcija, tipa mešač–odvajač (*eng. Mixer–settler*), sa znatno većim protocima (250 m^3/h).

Andrew Karr je razvio kolonu sa vibracionim pločama i koristio za ekstrakciju intermedijera penicilina, tzv. Karova kolona (1959. godine). U koloni su se nalazile ploče pričvršćene na jednoj ili više vertikalnih osovina koje su se pomerale delovanjem elektro-mehaničkog oscilatora postavljenog na vrh kolone. Ploče su sadržale veliki broj gusto raspoređenih otvora [4]. Ovakav način rada kolone uticao je na povećanje dodirne površine između faza, a samim tim i na povećanje koeficijenta prenosa mase [15].

Kolone sa vibrirajućim pločama (RPC) i kolone sa pulsirajućim pakovanim slojem (PPC), koje su koristile prednosti mešanja pomoću oscilacija, bili su uređaji koji su imali značajnu ulogu u industriji sve do ranih osamdesetih godina prošlog veka kada su brojne istraživačke grupe postale zainteresovane za primenu oscilirajućeg toka fluida u većem broju procesa [4]. Karova kolona se i danas koristi za ekstrakciju tečno - tečno [15].

Reaktori sa oscilirajućim tokom predstavljaju noviji tip reaktora čiji se rad zasniva na oscilovanju samog toka fluida. Nasuprot kolonama sa vibracionim pločama, ovaj tip reaktora se dosta razlikuje jer oscilovanje fluida u većoj meri poboljšava mešanje nego što utiče na povećanje kontaktne površine. Za razliku od kolona sa vibracionim pločama kod kojih su perforirane ploče (pregrade) postavljene na veoma malom rastojanju, pregrade (prstenovi) u reaktorima sa oscilirajućim tokom se nalaze na rastojanju ≈1,5*D*, a primarni mehanizam mešanja je, gore objašnjeno, generisanje vrtloga u zapremini tečnosti [15].

OSNOVNI KRITERIJUMI ZA PROJEKTOVANJE REAKTORA SA OSCILIRAJUĆIM TOKOM

Predloženi pristup projektovanju reaktora sa oscilirajućim tokom je zasnovan na održavanju geometrijske i dinamičke sličnosti, pri čemu se koriste različite bezdimenzione grupe i druge empirijske korelacije specifične za oscilirajuće tokove [15,26].

Geometrijska i dinamička sličnost

Geometrijska sličnost projektovanog reaktora sa oscilirajućim tokom se obezbeđuje održavanjem dve geometrijske vrednosti konstantnim: 1) rastojanja između pregrada, *L*, i 2) površine otvorenog dela pregrade (prstena), *S*:

$$L = 1{,}5D \tag{1}$$

$$S=\frac{d_0^2}{D^2} \tag{2}$$

gde je d_0 prečnik otvora, *D* unutrašnji prečnik reaktora. Površina otvorenog dela pregrada ima vrednost u opsegu 0,2–0,4, najčešće vrednost 0,25 kada prečnik otvora ima vrednost jedne polovine unutrašnjeg prečnika, *D* [15].

Rastojanje između pregrada utiče na oblik i dužinu vrtloga, dok d_0 kontroliše širinu vrtloga koji se formiraju u ćeliji reaktora sa oscilirajućim tokom. Ukoliko je rastojanje premalo, suzbija se formiranje vrtloga što ograničava njihov rast i redukuje preporučeno radijalno mešanje u svakoj ćeliji. Ukoliko je rastojanje veliko, javlja se suprotan efekat, vrtlozi formirani iza pregrade ne mogu efektivno ući u sledeću ćeliju, dolazi do stagnacije klipnog strujanja, vrtlozi bivaju rasuti i umanjeni [27,28]. Brunold i sardanici su predložili odnos dat jednačinom (1) [12,27].

Dinamika mešanja fluida oscilirajućim tokom definiše se pomoću nekoliko osnovnih bezdimenzionih brojeva: klasični Rejnoldsov broj, Re_n, oscilatorni Rejnoldsov broj, Re_o, i Strouhalov (*Strouhal*) broj, St_r [6,27].

Rejnoldsov broj je definisan na sledeći način:

$$Re_n = \frac{\rho u D}{\mu} \tag{3}$$

pri čemu *D* predstavlja unutrašnji prečnik reaktora, *ρ* gustinu fluida, *μ* dinamičku viskoznost i *u* srednju površinsku brzina fluida. Brunold i saradnici [11] su definisali jedan od dva bezdimenziona broja koji kontrolišu mehaniku fluida u reaktoru sa oscilirajućim tokom, oscilatorni Rejnoldsov broj:

$$Re_o = \frac{x_0 \omega D \rho}{\mu} \tag{4}$$

$$\omega = 2\pi f \tag{4a}$$

gde je x_0 amplituda oscilacija, *ω* ugaona brzina oscilacija, a *f* frekvencija oscilacija [15,27].

Amplituda (x_0) i frekvencija (*f*) oscilacija predstavljaju dva najznačajnija operativna parametra u reaktorima sa oscilirajućim tokom. Povećanje frekvencije i amplitude utiče da se i koeficijent prenosa mase $k_l a$, poveća [29]. S obzirom na to da su oscilacije obično oblika sinusoide, pomeraj, *x*, brzina, *v*, i ubrzanje, *a*, imaju sledeće vremenske zavisnosti [28,30]:

$$x = x_0 \sin(\omega t) \tag{5}$$

$$v = x_0 \omega \cos(\omega t) \tag{6}$$

$$a = -x_0 \omega^2 \sin(\omega t) \tag{7}$$

a maksimalna brzina u toku jednog ciklusa oscilacija je $x_0\omega$.

Za Re_o vrednosti u opsegu 100–300 vrtložni prstenovi se aksisimetrično formiraju u svim ćelijama između pregrada, strujanje pokazuje karakteristike bliske klipnom strujanju. Sa povećanjem vrednosti Re_o broja, simetrija se narušava, mešanje postaje intenzivnije i haotičnije i postaje slično mešanju u reaktorima sa mehaničnim mešanjem [27,28,30–32].

Drugi bezdimenzioni broj koji opisuje mehaniku fluida u reaktoru sa oscilirajućim tokom je Strouhalov broj, St_r, i predstavlja meru efektivnog širenja vrtloga. Definisan je kao odnos unutrašnjeg prečnika reaktora i dužine klipa za formiranje oscilacija [11,27,28]:

$$St_r = \frac{D}{4\pi x_0} \tag{8}$$

Sa povećanjem vrednosti ovog broja, povećava se i bezdimenziona srednja dužina vrtloga koji nastaju prilikom oscilovanja fluida [6,28].

Odnos *Re* brojeva toka sa oscilacijama i bez njih, odnosno, odnos njihovih brzina dat je sledećom jednačinom [23]:

$$\psi = \frac{Re_o}{Re_n} \tag{9}$$

Da bi se kvantifikovala aksijalna disperzija koriste se Pekletov broj:

$$Pe = \frac{u z}{De} \tag{10}$$

i Šmitov broj:

$$Sc = -\frac{\mu}{\rho De} \tag{11}$$

gde je *z* dužina reaktora, a *De* koeficijent aksijalne disperzije [11].

Radni uslovi

Kod reaktora sa oscilirajućim tokom, oscilirajući tok je superponiran na postojeći srednji tok. To znači da je nominalni Rejnoldsov broj toka fluida na ulazu vezan za srednju površinsku brzinu fluida, tj. određen je zapreminskim protokom, a oscilatorni Rejnoldsov broj je određen intenzitetom oscilacija. Uobičajno je da se površinska brzina podešava da bi se dobio željeni protok, čime se podešava vreme zadržavanja i na taj način se određuje kapacitet i dužina reaktora. Potom se biraju oscilatorni uslovi tako da bude veći od Re_n čime se obezbeđuje da superponirane oscilacije diktiraju režim mešanja [15]. Važno je napomenuti da postoje minimalne vrednosti Rejnoldsovog broja potrebne za postizanje strujanja: za ulazni tok $Re_n > 50$, i za oscilatorni tok [15,32,33]. Skorija istraživanja su pokazala da je odnos brzina, ψ, veoma bitan prilikom definisanja raspodele vremena zadržavanja u reaktoru sa oscilirajućim tokom. Preporučuje se da ovaj odnos brzina bude u opsegu $2 \leq \psi \leq 6$, da bi se u reaktoru ostvarilo strujanje približno klipnom strujanju [12,34]. Istraživanja su pokazala da se kod reaktora sa oscilirajućim tokom raspodela vremena zadržavanja dobro opisuje kaskadnim modelom strujanja, a da je za klipno strujanje, broj idealno izmešanih sudova u nizu manji od broja ćelija između pregrada. Broj teorijskih sudova u nizu $N \geq 10$ se smatra dovoljnim za postizanje strujanja koje se približava klipnom strujanju [15,34]. Srednje vreme zadržavanja se izračunava na osnovu površinske brzine (zapreminskog protoka), kao i kod klasičnih cevnih reaktora.

Izračunavanje gustine energije

U kontinualnim reaktorima sa oscilirajućim tokom, gubitak snage potiče i od osnovnog i od oscilirajućeg toka. Pad pritiska usled proticanja fluida kroz reaktor u kojem se nalaze pregrade se može izračunati primenom standardne jednačine za protok kroz otvore koja je proširena članom koji predstavlja ukupan broj identičnih pregrada, N_B:

$$\Delta P = N_B \frac{\rho\, u^2}{2C_0}\left(\frac{1}{S^2}-1\right) \tag{12}$$

gde je C_0 standardni koeficijent otvora i njačešće ima vrednost 0,6, a S predstavlja površinu otvorenog dela pregrade (prstena) definisanu jednačinom (2) [15].

Zbog uticaja oscilirajućeg toka, pad pritiska osnovnog toka ima veću vrednost u odnosu na pad pritiska kada je tok stacionaran, odnosno kada nema uticaja oscilacija. Odnos povećanja pritiska osnovnog toka se definiše na sledeći način [20]:

$$\zeta = \left[1+\left(\frac{4\psi}{\pi}\right)^3\right]^{1/3} \tag{13}$$

Snaga po jedinici zapremine, odnosno gustina snage ε_n, osnovnog toka je data jednačinom (14):

$$\varepsilon_n = \Delta P \frac{a_c}{V} u \zeta = \Delta P \frac{u}{z} \zeta \tag{14}$$

pri čemu a_c predstavlja površinu poprečnog preseka reaktora, V ukupnu zapreminu, a u površinsku brzinu [20]. Da bi se izračunao gubitak snage oscilatorne komponente toka, može se iskoristiti jednačina kvazi-stacionarnog toka [2,23,25], gde je prosečna snaga po jedinici zapremine, ε_V, definisana na sledeći način:

$$\varepsilon_V = \frac{2N_B \rho (x_0 \omega)^3}{3\pi C_0^2 z}\left(\frac{1}{S^2}-1\right) \tag{15}$$

Skorija istraživanja pokazuju da se, ukoliko je Strouhalov broj, St_r, veći od 0,2, gubitak snage može preciznije odrediti po modelu [15]:

$$\varepsilon_V = \frac{3N_B \rho \omega^3 x_0^2 l}{Sz} \tag{16}$$

gde je sa l označena dužina na kojoj se odvija mešanje usled vrtložnog strujanja i empirijski je utvrđeno da ima vrednost od 0,009.

Suma gubitka snage za osnovni tok (jednačina (14)) i gubitka snage oscilirajućeg toka (jednačine (15) ili (16)) predstavlja ukupan gubitak snage u ROT [15]:

$$\varepsilon_t = \varepsilon_n + \varepsilon_V \tag{17}$$

POBOLJŠANJE PRENOSA MASE U ROT

Zapreminski koeficijent prenosa mase, $k_l a$, zavisi od uslova strujanja, odnosno mešanja u reaktoru i fizičkih karakteristika fluida. U reaktoru sa oscilirajućim tokom, usled pojave vrtloga, koeficijent prenosa mase je značajno veći nego u klasičnim cevnim reaktorima, pri istim nominalnim protocima. U ROT rastojanje između pregrada je ključni projektni parametar koji utiče na dužinu vrtloga u ćelijama duž reaktora, pa time i na koeficijent prenosa mase. Ukoliko su pregrade previše blizu jedna drugoj, generisanje vrtloga može biti prigušeno što ograničava rast vrtloga i time štetno utiče na najbitniji mehanizam cirkulisanja fluida od zida reaktora do centra. Kao posledica ovoga javlja se mali koeficijent prenosa mase u sistemu. Ako su pregrade isuviše udaljene jedna od druge, vrtlozi koji se formiraju iza pregrade, ne mogu efektivno da pokriju zapreminu između dve predgrade. U ovom slučaju se stvaraju „slobodne“ zone u kojima se vrtlozi mogu rasformirati ili smanjiti, što takođe vodi ka slabijem ukupnom mešanju u uređaju. Jasno je da je optimalno rastojanje između pregrada u reaktoru sa oscilirajućim tokom ono pri kojem se postiže maksimalna dužina vrtložnog kretanja u ćelijama reaktora, bez njihovog prigušenja [35].

Ni i Gao [35] su eksperimentalno pokazali da koeficijent prenosa kiseonika između vazduha i vode raste sa povećavanjem rastojanja između pregrada u vertikalnim reaktorima sa oscilirajućim tokom prečnika 50 i 100 mm i visina 525 i 1050 mm, do vrednosti 1,8D, a da sa daljim povećanjem rastojanja vrednost koeficijenta prenosa mase opada. Autori su predložili da se reaktor sa oscilirajućim tokom projektuje tako da rastojanje između pregrada bude 1,5*D* [35].

Takođe, autori su utvrdili [35] da vrednost $k_l a$, raste sa povećavanjem frekvencije i amplitude oscilacija. Pri tom su uočili da za veće vrednosti amplitude, povećanje frekvencije rezultira znatno izraženijim rastom vrednosti $k_l a$, nego pri istoj promeni frekvencije za manje amplitude. Ovo ukazuje da promene amplitude oscilacija imaju veći uticaj na $k_l a$ nego promene frekvencije jer amplituda oscilacija kontroliše dužinu vrtloga duž kolone [20,35].

Oliviera i Ni [36] su pokazali da je vrednost zapreminskog koeficijenta prenosa mase u dvofaznom sistemu voda–vazduh u ROT četiri puta veća od vrednosti koja se ostvaruje u barbotažnoj koloni i da, ukoliko raspodeljivač gasa ima jedan otvor, $k_l a$ ima 13 puta veću vrednost. Ni i saradnici, kao alternativno objašnjenje za poboljšani prenos mase u reaktorima sa oscilirajućim tokom, iznose tvrdnju da ravnomerna raspodela brzine smicanja vodi ka stvaranju tanjih filmova tečnosti u reaktoru, pa otuda i povećanje vrednosti koeficijenta prenosa mase, k_l [23,37].

Na slici 5 je prikazana zavisnost zapreminskog koeficijenta prenosa mase od gustine energije u reaktoru sa mehaničkim mešanjem (RMM) zapremine 2 l i reaktoru sa oscilirajućim tokom (ROT) prečnika *D* = 50 mm u toku reakcije fermentacije ćelija kvasca *Saccharromyces cerevisiae.* Sa slike 5 se može uočiti da je koeficijent prenosa mase u reaktoru sa oscilirajućim tokom 75% veći nego u reaktoru sa mehaničkim mešanjem [38].

POBOLJŠANJE PRENOSA TOPLOTE U ROT

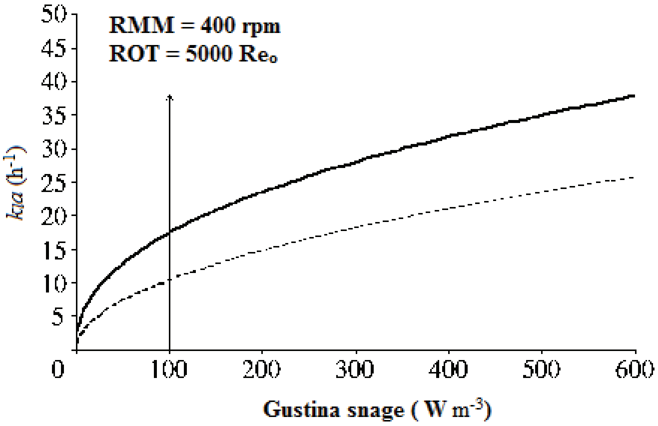
Prenos toplote prinudnom konvekcijom u cevnim sistemima takođe zavisi od uslova strujanja. Kada je strujanje fluida kroz cev potpuno turbulentno, brzina prenosa toplote je relativno velika zahvaljujući radijalnom mešanju koje se javlja pri ovakvom proticanju. Ukoliko su brzine strujanja fluida male ili srednje, odnosno strujanje je laminarno, nema dobrog radijalnog mešanja, te je brzina prenosa toplote smanjena [21]. Oscilatorno kretanje fluida kroz cevni reaktor sa pregradama daje mogućnost poboljšanja prenosa toplote, slično kao kod prenosa mase.

Mackley i Stonestreet [21] su predložili model kojim se za Re_n u opsegu od 100 do 1200 i Re_o u opsegu od 0 do 800 može predvideti vrednost Nuseltovog broja:

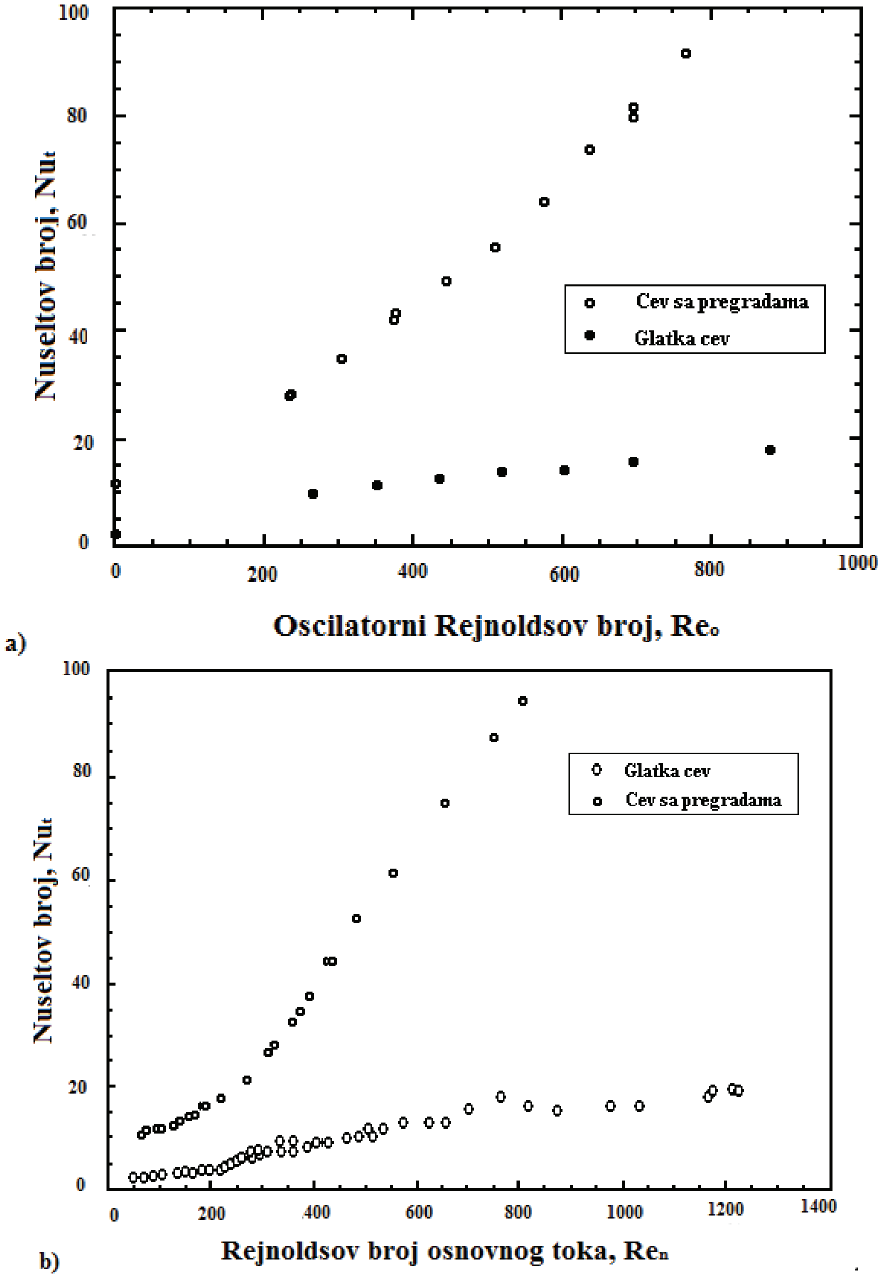
$$Nu = 0{,}35 Re_n^{1{,}3} Pr^{1/3} + 0{,}3\left[\frac{Re_o^{2{,}2}}{\left(Re_n + 800\right)^{1{,}25}}\right] \qquad (18)$$

Prvi član zbira u jednačini (18) odgovara stacionarnom strujanju koje doprinosi prenosu toplote i sličan je jednačini koju predlaže Dittus Boelter koja odgovara turbulentnom strujanju, s tim što eksponent od Re_n ima veću vrednost jer uključuje i uticaj prisustva pregrada. Drugi član se odnosi na povećanje *Nu* broja usled oscilirajućeg toka. Vrednosti *Pr* i *Nu* autori su određivali u svakom eksperimentu i njihova međusobna zavisnost nije potpuno utvrđena.

Glavna prednost uređaja sa oscilirajućim tokom je u tome što dobar prenos toplote može da se ostvari pri malim vrednostima Re_n. Autori su pokazali da je vrednost *Nu* broja u reaktorima sa oscilirajućim tokom 30 puta veća u odnosu na konvencionalne reaktore. Na slici 6a je prikazan uticaj prisustva pregrada na povećanje *Nu* broja pri oscilacijama u glatkoj cevi i u cevi sa pregradama za Re_n = 130. Na slici 6b je data zavisnost *Nu* od Re_n u glatkim i cevima sa pregradama bez oscilacija fluida [21]. Sa slike 6b se može uočiti i da samo prisustvo pregrada u uređaju utiče na povećanje *Nu* broja.



Slika 5. Zapreminski koeficijent prenosa mase u reaktoru sa mehaničkim mešanjem (RMM – isprekidana linija) i u reaktoru sa oscilirajućim tokom (ROT – puna linija) u zavisnosti od gustine energije. Adaptirano iz ref. [38] uz odobrenje izdavača Royal Society Publishing.
Figure 5. Volumetric mass transfer coefficient in STR (dashed line) and in OFR (solid line) depending on power density. Adopted from ref. [38] with the approval of publisher Royal Society Publishing.



Slika 6. Upoređivanje prenosa toplote u glatkim i cevima sa pregradama. a) Zavisnost Nu broja od Re_o za Re_n = 130; b) zavisnost Nu od Re_n, za Re_o = 0. Adaptirano iz ref. [21] uz odobrenje izdavača Elsevier.

Figure 6. Comparison of heat transfer efficiency for a smooth and a baffled tube. a) Nu number dependence on Re_o, for Re_n = 130; b) Nu number dependence on Re_n, for Re_o = 0. Adopted from ref. [21] with the approval of publisher Elsevier.

SMANJENJE BRZINE SMICANJA U ROT

Smicajni naponi predstavljaju veoma bitan faktor za bioprocese koji uključuju ćelije ili velike molekule kao što su enzimi, jer pri velikim naponima može doći do njihovog oštećenja ili deaktivacije. Brzina smicanja se definiše kao stepen promene brzine kojom jedan sloj fluida struji preko drugog, paralelnog sloja [38].

Ni i saradnici [18] su dali korelacije koje povezuju brzinu smicanja ($\bar{\gamma}$) i gubitak energije (ε) sa oscilatornim Rejnoldsovim brojem, Re_o, u ROT:

$$\bar{\gamma} = 6\times10^{-4} Re_o^{1,2}\ ,\ \ 251 \le Re_o \le 4021 \qquad (19)$$

gde je koeficijent korelacije 98%. Gubitak energije se može odrediti pomoću jednačine:

$$\varepsilon = 7,54\times10^{-13} Re_o^{3} \qquad (20)$$

Kombinovanjem jednačina (19) i (20) dobija se izraz koji povezuje brzinu smicanja i gubitak energije:

$$\bar{\gamma} = 62,6\varepsilon^{0.4} \qquad (21)$$

Srednja vrednost brzine smicanja u sudu sa mehaničkim mešanjem, $\dot{\gamma}_{ST}$, je direktno proporcionalna brzini mešanja. Metzner i Otto (1957. god) su predložili jednačinu za njeno izračunavanje koja važi za male vrednosti Rejnoldsovog broja [18]:

$$\dot{\gamma}_{ST} = kN_o \tag{22}$$

gde je sa N_o označena rotaciona brzina mešalice u rpm, a sa k konstanta koja zavisi od dizajna mešalice i tipa fluida. Calderbank i Moo-Young (1961) su predložili sledeću korelaciju kojom se k definiše kao:

$$k = B\left(\frac{4n}{3n+1}\right)^{\frac{n}{1-n}} \tag{23}$$

pri čemu je n indeks čija vrednost zavisi od reoloških karakteristika fluida, a B konstanta čija je vrednost 11.

U tabeli 2 su date srednje vrednosti brzine smicanja pseudo-plastičnog fluida u posudi sa mehaničkim mesanjem i koloni sa oscilirajućim tokom pri maksimalnoj vrednosti amplitude od 8 mm [18]. Pri tome vrednosti broja obrtaja mešalice odgovaraju frekvenciji oscilovanja, u cilju adekvatnog poređenja.

Tabela 2. Vrednost srednjih brzina smicanja u posudi sa mehaničkim mešanjem i u koloni sa oscilirajućim tokom [18]
Table 2. A comparison of the mean strain rate in a stirred tank and an oscillatory flow reactor [18]

Parametar	f / Hz; N_o / rpm			
	0,4; 24	0,8; 48	1,2; 72	1,6; 96
$\dot{\gamma}_{ST}$ / s	4,2	8,4	12,6	16,8
$\bar{\gamma}$ / s	3,5	7,1	10,1	13,3

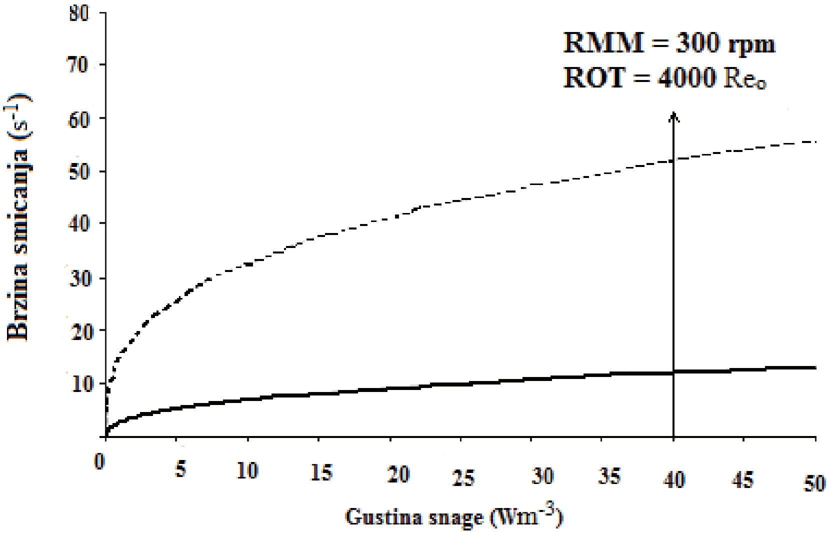
Evidentno je da je za istu pobudu po minuti vrednost srednje brzine smicanja niža u koloni sa oscilirajućim tokom prečnika 50 mm nego u posudi sa mehaničkim mešanjem prečnika 457 mm, s tim što ovakve brzine rotacije u posudi izazivaju mešanje malog intenziteta. Slika 7 pokazuje da je za isti unos energije od 40 W m^{-3}, srednja brzina smicanja u reaktoru sa oscilirajućim tokom 5 puta manja nego u reaktoru sa mehaničkim mešanjem [38].

Pored toga što su dimenzije uređaja sa mehaničkim mešanjem veće, srednja brzina smicanja fluida je veća. Uzevši u obzir ovu činjenicu i dimenzije posmatranih uređaja, zaključuje se da je kolona sa oscilirajućim tokom znatno bolji uređaj u pogledu smicajnih napona od reaktora sa mehaničkim mešanjem [18]. Prikazani rezultati ukazuju da su brzine smicanja u ROT, manje i od zabeleženih u pneumatskim reaktorima sa spoljašnjom recirkulacijom. Ovi uređaji su alternativa reakorima sa konvencionalnim mešanjem, za biohemijske procese, upravo zbog nižih smicajnih napona i većih brzina prenosa toplote i mase [39,40].

RASPODELA VREMENA ZADRŽAVANJA U ROT

U kontinualnim cevnim sistemima zahteva se uska raspodela vremena zadržavanja (RVZ), odnosno približno klipno strujanje, pa zbog toga RVZ predstavlja važan parametar koji utiče na rad reaktora [34]. Uslovi oscilatornog mešanja (frekvencija i amplituda) definišu i kvantifikuju raspodelu vremena zadržavanja u ROT [34].

Direktnim rešavanjem jednačina Navie–Stouksa (*Navier–Stokes*) može se dobiti tačna slika strujanja oscilirajućeg toka koja važi samo za aksisimetrični režim strujanja fluida (za Re_o < 250). S obzirom da se često



Slika 7. Srednja vrednost brzine smicanja u reaktoru sa oscilirajućim tokom, D = 50 mm (ROT – puna linija) i reaktoru sa mehaničkim mešanjem zapremine 2 l (RMM – isprekidana linija). Adaptirano iz ref. [38] uz odobrenje izdavača Royal Society Publishing.
Figure 7. Average shear rates for a 50 mm OFR (solid line) and 2 l STR (dashed line). Adopted from ref. [38] with the approval of publisher Royal Socity Publishing.

zahtevaju vrednosti Re_o veće od 250, raspodela vremena zadržavanja u ROT se najčešće opisuje pomoću sledećih približnih modela [34]:

1) disperzioni model, gde se reaktor posmatra kao kontinualni sistem i

2) kaskadni model, gde je reaktor podeljen na jednake, diskretne delove.

U većini radova koji se odnose na mešanje fluida pomoću oscilirajućeg toka koristi se disperzioni model sa jednim parametrom – koeficijentom aksijalne disperzije. Kvantifikovanje aksijalne disperzije je od praktičnog značaja za reaktore sa oscilirajućim tokom jer se dobijene vrednosti mogu iskoristiti za predviđanje raspodele vremena zadržavanja i za velike i za male reaktore [41]. Ispitivanja aksijalne disperzije u uređajima sa oscilirajućim tokom različitih dimenzija su pokazala tri zajedničke karakteristike:

1) vrednost koeficijenta aksijalne disperzije je mala, bliska onoj koja se javlja u uređajima sa približno klipnim strujanjem;

2) vrednosti koeficijenta aksijalne disperzije dobijene primenom različitih modela su približne;

3) ne postoji značajna razlika u aksijalnoj disperziji između šaržnih i kontinualnih operacija kada je Re_o veće ili jednako Re_n. U ovim uslovima uticaj oscilacija prevazilazi uticaj osnovnog toka na koeficijent aksijalne disperzije, *De*, osim u slučaju veoma niskih frekvencija [41].

Pretpostavlja se da se za dovoljno velike vrednosti Re_n može primeniti teorija Kolmogorova (*Kolmogoroff*, 1941) [42], pa se koeficijent aksijalne disperzije može izraziti kao:

$$De = l^{\frac{4}{3}} \varepsilon^{\frac{1}{3}} \tag{24}$$

gde l predstavlja karakterističnu dimenziju sistema, a ε gubitak energije za datu geometriju.

Ako se uzme da je gubitak energije približno jednak:

$$\varepsilon \approx x_0^3 \omega^3 \frac{\left(1-S^2\right)}{S^2 L} \tag{25}$$

gde je L bezdimenziono rastojanje između pregrada:

$$L = \frac{1}{N_B} \tag{26}$$

smenom jednačine (25) u jednačinu (24) dobija se sledeći izraz:

$$De \approx l^{4/3} \left(x_0 f\right) \left[\frac{\left(1-S^2\right)}{S^2 L}\right]^{1/3} \tag{27}$$

Bezdimenzioni disperzni broj može se izračunati iz sledeće relacije [5]:

$$\frac{1}{Pe} = \frac{De}{uL} = \frac{De}{L^2}\frac{L}{u} = \frac{De\tau}{L^2} \tag{28}$$

gde je sa τ označeno srednje vreme zadržavanja.

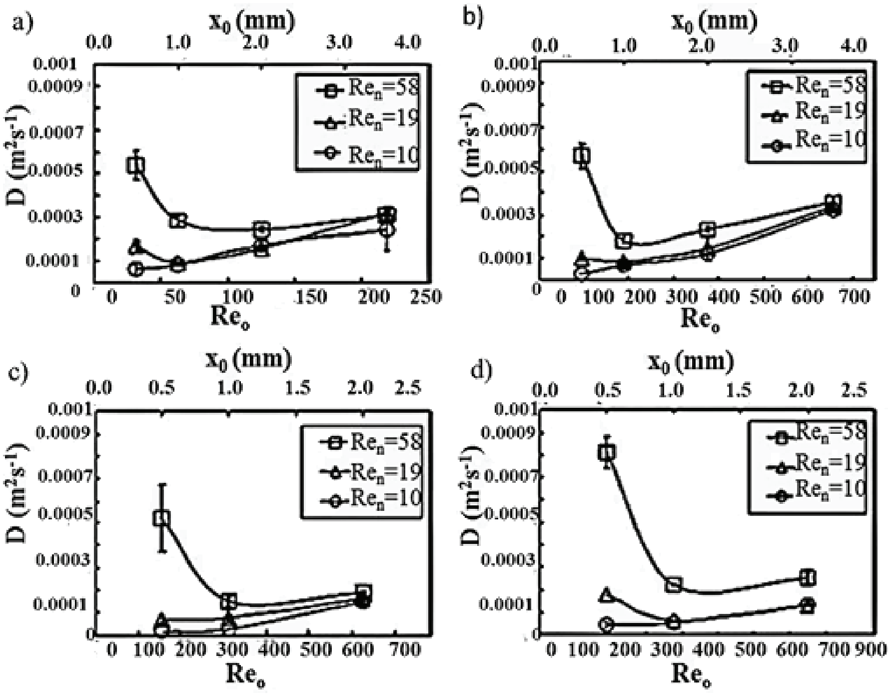
Zavisnost koeficijenta aksijalne disperzije od uslova oscilovanja u mezo-reaktoru (manje dimenzije) sa oscilirajućim tokom prikazana je na slici 8. Sa slike 8 se može zaključiti da koeficijent aksijalne disperzije blago raste sa porastom Re_o, za niske vrednosti Re_n. Međutim, za veću vrednost Re_n, *De* je značajno veći, zatim naglo opada sa porastom Re_o, da bi potom imao isti trend blagog porasta (ima minimalnu vrednost). Ovaj efekat je naročito izražen za više frekvencije oscilovanja (slika 8c i d).

Primena disperzionog modela se zasniva na određivanju srednjeg vremena zadržavanja statističkom analizom podataka, pod pretpostavkom da imaju Gausovu raspodelu. Koeficijent aksijalne disperzije se određuje na osnovu izmerenih vrednosti koncentracije obeležene supstance na izlazu iz reaktora, s pretpostavkom o savršenom impulsu na ulazu. Ukoliko impuls na ulazu nije savršen, koriste se vrednosti merenja u dve tačke [34].

Kaskadni model predstavlja drugi jednoparametarski model i pretpostavlja da fluid protiče kroz niz sudova sa idealnim mešanjem. Da bi se eksperimentalno odredio, ovaj model takođe zahteva primenu metode obeležene supstance za koju se pretpostavlja da u sistem ulazi kao idelna impulsna ili stepenasta promena [34].

Na slici 9 se može videti da raspodela vremena zadržavanja zavisi i od amplitude i od frekvencije oscilacija. Na slici 9 se može zapaziti da postoji optimalna oblast amplituda i frekvencija za koje je broj sudova u nizu (ili ćelija u reaktoru), *N*, najveći, odnosno aksijalna disperzija najmanja. Sa slike 9 se može zaključiti da je *N* veći u oblasti nižih frenkvencija i viših ili srednjih amplituda. Interesantno je uočiti i to da se *N* povećava sa povećavanjem Re_n, kao i da u tom slučaju dolazi do smanjenja uticaja frekvencije i amplitude (manje izražen maksimum). Treba napomenuti da je ovaj rezultat u suprotnosti sa rezultatima prikazanim na slici 8, gde se za veće vrednosti Re_n dobijala veća disperzija, a ne manja. To pokazuje da se raspodela vremena zadržavanja u ROT treba dodatno i detaljnije ispitati i da ona može da zavisi i od dodatnih faktora, kao što su geometrija sistema i sl.

Stonestreet i van der Veeken [34] su izveli kaskadni model za ROT koji se u osnovi zasniva na modelu koji je predložio Levenspiel [43], a na osnovu analize eksperimenata sa obeleženom supstancom i impulsnom pobudom. Oni su na osnovu kaskadnog modela i eksperimentalnih rezulatata dobili optimalan broj sudova sa idealnim mešanjem u nizu – *N*. Ako se sa *N* označi teorijski broj sudova sa idealnim mešanjem dobijen iz kaskadnog modela, a sa *M* broj realnih ćelija u reaktoru,



Slika 8. Koeficijent aksijalne disperzije u zavisnosti od vrednosti Re_o za Re_n = 10, 19 I 58 pri frekvencijama, f, od: a) 2, b) 6, c) 10 i d) 12 Hz. Adaptirano iz ref. [5] uz odobrenje izdavača Elsevier.
Figure 8. Axial dispersion coefficient as a function of Re_o, for Re_n = 10, 19 and 58 at different oscillation frequencies, f: a) 2, b) 6, c) 10 and d) 12 Hz. Adopted from ref. [5] with the approval of publisher Elsevier.

onda se koeficijent efikasnosti mešanja u ROT može predstaviti sledećom jednačinom:

$$\eta = \frac{N}{M} \qquad (29)$$

Koeficijent efikasnosti mešanja uvek ima vrednost manju od 1, odnosno teorijski broj sudova sa idealnim mešanjem dobijen primenom kaskadnog modela, *N*, je manji od realnog broja ćelija u ROT, *M* [34].

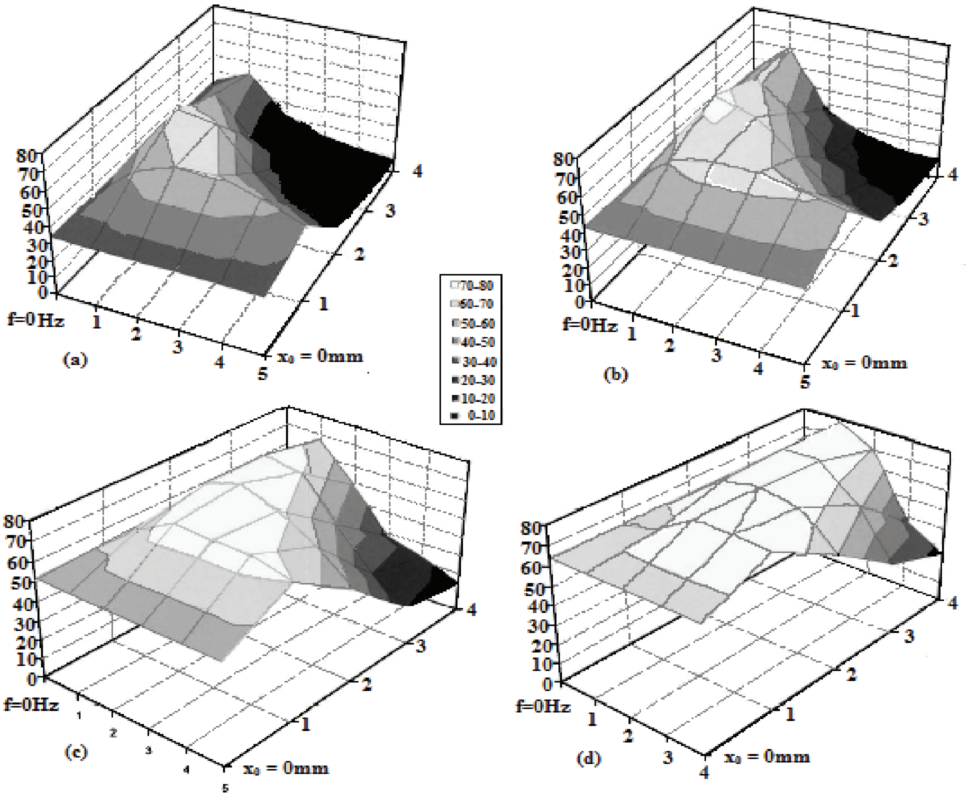
U reaktorima sa oscilirajućim tokom fluida u kojima je prisutna i čvrsta faza (reaktant, katalizator ili proizvod reakcije) slika strujanja je komplikovanija, jer pored vrtloženja fluida dolazi i do složenog kretanja čestica. Za ove sisteme jednoparametarski modeli raspodele vremena zadržavanja (disperzioni ili kaskadni) nisu dovoljno precizni u opisu slike strujanja čestica. U ovom slučaju adekvatniji su višeparametarski modeli strujanja, koji pored aksijalnog mešanja predviđaju postojanje stagnantnih zona čestica i njihovo ponašanje, koje se realno mogu očekivati u ROT [44,45].

PRIMERI INTENZIFIKACIJE PROCESA PRIMENOM REAKTORA SA OSCILIRAJUĆIM TOKOM

Zbog svojih performansi i uslova rada, reaktor sa oscilirajućim tokom našao je široku primenu u intenzifikaciji procesa. Pogodan je za reakcije sa dugim reakcionim vremenom (duže od 10 min) koje se u drugim, klasičnim uređajima odvijaju šaržno. Mehanizam mešanja i efikasni prenos toplote i mase u ovom tipu uređaja omogućava njihovu primenu u procesima u kojima se javljaju ćelije kultura mikroorganizama, kristalizaciji farmaceutskih i drugih proizvoda, dobijanju biogoriva, polimerizaciji, separacionim procesima, procesima prečišćavanja otpadnih voda i dr. [29].

Reaktor sa oscilirajućim tokom nudi poboljšano i uniformnije mešanje pri niskim vrednostima smicajnih napona u odnosu na konvencionalne uređaje. Ova prednost omogućava upotrebu u biohemijskim i biomedicinskim procesima koji uključuju ćelije kultura mikroorganizama koje su veoma osetljive na smicanje i za čiji rast je neophodan dobar prenos mase [16,29,38]. Vrednost koeficijenta prenosa mase kiseonika potrebnog za rast ćelija kvasca iz roda *Saccharemyces cerevisiae*, k_la, je u fermentatoru sa oscilirajućim tokom 75% veća nego u klasičnom reaktoru sa mehaničkim mešanjem [20]. Istraživanja su pokazala da i proizvodnja pululana iz obnovljivih, prirodnih izvora može da se ubrza upotrebom fermentatora sa oscilirajućim tokom. Upoređujući vrednosti koncentracije pululana dostignute za iste operativne uslove, zaključuje se da se u fermentatoru sa oscilirajućim tokom, u odnosu na fermentator sa mehaničkim mešanjem, postigne ista koncentracija pululana, ali za znatno kraće vreme (96 h u fermentatoru sa mehaničkim mešanjem, a 37 h u fermentatoru sa oscilirajućim tokom) [16].

U farmaceutskoj industriji jedna od ključnih operacija je vraćanje proteina u prvobitno konformacijsko



Slika 9. Uticaj amplitude i frekvencije na raspodelu vremena zadržavanja za Re_n *vrednosti: a) 95; b) 127; c) 190; d) 252. Adaptirano iz ref. [34] uz odobrenje izdavača Elsevier.*

Figure 9. Effect of amplitude and frequency on residence time distribution for the following Re_n *values: a) 95; b) 127; c) 190; d) 252. Adopted from ref. [34] with the approval of publisher Elsevier.*

stanje na kraju procesa [38], što se uglavnom odvija u rektorima sa mehaničkim mešanjem. Pri uvećanju razmera reaktora sa mehaničkim mešanjem dolazi do stvaranja zona „slabijeg“ mešanja, tj. smanjuje se efikasnost mešanja, što negativno utiče na vraćanje proteina u prvobitno konformacijsko stanje [22,38]. Lee i saradnici [46,47] su eksperimentalno pokazali da se vraćanje proteina u prvobitno konformacijsko stanje može vršiti efikasno u reaktoru sa oscilirajućim tokom. U ovom primeru, prednost reaktora sa oscilirajućim tokom u odnosu na konvencionalne reaktore ogleda se u tome što uvećanje razmera ovog reaktora nema uticaja na efikasnost mešanja, a samim tim i na stepen regeneracije proteina [22,38,47].

Efikasnost mešanja je jedan od najbitnijih faktora koji utiču na strukturu i kvalitet kristala nastalih kristalizacijom. Upoređivanjem morfologije kristala paracetamola dobijenih kristalizacijom u reaktoru sa mehaničkim mešanjem i šaržnog reaktora sa pulzacijama, dolazi se do zaključka da se u reaktoru kod kojeg se mešanje ostvaruje oscilovanjem fluida proizvode kristali pravilnijeg oblika sa manje grešaka u strukturi [48]. Ispitivanja kristalizacije α-glutaminske kiseline u reaktoru sa oscilirajućim tokom pokazuju da širina metastabilne zone opada sa povećanjem intenziteta mešanja i da je značajno manja u odnosu na zonu formiranu u reaktoru sa mehaničkim mešanjem [49].

Reaktor sa oscilirajućim tokom se pokazao i kao efikasan uređaj za proizvodnju biogoriva. U toku proizvodnje bioetanola iz celuloznih sirovina, enzimska saharifikacija celuloze do glukoze i fermentacija glukoze do etanola se u ROT odigravaju istovremeno, što predstavlja prednost. Stepen konverzije sirovine je približan stepenu konverzije ostvarenom u laboratorijskom šaržnom reaktoru, ali se postiže za 25 sati kraće [50]. Reakcija transesterifikacije biljnog ulja, kojom nastaje biodizel, se u rektoru sa oscilirajućim tokom odvija kontinualno 30 min na temperaturi od 50 °C. U šaržnom reaktoru se navedena reakcija dešava na višoj temperaturi i traje 60 min [51]. Biobutanol kao biogorivo potencijalno je bolje od etanola i može se dobiti aerobnom fermentacijom sirovina. Masnguta i Harvey su pokazali da proizvodnja biobutanola može biti unapređena ukoliko se aerobna fermentacija odvija u reaktoru sa oscilirajućim tokom. Najveća produktivnost postignuta u ovakvom bioreaktoru (0,22 g l^{-1} h^{-1}) je 38% veća od maksimalne vrednosti ostvarene u bioreaktoru sa mehaničkim mešanjem [52].

Reaktori sa oscilirajućim tokom su pogodni za intenzifikovanu proizvodnju polimera i postoji više aplikacija koje su komercijalizovane. Reaktor u kojem se pomeraju pregrade i time ostvaruje mešanje fluida, korišćen je za proizvodnju čestica reakcijom polimerizacije. Dimenzije i morfologiju čestica moguće je kontrolisati bez obzira da li reaktor radi šaržno ili kontinualno zahvaljujući dominaciji superponiranih oscilacija koje mešaju fluid u radijalnom pravcu, i obezbeđuju klipno ili približno klipno strujanje u kontinualnim sistemima. Ovakav vid mešanja u kombinaciji sa približno konstantnim intenzitetom turbulencija u reaktoru vodi ka ravnomernoj raspodeli veličina čestica sa velikim stepenom ponovljivosti [53].

Rezultati radova [2,4,54] pokazuju da proizvodnja polimetilmetakrilata i poliakrilamida u reaktoru sa oscilirajućim tokom ima visok stepen ponovljivosti kvaliteta proizvoda (~90%) sa približno Gausovom raspodelom veličine čestica, pri čemu je broj finih čestica konstantan i manji od 5%, za razliku od reaktora sa mehaničkim mešanjem gde je udeo finih čestica 8 do 10%. Veličina čestica, kao i raspodela veličine čestica se jednostavno može kontrolisati odabirom uslova oscilovanja, pri čemu hemijska reakcija ostaje nepromenjena [52]. Eksperimentalna istraživanja su pokazala da je lakše suspendovati čestice oscilacijama visokih frekvencija i malih amplituda, nego oscilacijama nižih frekvencija i velikih amplituda. U reaktoru sa oscilirajućim tokom ne samo da čestice ostaju suspendovane, već je moguće suspendovati ih za nekoliko sekundi ukoliko su istaložene [55].

Još jedan primer poboljšanja je reakcija između *n*-butilbromida i natrijum fenoftalata u prisustvu katalizatora koji prelazi iz jedne u drugu fazu u reaktoru kod kojeg se pomeraju pregrade i ostvaruje oscilovanje fluida. Ova reakcija počinje da se odvija pri manjem unosu energije (nakon jednog minuta) u odnosu na reaktor sa mehaničkim mešanjem, a pri tom se i postiže veći stepen konverzije [56,57].

Pored navedenih primena u reaktorskom inženjerstvu, ROT se mogu primeniti u procesima prečišćavanja otpadnih voda, kao i za druge separacione procese. U procesu prečišćavanja vode, flokulacija je operacija u kojoj se male čestice, u prisustvu polimera, spajaju gradeći flokule dovoljno velike da bi se mogle ukloniti filtracijom ili stabilizovati. Procenat flokulacije bentonita je u šaržnom flokulatoru sa pregradama, kod kojeg se mešanje fluida postiže delovanjem meha postavljenog sa donje strane uređaja (oscilatorno mešanje), približno isti kao i u flokulatoru sa mehaničkim mešanjem, s tim što se taj nivo u flokulatoru sa pregradama postiže pri znatno manjim vrednostima brzine smicanja [58].

Oslanjajući se na istraživanja Mackley i saradnika [59], u Univerzitetu u Kembridžu istraživači Fabiyi i Skelton [60] su 1999. godine ispitivali reakciju oksidacije koja se može primeniti u tretmanu otpadnih voda u fotokatalitičkom reaktoru sa pulsirajućim fluidom. Čestice, nosači katalizatora TiO_2 bile su suspendovane u tečnoj fazi pomoću pulzacija fluida izazvanih delovanjem dijafragme postavljene u dno reaktora, odakle je je uvođena i gasovita faza. Kataliza je inicirana ultraljubičastim zračenjem čiji se izvor nalazio van reaktora [60], a utvrđeno je da je efikasnost iskorišćenja fotona veća u odnosu na konvencionalne fotohemijske reaktore [5,23,32].

Separacioni procesi pomoću adsorpcije zastupljeni su u hemijskoj, petrohemijskoj i biohemijskoj industriji. Separacija adsorpcijom se zasniva na prenosu mase kroz jednu ili više kolona sa pakovanim slojem čestica ili granula od odgovarajućeg materijala – adsorbenta. Lau i saradnici su pokazali prednost primene oscilirajućeg toka u koloni sa pakovanim slojem sfernih čestica zeolita, u kojoj se suši etanol koji sadrži 3,2 mas.% vode. Za dati protok na ulazu, koncentraciju i temperaturu, prisustvo oscilirajućeg toka u procesu adsorpcionog sušenja etanola utiče na odlaganje vremena proboja, i smanjenje dužine neiskorišćenog pakovanog sloja. Primena oscilirajućeg toka poboljšava navedene parametre za 20% [61]. U tabeli 3 je dat pregled procesa u kojima su korišćeni uređaji sa oscilatornim mešanjem i način na koji se oscilacije generišu.

ZAKLJUČAK

Reaktori sa oscilirajućim tokom predstavljaju noviji tip uređaja u kojima se postiže vrlo dobro mešanje fluida koje ne zavisi od vrednosti Rejnoldsovog broja na ulazu, već samo od uslova, parametara oscilacija – frekvencije i amplitude. Ova karakteristika omogućava da se za male vrednosti brzine strujanja u ROT, koje bi u klasičnom cevnom reaktoru dale laminarno strujanje, dobije približno klipno strujanje fluida. Iz tog razloga su reaktori sa oscilirajućim tokom pogodni za reakcije koje dugo traju, te se veliki broj šaržnih procesa može prevesti u kontinualne, uz smanjenje unosa energije i dimenzija uređaja. Osim navedenog, mehanizam mešanja se kod ovog reaktora ne menja prilikom povećavanja razmera procesa, sa laboratorijskog na industrijski nivo, što nije slučaj kod reaktora sa mehaničkim mešanjem.

Kombinacija pregrada i oscilatornog kretanja stvara vrtložno proticanje fluida koje povećava efikasnost prenosa mase i toplote, a daje relativno male smicajne napone. Navedene pozitivne karakteristike omogućavaju intenzifikaciju procesa i komercijalizaciju reaktora sa oscilirajućim tokom u različitim industrijskim granama: prehrambenoj, farmaceutskoj i hemijskoj industriji, tretmanu otpadnih voda, itd.

Pored svih prednosti koje nudi, tehnologija reaktora sa oscilirajućim tokom ima svojih nedostataka. Pošto gasovita faza prigušuje oscilacije fluida, zapreminski udeo gasa u sistemu je ograničen do 15%, što znači da ovaj tip reaktora nije primenljiv u procesima u kojima se kao proizvod dobija gas ili je jedan od reaktanata u gasovitoj fazi. Prisustvo čvrste faze takođe utiče na širenje oscilacija fluida i intenzitet mešanja u ovim reaktorima. Istraživanja su pokazala da se ovaj tip reaktora može primeniti ukoliko udeo čvrste faze nije veći od 30% (što varira u zavisnosti od raspodele veličine čestica, gustine, itd.). Viskoznost fluida ili smeše fluida je još jedan faktor koji utiče na prostiranje oscilacija fluida. Ukoliko je viskoznost tečne faze veća od 0,5 Pa s, ovi reaktori se ne mogu primeniti. Osim ovih radnih ograničenja, ROT su složenije konstrukcije od običnih cevnih reaktora (prisustvo pregrada), a neophodno je i obezbediti dodatne uređaje za generisanje oscilacija, kao i za njihovu kontrolu. Ovo povećava cenu investicije za ROT, koja može biti opravdana u slučaju značajnih ostalih ušteda, navedenih u tekstu rada (Tabela 1).

Aktuelna istraživanja reaktora sa oscilirajućim tokom obuhvataju kompleksne višefazne procese sa česticama čvrste faze (kristalizacija, polimerizacija, imobilisani enzimi, katalizatori), procese koji uključuju tečnosti veoma velikih gustina i viskoznosti, i reakcije u kojima nastaje značajna količina gasa.

Spisak upotrebljenih oznaka

a – ubrzanje (m/s^2)
a_c – površina poprečnog preseka reaktora (m^2)
D – unutrašnji prečnik reaktora (m)
d_0 – prečnik otvorenog dela pregrada (m)
D_e – koeficijent aksijalne disperzije (m^2/s)
f – frekvencija oscilacija (Hz)
$k_l a$ – zapreminski koeficijent prenosa mase (mol/m^3)
l – dužina na kojoj se odvija mešanje usled vrtloženja (m)
L – rastojanje između pregrada (m)
M – broj aktuelnih ćelija u reaktoru
N – broj teorijskih sudova sa idealnim mešanjem
N_B – broj pregrada u ROT
N_o – broj obrtaja mešalice (rpm)
Nu – Nuseltov broj
ΔP – pad pritiska (Pa)
Pe – Pekletov broj
Pr – Prantlov broj
Re_n – Rejnoldsov broj toka bez oscilacija
Re_o – oscilatorni Rejnoldsov broj
S – površina otvorenog dela pregrada (m^2)
St_r – Strouhalov broj
Sc – Šmitov broj
t – vreme (h)
u – površinska brzina fluida (m/s)
x – pomeraj (m)
x_0 – amplituda oscilacija (mm)
z – dužina reaktora (m)
V – zapremina (m^3)
v – brzina (m/s)

Grčka slova

ρ – gustina fluida (m^3/kg)
μ – dinamička viskoznost fluida (Pas)
ω – ugaona brzina oscilacija (rad)
ψ – odnos brzine toka sa osilacijama i brzine toka bez oscilacija
τ – srednje vreme zadržavanja (h)
ζ – odnos povećanja pritiska
ε – gubitak energije (W/m^3)
ε_n – gubitak energije osnovnog toka (W/m^3)
ε_v – gubitak energije oscilatorne komponente toka (W/m^3)
ε_t – ukupni gubitak energije u ROT (W/m^3)
$\bar{\gamma}$ – brzina deformacije u reaktoru sa oscilirajućim tokom (1/s)
$\dot{\gamma}_{st}$ – srednja brzina deformacije u reaktoru sa mehaničkim mešanjem (1/s)
η – efikasnost mešanja

Konstante

C_0 – koeficijent u jednačini (12)
k – konstanta u jednačini (22)
B – konstanta u jednačini (23)
n – konstanta u jednačini (23)
l – konstanta u jednačini (24)

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SUMMARY

OSCILLATORY FLOW CHEMICAL REACTORS

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(Review paper)

Global market competition, increase in energy and other production costs, demands for high quality products and reduction of waste are forcing pharmaceutical, fine chemicals and biochemical industries, to search for radical solutions. One of the most effective ways to improve the overall production (cost reduction and better control of reactions) is a transition from batch to continuous processes. However, the reactions of interests for the mentioned industry sectors are often slow, thus continuous tubular reactors would be impractically long for flow regimes which provide sufficient heat and mass transfer and narrow residence time distribution. The oscillatory flow reactors (OFR) are newer type of tube reactors which can offer solution by providing continuous operation with approximately plug flow pattern, low shear stress rates and enhanced mass and heat transfer. These benefits are the result of very good mixing in OFR achieved by vortex generation. OFR consists of cylindrical tube containing equally spaced orifice baffles. Fluid oscillations are superimposed on a net (laminar) flow. Eddies are generated when oscillating fluid collides with baffles and passes through orifices. Generation and propagation of vortices create uniform mixing in each reactor cavity (between baffles), providing an overall flow pattern which is close to plug flow. Oscillations can be created by direct action of a piston or a diaphragm on fluid (or alternatively on baffles). This article provides an overview of oscillatory flow reactor technology, its operating principles and basic design and scale-up characteristics. Further, the article reviews the key research findings in heat and mass transfer, shear stress, residence time distribution in OFR, presenting their advantages over the conventional reactors. Finally, relevant process intensification examples from pharmaceutical, polymer and biofuels industries are presented.

Keywords: Process intensification • Oscillatory flow reactors • Batch to continuous processes • Oscillatory flow mixing

Laser ablation initiated fast discharge for spectrochemical applications

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Abstract

The results of an experimental study of the optical emission enhancement possibilities during the single pulse laser induced breakdown spectroscopy of the aluminum alloy are presented. This study is performed in air, argon and helium at different pressures with and without the additional fast electric discharge. The discharge was initiated by plasma plume created by laser ablation of target. The influences of various capacitors and discharge voltages on enhancement of the studied spectral line intensities were also studied. The application of the fast discharge through optical emission enhancement enables lowering of detection limits thus making this spectrochemical method comparable with the other analytical techniques.

Keywords: spectrochemistry, analytical spectrometry, laser induced breakdown spectroscopy, optical emission spectroscopy.

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The application of lasers for analytical purposes started only three years after their introduction [1,2]. The laser radiation with energies around 100 mJ and duration of several nanoseconds was focused on a submillimeter spot on a solid, liquid or gaseous sample inducing breakdown. Namely, the free electrons created through multi-photon ionization and inverse bremsstrahlung processes absorb energy from the laser pulse, colliding and freeing yet more electrons in an ionization cascade until thermally-hot, charge-neutral laser-induced plasma is produced. The spectral recordings of this plasma showed lines of all elements present in the sample. This fact opened the possibility for a new analytical technique – laser-induced breakdown spectroscopy – LIBS. The main advantages of LIBS are: 1) simplicity, 2) lack of sample preparation for the analysis of the gases, liquids and solids, 3) simultaneous multielement detection, 4) ability to detect high and low z elements, 5) only optical access to the target is required and 6) standoff analysis capabilities [2–4]. The unique capability of the laser plasma to sample materials and standoff possibility of LIBS enabled their use to monitor chemical, biological, radiological, nuclear and explosive threats from the safe distance. Besides the applications of LIBS in hazardous environment usually characteristic for chemical industry, other applications like rapid characterization of polymer type, composition of paints and varnishes or detection of impurities in petroleum, cosmetic or other chemical products are also numerous [2–4].

However, the detection limit was poor in comparison with other laboratory analytical techniques. The main reasons are low intensity of the spectral lines and very intense continuum radiation, which significantly lower signal to background – S/B ratio. The much faster decay of the background emission versus line emission opened the possibility for improvement of S/B ratio using gated photomultipliers or CCD cameras to record spectra. The development of the intensified CCD cameras enabled (besides gating) further gains in the recorded signal. Unfortunately, techniques such as inductively coupled plasma-mass spectrometry (ICP-MS) were still favorable.

These LIBS disadvantages were mainly overcome by enhancing optical emission with the use of the additional laser pulse in different double pulse technique configurations – DP-LIBS [2–6]. The first pulse is used to ablate the target and produce the plasma plume, while the second pulse is used to reheat the plasma and thus create a stronger emission. Currently, DP-LIBS technique is prevalent in broad range of applications such as analysis of samples under water [7] or in Mars conditions [8], detection of traces of various explosives [9] and many others.

In this work, single laser pulse techniques are tested as cheaper alternatives (to double pulse LIBS technique) for enhancing optical emission. For that purpose we performed analysis at different pressures in argon and helium in addition to atmospheric air. The use of the additional fast discharge initiated with a same laser pulse was also studied.

EXPERIMENTAL

Typical single pulse stand-off LIBS equipment consists of a Nd-YAG laser with focusing optics, light col-

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lection system (mostly fiber optics), detection system (like echelle spectrometer equipped with ICCD camera) and computer and electronics system for: a) synchronization of the laser, spectrometer and camera, b) detector gating and c) spectrum storage. In this experiment, see setup in Figure 1a, we used a Nd-YAG laser with energy of 50 mJ, pulse duration of 20 ns focused by use of a 5 cm diameter lens L_1 having a focal length of 10 cm. The aluminum alloy target was positioned 2 mm in front of the focal point, so the 0.2 mm spot, *i.e.*, laser fluence of ≈ 1.5 J/mm^2 was obtained. In order to perform the study in a controllable environment, the target was set inside a specially designed cross-shaped glass tube, see Figure 1b. The discharge part of the tube (having an internal diameter of 10 mm and tungsten electrodes 12 cm apart) was positioned perpendicular to the laser beam. This part of the tube has connectors for gas inlet and outlet and quartz windows for plasma observation. A laser beam entering through an upper glass window passes through the other branch of the tube and irradiates the target. A target surface was positioned in line with the bottom of the discharge tube, maximizing the recording volume of the plasma. In order to increase the optical signal reproducibility the target was rotated with a speed of ≈ 2 °/s.

Appropriate gas pressure in the tube was established with the help of a vacuum pump and gas handling systems consisting of gas bottle, regulation valve, needle valve and manometer. The fast pulse discharge is driven by a set of capacitors – C, charged with high voltage power supply – HV PS fired by the same laser pulse. By applying stabilized voltages up to 3 kV and using different C and current limiting resistor critically damped currents lasting several microseconds are realized.

A 1:1 plasma image was projected on the entrance slit of the 0.3 m imaging spectrometer Andor Schamrock 303, equipped with Andor ICCD iStar DH 720-18F-03 camera. The camera gating was performed by processing the Q switch signal from the laser with Stanford Research DG535 digital delay generator – DDG. The spectra were recorded at different delays between laser and current pulse (measured with Rogowski coil) and Tektronix TDS320 digital storage oscilloscope – DSO.

RESULTS

The measurements in atmospheric air were used as reference data for comparing optical signal intensities in different experimental conditions. This measurement was conducted without the use of air flow, additional fast discharge and even without use of the end windows at the discharge part of the tube. Consequently, the conditions similar to the ordinary single pulse LIBS were obtained. The spectra emitted from the layer 1.5 mm apart from the target surface was recorded with an input slit width of 10 micrometers and camera full vertical binning – FVB. Thus, spatially integrated intensities, along this most intense part of the plasma plume, were recorded. In order to obtain the best signal to noise ratio, all spectra recordings

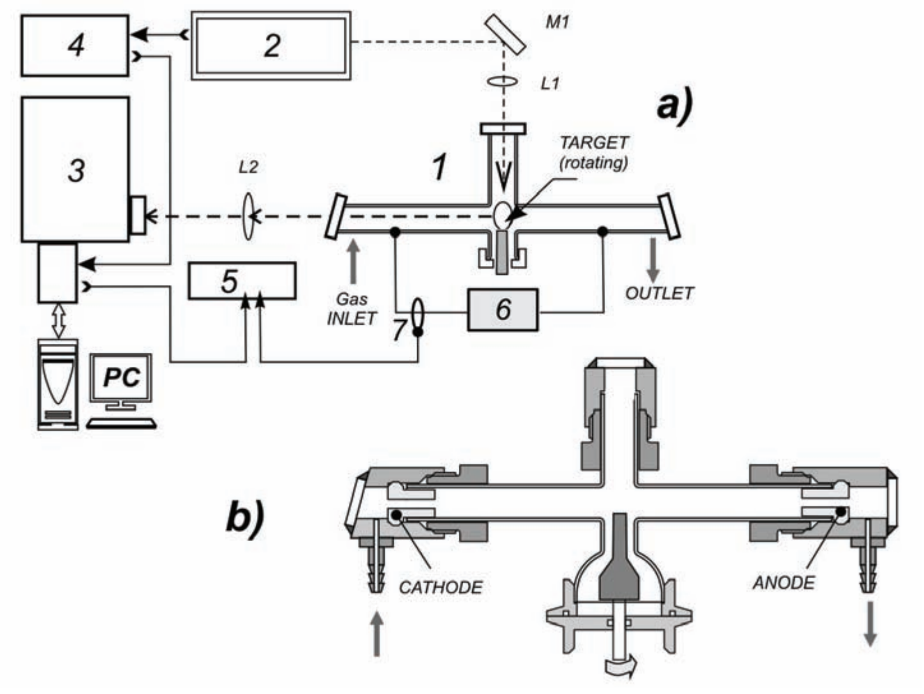

Figure 1. a) Experimental setup and b) detailed view of the glass tube (1) for laser-target interaction studies. 2 – Nd-YAG laser, 3 – imaging spectrometer, 4 – digital delay generator, 5 – digital storage oscilloscope, 6 – high voltage power supply, 7 – Rogowski coil.

(from 300 to 800 nm) were recorded with a delay of a 600 ns after a laser pulse with a gate of 8 μs with the accumulation of the ten pulses. Such gating is a standard LIBS procedure which prevents the recording of pronounced continuum radiation in first 600 ns and accumulation of optical or electrical noise after 8.6 μs in the studied case, *i.e.*, after the moment when the optical signal falls below a few % from their maximum value.

The effect of the surrounding atmosphere on optical signal intensity

The study of the surrounding atmosphere influence on the optical signal intensity spectra in argon and helium at 30 mbar was performed under the same experimental conditions. The part of the spectrum, presented in Figure 2a, clearly shows that the observed optical signals were higher for Ar and lower for He gas, compared to the signal obtained in an air atmosphere.

The enhancement was attributed to the lower ionization potential of Ar leading to the higher electron number density, as observed earlier by testing at atmospheric pressures [10]. The spectra in argon were also recorded at different pressures: 1, 5, 10, 20, 30, 50, 100, 150, 200, 300, 400, 700 and 1000 mbar. It is shown that the signal enhancement changes with the gas pressure, see Figure 2b. The optimum argon pressure in our case is 150 mbar. The enhancement is the same, not only for the most pronounced aluminum lines, but also for the lines of the different constituents of plasma. This is illustrated in Figure 2, where part of the spectra with characteristic lines of the aluminum, copper and magnesium are presented. Such enhancement is of fundamental importance for a more precise determination of alloy composition (in studied case), detection of impurity traces in pure metals or determination of various samples composition.

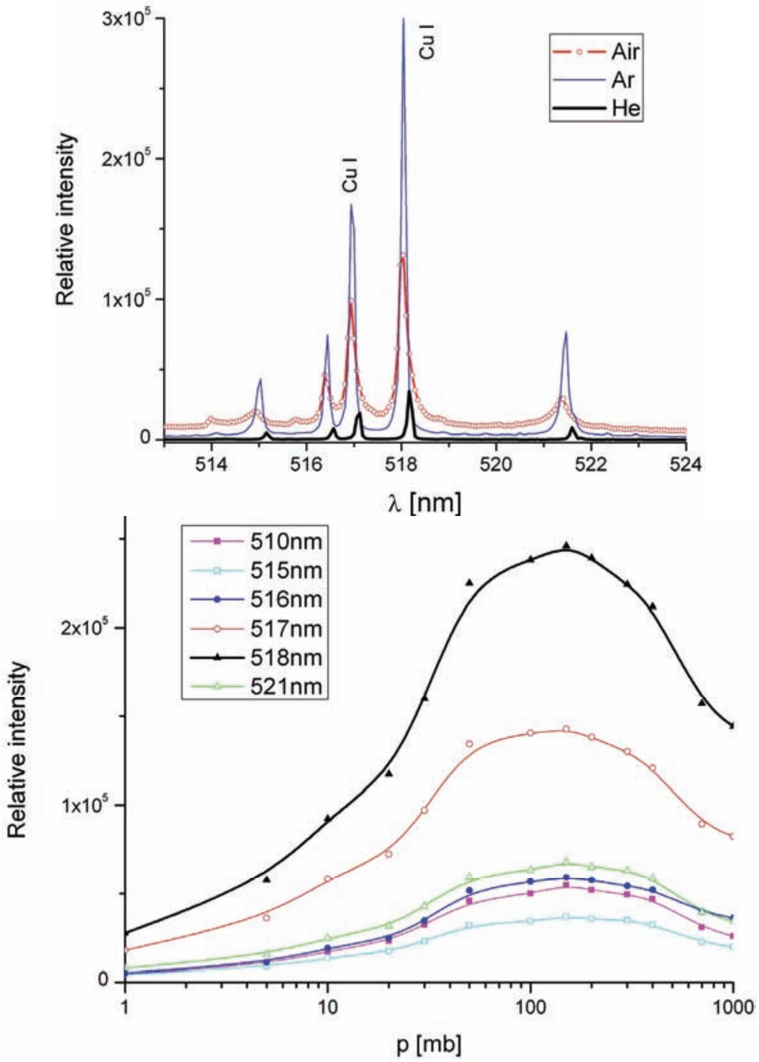

Figure 2. a) Part of the spectrum emitted from Al alloy target in air at 1 bar and in Ar or He at pressure of 30 mbar; b) relative line intensities for different argon pressures.

The effect of additional fast electric discharge – FED on signal intensity

The investigations of the optical signal enhancement by the use of the additional fast discharge, earlier performed only in air at atmospheric pressure [11] were broadened to studies in air, Ar and He at different pressures. In order to find the conditions for the enhancement of analytical line intensities, it is important to ensure that fast pulse discharge will be initiated by laser pulse after a minimum delay. This condition arises from the requirement that most of the evaporated target material will still be present inside the observation volume during the establishing of the discharge. Since the propagation of laser produced aluminum plasma is very fast, see, *e.g.*, [12], this delay time must be as small as possible. The duration of the evaporated material presence between the electrodes was first increased by lowering the flow rate of the surrounding gas by use of a needle valve inserted between the discharge tube and the vacuum pump. Moreover, the delay between the laser and the current pulse decreases with the increase of the applied voltage, which is limited by self-breakdown. Hence the necessity to determine the dependence of the self-breakdown voltage on pressure of used gases for our inter-electrode distance, *i.e.*, so-called Pashen curves, exists, see Table 1.

The initial study with argon at a pressure of 30 mbar and with a capacitor $C = 14.5$ μF charged up to $U = 2$ kV produced a significant but suboptimal increase of the line intensities, see Figure 2a. Although this voltage is almost half of the breakdown voltage (see Table 1), the delay between laser and current pulse is still smaller than the residence time of the target material in the discharge tube. Presence of target material during discharge is verified by measuring time evaluations of the emitted spectra. Measurements were performed with a fixed gate width of 0.2 μs, at different delay times with a variable step: 0.6 μs; 1–10 μs (step = 1 μs); 10––50 μs (step = 5 μs) ; 50–100 μs (step = 10 μs). Special attention was devoted to the characteristic lines of various plasma constituents: Mg I ($\lambda = 383$ nm); Cu I ($\lambda = 515$ nm); hydrogen H_α ($\lambda = 656$ nm); Al II ($\lambda = 705$ nm); and in II diffraction order Mg I ($\lambda = 766$ nm); Al I ($\lambda = 790$ nm).

In order to confirm the value of the spectral line intensity enhancement induced by the application of high voltage only, the comparison was performed by recording spectra at the same Ar gas pressure with two different gate widths. The first one corresponds to the time period before starting the discharge, while the other one includes variable times after starting FED. In the case when C = 14.5 μF and $U = 2$ kV the gates are 4 and 8 μs, respectively. In both cases we used a delay of 600 ns. These measurements, see Figure 3a, showed that the number of the spectral lines and their intensities are significantly increased. The reasons are more pronounced excitation of the Ar, as well as elements present in the target, glass tube and electrodes. Multiple ionization of all elements also occurred. With such a rich spectra it is difficult to resolve analytical lines due to overlap with other lines. This fact limits the application of these spectra for precise detection of alloy composition. Here, we must mention that all measurements are performed in the wavelength range 350–800 nm, due to spectrometer limitations. However, the appearance and intensities of the ion lines in the studied range are a strong indication that the use of the ion lines below 300 nm or above 800 nm under this conditions can be the same or even favourable compared to the other spectroscopic techniques, as also stated in description of LA-FED applications in atmospheric air for rapid detection of carbon in soils [13]. So, conclusion of inapplicability of these LA-FED conditions must be restricted only to the visible part of the spectra. In order to enable the application of LA-FED in the visible part of the spectra it was necessary to reduce the energy dissipated to the discharge. The first attempt of reducing the energy was by lowering the voltage, which caused the start of the discharge with significant and variable delay after the laser pulse i.e. pronounced non-reproducibility. Another attempt, to use a small capacitor of 0.1 μF lead to the establishment of the glow discharge due to the low RC constant of the charging circuit. Namely, after a first initiation of the discharge by a laser pulse, the capacitor was so rapidly recharged that transition to glow occurred. All spectral lines were very weak and synchronization of the camera with a laser pulse, *i.e.*, evaporation of the target material was impossible. On the contrary, the charging of a slightly bigger capacitor $C = 0.33$ μF up to 2 kV created a discharge with a delay of 20 μs. The spectral lines recorded are broader, but with almost the same peak intensity, see Figure 3b. These lines are superimposed on a very pronounced background even tens microseconds after the laser pulse. This significantly reduces signal to noise ratio and makes it inadequate for analytical applications.

Table 1. Measured dependence of breakdown voltage (U / V) in Ar and He

Athmosphere	*p* / mbar											
	0.75	1.5	2.25	3	4.5	6	7.5	11.25	15	22.5	30	37.5
Ar	600	950	1050	1200	1400	1600	1800	1950	2300	2700	3400	3800
He	2400	1000	600	800	950	1000	1050	1100	1250	1500	1650	1850

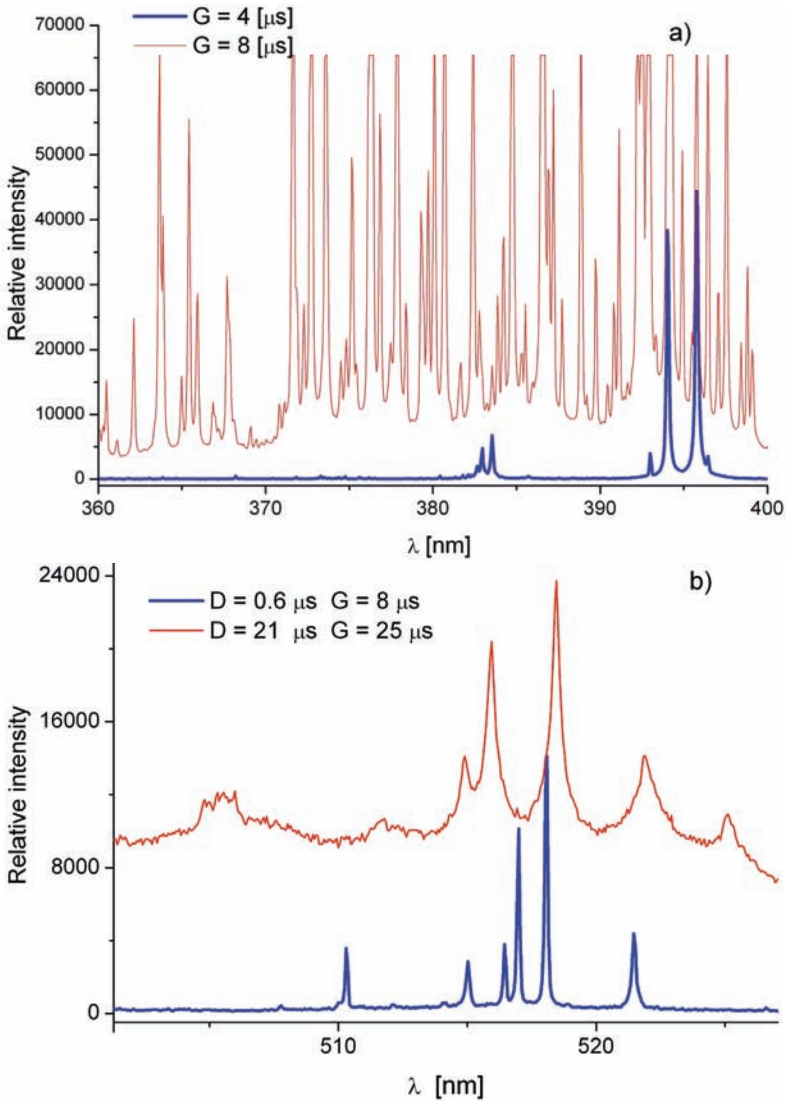

Figure 3. Example of the comparison of optical signals in 30 mbar of Ar with U = 2 kV at different delays – D and gates – G; a) D = 600 nm, C = 14.5 μF and b) C = 0.33 μF.

A series of measurements in air at a pressure of 10 mbars were also conducted, but the results were the same or worse than in argon. Namely, the obtained spectra are even more complicated, with lots of molecular band overlapping with the spectral lines of the target constituents.

In order to reduce the number of interfering spectral lines the investigation was extended to the use of the helium at pressure of 30 mbar. This pressure was chosen in order to facilitate comparison with measurements in argon. Given the influence of the voltage on the delay between the laser pulse and the discharge and the dependence of the self-breakdown voltage on pressure presented in Table 1, the capacitor voltage (C = 0.33 μF) did not exceed 1 kV. So, measurements of the spectra were performed in the same way as in argon but with different D and G values. Namely, the spectra characteristics for the ordinary SP-LIBS disappear 20 μs after a laser pulse, while after a 45 μs discharge in helium with the evaporated target material started.

Comparison of the spectra presented in Figure 4 shows that line intensities with application of fast discharge are more than two times greater than in SP-LIBS for the same gate G = 20 μs. Also, the prolonged duration of the plasma with discharge enables use of a larger gate width. Under these conditions, the order of magnitude increase of the line intensities is presented in Figure 4.

The enhancement of signals, shown in Figure 4, for He at 30 mbar and C = 0.33 μF, may be attributed to the increase of plasma length as well as to its prolonged duration. It should be stressed that significant enlargement of the radiation volume in the other direction also exists, whenever voltage is applied. It is clearly visible with the naked eye that the discharge fills

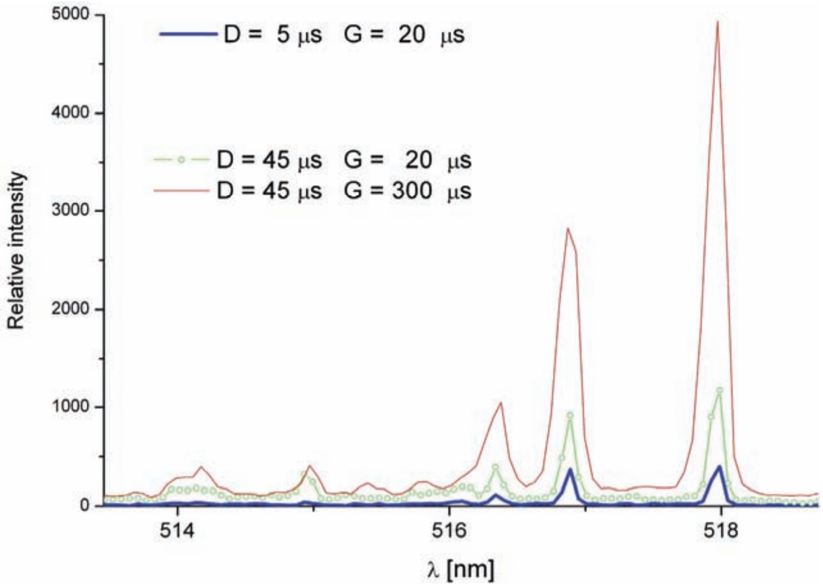

Figure 4. Example of the comparison of optical signals in He at p = 30 mbar, C = 0.33 μF and U= 1 kV at different delays – D and gate widths – G.

the whole discharge tube, while without applying voltage there is only a small plasma plume in front of the target. As stated before, all comparisons are performed for measurements at 1.5 mm with a slit width of 10 μm. This plasma volume increase additionally enhances the spectral line intensities in comparison to SP-LIBS, thus making this method comparable to other analytical techniques.

SUMMARY AND CONCLUSION

A laser initiated fast discharge, as an alternative method for enhancing the line intensities in a single pulse laser induced breakdown spectroscopy for the spectrochemical applications, was studied. Studies in various surrounding atmospheres with and without application of the fast electric discharge were enabled by constructing a cross shaped discharge tube with a 10 mm inner diameter and tungsten electrodes positioned 12 cm apart, see Figure 1b. The investigations were performed by measuring the time evaluation of the spectra from 350 to 800 nm with different delays and different gate widths. In all studied cases, the spectra were recorded by an imaging spectrometer equipped with a ICCD camera, only in the periods when a pronounced continuum radiation extinguish. The special attention was devoted to the recording of the characteristic lines of various elements present in targets like Mg I (λ = 383 nm); Cu I (λ = 515 nm); hydrogen H_α (λ = = 656 nm); Al II (λ = 705 nm); and in II diffraction order Mg I (λ = 766 nm); Al I (λ = 790 nm).

The measurements in different surrounding atmospheres without applying any voltage clearly show that the observed optical signals were higher for Ar and lower for He gas, compared to the signal obtained in an atmospheric air, see Figure 2a. The enhancement was attributed to the lower ionization potential of Ar leading to the higher electron number density, as observed earlier by testing at atmospheric pressures [10]. It is shown that the signal enhancement changes with the gas pressures, see Figure 2b. The optimum argon pressure in our case is 150 mbar.

Spectral line intensities enhancement with applications of the fast electrical discharge initiated by the same laser pulse was studied by charging capacitors of 14.5; 0.33 and 0.1 μF to different voltages between 1 and 3 kV. The voltage was determined from conditions: a) to be lower than self-breakdown voltage whose dependence on gas type and pressure was previously determined and b) high enough to minimize delay between the laser pulse and discharge starting. The second condition arises from the requirement that the most of evaporated target material would still be present within the observation volume during establishing of the discharge. The capacitance, gas type and pressure were determined from conditions: a) prevention of glow discharge development after laser initiation, b) lowering energy losses, due to the multiple ionizations of elements present in plasma, c) lowering of the background caused by overlapping of broad closely spaced spectral lines and, of course, d) enhancement of signal/ /background ratio by realization of more intense, longer lasting and larger volume plasma. It is shown (see Figure 3a) that discharging of the 14.5 μF capacitor previously charged up to 2 kV in 30 mbar of argon leads to intense spectra inappropriate for analytical application. This conclusion holds only for the recorded part of the spectra between 350 and 800 nm, while observation and intensities of the ion lines range are a strong indication that the use of the ion lines below 300 nm or above 800 nm under these conditions can be the same or even favourable to the other spectroscopic techniques.

Decreasing of a number of interfering lines in the visible part of the spectra by use of the He at *p* = 30 mbar and capacitor of 0.33 μF charged up to only 1 kV showed, see Figure 4, that S/B ratio can thus be increased by one order of magnitude. Recording of spectra from the whole plasma volume, which is also significantly increased, will lead to the further enhancement of optical signals thus making LA-FED comparable with other analytical techniques.

The optimization of the working conditions (gas type and pressure), as well as the use of small nF capacitance and modifications of power supply in order to obtain voltages above 10 kV and periodical nanosecond current pulses which most probably lead to even greater optical signal enhancement, is in progress.

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IZVOD

LASERSKOM ABLACIJOM INICIRANO BRZO ELEKTRIČNO PRAŽNJENJE ZA SPEKTROHEMIJSKE PRIMENE

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(Naučni rad)

U ovom radu ispitivane su različite metode za povećavanje intenziteta spektralnih linija elemenata uzorka pri interakciji laserskog impulsa sa metom od legure aluminijuma. Povećanje optičke emisije osnovni je preduslov za snižavanje praga detekcije spektroskopije laserski indukovanog proboja sa jednim laserskim impulsom. Ispitivanja su vršena pri različitim pritiscima argona, helijuma ili vazduha, sa ili bez dodatnog brzog električnog pražnjenja. Konstruisana je i korišćena cev za pražnjenje u obliku krsta, unutrašnjeg prečnika 10 mm, sa rastojanjem izmedju elektroda 12 cm. Snimana je vremenska evaluacija spektara od 350 do 800 nm sa različitim vremenima kašnjenja i različitim širinama vremenskih prozora. Merenja su vršena pomoću spektrometra opremljenog ICCD kamerom, samo u vremenima posle gašenja intenzivnog kontinualnog zračenja. Merenja u različitim okolnim gasovima na istom pritisku bez električnog pražnjenja pokazala su da su optički signali u Ar veći, a u He manji od signala dobijenih u vazduhu. Maksimalni intenziteti dobijeni su pri pritisku Ar od 150 mbar. Ovo povećanje je najverovatnije prouzrokovano povećanjem gustine elektrona usled nižeg jonizacionog potencijala argona. Istraživanja uticaja brzog električnog pražnjenja na intenzitet zračenja vršena su pražnjenjem kondenzatora kapaciteta 14,5; 0,33 ili 0,1 μF, prethodno napunjenih do napona izmedju 1 i 3 kV. Napon kondenzatora odredjivan je iz uslova da njegova vrednost bude: a) manja od napona samookidanja i b) dovoljno velika radi minimiziranja kašnjenja izmedju laserskog impulsa i pojave pražnjenja. Drugi uslov proizilazi iz zahteva da ispareni materijal mete bude još uvek prisutan u posmatranoj zapremini tokom uspostavljanja pražnjenja. Kapacitet kondenzatora, kao i vrste i pritisci okolnog gasa birani su kako bi se: a) sprečilo uspostavljanje tinjavog pražnjenja, b) smanjili gubici energije usled višestrukih jonizacija elemenata u plazmi, c) smanjilo pozadinsko zračenje nastalo usled preklapanja brojnih spektralno bliskih linija, d) produžilo vreme trajanja i e) povećala zapremina plazme. Optimalno povećanje integralnog intenziteta analitičkih linija od preko dva reda veličine dobijeno je u He pri p = 30 mbar sa kondenzatorom od 0,33 μF napunjenim do 1 kV, vidi sliku 4. Treba napomenuti da je iniciranjem pražnjenja u argonu (p = 30 mbar; C = 14.5 μF; U = 1 kV) dodatno povećana brojnost i intenzitet linija. Zbog preklapanja linija, analitička primena pražnjenja pri ovim uslovima onemogućena je u posmatranoj oblasti spektra, što ne mora biti slučaj u ostalim oblastima.

Ključne reči: Spektrohemija • Analitička spektrometrija • Spektroskopija laserski indukovanog proboja • Optička emisiona spektroskopija

Hemijski sastav i antioksidativna aktivnost etarskog ulja *Thymus serpyllum* L.

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Izvod

Cilj ovog rada bio je ispitivanje hemijskog sastava i antioksidativnog potencijala etarskog ulja majčine dušice (*Thymus serpyllum* L.). Etarsko ulje majčine dušice izolovano je iz osušene herbe *T. serpyllum* postupkom hidrodestilacije. Za hidrodestilaciju etarskog ulja *T. serpyllum* korišćen je originalni poluindustrijski destilacioni uređaj SP-130 koji radi na principu destilacije vodom i vodenom parom. Gasnohromatografskom analizom određene su najzastupljenije komponente kod etarskog ulja *T. serpyllum*: *trans*-nerolidol (24,2%), germakren D (16,0%), timol (7,3%), δ-kadinen (3,7%) i β-bisabolen (3,3%). Etarsko ulje je pokazalo značajno bolju sposobnost neutralizacije slobodnih DPPH radikala (IC_{50} = 0,503 µL/ml) u poređenju sa sintetskim antioksidansima BHA i BHT.

Ključne reči: Thymus serpyllum L., hidrodestilacija, etarsko ulje, hemijski sastav, antioksidativna aktivnost.

NAUČNI RAD

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Majčina dušica (*Thymus serpyllum*) pripada rodu *Thymus* koji u svetu obuhvata oko 300–400 vrsta, sa velikim brojem podvrsta, varijeteta, subvarijeteta i formi, a u flori Srbije zastupljena je 31 vrsta ovog roda [1]. Predstavlja omiljeni lek ne samo u narodnoj nego i naučnoj medicini. Ulazi u sastav velikog broja biljnih preparata koji se izrađuju bilo u apotekama bilo u farmaceutskoj industriji zbog svojih farmakoloških svojstava. Koristi se kao antiseptik, anthelmintik, karminativ, ekspektorans, sedativ, tonik, konzervans, aromatik, stomahik i antispazmotik. Majčina dušica ima raznovrsnu primenu u medicini, farmaciji, prehrambrenoj i kozmetičkoj industriji, zatim u industriji alkoholnih i bezalkoholnih napitaka i industriji boja i lakova [2,3].

Majčina dušica sadrži etarsko ulje koje pokazuje fungicidno, antiseptičko i antioksidativno dejstvo. Etarsko ulje iz herbe se koristi u parfemima, sapunima i pastama za zube. Sadržaj etarskog ulja *T. serpyllum* varira u velikoj meri u zavisnosti od porekla i načina dobijanja i kreće se u rasponu približno od 0,1 do 0,6% [4], ili između 0,1 i 1% [5,6]. Na hemijski sastav i prinos etarskog ulja herbe *T. serpyllum* utiče geografsko poreklo, faza razvoja biljke, vreme berbe, stanište i klimatski uslovi gde vrsta raste [7]. Hemijski polimorfizam je karakterističan za biljke koje pripadaju rodu *Thymus*, i mogu se razlikovati geraniolni, germakren-D, citralni, linaloolni, (*E*)-kariofilenski, α-terpinilacetatni, karvakrolni, timolni i mnogi drugi hemotipovi [8–10]. Određivanje sadržaja etarskog ulja *T. serpyllum* poreklom iz Srbije sa padina Kopaonika, pokazalo je da etarsko ulje sadrži visok procenat seskviterpena (60,5%), dok je sadržaj monoterpenskih komponenti bio znatno niži (37,9%). Dominantna komponenta iz klase seskviterpena bio je *trans*-kariofilen (27,7%), a zatim i γ-murolen (10,5%), α-humulen (7,5%), β-bisabolol (2,6%) i trans-nerolidol (2,4%) [11].

U prehrambenoj industriji koriste se sintetski i prirodni antioksidansi. Oni su prisutni u mnogim namirnicama i imaju ulogu aditiva jer štite hranu od oksidacije. Međutim, zbog toksikoloških razloga prekomerna upotreba sintetskih antioksidanasa je dovedena u pitanje, a zahtevi potrošača su usmereni ka korišćenju prirodnih [12–14]. Fenolni monoterpenoid u etarskom ulju majčine dušice, timol i karvakrol najviše doprinose prijatnom mirisu njegovog etarskog ulja, a poznato je i da inhibiraju lipidnu peroksidaciju i ispoljavaju veoma snažno antimikrobno dejstvo na različite vrste mikroorganizama. U nekoliko radova je proučavano antimikrobno i antioksidativno dejstvo etarskog ulja i ekstrakata tamjana [15–22]. U radu Lee i sar. [22] pokazano je da glavne komponente ekstrakta *T. vulgaris*, posebno eugenol, timol i karvakrol, imaju jaču antioksidativnu aktivnost od sintetskih antioksidanasa kao što su butil-

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ovani hidroksitoluen (BHT) i α-tokoferol. U radu Youdim i sar. [23] etarsko ulje *T. vulgaris* je pokazalo bolje antioksidativno dejstvo na usporavanje lipidne peroksidacije ulja žutog noćurka od askorbilpalmitata i sličnu aktivnost kao α-tokoferol.

Postoji mnogo objavljenih radova o hemijskom sastavu etarskog ulja iz biljaka koje pripadaju rodu *Thymus* [24–26], međutim samo nekoliko studija se bavilo ispitivanjem hemijskog sastava etarskog ulja *T. serpyllum* [11,20,26–30]. Koliko je poznato autorima postoje dva objavljena rada o ispitivanju antioksidativne aktivnosti etarskog ulja *T. serpyllum* poreklom iz Pakistana [28] i poreklom iz Hrvatske [29]. Cilj ovog rada bio je ispitivanje potencijalne antioksidativne aktivnosti etarskog ulja *T. serpyllum* poreklom iz Srbije, kao i ispitivanje njegovog hemijskog sastava.

EKSPERIMENTALNI DEO

Biljni materijal

Biljni materijal *Thymus serpyllum* L. je prikupljen u fazi cvetanja tokom juna 2012. godine u centralnoj Srbiji, na planini Pasjača, u blizini naselja Žitorađe. Prikupljeni biljni materijal je sušen u hladu i samleven do granulacije 0,5 mm.

Izolacija etarskog ulja

Za potrebe ovog rada etarsko ulje majčine dušice izolovano je iz osušene herbe *T. serpyllum* postupkom hidrodestilacije. Za hidrodestilaciju etarskog ulja *T. serpyllum* korišćen je poluindustrijski destilacioni uređaj SP-130 koji radi na principu destilacije vodom i vodenom parom. Temperatura u toku hidrodestilacije u sudu SP-130 se kretala od 100–102 °C, na atmosferskom pritisku, a ceo proces hidrodestilacije je trajao 5 h.

Gasnohromatografska karakterizacija etarskog ulja

Ispitivanje hemijskog sastava etarskog ulja izvedeno je primenom gasne hromatografije uz plameno-jonizujuću detekciju (GC/FID) i kombinacijom gasne hromatografije i masene spektrometrije (GC/MS).

Klasična analitička gasnohromatografska analiza (GC/FID) urađena je na Hewlett–Packard gasnom hromatografu, model HP-5890 Series II, opremljenom *split–splitless* injektorom povezanim sa HP-5 kolonom (25 m×0,32 mm, debljine filma 0,25 µm) i plameno-jonizujućim detektorom (FID). Kao noseći gas korišćen je vodonik (1 ml/min). Etanolni rastvor uzorka etarskog ulja (1 µl, 1% rastvor) injektiran je u *split*-režimu (1:30). Temperatura injektora iznosila je 250 °C, a detektora (FID) 300 °C, dok je temperatura kolone menjana u linearnom režimu temperaturskog programiranja od 40–260 °C (2 °C/min), a potom držana konstantnom na 260 °C 15 min. Za kvantifikacione svrhe procenti površina pikova dobijeni integracijom sa odgovarajućeg hromatograma (GC/FID) uzeti su kao osnova.

Isti analitički uslovi korišćeni su i za potrebe GC/MS analize rađene na HP G 1800C Series II GCD analitičkom sistemu (Hewlett-Packard, Palo Alto, CA, USA), s tim što je tu rađeno sa HP-5MS kolonom (30 m×0,25 mm×0,25 µm) i što je kao noseći gas korišćen helijum. Temperatura transfer linije iznosila je 260 °C. Maseni spektri snimani su u EI režimu (70 eV), u opsegu *m*/*z* 40–450. Uzorak 1% EtOH rastvora etarskog ulja (0,2 µl) injektiran je u split-režimu (1:30).

Identifikacija pojedinačnih komponenata vršena je masenospektrometrijski i preko Kovačevih indeksa, uz korišćenje različitih baza masenih spektara (NIST/ /Wiley), različitih načina pretrage (PBM/NIST/AMDIS, ver. 2.1) i raspoloživih literaturnih podataka [31].

Određivanje antioksidativne aktivnosti etarskog ulja (DPPH metoda)

Relativna antiradikalska aktivnost etarskog ulja ispitivana je pomoću stabilnog slobodnog radikala DPPH (1,1-difenil-2-pikrilhidrazil) koji se često koristi u proceni antioksidantne aktivnosti. DPPH radikal apsorbuje na 517 nm (ljubičaste je boje), pri čemu se apsorbancija znatno smanjuje kada je ovaj radikal izložen hvatačima slobodnih radikala jer dolazi do transfera atoma vodonika antioksidansa na DPPH. Pri tom, smanjena apsorbancija na 517 nm ukazuje na antioksidantni potencijal ekstrakta/etarskog ulja [32].

Pri proceni antioksidantne spospobnosti etarskog ulja majčine dušice korišćene su radne koncentracije rastvora ulja u opsegu 0,052–3,333 µL/mL. Po 10 µL rastvora određene koncentracije etarskog ulja je mešano sa 190 µL metanola i 100 µL metanolnog rastvora DPPH (67,2 µmol/L). Sve probe pripremljene su u mikropločama. Za svaku koncentraciju pripremljene su radne probe u tri ponavljanja i jedna korekcija (slepa proba, tj. apsorbanca ekstrakta bez DPPH reagensa), dok je za celu ploču rađena jedna kontrola (maksimalna količina DPPH, tj. nije dodat ekstrakt).

Nakon 60 min inkubacije na sobnoj temperaturi, u mraku, apsorbancije dobijenih rastvora očitavane su spektrofotometrijski na 517 nm (čitač mikroploča Multiskan Spectrum, Thermo Corporation).

Kapacitet hvatanja DPPH·radikala (DPPH-RSC) računat je na osnovu sledeće jednačine:

$$\%DPPH-RSC=100-100\frac{(A_{\mathrm{sr}}-A_{\mathrm{kor}})}{A_{\mathrm{kontrola}}}$$

gde je A_{sr} srednja vrednost apsorbancija tri radne probe, A_{kor} apsorbancija samog ekstrakta (bez reagenasa) i A_{kontrola} absorbancija DPPH. Za dobijene vrednosti za RSC (inhibicije) nacrtane je kriva zavisnosti inhibicije od koncentracije etarskog ulja u programu Origin 8 (Origin Lab Corporation), iz kojih je određena IC_{50} vrednost (koncentracija ispitivanog ulja pri kojoj je neutralisano 50% radikala).

REZULTATI I DISKUSIJA

Hemijski sastav etarskog ulja *Thymus serpyllum* L.

Izolovano etarsko ulje *T. serpyllum* L. je tečnost svetlo žute boje, mirisa karakterističnog za rod *Thymus*. Težina osušene biljne mase koja je tretirana iznosila je 5,5 kg. Nakon obezvodnjavanja sa anhidrovanim Na_2SO_4 dobijeno je 4,1 g čistog etarskog ulja. Ostvaren prinos etarskog ulja primenom destilacionog uređaja SP-130 iznosio je ~0,1 %. U istraživanjima *T. serpyllum* poreklom iz Srbije sa padina Kopaonika, sadržaj etarskog ulja je iznosio 3 ml/kg osušene biljke (~0,3%) [11]. U radu Raal i sar. prinos etarskog ulja *T. serpyllum* poreklom iz Estonije sa 20 različitih lokaliteta kretao se u opsegu 0,6-4,4 ml/kg, pri čemu je samo prinos dobijen sa jednog lokaliteta bio u skladu sa standardima Evropske farmakopeje (3 ml/kg) [26]. Destilacijom pomoću vodene pare iz *T. serphyllum* poreklom iz Pakistana ostvaren je prinos od 0,48% [20] i od 29,0 g/kg [28]. Varijacije u prinosima etarskog ulja *T. serpillum* mogu se objasniti različitim agroklimatskim uslovima regiona.

Hemijski sastav etarskog ulja *Thymus serpyllum* L. prikazan je u tabeli 1. Najzastupljenije komponente u etarskom ulju su: *trans*-nerolidol (24,2%), germakren D (16,0%), timol (7,3%), δ-kadinen (3,7%) i β-bisabolen (3,3%) što čini 54,5% od ukupno 65 identifikovanih komponenti. Komponente etarskog ulja su grupisane u pet grupa: monoterpenski ugljovodonici, oksidovani monoterpeni, seskviterpenski ugljovodonici, oksidovani seskviterpeni i ostala jedinjenja. Seskviterpenski ugljovodonici (19 jedinjenja, 35,1%) i oksidovani seskviterpeni (15 jedinjenja, 34,8%) predstavljaju najzastupljeniju grupu hemijskih jedinjenja. Oksidovani seskviterpen *trans*-nerolidol sa 24,2% i seskviterpenski ugljovodonik germakren D sa 16,0% su najzastupljenije komponente u ispitivanom etarskom ulju. Klasa monoterpenskih ugljovodonika sadrži 10 jedinjenja (9,8%) od kojih su *p*-cimen (2,1%), mircen (1,6%), trans-β-ocimen (1,5%) i γ-terpinen (1,5%) najzastupljeniji. Etarsko ulje takođe sadrži značajnu količinu oksidovanih monoterpena (19,4%) od kojih su timol (7,3%), *cis*-tujon (1,9%), geraniol (1,4%), 1,8-cineol (1,4%) i terpinil-acetat (1,0%) najzastupljeniji. Ostala jedinjenja među kojima se nalaze i dva jedinjenja koja nisu indentifikovana su zastupljena sa 0,9%. U herbi majčine dušice ukupno je indentifikovano 65 komponenti što predstavlja 99,5% ispitivanog etarskog ulja. Na slici 1 prikazan je normalizovani hromatogram ispitivanog etarskog ulja.

Tabela 1. Hemijski sastav etarskog ulja Thymus serpyllum L.; KIE – eksperimentalno određen Kovačev indeks, KIL – Kovačev indeks dat literaturnim podacima, RRT – relativno retenciono vreme (FID) odgovarajuće komponente u odnosu na odabranu referentnu komponentu, CI – koncentracioni indeks
Table 1. Chemical composition of essential oil of Thymus serpyllum L.

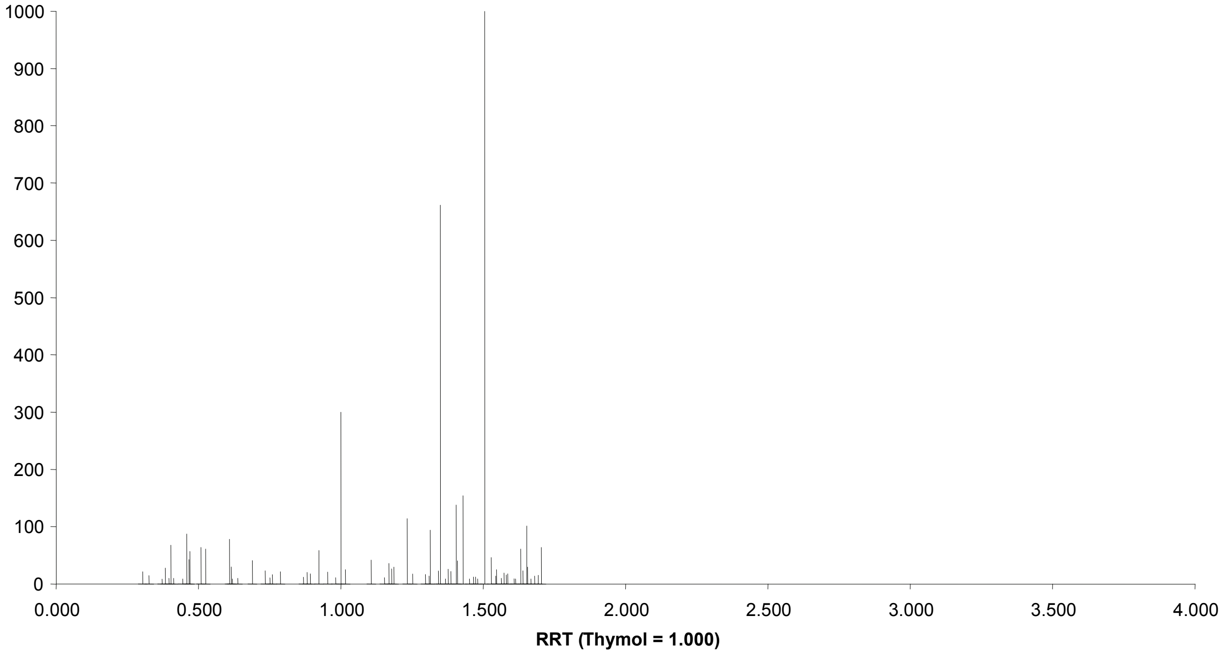
Komponenta	*KIE*	*KIL*	*RRT*	*CI*	mas.%
Monoterpenski ugljovodonici (9,76%)					
α-Pinen	924,9	932	0,304	21	0,51
Kamfen	938,0	946	0,326	15	0,35
Sabinen	973,1	969	0,372	9	0,21
β-Pinen	974,0	974	0,384	28	0,67
Mircen	989,8	988	0,403	68	1,64
α-Terpinen	1011,1	1014	0,444	9	0,21
p-Cimen	1018,8	1020	0,459	87	2,11
Limonen	1021,8	1024	0,466	43	1,03
trans-β-Ocimen	1047,1	1044	0,509	64	1,55
γ-Terpinen	1052,4	1054	0,525	61	1,48
Oksidovani monoterpeni (19,45%)					
1,8-Cineol	1022,7	1026	0,471	57	1,38
cis-Tujon	1099,8	1101	0,609	78	1,89
Linalol	1102,6	1095	0,615	30	0,72
trans-Tujon	1115,0	1112	0,619	9	0,21
α-Kamfolenal	1124,9	1122	0,638	10	0,24
Kamfor	1134,1	1141	0,690	41	0,99
Borneol	1159,6	1165	0,734	23	0,56
Mentol	1170,3	1167	0,752	11	0,26
Terpinen-4-ol	1172,4	1174	0,760	16	0,40
α-Terpineol	1187,9	1186	0,788	21	0,52
Timol methil etar	1232,9	1232	0,869	12	0,29

Tabela 1. Nastavak
Table 1. Continued

Komponenta	*KIE*	*KIL*	*RRT*	*CI*	mas.%
Oksidovani monoterpeni (19,45%)					
Karvakrol metil etar	1242,8	1241	0,882	20	0,49
Timokinon	1246,7	1248	0,894	18	0,43
Geraniol	1263,0	1257	0,923	59	1,42
Geranial	1274,7	1264	0,954	21	0,50
Bornil acetat	1279,1	1287	0,981	11	0,27
Timol	1303,9	1289	1,000	300	7,26
Karvakrol	1313,3	1298	1,017	25	0,61
Terpinil acetat	1343,9	1346	1,107	42	1,01
Seskviterpenski ugljovodonici (35,07%)					
α-Kopaen	1363,8	1374	1,153	11	0,27
β-Bourbonen	1371,4	1387	1,169	36	0,87
β-Kubeben	1379,2	1387	1,179	26	0,64
β-Elemen	1382,0	1389	1,186	29	0,71
β-Kariofilen	1408,3	1417	1,233	114	2,76
β-Kopaen	1415,5	1430	1,253	18	0,42
α-Humulen	1439,8	1452	1,297	17	0,40
Allo-aromadendren	1453,6	1458	1,310	14	0,34
(*E*)-β-Farnesen	1457,7	1454	1,314	94	2,28
γ -Murolen	1466,0	1478	1,343	22	0,54
Germakren D	1472,0	1484	1,349	662	16,02
Epi-bicikloseskvifelandren	1481,2	1493	1,367	9	0,22
Biciklogermakren	1487,1	1500	1,377	26	0,63
α-Murolen	1489,8	1500	1,387	22	0,52
β-Bisabolen	1501,0	1505	1,405	138	3,33
trans-Kalamenen	1510,4	1521	1,410	40	0,97
δ-Kadinen	1512,9	1522	1,429	154	3,73
α-Kadinen	1525,8	1537	1,452	9	0,21
α-Kalakoren	1552,5	1544	1,481	9	0,21
Oksidovani seskviterpeni (34,77%)					
cis-Seskvisabinen-hidrat	1536,9	1542	1,466	12	0,29
Elemol	1543,1	1548	1,474	12	0,29
trans-Nerolidol	1560,5	1561	1,505	1000	24,20
Kariofilen-oksid	1569,6	1582	1,528	46	1,12
Tujopsan-2-α-ol	1575,0	1586	1,544	14	0,34
Viridiflorol	1577,8	1592	1,547	25	0,61
β-Kopaen-4-α-ol	1583,4	1590	1,564	10	0,24
Humulen-epoksid II	1594,7	1608	1,574	19	0,46
β-Oplopenon	1596,5	1607	1,581	16	0,39
1,10-Di-epi-kubenol	1602,2	1618	1,586	18	0,43
Epi-α-kadinol (τ-kadinol)	1630,0	1638	1,632	61	1,47
α-Murolol (torejol)	1636,1	1644	1,640	23	0,57
α-Kadinol	1643,6	1652	1,653	101	2,45
Helifolenol A	1674,7	1674	1,693	15	0,37
Germakra-4(15),5,10(14)-trien-1-α-ol	1681,3	1685	1,704	64	1,54
Ostalo (0,91%)					
1-Okten-3-ol	985,7	974	0,397	10	0,24
3-Oktanol	1002,1	988	0,413	10	0,24

Tabela 1. Nastavak
Table 1. Continued

Komponenta	*KIE*	*KIL*	*RRT*	*CI*	mas.%
		Ostalo (0,91%)			
Nije identifikovana	1615,7	–	1,609	9	0,22
Nije identifikovana	1619,3	–	1,614	9	0,21
		Ukupno: 99,96%			



Slika 1. Normalizovani hromatogram etarskog ulja Thymus serpyllum L.
Figure 1. Normalised chromatogram of the essential oil of Thymus serpyllum L.

U radu koji se bavio analizom etarskog ulja *T. serpyllum* sa područja Kopaonika indentifikovano je ukupno 26 komponenti, što je predstavljalo 98,4% etarskog ulja [11]. U pomenutom radu sadržaj seskviterpenskih komponenti iznosio je 60,5% (dominantno jedinjenje je bio *trans*-kariofilen sa 27,7%), dok je sadržaj monoterpenskih komponenti iznosio 37,9% (dominantno jedinjenje je bio α-pinen sa 6,9%). U ovom radu sadržaj seskviterpenskih komponenti iznosio je 69,84% (dominantna jedinjenja su *trans*-nerolidol sa 24,20% i seskviterpenski ugljovodonik germakren-D sa 16,02%), dok je sadržaj monoterpenskih komponenti iznosio 29,21% (dominantno jedinjenje je bio timol 7,26%).

Uporedna analiza rezultata hemijskog sastava etarskog ulja *T. serpyllum* dobijenih u okviru ispitivanja u ovom radu sa rezultatima iz literaturnih podataka [11,20,26–30] ukazuju na velike suprotnosti i razlike kada se govori o dominantnim jedinjenjima u etarskom ulju, što je najverovatnije posledica uticaja različitih klimatskih i drugih faktora na biosintezu ovih sekundarnih metabolita. Kako je prikazano u tabeli 2, timol i karvakrol, u literaturi pominjani kao dominantne komponente etarskog ulja *T. serpyllum* [33,34] nisu glavne komponente etarskog ulja poreklom iz Srbije, Rusije i Estonije, već samo za etarsko ulje poreklom iz Pakistana i Hrvatske. Interesantno je da je najveća podudarnost sa našim etarskim uljem *T. serpyllum* po pitanju dominantne komponente, nađena u etarskom ulju *T. serpyllum* poreklom iz Rusije, sa područja Altaja [30] (planina Kolivan, 150 m n.v.), kod koga su glavni sastojci ulja bili *trans*-nerolidol (29,8%), 1,8-cineol (14,0%), *cis*-β-terpineol (8,2%), *β*-mircen (4,0%), kamfor (4,0%) i *p*-cimen (3,8%).

Antioksidativana aktivnost etarskog ulja *Thymus serpyllum* L.

Antioksidantna sposobnost etarskog ulja majčine dušice je određivana njegovom sposobnošću da neutrališe DPPH, tj. koliko su komponente etarskog ulja sposobne da doniraju atom vodonika i prevedu DPPH radikal u njegovu redukovanu formu DPPH-H. IC_{50} vrednosti su date u tabeli 3 i iz njih možemo zaključiti da je etarsko ulje majčine dušice ispoljilo znatno bolju aktivnost u odnosu na sintetske antioksidanse BHA, a naročito u odnosu na BHT.

Tabela 2. Uporedna analiza hemijskog sastava (%) etarskog ulja Thymus serpyllum L.
Table 2. Comparative analysis of the chemical composition (%) of essential oils of Thymus serpyllum L.

Literatura	Rezultati istraživanja	[30]	[20]	[27]	[11]	[29]	[28]
Metoda izolovanja etarskog ulja	Destilacija vodom i vodenom parom SP-130	Destilacija vodom	Destilacija vodenom parom	Destilacija vodom -Clevenger	Destilacija vodom-Clevenger	Destilacija vodom-Clevenger	Destilacija vodom-Clevenger
Poreklo	Srbija	Rusija	Pakistan	Turska	Srbija	Hrvatska	Pakistan
Monoterpenski ugljovodonici	9,76	9,5	93,69	81,9	37,9	12,2	22,5
Oksidovani monoterpeni	19,45	41,8				79,4	63,0
Seskviterpenski ugljovodonici	35,07	5,9	–	10,86	60,5	3,5	8,17
Oksidovani seskviterpeni	34,77	40,6				–	2,37
Ostalo	0,91	1,9	–	6,7	–	–	–
Dominantna jedinjenja	trans-Nerolidol (24,2%), germakren D (16,02%), timol (7,26%), δ-kadinen (3,73%) i β-bisabolen (3,33%)	trans-Nerolidol (29,8 %), 1,8-cineol (14,0%), cis-βterpineol (8,2%), β-mircen (4,0%), kamfor (4,0%), *p*-cimol (3,8%)	Timol (53,3%), karvakrol (10,4%), *p*-cimen (8,8%), karen (5,1%), kamfor (4,9%)	2,4,6-Trimetilanizol (73,41%), 3,5-dimetil benzoeva kiselina (5,38%), β-bisabolen (3,67%)	*trans*-Kariofilen (27,7%), γ-murolen (10,5%), α-humulen (7,5%), α-pinen (6,9%), 3-oktanon (6,6%), timol (5,6%), kamfor (3,6%)	Timol (30%), karvakrol (49,4%), γ-terpinen (5,3%), *p*-cimen (5,2%), kariofilen (3,5%)	Karvakrol (44,4%), *o*-cimen (14,0%), α-terpineol (6,47%), α-pinen (6,06%), β-kariofilen (5,25%), 1,8-cineol (3,44%)
Broj ukupno indentifikovanih jedinjenja	65	42	20	35	26	8	35
Ukupno	99,96	96,6	93,69	99,47	98,4	95,1	96,0

Tabela 3. DPPH antiradikalska aktivnost (RSC) etarskog ulja Thymus serpyllum L.
Table 3. DPPH radical-scavenging activity of essential oils of Thymus serpyllum L.

Agens	DPPH IC_{50}
Etarsko ulje	0,50 μL/mL
BHA	0,98 μg/mL
BHT	5,58 μg/mL

U postojećoj literaturi postoje dva objavljena rada o ispitivanju antioksidativne aktivnosti etarskog ulja *T. serpyllum*. Etarsko ulje *T. serpyllum* poreklom iz Hrvatske [29] pokazalo je slabiju sposobnost da neutrališe DPPH radikale od BHA, BHT, tokoferola, askorbinske kiseline i etarskog ulja *T. vulgaris.* Takođe, etarsko ulje *T. serpyllum* poreklom iz Pakistana [28] pokazalo je slabiju sposobnost da neutrališe DPPH radikale od BHT i timola.

Kako je prikazano u tabeli 4, rezultati ispitivanja antioksidativne aktivnosti etarskog ulja *T. serpyllum* dobijenog postupkom vode i vodene pare uređajem SP-130 je pokazalo bolju sposobnost neutralisanja

Tabela 4. Empirijsko istraživanje i literaturni pregled sposobnosti neutralisanja DPPH radikala korišćenjem etarskog ulja roda Thymus
Table 4. Empirical research and literature review about ability to neutralize DPPH radicals using essential oils of the genus Thymus

Etarsko ulje i poreklo	Metoda dobijanja	Antioksidativna aktivnost	Literatura
T. serpyllum, Srbija	Destilacija vodom i vodenom parom uređajem SP-130	etarsko ulje *T. serpyllum* > BHA > BHT	Ovde
T. serpyllum; T. vulgaris Hrvatska	Destilacija vodom-Clevenger	askorbinska kiselina> tokoferol > BHT > BHA > etarsko ulje *T. serpyllum* > etarsko ulje *T. vulgaris*	[29]
T. serpyllum; T. linearis Pakistan	Destilacija vodom-Clevenger	BHT > timol > etarsko ulje *T. serpyllum* > etarsko ulje *T. linearis* > karvakrol	[28]
T. vulgaris, Jemen	Destilacija vodom-Clevenger	BHT > etarsko ulje *T. vulgaris*	[35]
T. vulgaris, Slovačka	Komercijalno etarsko ulje	BHT > askorbinska kiselina> etarsko ulje *T. vulgaris*	[36]

DPPH radikala od sintetskih antioksidanasa BHT i BHA, što nije podudarno sa rezultatima drugih istraživača. Obzirom na dobijene rezultate verovatno antioksidativna aktivnost etarskih ulja roda *Thymus* se ne može pripisati dominantnim komponentama timolu i karvakrolu kao što ističu drugi istraživači. U našem slučaju se verovatno radi o sinergizmu između većeg broja komponenti prisutnih u etarskom ulju u manjim količinama (65 komponenti). Glavne komponente u ispitivanom etarskom ulju bile su *trans*-nerolidol i germakren D. Buduća istraživanja bi trebalo usmeriti na ispitivanje antioksidativne aktivnosti *trans*-nerolidola i germakrena D kako bi se utvrdio njihov doprinos izuzetno jakoj antioksidativnoj sposobnosti etarskog ulja dobijenog postupkom vode i vodene pare uređajem SP-130.

ZAKLJUČAK

U ispitivanom etarskom ulju indentifikovano je 65 komponenti. Za razliku od podataka iz literaturnih izvora, timol i karvakrol nisu glavne komponente u ispitivanom etarskom ulju *T. serpyllum* dobijenom metodom destilacije vodom i vodenom parom uređajem SP-130. Glavne komponente u ispitivanom ulju bile su *trans*-nerolidol, germakren D, timol, δ-kadinen i β-bisabolen. Uporedna analiza rezultata hemijskog sastava etarskog ulja *T. serpyllum* dobijenog u okviru ispitivanja u ovom radu sa rezultatima iz literature je otkrila velike varijacije u hemijskom sastavu etarskih ulja. Sastav kao i antioksidativna aktivnost etarskih ulja roda *Thymus* zavisi od različitih faktora kao što su način gajenja ili sakupljanja, kvalitet obrađene biljne sirovine, geografsko poreklo, klimatski uslovi, postupci obrade i optimizacije tehnoloških postupaka dobijanja (destilacija, ekstrakcija).

Etarsko ulje *T. serpyllum* je ispoljilo značajnu sposobnost neutralizacije slobodnih DPPH radikala, snažnije od BHT i BHA i može se koristiti kao antioksidantna komponenta u obliku dijetetskih preparata za sprečavanje ili usporavanje oksidativnog stresa prouzrokovanog slobodnim radikalima ili kao potencijalni prirodni antioksidans u prehrambenoj industriji umesto sinteskih antioksidanasa BHT i BHA.

Etarsko ulje dobijeno ovim postupkom se može koristiti u prehrambenoj industriji ne samo zato što štiti potencijalni proizvod od oksidacije već i zbog potencijalne biološke i farmakološke aktivnosti, koje je neophodno detaljnije ispitati. Rezultati su pokazali da etarsko ulje *T. serpyllum* može predstavljati važan izvor za proizvodnju i primenu u prehrambenoj industriji nutritivnih suplemenata, komponenata funkcionalne hrane ili prirodnih antioksidanasa.

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SUMMARY

CHEMICAL COMPOSITION AND ANTIOXIDATIVE ACTIVITY OF ESSENTIAL OIL OF *Thymus serpyllum* L.

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(Scientific paper)

Wild thyme (*Thymus serpyllum* L.) is a popular remedy regarding both tradetional and conventional medicine. It is used as the antiseptic, aromatic, expectorant, stomachic, antispasmodic, carminative and preservative substance. For the purpose of this paper, wild thyme essential oil was isolated from the dried herb *T. serpyllum* by hydrodistillation. Original semi-industrial distillation device SP-130 performing distillation by water and steam was used for the hydrodistillation of *T. serpyllum* essential oils. The temperature during the hydrodistillation in the device SP-130 ranged from 100–102 °C at atmospheric pressure, and the whole process lasted 5 hours. The isolated essential oil is a liquid of light yellow colour and the odour characteristic of the genus *Thymus*. Obtained yield of essential oil was 0.08 %, with 65 components identified in the tested essential oil. The most represented chemical groups are sesquiturpene hydrocarbons with 35.1%, and oxygenated sesquiturpenes with 34.8%. The main components of essential oil of *T. serpyllum* were: *trans*-nerolidol (24.2%), germacrene D (16.0%), thymol (7.3%), δ-cadinene (3.7%) and β-bisabolene (3.3%). The essential oil showed significantly better ability to neutralize DPPH free radicals (IC_{50} = 0.503 µL/mL) compared with synthetic antioxidants BHA and BHT. Synthetic chemical compounds such as BHA and BHT are used in food industry as antioxidants due to their ability to prolong the shelf-life of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity, colour changes, degradation of the flavor and loss of nutrient value. In recent years, there is a considerable interest in finding natural compounds that could replace sinthetic antioxidants because of adverse toxicological reports on many synthetic compounds. Lamiaceae herbs and their essential oils or extracts application has proven to be the effective preservation agents for the extension shelf-life of foodstuffs, indicating their potential use in food industry as functional ingredients and food additives. The antioxidant activity of extracts from some Lamiaceae herbs is comparable to that of the most common synthetic antioxidants BHT and BHA. Free radicals are a major cause of many degenerative diseases, such as atherosclerosis, cancer, cardiovascular diseases, inflammatory bowel diseases, skin aging, old age dementia and arthritis. Epidemiological data and randomized clinical trials provide sample indications that antioxidants play a fundamental role in the prevention of cancer and cardiovascular diseases. They act as scavengers of reactive oxygen species and metal chelators that protect human cells and reduce oxidative damages. Natural antioxidants are important in the food industry because they can have a double functionality, that is, they can be useful as a food preservation agents while providing important health benefits for humans by maintaining our health and preventing disease. This investigation showed that the essential oil of *T. serpyllum* can be an important source for the production and application in the food industry as nutritional supplements, functional food components or natural food antioxidants.

Keywords: *Thymus serpyllum* L. • Hydrodistillation • Essential oil • Chemical composition • Antioxidative activity